Synthesis Proposal of Immunosuppressive 9,10-secosteroid A from the Gorgonian Verrucella Umbraculum

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Abstract: The proposal of a total synthesis plan of one of a series of four biologically significant 9,10-secosteroids recently isolated from Verrucella umbraculum, together with several alternative procedures to the synthesis plan, using widely available chemicals, is described in this work. Immunosuppressive Verrucellol A is a 9,10-secosteroid with a bicyclic skeletal framework and an aromatic moiety. Herein, the reported proposal commences via the synthesis of two of the three structural constituents – the α, β-unsaturated aryl ketone vinyl, and the olefin side chain. Later, the main bicyclic ring structure is constructed via a Robinson Annulation reaction, followed by the introduction of the olefin side chain by Grignard reductive addition. Ultimately, an epoxidation-reductive ring opening would complete the synthesis plan.

1 INTRODUCTION

The polycyclic structures of a series of Verrucella 9,10-secosteroids resemble that of vitamin D (Maestro, Molnár, Carlberg 2019), with the two rightmost steroidal saturated rings preserved, and a cleavage of C-9, C-10 bond present. The C-5, C-6 and C-7, C-8 olefin functional groups are reduced, and characteristically, a 3-hydroxy-10-methyl disubstituted benzene ring is attached to the fragmented third ring, that is, in a steroidal framework. Variable saturated and unsaturated substituents are connected to the five-membered ring of the bicyclic system in this series of 9,10-secosteroids, with irregular decorations of hydrogen and hydroxyl groups at various regions. Specifically, four 9,10-secosteroids, together with their twelve derivatives, are isolated utilizing acetone extraction and repeated column chromatography from the Gorgonian *Verrucella umbraculum* collected from the Yongxing Islands (Li, Sun, Tang, Su, Zheng, Zhang 2021). Interestingly, in the immunomodulation assay, the majority of the secosteroids and their derivatives displayed an inhibitory effect towards the proliferation of $CD4^+$ T cells, or commonly known as the T helper cells.

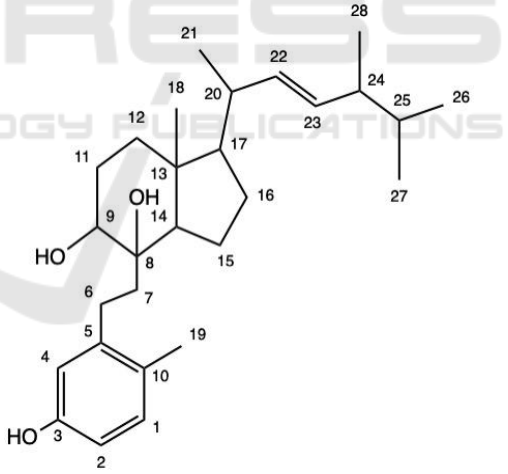

Figure.1: Target Molecule with carbon atoms numbered.

Amongst them, Verrucellol A, which features a C-22, C-23 unsaturated hydrocarbon side chain and an 8,9-diol moiety, displayed particular immunosuppressive efficacy, as compared with its 9-keto and 15-hydroxy counterparts (Li, Sun, Tang, Su, Zheng, Zhang 2021). Therefore, the potential of using Verrucellol A upon treating several autoimmune diseases has been derived, including Type 1 diabetes (Haskins, Cooke 2011), and systemic lupus erythematosus (SLE)(He et al 2016), as both these

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disorders feature $CD4^+$ T cells malfunction. The existing immunosuppressive pharmaceuticals are mainly glucocorticoids, which are sometimes prone to a list of psychological and cognitive adverse effects (Judd et al 2014, Correction 2014). This highlights the potential of using 9,10-Verrucellol A in replacement of the existing glucocorticoid treatments (once the drug efficacy and toxicity are determined with further investigations), as quite significant structural differences are present between the two genres of immunomodulatory molecules (Maestro, Molnár, Carlberg 2019, Li, Sun, Tang, Su, Zheng, Zhang 2021). Owing to the immunosuppressive effects Verrucellol A features towards the proliferation of $CD4^+$ T cells, and its better performance than its counterparts, and the existing adverse effects of glucocorticoids immunomodulatory treatments, we were motivated to propose a synthesis plan of this molecule. Several suggestions of reaction conditions are also included at critical procedures of the synthesis.

