Scalable Synthesis of (−)-Thapsigargin

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Supporting Information

ABSTRACT: Total syntheses of the complex, highly oxygenated sesquiterpenes thapsigargin (1) and nortrilobolide (2) are presented. Access to analogues of these promising bioactive natural products has been limited to tedious isolation and semisynthetic efforts. Elegant prior total syntheses demonstrated the feasibility of creating these entities in 36–42 step processes. The currently reported route proceeds in a scalable and more concise fashion by utilizing two-phase terpene synthesis logic. Salient features of the work include application of the classic photosantonin rearrangement and precisely choreographed installation of the multiple oxygenations present on the guaianolide skeleton.

The structure of thapsigargin (1, Figure 1A) was elucidated in 1978,1 but its use can be anecdotally traced back to ancient times as a popular folk medicine.2 As one of the most highly oxidized members of the venerable guaianolide sesquiterpene family, 1 represents a classic target for total synthesis.4 One measure of the difficulty in approaching a total synthesis of this natural product is the percentage of skeletal carbon atoms that bear an oxygen atom (53%). For reference, notoriously difficult targets such as ingenol,3 phorbol4 and Taxol5 range from 25 to 42%. For this reason, the sole successful effort to reach 1 and the related natural product nortrilobolide (2)6 required 427 and 368−10 steps, respectively.

As part of a continuing collaboration with LEO Pharma11 the Scripps team was presented with the challenge of inventing a scalable and modular route to this family of natural products (namely, 1 and 2) due to their promising therapeutic potential. Thapsigargin is a potent inhibitor of the SERCA-pump protein, with potential for applications in a variety of medicinal areas.8,12−14 For example, a prodrug derivative of 1 is currently in late-stage clinical trials for a variety of cancers.15,16 In this article, the execution of a concise, scalable, two-phase total synthesis of 1 and 2 is presented featuring a single-step construction of the carbon framework (cyclase-phase17) followed by precisely choreographed oxygenation events (oxidase phase).

Figure 1. (A) A two-phase approach to thapsigargin (1) and nortrilobolide (2); (B) an inspiring precedent from Barton; (C) a graphical representation of the oxidase phase challenge.
an effort to access oxidized guaianolides such as 1 from 7 (Figure 1C).24 The primary challenge for this work was therefore a rapid and efficient installation of six additional oxygen atoms onto 7 in the correct stereochemical configuration to access a guaiane of "level 9" oxidation pattern.17 While numerous routes are plausible to access 1 and 2 from the bottom of the oxidase pyramid (Figure 1C), outlined in this article is the optimized route and the logic associated with the timing of each oxidation event.

Our synthetic endeavors began with the known Robinson annulation between (+)-dihydrocarvone (4) and ethyl vinyl ketone to deliver the decalin 7.25 Exposure of the reaction mixture to an oxygen atmosphere after the annulation event results in the diastereoselective installation of the alcohol at the γ-position (C-6). This sequence to prepare 7 could be completed on a 30-g scale, providing ample material for the ensuing oxidase phase.

Having constructed the skeletal carbons of 1 in a single step, we next targeted dienone 8, the requisite intermediate for the key photochemical rearrangement. While the installation of the C1−C2 unsaturation with DDQ gave inconsistent results on a gram scale, a one-pot bromination/elimination sequence reliably produced diene 8 from 7 in 85% yield on a gram scale. Dehydration of 8 with Burgess reagent, followed by chemo- and diastereoselective (5:1 dr) dihydroxylation of the terminal olefin with AD-mix-α afforded diol 10 in 60% yield.26 Crystalline derivative 9 was synthesized (four steps from 8), which indirectly confirmed the stereochemical configuration of 10.

