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Total Synthesis of Thapsigargin, a Potent SERCA Pump Inhibitor

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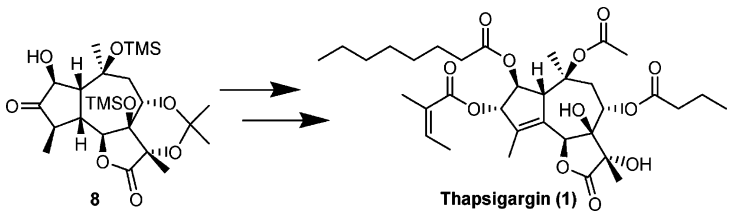
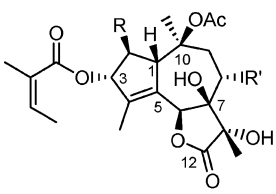
ABSTRACT



The enantioselective total synthesis of thapsigargin, a potent, selective inhibitor of the Ca^{2+} pump SERCA, is described. Starting from ketoalcohol 8, key steps involve regioselective introduction of the internal olefin at C4–C5, judicious protecting group choice to allow chelation-controlled reduction at C3, and chemoselective introduction of the angelate ester function at C3-*O*. A selective esterification approach completes the total synthesis in a total of 42 steps and 0.61% overall yield (88.6% average yield per step).

Thapsigargin (Tg, **1**) belongs to a family of 17 sesquiterpene lactones isolated by Christensen et al. from the Mediterranean plant species *Thapsia*.[1] These highly oxygenated guianolides have a common tricyclic core with seven or eight stereocenters, displaying a variety of acyl groups (Figure 1).



Thapsigargin (1)
R = Octanoyl, R' = *n*-Butanoyl
Trilobolide **(2)**
R = H, R' = *(S)*-2-MeButanoyl
Nortrilobolide **(3)**
R = H, R' = *n*-Butanoyl
Thapsivillosin F **(4)**
R = H, R' = Senecioyl

Figure 1. Thapsigargin and related guianolides.

Although formidable structures, they have only recently become target molecules for the synthetic community.[2] In contrast, their relevance in biology and oncology programs is demonstrated by almost 10 000 publications over the past 10 years.[3] Thapsigargin has significant biological activity; it has long been recognized as a potent histamine liberator[1,4] and as a selective, irreversible inhibitor of sarco-endoplasmic reticulum Ca^{2+} ATP dependent pumps (SERCAs) up to subnanomolar concentrations (K_D = 0.4 nM),[5] rendering it a useful tool in the probing of intracellular Ca^{2+} signaling pathways. The lipophilic nature of Tg allows it to cross cell membranes and inhibit the SERCA, locking it into a conformation which has poor affinity for Ca^{2+} and ATP,[6]

(1) Rasmussen, U. S. B.; Christensen, S. B.; Sandberg, F. *Acta Pharm. Suec.* **1978**, *15*, 133.

(2) (a) Kaliappan, K. P.; Nandurdikar, R. S. *Org. Biomol. Chem.* **2005**, *3*, 3613. (b) Manzano, F. L.; Guerra, F. M.; Moreno-Dorado, F. J.; Jorge, Z. D.; Massanet, G. M. *Org. Lett.* **2006**, *8*, 2879. (c) 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. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12073. (d) Oliver, S. F.; Högenauer, K.; Simic, O.; Antonello, A.; Smith, M. D.; Ley, S. V. *Angew. Chem., Int. Ed.* **2003**, *42*, 5996.

(3) Scifinder search engine (V2006), 2005 American Chemical Society. (Search string 'thapsigargin'; date, 11/27/06).

(4) Patkar, S. A.; Rasmussen. U.; Diamant, B. *Agents Actions* **1979**, *9*, 53.

(5) (a) Ali, H.; Christensen, S. B.; Foreman, J. C.; Pearce, W.; Piotrowski, W.; Thastrup, O. *Br. J. Pharmacol.* **1985**, *85*, 705. (b) Sagara, Y.; Inesi, G. *J. Biol. Chem.* **1991**, *266*, 13503. (c) Davidson, G. A.; Varhol, R. J. *J. Biol. Chem.* **1995**, *270*, 11731.

(6) Toyoshima, C.; Nomura, H. *Nature* **2002**, *418*, 605.