2 RETROSYNTHETIC ANALYSIS

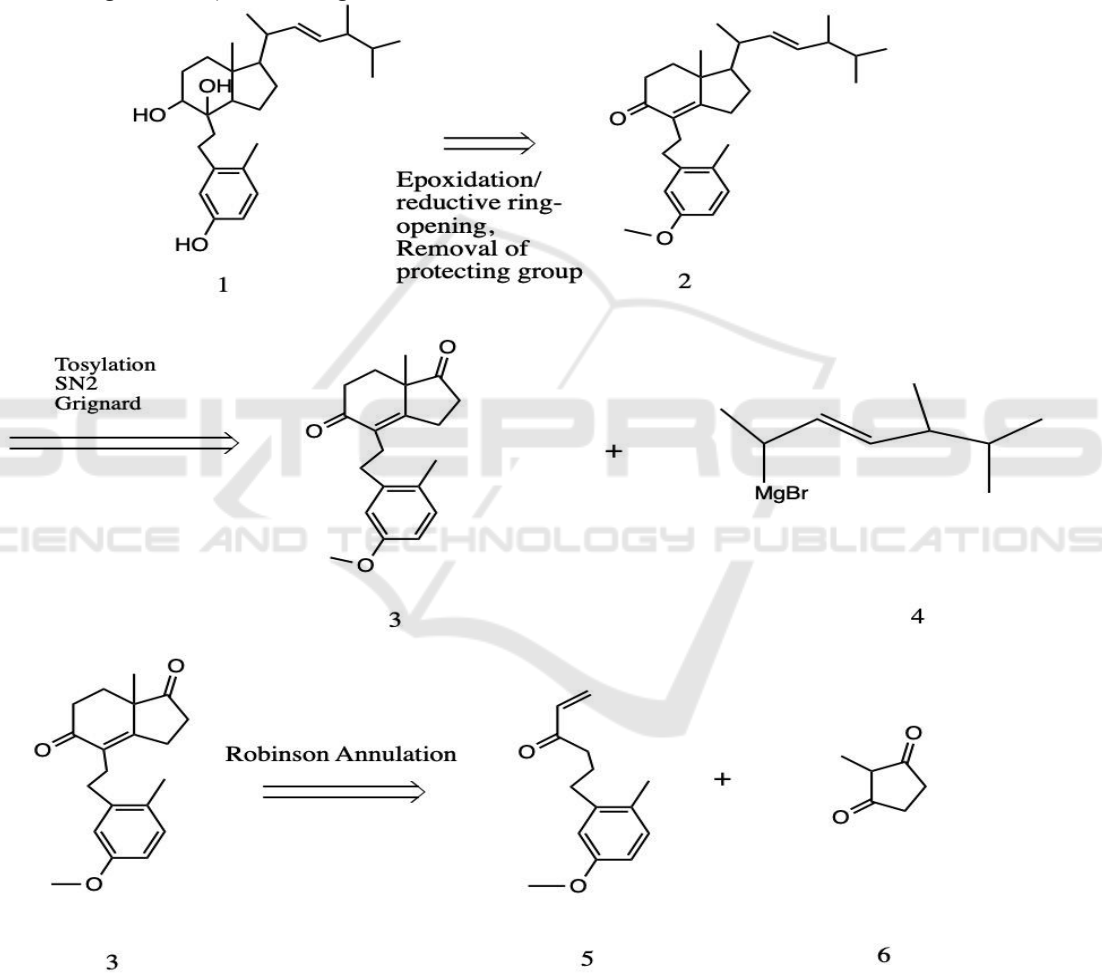

Figure 2: Retrosynthesis of 9,10-Verrucellol A.

