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Toward the Synthesis of Thapsigargin: Enantioselective Synthesis of 7,11-Dihydroxyguaianolides

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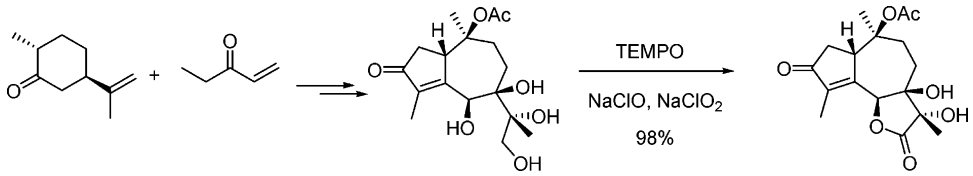
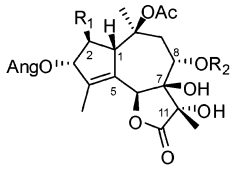
ABSTRACT



The enantioselective synthesis of a 7,11-dihydroxyguaianolide bearing the stereochemistry present in thapsigargin, a potent and selective inhibitor of the Ca^{2+} SERCA-ATPase pumps, is described. Starting from (+)-dihydrocarvone, the synthesis presents two key steps. The first one involves the photochemical rearrangement of a γ,δ-unsaturated ketone eudesmane into the corresponding guaiane. The second step consists of the regioselective oxidation of an unprotected tetrahydroxylated ketone to provide a dihydroxylactone with the required stereochemistry.

Thapsigargin (Tg, **1**) was first isolated by Christensen and co-workers from the roots of *Thapsia garganica* (Umbelliferae).[1] This compound belongs to a family of guaianolides whose structures differ in the nature of their substituents on C-2 and C-8 (Figure 1).[2]

Tg presents a remarkable histamine-releasing ability, inducing within 4−5 h erythema, the appearance of small vesiculae, and intense itching, which remain for several days when applied directly on the skin.[3] However, the most important activity displayed by Tg is its capacity to inhibit selectively the sarco- and endoplasmic reticulum Ca^{2+}-dependent ATPases (SERCA).[4] This inhibition of the SERCA pumps leads to depletion of the Ca^{2+} pool and a capacitance influx of extracellular Ca^{2+}, resulting in a sustained elevation of the cytosolic Ca^{2+} concentration, leading to DNA fragmentation and apoptosis of the treated cells.[5] Especially significant is its capacity to induce apoptosis in human androgen-independent prostatic cancer cell lines.[6]



1 Thapsigargin R_1 = O-Oct R_2 = But
2 Trilobolide R_1 = H R_2 = (S)-2-MeBut
3 Nortrilobolide R_1 = H R_2 = But
4 Thapsivillosin F R_1 = H R_2 = Sen

Oct = octanoyl But = butanoyl
Sen = senecioyl Ang = angeloyl

Figure 1. Structure of thapsigargin (**1**) and guaianolides synthesized by the Ley group (**2-4**) (vide infra).

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This phenomenon is even found in primary human prostatic cancer cell cultures in which about 98% of the cells are in G_0. This ability to kill proliferatively quiescent G_0 cells by inhibiting the ubiquitous SERCAs means that Tg cannot be administered systemically due to the toxicity to the host. To prevent this problem, Christensen, Isaacs, et al. have formulated a Tg-based inactive prodrug with a carrier peptide which targets specifically PSA, a prostate-specific serine protease active only in the extracellular fluid of prostatic cancer cells and inactive in blood serum. This prodrug selectively hydrolyzes the amide bond between a Gln and a Leu residue, liberating the active drug only in the extracellular fluid of PSA-secreting prostatic cells, thus targenting only prostatic cancer cells.[7]

The use of such an interesting compound is limited by dependence on natural sources, and the availability of synthetic thapsigargin-related compounds would be welcomed by those involved in the treatment of this severe illness.

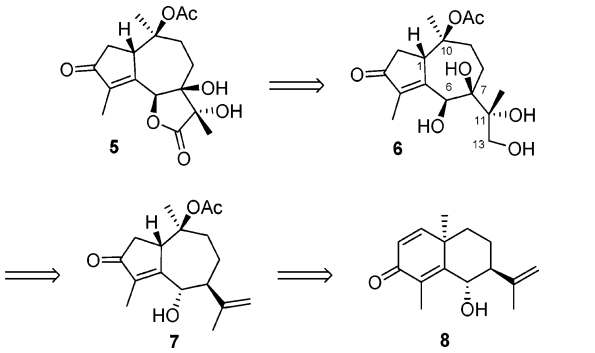
Some efforts aimed at the synthesis of this type of compounds have been reported. Christensen and Ley have carried out several SAR studies in which some Tg-related compounds have been synthesized.[8] Recently, Ley and coworkers have reported an impressive synthesis of three members of the thapsigargin series: trilobolide (**2**), nortrilobolide (**3**), and thapsivillosin F (**4**) (Figure 1).[9]

Herein, we present our methodology for the synthesis of the 7,11-dihydroxyguaianolide **5**, which possesses five of the eight chiral centers present in thapsigargin itself. This compound is a suitable intermediate for the total synthesis of thapsigargin as the functional groups present in C-3 and C-7 would enable access to C-2 and C-8 in which an octanoyl and a butanoyl group are present, respectively. Moreover, recently it has been reported that it is not necessary for the C-2 carbon to be oxygenated and that trilobolide and other 2-deoxythapsigargins are alternative to Tg as starting materials for the development of SERCA inhibitors.[10]

The initial study of the target molecule displayed several difficulties that should be solved. In addition to the high degree of oxidation in such a small backbone, Tg and all guaianolides isolated so far from plants from the Umbelliferae family present the stereochemistry of the five- and seven-membered rings fusion opposite to that found in the rest of guaianolides isolated from other families of plants.

