JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

A Concise, Efficient and Scalable Total Synthesis of Thapsigargin and Nortrilobolide from (*R*)-(−)-Carvone

Dezhi Chen and P. Andrew Evans*

Department of Chemistry, Queen's University, 90 Bader Lane, Kingston, Ontario K7L 3N6, Canada

Supporting Information

ABSTRACT: A concise, efficient and scalable synthesis of thapsigargin and nortrilobolide from commercially available (*R*)-(−)-carvone was developed. Our synthetic strategy is inspired by nature's carbon−carbon bond formation sequence, which facilitates the construction of a highly functionalized sesquiterpene lactone skeleton in five steps *via* an enantioselective ketone alkylation and a diastereoselective pinacol cyclization. We envision that this strategy will permit the construction of other members of the family, structural analogs and provide a practical synthetic route to these important bioactive agents. In addition, we anticipate that the prodrug Mipsagargin, which is currently in late-stage clinical trials for the treatment of cancer, will also be accessible *via* this strategy. Hence, the limited availability from natural sources, coupled with an estimated demand of one metric ton per annum for the prodrug, provides a compelling mandate to develop practical total syntheses of these agents.

Thapsigargin (**1**) was isolated from the Mediterranean plant *Thapsia garganica* L. by Christensen and co-workers in 1978, which along with a number of structurally related guaianolides are collectively called thapsigargins (Figure 1A).[1,2] The structure of **1** was elucidated through extensive spectroscopic studies and X-ray crystallographic analysis of various derivatives, which revealed a hexaoxygenated guaianolide core functionalized with acetyl, butyryl, angelyl and octanyl esters in addition to two free tertiary hydroxyl groups.[3] The significance of thapsigargin (**1**) in molecular biology and oncology is attested to by over 17 000 publications over the past 40 years.[4] For instance, **1** is categorized as a highly selective subnanomolar inhibitor of intracellular calcium ion transport enzymes, which are termed sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCAs).[5] The remarkably high level of selectivity toward SERCAs makes **1** a particularly useful tool to investigate a variety of Ca^{2+}-dependent cellular processes.[6] More significantly, the induction of cell apoptosis is also dependent on Ca^{2+} signals, in which the strategic application of thapsigargin (**1**) to promote the induction of programmed cancer cell death in a proliferation independent manner has led to the development of novel cancer therapeutics.[7] For example, the prodrug, Mipsagargin (**5**), which has a polypeptide chain at the C-8 position of **1**, is currently in late stage clinical trials for the treatment of liver, brain, prostate and kidney cancer (Figure 1B).[8] Furthermore, it is anticipated that the clinical demand for this agent is likely to exceed one metric ton per annum.[1,9] The intriguing structural complexity and biological importance of thapsigargin (**1**) have attracted degradation and derivatization studies,[1] in addition to total synthesis efforts.[9−11] Nevertheless, the development of a commercially feasible synthetic route to this complex chemical structure still represents a significant and ongoing challenge. Herein, we now disclose a concise, efficient and scalable strategy to access the highly oxidized guaianolide skeleton, which enables the total synthesis of thapsigargin (**1**) and nortrilobolide (**3**) from commercially available (*R*)-(−)-carvone (**10**).