In the analysis of Verrucella 9,10-Secosteroid's structure, so as to devise the retrosynthetic strategy, it is noticed that if the diol at C-8 and C-9 on molecule **1** can be converted to an α, β-unsaturated ketone, the structure is very close to a product of Robinson Annulation. On the other hand, since the alcohol on the benzene ring can be very reactive to bases, it is decided to protect it with a methyl group until the last step of synthesis. For the olefin side chain at the top right, it can be added to the 5-membered ring via a Grignard reaction. It is concluded that the alkyl group should be added after the Robinson Annulation for a cleaner reaction. Since molecule **6** is commercially available, the main goal is to synthesize molecule **4**

and molecule **5**. It is envisioned that molecule **4**, which has an olefin on it, can be assembled through a Wittig reaction. After that, the Riley oxidation and a substitution reaction can contribute to synthesize molecule **2**. Considering the fact that an α, β-unsaturated ketone is quite electrophilic, it is decided to add the terminal olefin last when constructing molecule **5**. Thus, the synthesis plan is proposed to start with a benzene derivative **11**, then extend the carbon chain and make a Weinreb amide, which an addition of Grignard reagent can result in the desired molecule **5**. (Schematic diagram of retrosynthetic strategy see Figure 2.)

3 FORWARD SYNTHESIS PROPOSAL

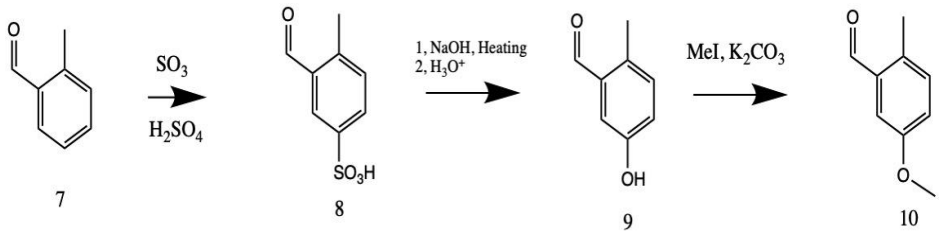

Figure 3: Synthesis of ether-protected aryl constituent 10.

The proposal of the synthesis of Verrucellol A begins with the regioselective synthesis of ether-protected aryl constituent **10**. Due to the commercial availability of 2-methyl benzaldehyde **7**, this is chosen as the starting material of the procedure. By regioselectively sulfonating the *meta*- position with respect to the aldehyde, followed by reflux heating with NaOH and then an acidic workup, the *meta*-regioselective hydroxylation of the benzene derivative can be achieved. In order to prevent the phenol **9** from further reactions with alkaline conditions described below, the use of methyl ether as its protection is incorporated (MeI, K_2CO_3, acetone, reflux) (Greene, Wuts 1999a). This series of processes (see Figure 3) should give the ether-protected aryl constituent **10** in quite good yields.

