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Concise Synthesis of Thapsigargin from Nortrilobolide

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ABSTRACT

Herein, we describe an expedient synthesis of the hexaoxygenated guaianolide thapsigargin (**1**), a potent inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), from the natural product nortrilobolide (**2**). This protocol involves three key steps: the one-pot substitution-oxidation reaction of an allylic ester into its corresponding α,β-unsaturated ketone, subsequent stereoselective α´-acyloxylation in the presence of $Mn(OAc)_3$ and a highly stereoselective ketone reduction.

Thapsigargin (**1**) is a biologically active hexaoxygenated guaianolide belonging to the sesquiterpene family which has been intensively studied during the past decades (Figure 1).[1] This natural product, isolated from *Thapsia garganica* L. (Apiaceae), an umbelliferous plant growing in the Mediterranean area, is a potent inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA).[2] More than forty years of collaborative and intensive research to decipher its chemistry and pharmacology has recently led to several targeted prodrugs, by conjugation of thapsigargin to different peptides, for possible treatment of various cancer types such as prostate and liver cancer.[3] Among these prodrugs, G202, which was named mipsagargin by the USAN council, is currently in phase II clinical trial for patients with hepatocellular carcinoma.[4] As it is foreseen that the annual demand for thapsigargin will become an amount of approximately 1 ton per year, there is consequently a need to develop short and efficient synthetic or semisynthetic methods to prepare thapsigargin (**1**) and potential analogues on a large scale. Several chemical studies have been reported for the preparation of thapsigargin and related guaianolides but this has required considerable synthetic effort, providing methods that are not suitable for large scale synthesis.[5] For example, Ley and co-workers recently published the total synthesis of thapsigargin from (*S*)-carvone in 42 steps.[6]

Pentaoxygenated guaianolides such as nortrilobolide (**2**) can be isolated from the genera *Thapsia* and *Laser* (Figure 1).[7] We speculated that a probable pathway for accessing hexaoxygenated thapsigargin could involve the use of nortrilobolide as a starting material. Indeed, although direct oxygenation of the C-2 position in derivative **2** is not feasible, an appropriate transformation of C-3 into a ketone could possibly allow the stereoselective α´-acyloxylation of C-2 which after subsequent chemical modifications would provide thapsigargin (**1**).

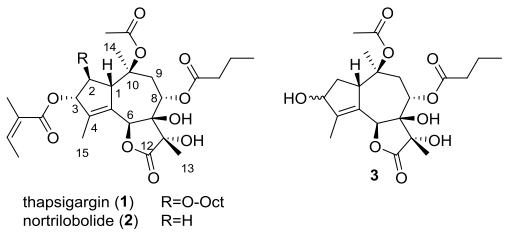

Figure 1. Structures of thapsigargin (**1**), nortrilobolide (**2**) and epimeric alcohol intermediates **3**. Oct = octanoyl.

Herein, we wish to report an expedient semisynthetic protocol for the preparation of compound **1** from natural product **2** in only 4 steps and 21% overall yield. This concise synthesis highlights two key transformations: a one-pot cleavage of the angelate ester and subsequent oxidation of the alcohol intermediate to its corresponding ketone as well as a stereoselective α´-acyloxylation.

First, the angelate moiety was cleaved upon treatment of nortrilobolide (**2**) with acid (AcOH, HF, TFA, *p*-TsOH·H_2O) in the presence of water and acetonitrile to afford the corresponding epimeric alcohols **3**. However, these allylic alcohols were extremely sensitive substrates and tended to decompose and/or