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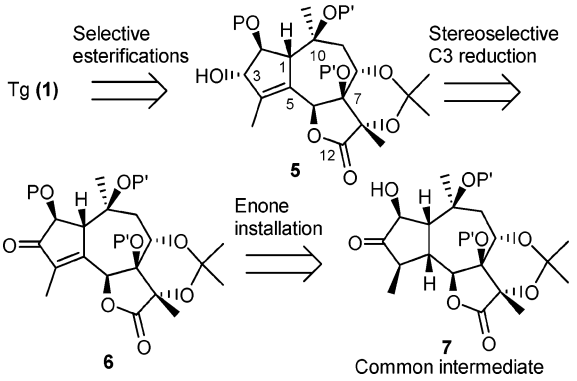
thus preventing sequestering of Ca^{2+} from the cytosol, allowing a massive secondary influx of extracellular Ca^{2+} leading to apoptosis.[7]

There has been a recent discovery that a prodrug, comprising Tg linked through C8 to a peptide specifically cleaved by a prostate-specific antigen (a serine protease), may allow site-specific targeting of Tg to prostate cancer cells.[8] The notion of thapsigargin as a real and viable prostate cancer treatment has therefore greatly elevated the need for its chemical synthesis.

The synthetic approach established during our syntheses of the related natural products,[2c,d] trilobolide **2**, nortrilobolide **3**, and thapsivillosin F **4**, recognizes that the bulk of the structural variation in the family exists in the five-ring cyclopentene unit. Thus, our approach to the synthesis of the thapsigargins centers upon a late-stage divergent strategy to this motif, via a common intermediate (Scheme 1). It was

Scheme 1. Late-Stage Retrosynthetic Analysis of Thapsigargin

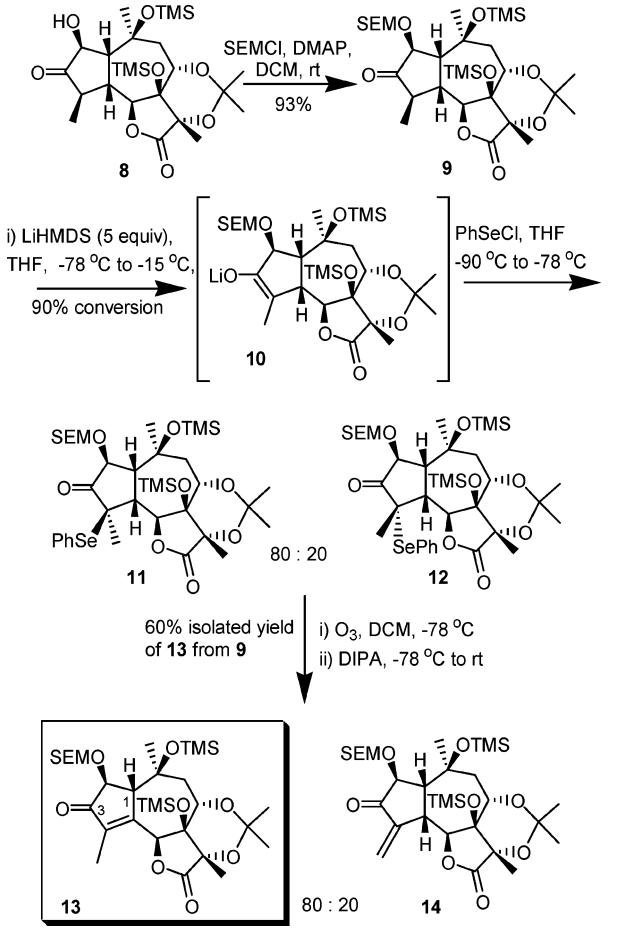


envisaged that this approach could allow access to all 17 natural compounds in the series as well as to late-stage analogues for structure−activity relationship analysis.[2c]

Several problems remained, however, before synthesis of thapsigargin **1** could be accomplished. First, introduction of the internal double bond at C4−C5 had only previously been achieved by an unusual catalytic selenium-mediated reaction which led to concomitant deoxygenation at C2.[2c,d] Second, establishing the C3 stereochemistry via reduction proved challenging with the presence of bulky functionality at C2. Finally, a highly selective esterification−deprotection routine would have to be implemented to reach the final natural product.

The synthesis began with the installation of the SEM ether at C2-*O* of our previously reported intermediate **8**[2c,d] in 93% yield (Scheme 2). This protecting group was selected for

Scheme 2. Selenide Introduction and Enone Installation



several reasons. First, model studies had suggested that a bulky group was necessary to direct enolization to the C4 position rather than C2; this would be pivotal to double bond installation. Second, it was deemed important to have a chelating group to deliver a hydride source to the ketone at C3 from the exo face of the five-membered ring, circumventing steric encumbrance at C2. The increased lability of SEM over a MOM ether was also pertinent due to our inability to remove MOM from the C2-*O* position in various intermediates during previous studies.