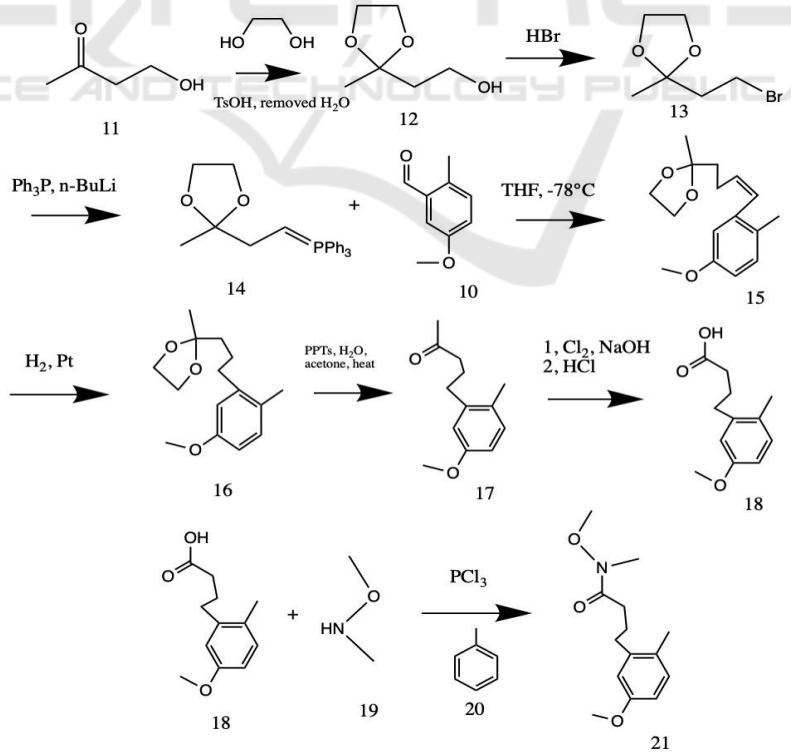

Figure 4: Forward Synthesis of Weinreb Amide **21.**

The synthesis of ether-protected aryl ketone vinyl **5**, which is vital to the construction of the six-membered ring of the bicyclic skeleton, commences with commercially available 4-hydroxy-2-butanone **11**. In order to install the carbon chain from C-5 to C-8 (same labelling as in Verrucellol A molecule) via a Wittig reaction, it is necessary to substitute the hydroxyl group of butanone **11** into a PPh_3 moiety, making it the Wittig reagent **14**. This can be actualized by ketal protection of the carbonyl (ethylene glycol, tosylic acid, benzene, reflux) (Greene, Wuts 1999b), and then bromination utilizing HBr; the bromine substituted ketal **13** can then be treated with PPh_3, followed by n-butyllithium deprotonation, which yields the desired phosphonium ylide **14**. The Wittig olefination can thus be commenced by adding the ether-protected aryl constituent **10** to ylide **14** in THF (Maryanoff, Reitz 1989), generating the 6,7-*cis* unsaturated aromatic ketal **15**. This ketal **15** can then be reduced by hydrogen gas over platinum so as to establish the saturated C-6, C-7 σ system. Platinum has been selected as the transition-metal catalyst as it is almost inactive towards other reactions to olefin than hydrogenation, such as olefin *cis-trans* isomerization (Bond, Wells 1965). The saturated ketal **16** is then subjected to deprotection at carbonyl to yield the aryl ketone **17** (PPTs, H_2O, acetone, heat) (Greene, Wuts 1999b). As linking the vinyl moiety to the aromatic ketone **17** via Grignard addition with Weinreb amide **21** is intended, the conversion of aryl ketone **17** into its carboxylic acid derivative **18** is a sound decision. It is envisioned that this functional group interconversion can be realized by a chloroform oxidation (excess Cl_2, NaOH, methyl alcohol, then HCl workup), for comparing with other halogen counterparts, hypochlorite facilitates the reaction kinetics most, raising the yield and simplifying the separation procedures rather significantly (Fuson, Bull 1934, VanArendonk, Cupery 1931). The desired Weinreb amide **21** can be yielded from the carboxylic acid 18 in a one-pot, transition-metal-free reaction with *N, O*-dimethylhydroxylamine **19** (PCl_3, toluene **20**)(Huang, Hu, Niu, Wang, Xu, Su, Fu 2013). This method is no longer prone to moisture and air sensitivity during storage, which may sometimes become problematic (Niu, Zhang, Huang, Xu, Wang, Hu 2009). (Schematic diagram see Figure 4.)

Figure.5 Grignard Addition to Weinreb Amide **21** to form molecule **5.**

Then, the ether-protected aryl ketone vinyl **5** can be easily granted by adding the vinyl Grignard **22** to the Weinreb amide **21** in THF followed by an HCl workup, as the formation of a stabilized O-Mg-O chelating intermediate in this process prevents double addition of vinyl moiety. This is an intermediate that can be easily removed by an acidic workup to generate the desired α, β-unsaturated ketone **5** (Nahm, Weinreb 1981). (see Figure 5)