With diol 10 in hand, the stage was set for the next oxidation at C-8. Selective protection of the primary alcohol followed by in situ allylic C−H oxidation using SeO227 diastereoselectively delivered allylic alcohol 11. Additional reagents for this oxidation were evaluated. In most cases, decomposition was observed. Under electrochemical conditions,28 for example, the major product observed was peroxide 12, with oxidation taking place at the undesired C-6 position. The TBS group proved crucial to the success of this oxidation, as the free diol, cyclic carbonate, or ester at the same position were all found to either hamper or diminish the reactivity. Having synthesized allylic alcohol 11, Mitsunobu inversion with butyric acid allowed for the smooth installation of the butyrate with the desired stereochemical configuration at C-8. Interestingly, the desired major C-11 stereoisomer reacted preferentially in this trans-

Scheme 1. Concise Synthesis of (−)-Thapsigargin (1)24

Reagents and conditions: (a) EVK (1.2 equiv) 15% KOH/MeOH; then O2 (1 atm) (50%); (b) TMSOTf (2.5 equiv), Et3N (5 equiv), CH2Cl2, 1.5 h, 0 °C; then NBS (1.3 equiv), 2 h; then DBU (10 equiv), THF, 50 °C, 16 h; then HCl (3M), 3 h, 23 °C (85%); (c) Burgess reagent (1.6 equiv), MeCN, 5 h, 80 °C; then K2CO3 (2 equiv), H2O, 25 min, 23 °C; then tBuOH, AD-mix-α, 24 h, 0 °C (MeCN/tBuOH/H2O 1:1:1) (60% dr. 5:1); (d) TBSCI (2 equiv), imidazole (2 equiv), dioxane, 5 h, 23 °C, then NaHCO3, (10 equiv), SeO2 (10 equiv), 24 h, 105 °C (52%); (e) PbO2 (2 equiv), DEAD (2 equiv), butyric acid (2 equiv), 16 h, 0–23 °C (60%); (f) hv, AcOH (0.01 M), 14 h, 23 °C (50%); (g) KMnO4 (2.1 equiv), octanoic acid (35 equiv), octanoic anhydride (9.5 equiv), PhH, 20 h, 85 °C (67%); (h) OsO4 (10 mol %) NMO (2 equiv), citric acid (2 equiv), 36 h, 50 °C (acetone/tBuOH/H2O 1:1:1) 13 h, 50 °C (33%); (i) PySO3 (20 equiv), pyridine (30 equiv), DMSO, DIPEA (20 equiv), CH3Cl, 46 min, 0 °C (43%); (j) Zn(BH4)2 (11.7 equiv), EtOH, 1 h, −20 °C (88%); (k) PhCOCI (3 equiv), angelic acid (3 equiv), PhMe, 72 h, 90 °C (59%). EVK = ethyl vinyl ketone, TMSOTf = trimethylsilyl trifluoromethanesulfonate, NBS = N-bromosuccinimide, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TBSCI = tert-butyldimethylsilyl chloride, DEAD = diethyl azodicarboxylate, DIPEA = diisopropylethylamine, DMSO = dimethyl sulfoxide.
formation, serendipitously leading to a diastereomerically enriched product (13, 10:1 dr).