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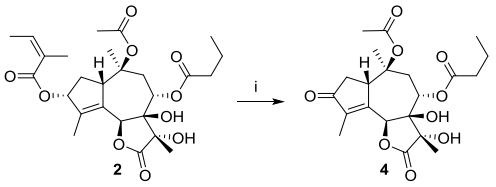
quickly dehydrate forming several by-products which explained the observed high conversion of the starting material but relatively low isolated yield.[8] Due to the labile nature of **3** it was deemed crucial to trap this intermediate during angelic ester cleavage and it was expected that this could be accomplished by oxidizing the alcohol as soon as it was formed. A one-pot, two-step procedure as illustrated in Scheme 1 was therefore developed. We envisioned chromium(VI) oxide as the reagent of choice due to its solubility in aqueous acidic media, ease of handling and removal and noteworthy its inertness when mixed with nortrilobolide (**2**). Aqueous hydrogen fluoride (HF) was chosen as the acidic medium for the reaction. Preliminary attempts showed that the use of an equivalent amount of CrO_3 was not sufficient to fully convert the starting material (Table 1, entry 1). To our delight, an increase to 2.5 equivalents of chromium(VI) oxide provided the desired ketone **4** in 60% after 6.5 h at 85 °C (Table 1, entry 2). The reaction was enhanced by the use of microwave (MW) irradiation (Table 1, entries 3–6) leading to the desired ketone in yields ranging from 61–68%. Finally, the use of 1.4 equivalents of the oxidizing reagent under MW conditions at 95 °C for 2 h in the presence of 2 equivalents of HF gave compound **4** in 74% yield (Table 1, entry 7). Pleasingly, this reaction could be easily performed on a gram scale.



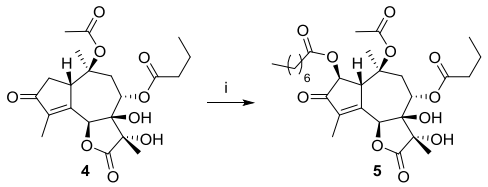
Scheme 1. Reagents and conditions: (i) HF (2 equiv), CrO_3 (1.4 equiv), CH_3CN, MW, 95 °C, 2 h, 74%.

Table 1. Synthesis of ketone **4** from nortrilobolide (**2**).

Entry	HF (equiv)	CrO_3 (equiv)	Temp. (°C)	Time (h)	Yield (%)
1	5	0.3	60[a]	16	20[c]
2	5	2.5	85[a]	6.5	60[d]
3	5	2.5	80[b]	1.5	61
4	5	2.5	85[b]	1.5	67
5	5	2.5	100[b]	0.5	68
6	2.5	1.25	100[b]	1	68
7	**2**	**1.4**	**95[b]**	**2**	**74**

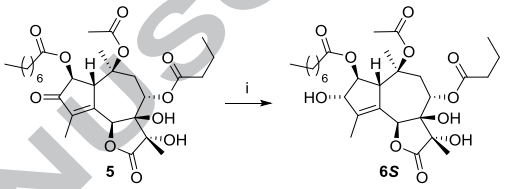
[a] Heating in an oil bath. [b] Under microwave conditions. [c] TLC showed the presence of the intermediate alcohols **3**, the starting material **2** as well as several by-products. [d] Starting material **2** (12%) was recovered after purification by chromatography.

The next challenge was the stereoselective introduction of the octanoyl backbone at the C-2 position of the ketone intermediate **4** via α′-acyloxylation. Several methods for selective α-oxygenation of carbony groups have been reported including oxidation with heavy metals,[9] hypoiodite catalyzed α-oxyacylation,[10] and sigmatropic rearrangement of acyloxyenamines.[11] We recently obtained successful results[8] using manganese(III) acetate.[12] Thus, ketone **4** was heated for 7 h at 120 °C in a mixture of dry benzene–octanoic acid (5:1) in the presence of 2.5 equivalents of $Mn(OAc)_3{\cdot}2H_2O$ using a Dean-Stark apparatus to give α´-acylated ketone **5** in 51% yield (Scheme 2).[13] Notably, this procedure resulted in the formation of **5** with the desired C-2 stereochemistry as confirmed by ^{1}H NMR comparison with a pure isolated sample.[14]



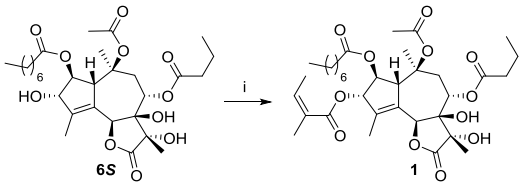
Scheme 2. Reagents and conditions: (i) $Mn(OAc)_3{\cdot}2H_2O$ (2.5 equiv), dry benzene–octanoic acid (5:1), 120 °C, Dean-Stark, 7 h, 51%.