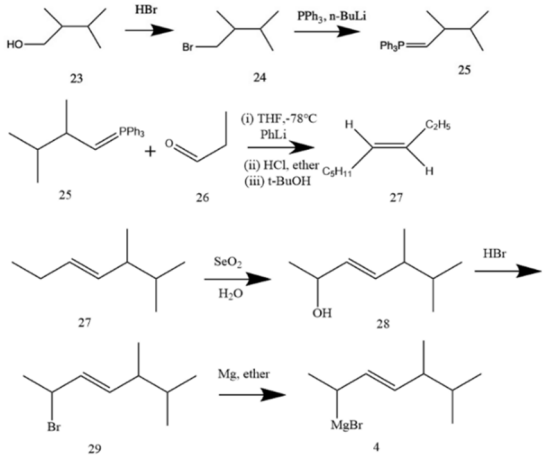

Figure 6: The Synthesis of Grignard Reagent **4.**

The synthesis of the olefin side chain (see Figure 6) and its attachment to the main framework could be initiated by introducing 2,3-dimethylbutanol **23**, which is widely accessible. In an attempt to prepare a proper ylide for future reactions, a triphenylphosphine group could substitute the OH group on the α carbon. A proposed method is to substitute via HBr as such reactions take place under relatively milder conditions. The yielded 1-bromo-2,4-dimethylbutane **24** could subsequently react with PPh_3 under strongly basic conditions (for example, with the presence of n-BuLi), therefore producing the desired ylide **25**. A Schlosser-modified Wittig reaction with propionaldehyde **26** could then be conducted to give 5,6-dimethyl-3-heptene **27**, which features the desired *E*-stereoselectivity (Sondheimer, Mechoulam 1957, Schlosser, Christmann 1966) with a subsequent workup. With Schlosser modification, at a low temperature and basic conditions, the oxaphosphetane intermediate formed (Vedejs, Marth 1990, Vedejs, Snoble 1973) favors the formation of *E*-stereoisomer concomitantly due to the stabilizing effect of lithium to the ylide and the repulsion between the bulky moieties.

The olefin side chain could be attached to a bicyclic ring via a Grignard reaction. To prepare the Grignard reagent **4**, allylic hydroxylation by selenium dioxide could be applied in an attempt to oxidize the C-20 and prepare an enol. Such method introduces a hydroxyl group selectively without any structural rearrangement, whereas most other methods of oxidizing allylic carbons do not have this advantage of regioselectivity and would often prefer reacting with the tertiary carbon, therefore leading to undesired products. Studies have shown that it is a sterically-driven reaction (Stephenson, Speth 1979). There are two allylic carbons where an addition could take place—a secondary carbon, C-20, and a tertiary carbon, C-24. Therefore, the addition of OH group would, presumably, take place on C-20. To complete the preparation of the olefin side chain, the OH group is substituted by Br so as to proceed to prepare the Grignard reagent **4**. With ether as a solvent, magnesium reacts with 2-bromo-5,6-Dimethyl-3-heptene **29** to give the final Grignard reagent **4** (Seyferth 1979).

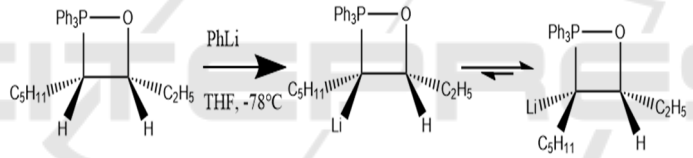

Figure.7: Schlosser Modification Mechanism.