With the C-8, C-11, and C-12 oxidations in place, the key photochemical rearrangement was attempted. Concentration was found to be critical: after considerable experimentation, it was found that irradiating a 0.01 M solution of 13 in glacial acetic acid with a Hg lamp furnished enone 14 in 50% yield on a 2.5 g scale, thereby stereoselectively constructing the guaianolide skeleton. The C-10 acetate was concomitantly installed in the same process. It should also be noted that installation of the C-8 oxidation after the photochemical rearrangement proved unsuccessful (see Supporting Information for details). With the [5,7] ring system along with several key oxidations in place, the final four oxidation events were executed. Treating 14 with potassium permanganate in the presence of octanoic acid and octanoic anhydride in refluxing benzene furnished the desired α-octanoylated enone 15. Dihydroxylation of 15 with stoichiometric osmium tetroxide was, while effective, a costly and unscaleable method for the installation of C-6 and C-7 oxygen atoms. After extensive experimentation, it was found that a modified Upjohn procedure with citric acid rendered the reaction catalytic at elevated temperature (50 °C). Under these reaction conditions, 16 was obtained in 33% yield along with 23% of the TBS protected tetra-ol. With tetra-ol 16 in hand, lactonization under Parikh–Doering conditions afforded lactone 17 presumably via the intermediacy of a lactol. A gram scale synthesis of lactone 17 (500 mg synthesized) was therefore achieved in only nine steps, marking the completion of a formal synthesis of 1. For safety reasons, lactone 17 was selected as the end point of the gram scale synthesis, given that the penultimate intermediate 18, as well as the final natural product, are known to be highly toxic at very low concentrations. An analytically pure sample of 1 was synthesized from 17 via zinc borohydride reduction, followed by an acylation with the mixed anhydride of angelic acid and benzoyl chloride. This constitutes a 11-step synthesis of 1 with an overall yield of 0.137%. In addition, an alternative 14-step synthesis to 1 has been developed (see Supporting Information for details). Despite being a slightly longer sequence, this route features a higher yielding photorearrangement (80%) and dihydroxylation (85%), bringing the overall yield to 0.637%. Furthermore, this sequence potentially enables the facile installation of a variety of esters via simple acylation reactions after the photorearrangement.

The same route could be repurposed to allow for facile access to nortrilobolide (2) as depicted in Scheme 2. Oxidation of the photoisomerized intermediate 14 via dihydroxylation with subsequent silyl ether cleavage delivered tetra-ol 19 in 48% yield. Parikh–Doering oxidation provided lactone 20 in 80% yield. Diastereoselective reduction furnished the corresponding alcohol, which was acylated in the presence of neat angelic anhydride to give 2 in 48% yield over two steps. Overall, this sequence constitutes a 10-step synthesis of (−)-nortrilobolide and is another example of the flexibility inherent to the two-phase approach for readily delivering analogues and related members in a highly oxygenated terpene class.

As mentioned above, the order and nature of functionalizations en route to 1 and 2 in the oxidase phase were critical to the success of this approach. Of the eight oxygenated carbon atoms (C-2, C-3, C-6, C-7, C-8, C-10, C-11, and C-12), C-3 and its adjacent unsaturation were installed at the outset as they enabled the cyclase phase to proceed rapidly. The C-6 oxygen was installed initially and then dehydrated as a means to allow access to C-7 via downstream dihydroxylation. Similarly, C-11 and C-12 could be installed using the native olefin functionality of dihydrocarvone also via dihydroxylation. The allylic C–H bond at C-8 was then incorporated (vide supra) before photorearrangement due to empirical findings. The C-10 oxygen emerged as a consequence of the ensuing rearrangement. The key Mn-mediated oxidation at C-2 is preceded on substrates such as 19 however, a significantly higher yield was obtained with 14 via a KMnO₄ mediated octanoylation. The precise choreography of oxidation events potentially enables the facile access to a number of analogues with varying oxidation states as well as the remaining members of the thapsigargin family of natural products. To name a few examples, this route enables the access of the C8 oxygen in both stereochemical configurations. Additionally, different nucleophiles can be utilized in the Mitsunobu inversion to install a variety of functional groups. Lastly, different acids/ anhydrides can be utilized in the α-oxidation event at C2 to append a number of different esters.

The route described here benefits from an effective strategy rather than a recent methodological advance, with Sharpless AD being the newest method employed. Rather, it was the two-phase approach that enabled scalable access to these highly oxygenated and complex natural products. Indeed, a 2015 review on these terpenes stated: “Approaches utilizing semisynthesis or total synthesis are currently far from being economically feasible.” Total synthesis is now a potentially viable option for both the scalable procurement of 1 and a variety of designed analogues with deep-seated modifications.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.6b00313.
Experimental procedures, analytical data (1H and 13C NMR, MS) for all new compounds (PDF)
Crystallographic information file (CIF)

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Notes

The authors declare no competing financial interest.

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