Enolization at C4 initially involved treatment of **9** with NaHMDS and attempted trapping with TMSCl. The resulting silyl enol ether, however, was highly unstable, so a protocol employing in situ reaction of the lithium enolate proved preferable. Treatment of **9** with 5 equiv of LiHMDS under strict temperature control allowed conversion to the desired lithium enolate without enolization of the C2 position. Reaction at −90 to −78 °C was optimal for approach of the electrophile (phenyl selenyl chloride) from the less-hindered (exo) face of the 5-7 fused bicycle. This resulted in a pleasing 80:20 mixture of selenide epimers **11** and **12**.

A selenide oxidation−elimination process was then implemented to regioselectively install the internal double bond. Statistically, however, removal of one of the three methyl protons by the intermediate selenoxide (leading to the

(7) (a) Thastrup, O.; Cullen, P. J.; Drøbak, B. K.; Hanley, M.; Dawson, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2466. (b) Futami, T.; Miyagashi, M.; Taira, K. *J. Biol. Chem.* **2005**, *280*, 826.

(8) (a) Jakobsen, C. M.; Denmeade, S. R.; Isaacs, J. T.; Gady, A.; Olsen, C. E.; Christensen, S. B. *J. Med. Chem.* **2001**, *44*, 4696. (b) Christensen, S. B.; Andersen, A.; Kromann, H.; Trieman, M.; Tombal, B.; Denmeade, S.; Isaacs, J. T. *Bioorg. Med. Chem.* **1999**, *7*, 1273. (c) Denmeade, S. R.; Jakobsen, S.; Janssen, S.; Khan, S. R.; Garrett, E. S.; Lilja, H.; Christensen, S. B.; Isaacs, J. T. *J. Natl. Cancer Inst.* **2003**, *95*, 990.

undesired exo double bond) would be more favorable than removal of the required single ring-junction proton (Figure 2). We were pleased to find that treatment of a mixture of

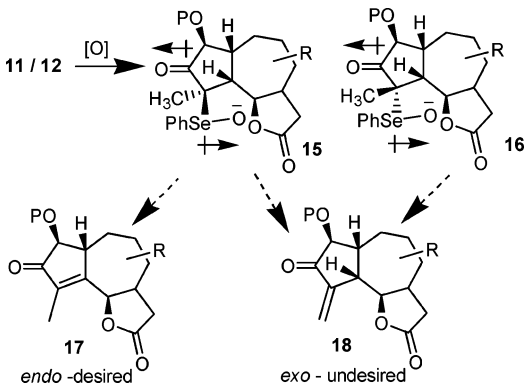


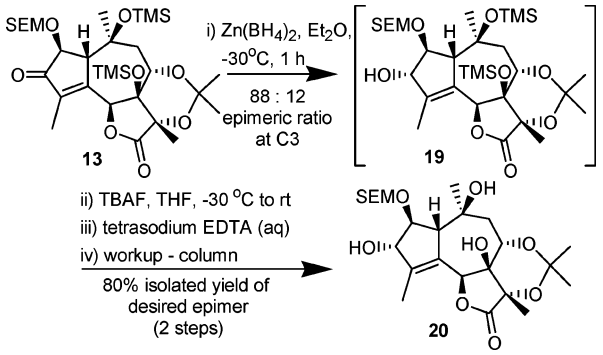
Figure 2. Possible fates of Tg selenoxides.

selenides **11/12** with ozone, followed by the addition of diisopropylamine, resulted in clean oxidation−elimination to translate the 80:20 mixture of C4 epimers to an 80:20 mixture of endo/exo products (Scheme 2). This result suggests that none of the desired isomer **15** takes part in proton abstraction from the methyl group, possibly due to minimization of dipole−dipole interactions between the selenoxide and the C3 ketone, encouraging the selenoxide closer to the internal proton.[9] The double bond isomers were then separated by flash chromatography to afford a good yield of the desired endo isomer (60% isolated yield of **13** from **9**).