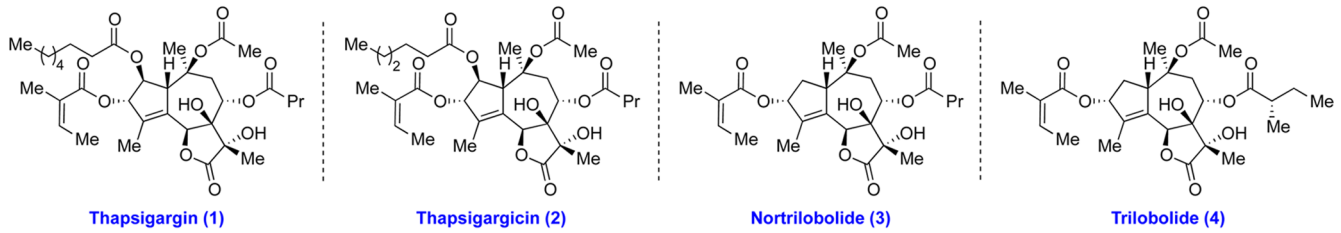
The total synthesis of thapsigargin (**1**) has a number of inherent challenges, namely, the stereoselective construction of a hexaoxygenated 5−7−5 tricyclic guaianolide skeleton functionalized with eight stereogenic centers, the installation of four different ester groups, a *trans*-vicinal tertiary diol and an internal tetrasubstituted olefin. An efficient synthesis of **1** requires the strategic introduction of these oxygen substituents to minimize redox chemistry, protecting group and functional group manipulations. To this end, we devised a divergent strategy, wherein the common synthetic intermediate **6** would access both thapsigargin (**1**) and the other members of the thapsigargin family, for example, nortrilobolide (**3**) (Scheme 1A). Inspired by the proposed biosynthetic pathway for thapsigargin (**1**),[12] we envisioned that the disconnection of the carbon−carbon bond at C-6/C-7 would provide a novel approach and minimize functional group transformations (Scheme 1B). The assembly from (*R*)-(−)-carvone (**10**) and the methylerythritol derivative **9** (see Supporting Information) combines a ten- and a five-carbon fragment,[13] which is consistent with the logic of nature's strategic combination of building blocks in terpene biosynthesis. Hence, the proposed abiotic approach would utilize a transition-metal-catalyzed enantioselective alkylation reaction to forge the 15-carbon framework **8** necessary for a metal-mediated aldehyde/ketone pinacol coupling reaction to construct the guaianolide skeleton **7** *en route* to the common synthetic intermediate **6** (Scheme 1C). This biosynthetically inspired process could incorporate the necessary stereochemistry and functionalities for the synthesis of thapsigargin (**1**) in a concise manner. Our hypothesis would provide rapid and scalable access to **6**, which serves as a key intermediate for the preparation of **1** and related members of the family that should permit detailed structure−activity relationship studies on these important agents.

The synthesis commences with the regioselective allylic chlorination[14] of the commercially available monoterpene, (*R*)-(−)-carvone (**10**), followed by a one-pot reduction and *in situ*

Received: February 18, 2017

DOI: 10.1021/jacs.7b01734
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

A. Molecular Structure of Thapsigargin (**1**), Thapsigargicin (**2**), Nortrilobolide (**3**) and Trilobolide (**4**).



B. Mipsagargin (**5**), a Prodrug in Phase II Clinical Trials.

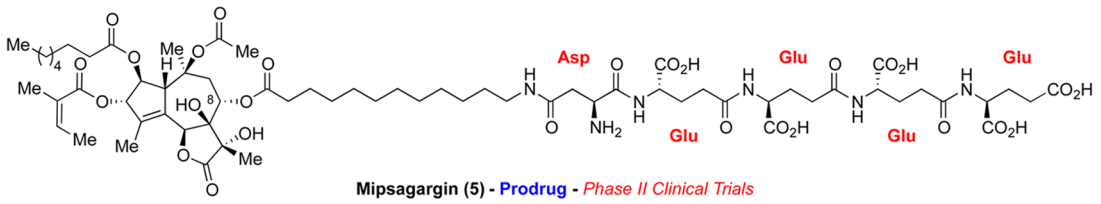
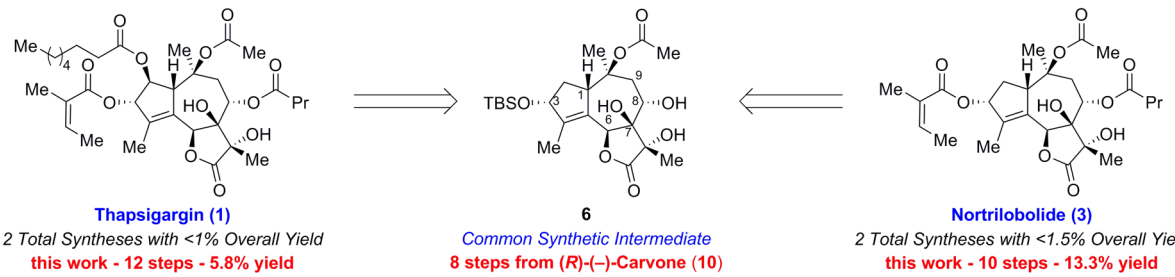