Stereoselective reductions of similar ketones to give the α-alcohols have previously been performed using zinc borohydride.[6,15] In this case, treatment of acylated ketone **5** with $Zn(BH_4)_2$ in THF provided, after subsequent treatment with disodium dihydrate EDTA, two epimeric alcohols **6*S*** and **6*R*** in a 4:3 ratio.[16] To our delight, the target alcohol **6*S*** was isolated in 87% yield when the reaction was performed in dry Et_2O with only trace amounts of **6*R*** (Scheme 3).



Scheme 3. Reagents and conditions: (i) $Zn(BH_4)_2$ (11.6 equiv), dry Et_2O, -20 °C, 3.5 h, 87%.

Finally, angeloylation of alcohol **6*S*** furnished thapsigargin (**1**) in 65% yield (Scheme 4).[17]



Scheme 4. Reagents and conditions: (i) benzoyl chloride (3.5 equiv), angelic acid (3.5 equiv), TEA (3.5 equiv), dry PhMe, 90 °C, 72 h, 65%.

In conclusion, an expedient synthesis of thapsigargin (**1**) has been performed in 4 steps starting from the natural product nortrilobolide (**2**). It is noteworthy that the strategy does not involve any protection/deprotection steps. Further optimization and studies concerning the construction of new thapsigargin derivatives are currently in progress.

Acknowledgments

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13. A mixture of starting material **4** and the corresponding 2-acetoxy ketone derivative were also recovered after purification. These two compounds coelute and cannot be separated by chromatography on silica gel. For more details, see ESI as well as ref 8.
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16. Two other by-products were obtained during the reduction with zinc borohydride in THF. For more details, see ESI.
17. The mixed angelic anhydride was prepared according to a modified procedure described in: Liang, X.; Grue-Sørensen, G.; Petersen, A. K.; Högberg, T. *Synlett* **2012**, 2647–2652. Moreover, starting material **6S** (12%) was also recovered after purification by column chromatography.

Supplementary Material

Experimental procedures and copies of NMR spectra for all relevant synthesized compounds.

Graphical Abstract

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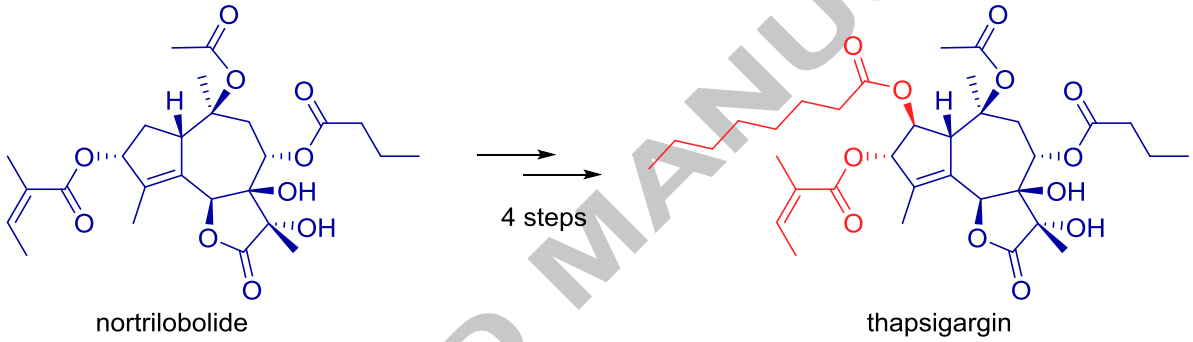

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