To stereoselectively reduce the C3 ketone, it was thought that the inherent bulk of the seven-membered ring at C1 may lead to attack of a hydride source at the exo face, in accord with our trilobolide synthesis.[2d] Unfortunately, treatment of **13** with sodium borohydride (in methanol or THF) or under Luche conditions produced the undesired C3-(*R*) epimer almost exclusively. It was clear that the bulky C2 substituent overrides the steric bias of the bicycle, forcing reduction from the endo face. The use of zinc borohydride, however, reversed the stereoselectivity, resulting in an 88:12 ratio in favor of **19** by ^{1}H NMR (Scheme 3). This pleasing result was marred by the reduction product being highly unstable to isolation, presumably due to the presence of strongly Lewis acidic zinc residues.[10] However, after extensive experimentation, a satisfactory protocol was developed, involving telescoped reduction and TBAF addition to remove the silyl groups, coupled with washing with aqueous tetrasodium EDTA. This enabled isolation of **20**, affording a reliable 80% yield over the two steps.

A strategic esterification−deprotection strategy was all that was necessary to complete the synthesis. Literature reports

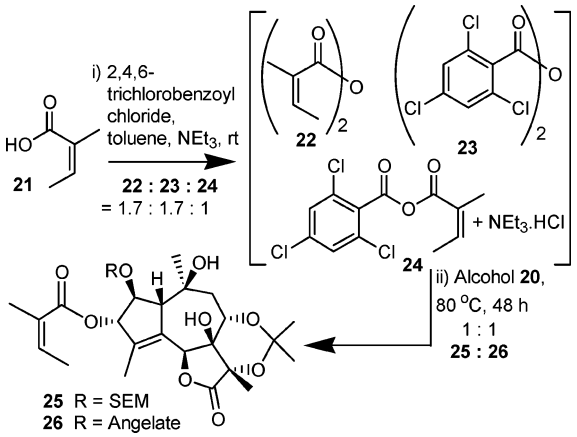
Scheme 3. Chelation-Controlled Reduction of **13**



suggest that angelate ester formation should be best performed using the Yamaguchi mixed anhydride, generated in situ from the acid chloride and angelic acid,[11] designed to prevent double bond isomerization to the thermodynamically favored tiglate ester.

Unfortunately, applying this protocol to **20** led to partial SEM deprotection, resulting in a 50:50 mixture of **25** and bisangeloyl product **26** (Scheme 4). On examining this

Scheme 4. Problematic Angelate Ester Formation



reaction further, we were surprised to find that ^{1}H NMR of the acylating mixture before addition of **20** revealed a 1:1.7:1.7 mixture of desired anhydride **24**, angelic anhydride **22** (proven in our hands to be far less reactive in acylation reactions), and 2,4,6-trichlorobenzoic anhydride **23**.[12] It was found that using an excess of the acyl chloride, slow addition of angelic acid, and flash chromatography allowed isolation of pure mixed anhydride **24**. Reacting this with **20** in the presence of $NaHCO_3$ circumvented loss of SEM, affording 52% yield of monoangelate **25** and 6% yield of unreacted **20** (Scheme 5). We believe the modest yield to be due to the low reactivity of **20**, with the remainder being lost to

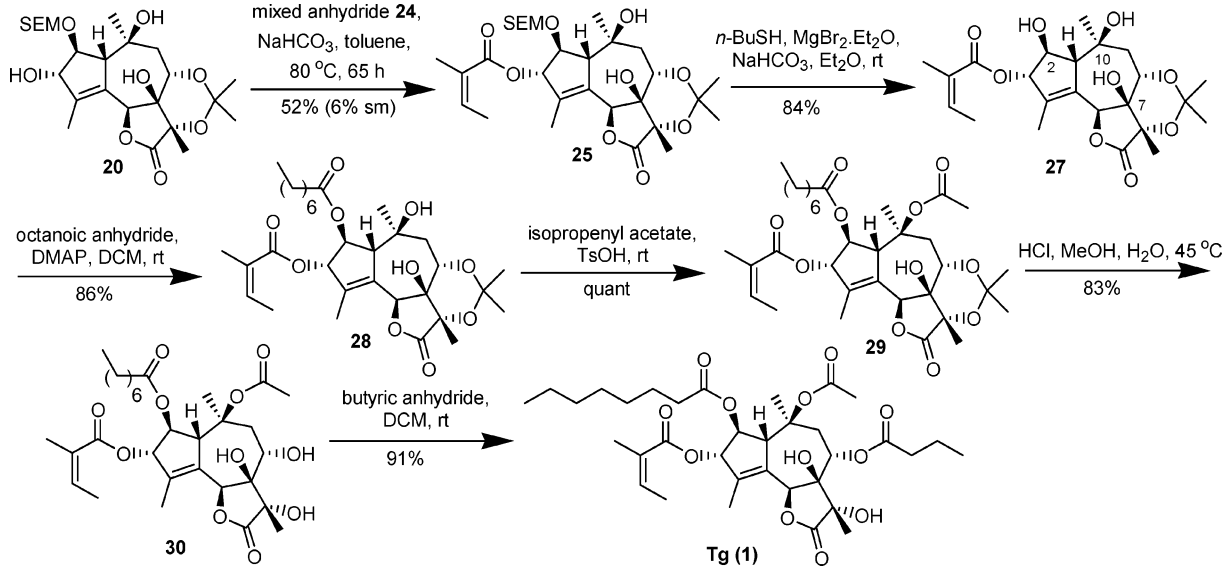
(9) For related discussions relating to dehydrosulfenylations, see: Trost, B. M.; Salzmann, T. N.; Hiroi. *J. Am. Chem. Soc.* **1976**, *98*, 4887.