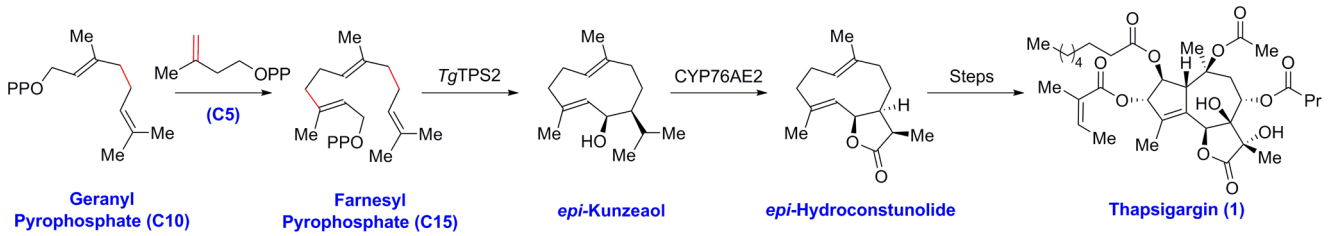
Figure 1. Structurally and biologically interesting thapsigargin derivatives.

Scheme 1. Biosynthetic Inspiration, Strategic and Retrosynthetic Analysis

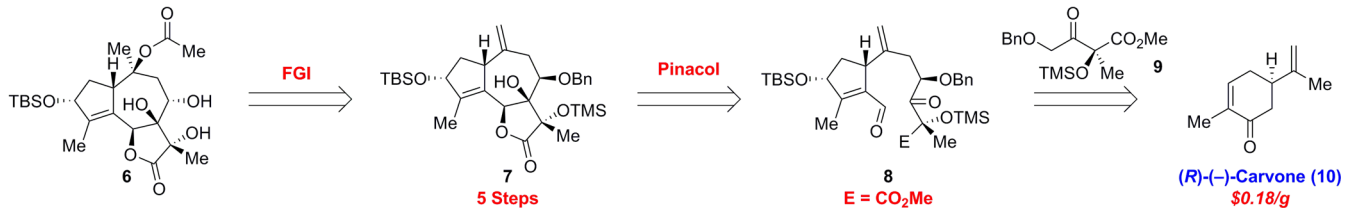
A. Strategic Analysis and Identification of a Common Synthetic Intermediate