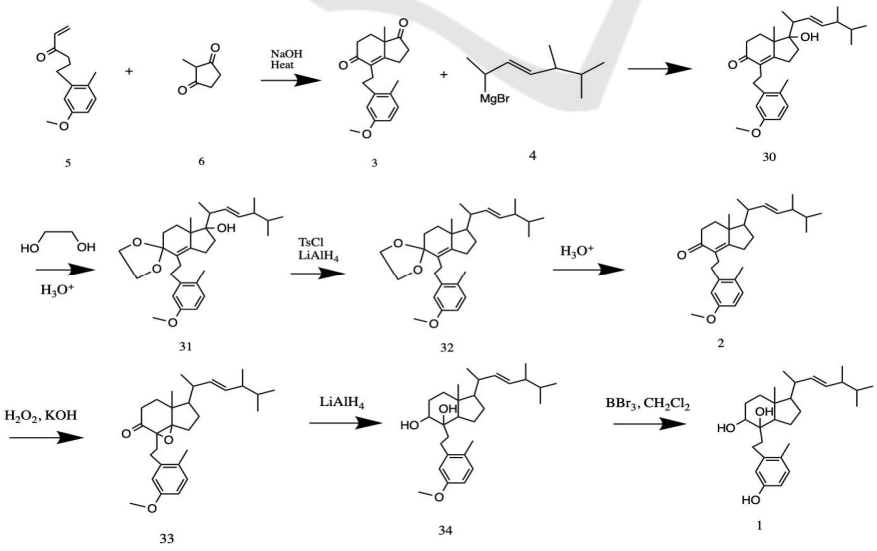

Figure.8: Robinson Annulation and Reductive Ring Opening to reach target molecule.

With molecule **4** and **5** synthesized, it is ready to assemble the target molecule. The proposed method commences by executing a Robinson annulation with molecule **5** and **6** and results in molecule **3** (Li 2009). There are currently two carbonyls on molecule **3**. Since the α, β-unsaturated ketone is conjugated, its resonance effect makes it less likely to react with Grignard reagent **4** than the other ketone (at C-17). Therefore, the addition of Grignard reagent **4** will be more likely to happen at C-17. In order to eliminate the extra OH group (at C-17) formed during Grignard reaction, the ketone at C-9 is first protected with ethylene glycol (ethylene glycol, tosylic acid, benzene, reflux) (Greene, Wuts 1999b); then, the hydroxy group at C-17 is tosylated to make it a better leaving group, followed by the use of $LiAlH_4$ as a base to substitute the tosyl group with an H, resulting in molecule **2**. In order to prevent the α, β-unsaturated ketone from reacting with $LiAlH_4$, ketal (ethylene glycol, tosylic acid, benzene, reflux) is used to protect it until OH group at C-17 is eliminated; molecule **32** is then subjected to de-protection (PPTs, H_2O, acetone, heat) (Greene, Wuts 1999b). The next step of the synthesis is to reduce the ketone to an alcohol and add an OH to the olefin at α-position. Such procedure is proposed as it achieves both of them through a reductive epoxide opening reaction. H_2O_2 and KOH are used to construct an epoxide at the α-position, then $LiAlH_4$ will be added. Since ketone is an electron withdrawing group, it makes the olefin at C-8 more reactive to nucleophilic epoxidation than the olefin at C-22 with alkyl electron donating groups around it. $LiAlH_4$ first reduces the carbonyl to an alcohol, then the Al will chelate with both oxygen atoms; therefore, when the $LiAlH_4$ attacks the epoxide, it is envisioned to attack C-14, subsequently opening the epoxide ring and forming molecule **34** (Diehl 1937). The last step of the synthesis is to de-protect the MeO group (BBr_3, CH_2Cl_2) on the benzene ring, which gives us the target molecule (Greene, Wuts 1999a).

4 EVALUATIONS

The purpose of this proposal is to offer an insight into the synthesis of a potentially biologically significant molecule, so experimentations are necessary when realizing this scheme in laboratories. To further elaborate the synthesis, efforts could be endeavored to select a route that features diastereoselectivity of 9,10-Verrucellol A. Nonetheless, we will include below several suggestions which could be taken into account if unexpected yields are derived from particular procedures.

As sometimes, too reactive reagents (such as strong acids and alkalis, high temperatures and pressures, repetitive heating and so on) might lead to undesired functional group interconversions, which is problematic. One potential manifestation of this repercussion lies with the regioselective synthesis of ether-protected aryl constituent **11**. Included in the aforementioned synthesis scheme is the utilization of the Dow process (sulfonation, NaOH reflux, workup) with 2-methyl benzaldehyde **20**, but from practical perspectives, the high temperature in alkaline conditions may degrade the desired aldehyde functional group.