Our proposed retrosynthesis is outlined in Scheme 1. We envisaged a photochemical rearrangement from the corresponding eudesmane to afford a guaiane,[11] which after the appropriate transformations would lead to a tetrahydroxylated guaiane. The structural complexity of this tetrahydroxylated compound provided an excellent opportunity to explore the synthetic utility of the TEMPO/NaClO/$NaClO_2$ oxidation methodology developed in our laboratory.[12]

Scheme 1. Retrosynthetic Analysis of the 7,11-Dihydroxyguaianolide **5**



We were mindful that tetraol **6** should be oxidized to the corresponding γ-lactone avoiding the cleavage of the C-11/C-13 bond that is observed when 1,2-diols are treated with most oxidant systems.

The synthesis of guaianolide **5** commenced with the one-pot Robinson annulation and further O_2/KOH γ-hydroxylation between (+)-dihydrocarvone **9** and ethyl vinyl ketone **10**, affording the hydroxyenone **11** in 70% yield (Figure 2).[13] The dehydrogenation of **11** was accomplished with DDQ in refluxing 1,4-dioxane, furnishing the dienone **8** in 70% yield. When **8** was irradiated with a Hg-medium pressure lamp for 6 h in acetic acid, not only the desired guaiane **12** was formed but important amounts of the cyclic ether **13** and the tricyclic enone **14** were also isolated.

Attempts to avoid the formation of undesired **13** and **14** proved unsuccessful. Protection of the hydroxyl group of **8**, as its acetate or MEM derivative, effectively suppressed the

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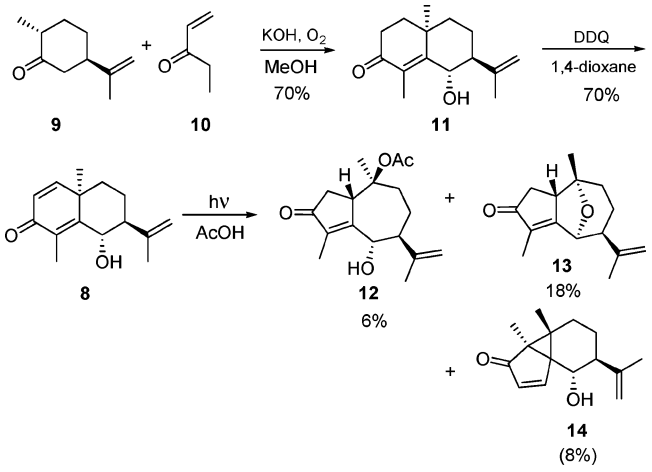

Figure 2. Photochemical rearrangement of **8**.

formation of the cyclic ether, but the efficiency of the photorearrangement was still low.

Since the irradiation of **8** yielded important amounts of **13** and **14**, respectively, we decided that a feasible alternative was to carry out the photochemical rearrangement on a substrate lacking any functional group at C-6 as in the case of **15**.[14] The existence of the double bond C4−C5 would allow the functionalization of C-6 of the guaiane framework in subsequent steps (Figure 3).

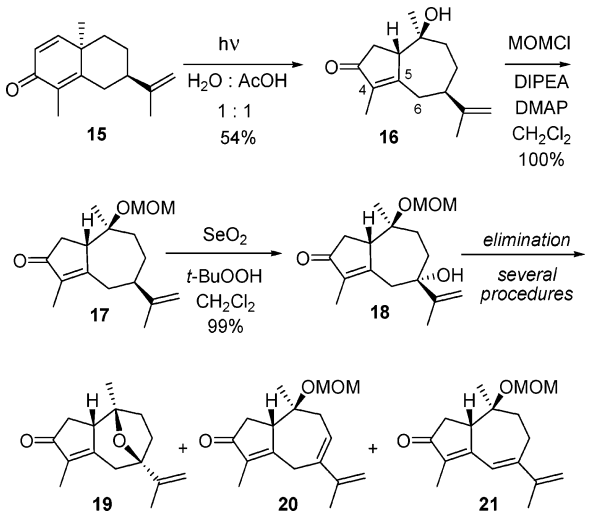

Figure 3. Second strategy: photochemical rearrangement in an unfunctionalized C-6 eudesmane.

Nevertheless, all the attempts to use the α,β-unsaturated ketone group to access to C-6 were unfruitful. As an example, treatment of **16** under Corey's conditions (*t*-BuOOH, Pd-$(OH)_2$/C)[15] to form the corresponding enedione did not proceed at all, the starting material being totally recovered.