B. Proposed Biosynthetic Pathway for Thapsigargin



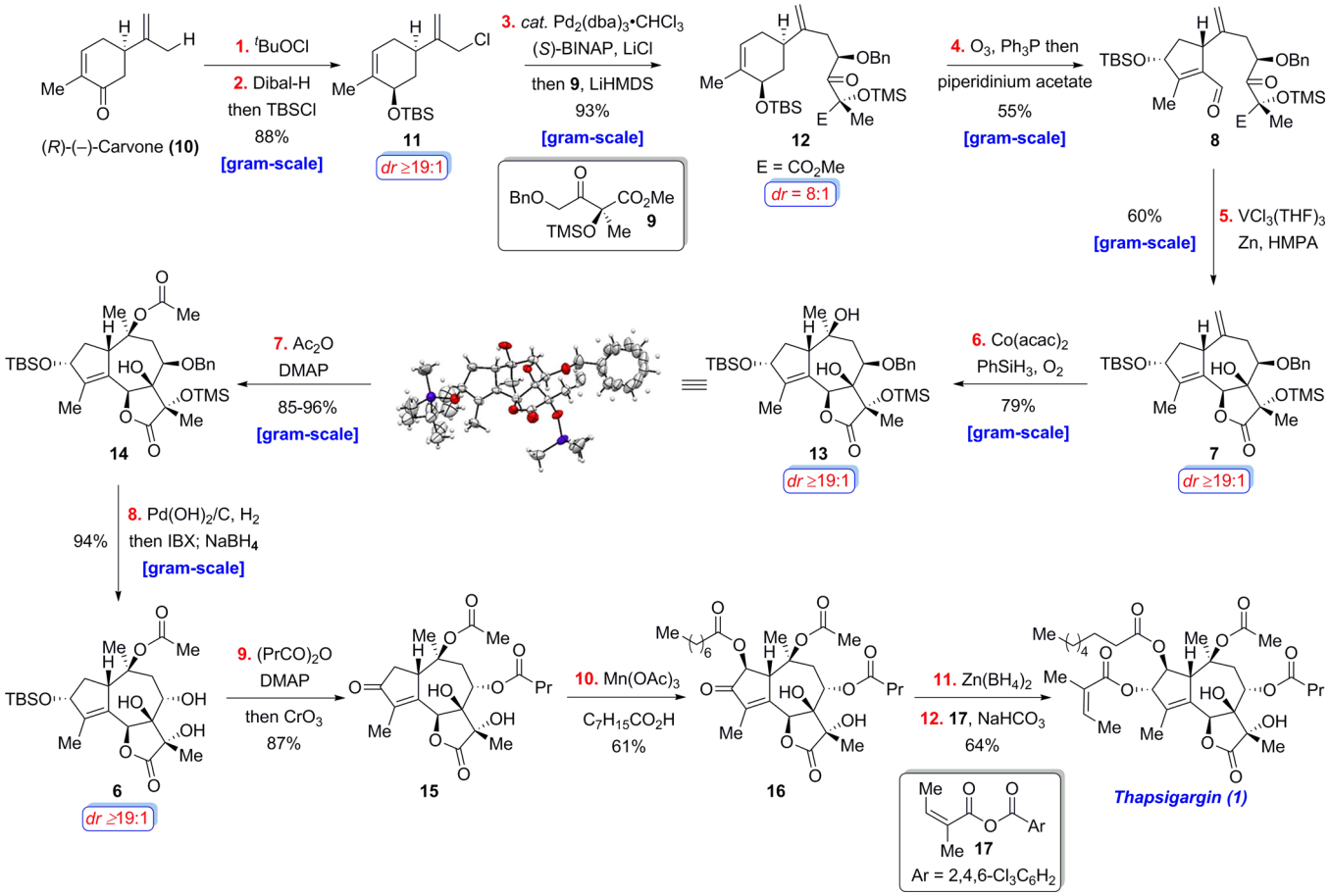
C. Biosynthetically Inspired Retrosynthetic Analysis



protection of the secondary alcohol to provide the *tert*-butyldimethylsilyl ether **11**[15] in excellent overall yield (Scheme 2). The asymmetric alkylation of **11** with the lithium enolate of ketone **9**, which was generated with lithium hexamethyldisilazide, was achieved in the presence of lithium chloride and the chiral catalyst derived from $Pd_2(dba)_3 \cdot CHCl_3$ and (*S*)-BINAP to furnish the coupled product **12** in 93% yield and with 8:1 diastereoselectivity.[16] The ring contraction of the cyclohexene in **12** was then accomplished *via* selective ozonolysis of the more electron-rich olefin followed by an *in situ* intramolecular aldol condensation catalyzed by piperidinium acetate,[13b,17] to afford the cyclopentene derivative **8** in moderate yield. This one-pot transformation permits the installation of the internal tetrasubstituted double bond present in the natural product and simultaneously provides the intermediate required for the sequential pinacol coupling/lactonization cascade reaction. To this end, a range of reaction

DOI: 10.1021/jacs.7b01734
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Scheme 2. 12-Step Total Synthesis of Thapsigargin (1) from (*R*)-(−)-Carvone



conditions were investigated to implement the proposed sequence, in which the well-established samarium(II) iodide mediated pinacol coupling with a range of additives provided none of the desired adduct.[18] Alternatively, treatment of **8** with low valent titanium reagents delivered reduced side products.[19] Gratifyingly, more encouraging results were obtained when **8** was slowly added to a solution of the dimeric vanadium complex, $[V_2Cl_3(THF)_6]_2[Zn_2Cl_6]$, which was prepared *in situ* in the presence of hexamethylphosphoramide (HMPA), to afford the sesquiterpene lactone **7** in 60% yield and with ≥19:1 diastereoselectivity.[20] Hence, this five-step sequence provides a scalable and stereochemically versatile approach to the highly oxidized guaianolide skeleton **7**, which will enable the construction of a wide array of structural analogs.