(10) The use of polymer-supported zinc borohydride for a prolonged period followed by filtration returned only starting material.

(11) Hartmann, B.; Kanazawa, A. M.; Deprés, J. P.; Greene, A. E. *Tetrahedron Lett.* **1991**, *32*, 5077.

(12) For a related observation, see: Dhimitruka, I.; SantaLucia, J., Jr. *Org. Lett.* **2006**, *8*, 47.

Scheme 5. Selective Esterifications/Deprotections to Complete the Synthesis of Thapsigargin (**1**)



decomposition; under these new conditions, the epimeric C3-(*R*) alcohol affords 98% yield of its monoangelate.[13] Critically, no bisangeloyl material could be detected in either case. This should therefore be an improved procedure for others wishing to generate angelate esters under mildly basic conditions.

Despite literature reports of facile angelate to tiglate isomerization,[14] treatment of **25** with a Lewis acid and *n*-butane thiol[15] resulted in SEM deprotection with no detectable isomerization.[16] Selective esterification at C2-*O* was achieved with octanoic anhydride and DMAP, leaving the C7 and C10 alcohols unaffected. The C10-*O* position could then be acetylated using neat isopropenyl acetate and catalytic toluene sulfonic acid, also leaving the C7-*O* free. Finally, acetonide cleavage to give triol **30** and selective butyrate formation completed the total synthesis of thapsigargin **1** (17 mg scale), which was identical to the natural product by standard spectroscopic techniques.[17]

In conclusion, the total synthesis of thapsigargin has been achieved in a total of 42 steps and in 0.6% overall yield. This route has proved highly efficient, with an average yield of 88.6% per step and the ability to generate 10 mg of thapsigargin for every 380 mg of (*S*)-carvone as the readily available starting material.[2c,d] We believe that this synthetic route has the potential to address a need for greater quantities of thapsigargin and its analogues[18] within the biological community.

Acknowledgment. This work was supported by a GSK/Insight Faraday CASE Studentship (S.P.A.), E.P.S.R.C Research Fellowships (M.B., E.C.), the Novartis Research Fellowship (S.V.L.), a Royal Society URF (M.D.S.), and the Deutsche Forschungsgemeinschaft (F.W.). We also thank Prof. S. B. Christensen (Danish University of Pharmaceutical Sciences) for his kind donation of a sample of natural thapsigargin for reference purposes.

Supporting Information Available: Experimental procedures, full characterization, and copies of $^{1}H/^{13}C$ NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL062947X

(13) See experimental data; compound **25a**, Supporting Information.

(14) (a) Beeby, P. J. *Tetrahedron Lett.* **1977**, 3379. (b) Bal-Tembe, S.; Bhedi, D. N.; de Souza, J. N.; Rupp, R. H. *Heterocycles* **1987**, *26*, 1239.

(15) Vakalopolous, A.; Hoffmann, H. M. R. *Org. Lett.* **2000**, *2*, 1447.

(16) The ease of deprotection possibly indicates an interaction among the angelate ester, the SEM oxygens, and Lewis acid.

(17) ^{1}H NMR spectra of natural *and* synthetic thapsigargin in $CDCl_3$ were found to be highly concentration dependent. Mixing experiments with the natural product confirmed the structure of the synthetic material (see Supporting Information). Full details will be disclosed in due course.

(18) Søhoel, H.; Liljefors, T.; Ley, S. V.; Oliver, S. F.; Antonello, A.; Smith, M. D.; Olsen, C. E.; Isaacs, J. T; Christensen, S. B. *J. Med. Chem.* **2005**, *48*, 7005.