Other approaches based in the isomerization of the C-4/C-5 double blond to C-5/C-6 (formation of the enol acetate, protection of the carbonyl group, selective bromination) did not work.

We then tried a different approach. When enone **17** was treated with selenium dioxide and *tert*-butyl hydroperoxide, an alcohol group was quantitatively introduced at C-7 leading to **18**.

Nevertheless, attempted elimination reactions under different protocols mainly led to the cyclic ether **19**, and only minor amounts of the elimination products **20** and **21** were observed in some cases (Figure 3).

Taking into account the difficulties found to set a double bond between C-6 and C-7 in the guaiane skeleton, we decided to install such a double bond prior to the irradiation (Figure 4). Thus, starting from hydroxydienone **8**, exposure to Burgess reagent produced smoothly the fully conjugated alkenone **22** in quantitative yield.[16] The terminal double bond C11−C13 was then submitted to treatment with AD-mix α in a 1:1 *t*-BuOH/H_2O mixture, giving rise to diol **23** (4:1 selectivity favoring the required 11-*S*-epimer). After purification by preparative HPLC, diol **23** was dissolved in acetic acid and irradiated for 2.5 h, yielding guaiane **24**. The high yield of this process is remarkable given the precedented sensitivity of this type of photorearrangements to the conditions in which the substrate is irradiated (time, concentration, solvent, etc.).

With guaiane **24** in hand, the stereoselective functionalization of the double bond C6−C7 became the new task. After some experimentation, it was found that treatment with osmium tetraoxide and *N*-methylmorpholine *N*-oxide in the presence of methanesulfonimide produced the tetrahydroxylated compound **6** in 61% yield. Despite the existence of four free hydroxyl groups, this compound could easily be purified by column chromatography using mixtures of acetone and hexanes. Finally, we arrived at one of the key steps of the synthesis in which the highly hydroxylated compound **6** should be submitted to selective oxidation of the primary alcohol to the corresponding carboxylic acid, without affecting any of the three remaining hydroxyl groups.[17] Although we were aware of the smoothness of the oxidations with the system TEMPO/NaClO/$NaClO_2$, we were not totally confident that the presence of four hydroxyl groups in four contiguous centers of the molecule would resulted in the clean formation of the lactone. To our delight, when we treated the tetraol **6** with such an oxidant system, we observed a very smooth and quantitative formation of lactone **5**.

Diagnostic strong NOE enhancements between H-6 and H-8α, H-6 and 3H-15, and H-6 and 3H-14 served to confirm the relative stereochemistry of H-6 as α. In addition, the relative configuration β of the hydroxyl group at C-7 was

(14) **15** was prepared by a standard Robinson annulation reaction as in **11** avoiding the presence of O_2 and further treatment with DDQ.

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(17) A similar approach was successfully employed by the Ley group by using TPAP (see ref 9a).

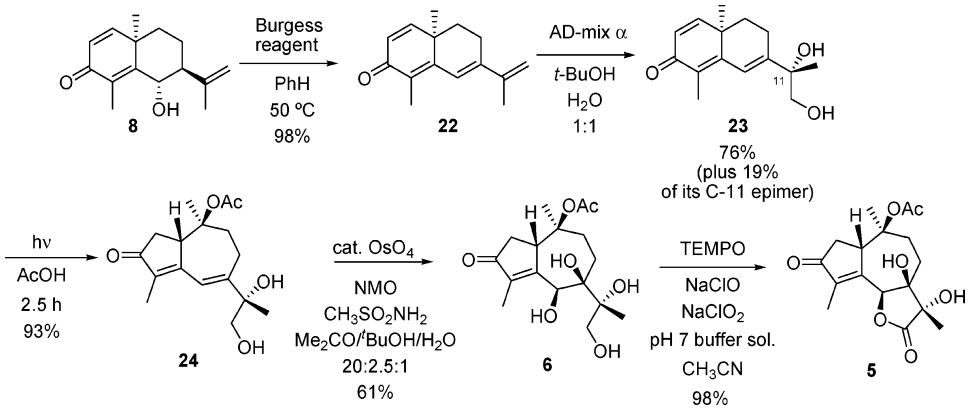

Figure 4. Synthesis of 7,11-dihydroxyguaianolide **5**.

stated from the cis course of the dihydroxylation reaction. The stereochemistry at C-11 was nevertheless more difficult to be determined as no significative NOE effect was observed.

Thus, from 11-*epi*-**23** the same synthetic sequence was followed in order to confirm the absolute stereochemistry at C-11. 11-*epi*-**5** was synthesized and submitted to NOEdiff experiments. A correlation between H-6, 3H-14, and 3H-13 allowed us to determine an *R* configuration for C-11 in 11-*epi*-**5** and consequently a *S* configuration for C-11 in **5**.

In summary, we have enantioselectively synthesized 7,11-dihydroxyguaianolide **5**, an important intermediate in the synthesis to thapsigargin in six steps starting from (+)-dihydrocarvone and ethyl vinyl ketone. An investigation to access to thapsigargin itself from **5** is currently in progress.

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Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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