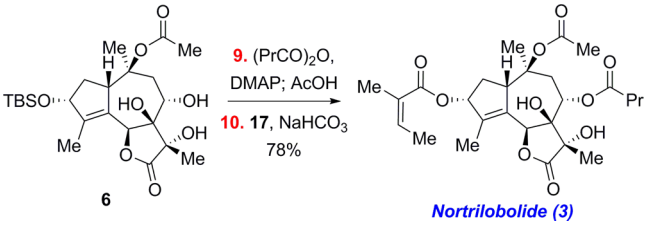
The installation of the tertiary acetate at the C-10 position was then accomplished by a selective cobalt-catalyzed Mukaiyama hydration of the less sterically hindered olefin and acetylation of the resulting tertiary alcohol.[21] Treatment of **7** with cobalt(II) acetylacetonate $[Co(acac)_2]$ catalyst and excess phenylsilane ($PhSiH_3$) under an oxygen atmosphere provided **13** in 79% yield and with ≥19:1 diastereoselectivity. Single-crystal X-ray diffraction analysis of **13** confirmed both the relative configuration and structural assignment of this key tricyclic intermediate. The selective acetylation of the C-10 tertiary alcohol in **13** at elevated temperature then afforded the desired tertiary acetate **14** in excellent yield. The stereochemistry at the C-8 position was then adjusted and the requisite ester was introduced using the following sequence. Selective hydrogenation of the benzyl ether in **14** with palladium hydroxide under a hydrogen atmosphere and the oxidation of the resulting secondary hydroxyl group with 2-iodoxybenzoic acid (IBX) followed by sodium borohydride reduction, afforded **6** in 94% overall yield and with ≥19:1 diastereoselectivity on gram scale. In addition, these conditions conveniently cleave the trimethylsilyl protecting group. The selective acylation of **6** proceeded cleanly, which permits the *in situ* Jones oxidation of the *tert*-butyldimethylsilyl ether to furnish the key *α*,*β*-unsaturated cyclopentenone **15** in 87% yield and thus sets the stage for the completion of the synthesis.[22]

The advancement of **15** to thapsigargin (**1**) was achieved *via* a modification of Christensen's three-step protocol.[9] The octanoyloxy side chain at the C-2 position in **15** was introduced stereoselectively using manganese(III) acetate $[Mn(OAc)_3]$ as the oxidant in benzene and octanoic acid as a mixed solvent system to afford **16** in modest yield. Diastereoselective reduction of the *α*,*β*-unsaturated ketone in **16** with zinc borohydride at −20 °C, followed by the angeloylation of the sterically hindered C-3 alcohol using anhydride **17** in the presence of sodium bicarbonate afforded the natural product, thapsigargin (**1**), in 64% yield.[10b]

Nortrilobolide (**3**), which differs from thapsigargin (**1**) at the C-2 position, was isolated from the same plant *Thapsia garganica* L. by Smitt and Christensen in 1991.[2b] Although there is no oxygenated substituent at the C-2 position, it has been reported that **3** exhibits equipotent inhibition of SERCAs to that of thapsigargin (**1**).[23] Hence, nortrilobolide (**3**) represents an important lead for the development of new anticancer agents, which can be prepared using the rapid and scalable synthesis of the common synthetic intermediate **6**. The selective acylation of the C-8 secondary alcohol in **6** followed by the *in situ* deprotection of the *tert*-

DOI: 10.1021/jacs.7b01734
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Scheme 3. 10-Step Total Synthesis of Nortrilobolide (3) from (*R*)-(−)-Carvone



butyldimethylsilyl protecting group under mild acidic conditions furnished an allylic alcohol intermediate, which was subjected to the same Yamaguchi acylation described above to provide nortrilobolide (**3**) in 78% yield over two steps (Scheme 3).[10a]