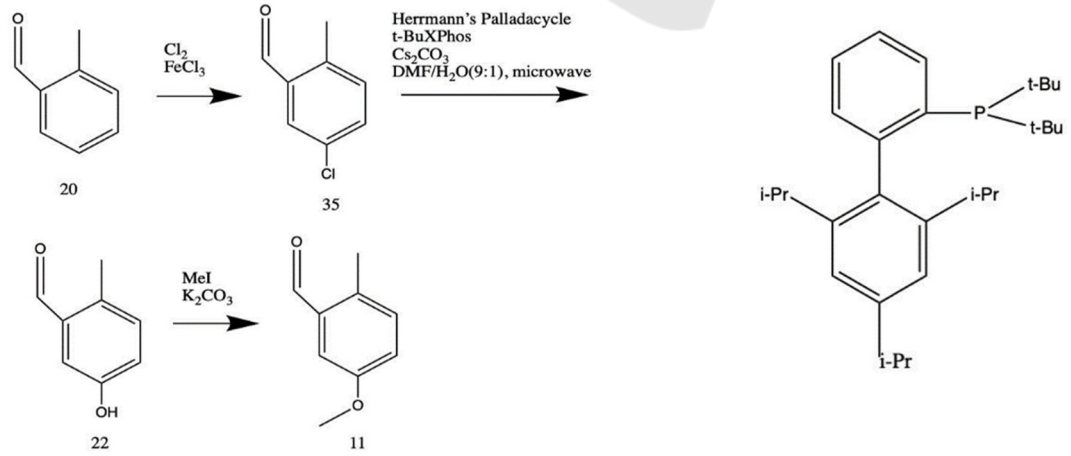

Figure.9: Pd-catalyzed synthesis of ether-protected aryl constituent 11 (left); with structure of t-BuXPhos ligand elucidated (right).

Instead, a palladium-catalyzed, microwave-assisted alkaline hydroxylation of aryl chloride **35** (see Figure 9), with the help of Herrmann's Palladacycle (Herrmann, Brossmer, Reisinger, Riermeier, ÖFele, Beller 1997) and t-BuXPhos ligand, can be incorporated (Yu, Chen, Huang, Chern 2012). This method has been proved to maintain high yields even with aldehyde substituents present on the benzene ring, as milder reaction conditions are featured. Therefore, the modified reaction cascade would be as follows: aryl chlorination (liquid Cl_2, $FeCl_3$), then palladium-catalyzed hydroxylation (Herrmann's Palladacycle, t-BuXPhos, Cs_2CO_3, DMF/H_2O (9:1), microwave) (Yu, Chen, Huang, Chern 2012), followed by methyl ether protection (MeI, K_2CO_3, acetone) (Greene, Wuts 1999a), giving the desired ether-protected aryl constituent **11**.

It should be noted that in the synthesis proposal, it has been hypothesized that for molecule **3**, the ketone at C-17 reacts more readily with Grignard moiety than the α, β-unsaturated ketone at C-9. Nonetheless, if proved otherwise in experimentations (by getting more product molecules whose bicyclic system is linked to the olefin side chain at C-9), a back-up synthesis route can be adopted: the α, β-unsaturated ketone at C-9 is first protected (ethylene glycol, tosylic acid, benzene, reflux) (Greene, Wuts 1999b), followed by reductive addition with Grignard reagent **4** in THF, which yields molecule **31** directly. The synthesis can then proceed in the same cascade as shown in Figure 8.

5 CONCLUSIONS

In summary, because of its biochemical significance and potential pharmacological efficacy, a synthesis scheme of 9,10-Verrucellol A has been proposed, using widely accessible starting materials and a relatively straightforward synthesis route, which features constructing the bicyclic skeleton with a Robinson Annulation, followed by joining the olefin side chain by a Grignard addition, and approaching the structure of target molecule with several fine adjustments. A number of proposed reaction conditions have also been included herein. This paper could act as a foundation for further investigations, such as diastereoselective synthesis of 9,10-Verrucellol A; *in vivo* assessments of the target's pharmacological efficacy could also be endeavored to evaluate its practical use.

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