Overall, the total syntheses of thapsigargin (**1**) and nortrilobolide (**3**) were accomplished in 12 and 10 steps, respectively (longest linear sequence: 5.8% and 13.3% overall yield) from commercially available (*R*)-(−)-carvone (**10**). Notably, the total syntheses were accomplished in less than one-third of the number of steps required by Ley and co-workers (42 and 36 steps, respectively) and significantly more efficient (40 and 30 times, respectively) than the syntheses recently reported by Baran and co-workers. Furthermore, the five-step synthesis of 7 represents one of the shortest approaches to the guaianolide skeleton developed to date,[24] which we anticipate will allow the rapid preparation of a library of simplified thapsigargin analogs for detailed structure−activity relationship studies. In addition, it is envisioned that this approach could provide the basis for a manufacturing route to this important agent, particularly given the brevity and scalability of the synthesis. Finally, we believe that this strategy will provide a useful guide for the construction of related polyoxygenated terpenes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b01734.

Experimental procedures, spectral data and copies of spectra for all new compounds (PDF)
Crystallographic data (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*Andrew.Evans@chem.queensu.ca

ORCID

P. Andrew Evans: 0000-0001-6609-5282

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Dedicated to Professor Stephen F. Martin for his outstanding contributions to natural product chemistry. We sincerely thank the National Sciences and Engineering Research Council (NSERC) for a *Discovery Grant* and Queen's University for generous financial support. NSERC is also thanked for supporting a *Tier 1 Canada Research Chair* (P.A.E.).

■ REFERENCES

(1) For a recent review on thapsigargin, see: Doan, N. T. Q.; Christensen, S. B. *Curr. Pharm. Des.* **2015**, *21*, 5501.

(2) For the isolation of the thapsigargins, see: (a) Rasmussen, U.; Christensen, S. B.; Sandberg, F. *Acta Pharm. Suec.* **1978**, *15*, 133. (b) Smitt, U. W.; Christensen, S. B. *Planta Med.* **1991**, *57*, 196. (c) Liu, H.; Jensen, K. G.; Tran, L. M.; Chen, M.; Zhai, L.; Olsen, C. E.; Søhoel, H.; Denmeade, S. R.; Isaacs, J. T.; Christensen, S. B. *Phytochemistry* **2006**, *67*, 2651.

(3) (a) Christensen, S. B.; Larsen, I. K.; Rasmussen, U.; Christophersen, C. *J. Org. Chem.* **1982**, *47*, 649. (b) Christensen, S. B.; Norup, E. *Tetrahedron Lett.* **1985**, *26*, 107.

(4) SCIFINDER, American Chemical Society (Searched by research topic 'thapsigargin' on February 18, 2017).

(5) Thastrup, O.; Cullen, P. J.; Drøbak, B. K.; Hanley, M. R.; Dawson, A. P. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 2466.

(6) Treiman, M.; Caspersen, C.; Christensen, S. B. *Trends Pharmacol. Sci.* **1998**, *19*, 131.

(7) Christensen, S. B.; Skytte, D. M.; Denmeade, S. R.; Dionne, C.; Moller, J. V.; Nissen, P.; Isaacs, J. T. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 276.

(8) For the synthesis of Mipsagargin (**5**), see: Lynch, J. K.; Hutchison, J. J.; Fu, X.; Kunnen, K. Methods of Making Cancer Compositions. WO 2014145035A1, September 18, 2014.

(9) Crestey, F.; Toma, M.; Christensen, S. B. *Tetrahedron Lett.* **2015**, *56*, 5896.

(10) For total syntheses of thapsigargin (**1**) and nortrilobolide (**3**), see: (a) Oliver, S. F.; Högenauer, K.; Simic, O.; Antonello, A.; Smith, M. D.; Ley, S. V. *Angew. Chem., Int. Ed.* **2003**, *42*, 5996. (b) Ball, M.; Andrews, S. P.; Wierschem, F.; Cleator, E.; Smith, M. D.; Ley, S. V. *Org. Lett.* **2007**, *9*, 663. (c) Andrews, S. P.; Ball, M.; Wierschem, F.; Cleator, E.; Oliver, S.; Högenauer, K.; Simic, O.; Antonello, A.; Hünger, U.; Smith, M. D.; Ley, S. V. *Chem. - Eur. J.* **2007**, *13*, 5688. (d) Chu, H.; Smith, J. M.; Felding, J.; Baran, P. S. *ACS Cent. Sci.* **2017**, *3*, 47.

(11) For synthetic approaches to the thapsigargins, see: (a) Ley, S. V.; Antonello, A.; Balskus, E. P.; Booth, D. T.; Christensen, S. B.; Cleator, E.; Gold, H.; Högenauer, K.; Hünger, U.; Myers, R. M.; Oliver, S. F.; Simic, O.; Smith, M. D.; Søhoel, H.; Woolford, A. J. A. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 12073. (b) Kaliappan, K. P.; Nandurdikar, R. S. *Org. Biomol. Chem.* **2005**, *3*, 3613. (c) Manzano, F. L.; Guerra, F. M.; Moreno-Dorado, F. J.; Jorge, Z. D.; Massanet, G. M. *Org. Lett.* **2006**, *8*, 2879. (d) Tap, A.; Jouanneau, M.; Galvani, G.; Sorin, G.; Lannou, M.-I.; Férézou, J.-P.; Ardisson, J. *Org. Biomol. Chem.* **2012**, *10*, 8140. (e) Marín-Barrios, R.; García-Cabeza, A. L.; Moreno-Dorado, F. J.; Guerra, F. M.; Massanet, G. M. *J. Org. Chem.* **2014**, *79*, 6501. (f) Doan, N. T. Q.; Crestey, F.; Olsen, C. E.; Christensen, S. B. *J. Nat. Prod.* **2015**, *78*, 1406.

(12) Andersen, T. B.; Martinez-Swatson, K. A.; Rasmussen, S. A.; Boughton, B. A.; Jørgensen, K.; Andersen-Ranberg, J.; Nyberg, N.; Christensen, S. B.; Simonsen, H. T. *Plant Physiol.* **2017**, DOI: 10.1104/pp.16.00055.

(13) (a) Yang, H.; Gao, Y.; Qiao, X.; Xie, L.; Xu, X. *Org. Lett.* **2011**, *13*, 3670. (b) Hu, X.; Xu, S.; Maimone, T. J. *Angew. Chem. Int. Ed.* **2017**, *56*, 1624.

(14) Nakamura, E.; Aoki, S.; Sekiya, K.; Oshino, H.; Kuwajima, I. *J. Am. Chem. Soc.* **1987**, *109*, 8056.

(15) Pisoni, D. S.; Gamba, D.; Fonseca, C. V.; Da Costa, J. S.; Petzhold, C. L.; De Oliveira, E. R.; Ceschi, M. A. *J. Braz. Chem. Soc.* **2006**, *17*, 321.

(16) Braun, M.; Meier, T.; Laicher, F.; Meletis, P.; Fidan, M. *Adv. Synth. Catal.* **2008**, *350*, 303.

(17) Wolinsky, J.; Slabaugh, M. R.; Gibson, T. *J. Org. Chem.* **1964**, *29*, 3740.

(18) Szostak, M.; Fazakerley, N. J.; Parmar, D.; Procter, D. J. *Chem. Rev.* **2014**, *114*, 5959.

(19) McMurry, J. E. *Chem. Rev.* **1989**, *89*, 1513.

(20) Raw, A. S.; Pedersen, S. F. *J. Org. Chem.* **1991**, *56*, 830.

(21) Isayama, S.; Mukaiyama, T. *Chem. Lett.* **1989**, *18*, 1071.

(22) Evans, P. A.; Roseman, J. D.; Garber, L. T. *Synth. Commun.* **1996**, *26*, 4685.

(23) Søhoel, H.; Jensen, A.-M. L.; Møller, J. V.; Nissen, P.; Denmeade, S. R.; Isaacs, J. T.; Olsen, C. E.; Christensen, S. B. *Bioorg. Med. Chem.* **2006**, *14*, 2810.

(24) For a recent review, see: Santana, A.; Molinillo, J. M. G.; Macías, F. A. *Eur. J. Org. Chem.* **2015**, 2093.

DOI: 10.1021/jacs.7b01734
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX