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(54) Title: NOVEL THAPSIGARGIN ANALOGUES AND METHODS OF PREPARING THEM

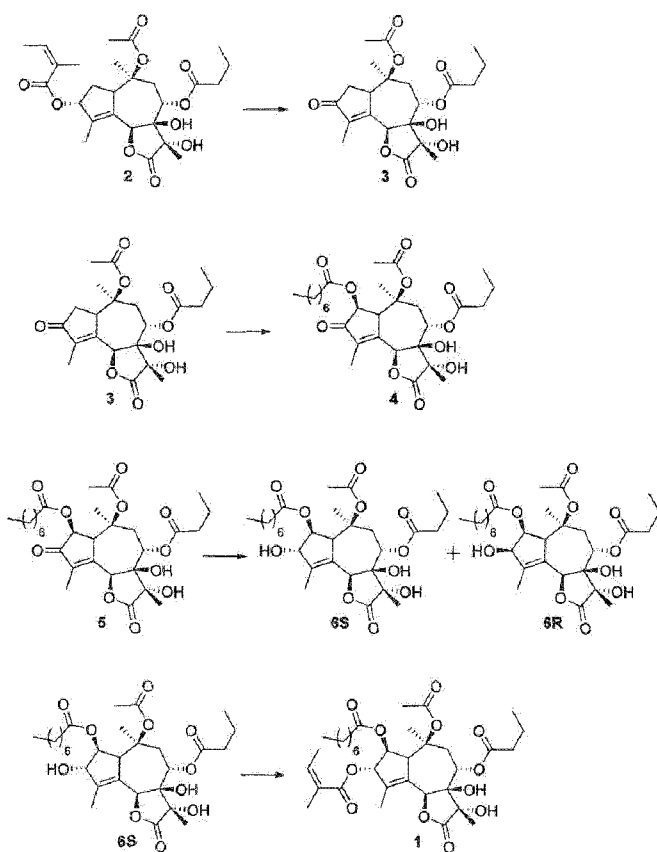


Fig 4

(57) Abstract: Present invention relates to a novel and efficient method of preparing thapsigargin analogues. Present invention also relates to specific compounds and their use for the preparation of thapsigargin analogues.



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Novel Thapsigargin analogues and methods of preparing them

Introduction

Present invention relates to novel thapsigargin analogues and a novel method of preparing thapsigargin analogues to accommodate for a shortened and efficient synthetic route of various thapsigargin analogues enabling for large scale synthesis of thapsigargin analogues from other natural products.

Background of Invention

In the treatment of various diseases, drugs based on various natural products have since long been used and are still very important in particular but not exclusively in the field of chemotherapeutics and antiparasitic agents. A well-known example is paclitaxel, marketed as Taxol, which is used in the treatment of various cancer types such as lung, ovarian, breast, head and neck cancer, and advanced forms of Kaposi's sarcoma.

Another natural product that is expected to become useful in medicine is thapsigargin, which is one of the secondary metabolites found in *Thapsia garganica* L. Apiaceae, of which populations are found in the Mediterranean area. Thapsigargin, a sesquiterpene lactone is a highly specific inhibitor of the sarcoplasmic and endoplasmic reticulum Ca^{2+} -ATPase (SERCA) pump. By inhibiting the SERCA pump, thapsigargin raises the cytosolic (intracellular) calcium concentration by blocking the ability of the cell to pump calcium into the sarcoplasmic or the endoplasmic reticula, which causes these stores to become depleted. Store-depletion can secondarily activate plasma membrane calcium channels, allowing an influx of calcium into the cytosol.

Thapsigargin specifically inhibits the fusion of autophagosomes with lysosomes; the last step in the autophagic process. The inhibition of the autophagic process in turn induces stress on the endoplasmic reticulum, which ultimately leads to cellular death.

A prodrug of thapsigargin has been indicated to be useful in treatment of various cancer types such as but not exclusively prostate, breast, liver, kidney cancer and in glioblastoma multiforme.

Several derivatives of thapsigargin have lately been developed, which can be targeted towards prostate cancer cells or endothelial cells in neovascular tissue in tumours. One class of analogues is so called prodrugs, meaning that the thapsigargin scaffold has

been derivatized to include structural moieties, which mask the pharmacological activities of the compound unless the prodrug is cleaved in specific cell or organ by physiological means e.g. enzymatic cleavage to release a pharmacological active compound e.g. a derivative of thapsigargin, which still possesses the apoptotic activity. This method is commonly used in drug development to target active compounds, wherein the use of the compound *per se* would cause an intolerable systemic toxicity.

In the case of thapsigargin prodrugs, two examples can be noted within the field of treatment of cancers. G115 and G202 are two examples that have been developed with the purpose of being hydrolyzed by two different enzymes. G115 is to be hydrolyzed by PSA (Prostate-specific antigen) which is a glycoprotein enzyme. G202 is to be hydrolyzed by PSMA (Prostate-specific membrane antigen). Consequently, the prodrugs have been tailored to be cleaved by the specific enzymes and thereafter allowing thapsigargin to perform its action.

As there is great potential use of these drugs in medicine it is foreseen that the annual demand for thapsigargin will become an amount to about 1 ton. Even though the plant *T. garganica* is considered a weed, its natural growth and spread will not match the annual demand and consequently, the natural population could potentially face extinction. In search of sustainable alternatives for the demand of natural products, synthetic or semi-synthetic methods have been employed. A similar problem concerning sustainable supply of paclitaxel was solved by developing a semi-synthetic route starting from deacetyl-baccatin, which may be isolated from the needles of *Taxus baccatus*.

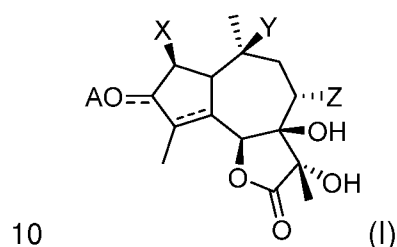
Consequently, there is an unmet need to have a complete or partially synthetic method of preparing thapsigargin analogues in a short and efficient manner in order to meet the demand for natural products or analogues thereof.

In the art reports have been made on the synthesis of thapsigargins and trilobolides, where e.g. (S)-carvone has been used as a starting point in the synthesis (Oliver, S. F., *et al.*, *Angew. Chem. Int. Ed.*, **2003**, 42, 5996–6000). Total synthesis of thapsigargin and analogues has been presented (Andrews S. P. *et al.*, *Chem. Eur. J.*, **2007**, 13, 5688–5712). However, the synthesis is lengthy and with a very low overall yield rendering the method unsuitable for large scale synthesis.

Summary of the Invention

Present invention relates to thapsigargin analogues, methods of preparing said analogues and use of said analogues as intermediates in method for preparing other thapsigargin analogues.

- 5 The invention relates to a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein Formula (I) is



wherein the dotted bonds represent an optional bond and wherein the dotted bond between the carbon and the oxygen represents an optional bond thus forming a carbonyl functional group and when the dotted bond present, A is absent;

15

A is -H or alkyl such as methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *i*-pentyl, *sec*-pentyl, neopentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl or an acyl group such as e.g. formyl, acetyl, propionyl, *n*-butyryl or an α,β -unsaturated acyl group which may be further optionally substituted such as angeloyl, crotonyl, acryloyl, 3,3-dimethylacryloyl, (*Z*) or (*E*)-2-methylbut-2-enoyl, etc. A may also be a trialkylsilyl group or a diarylalkylsilyl group such as trimethylsilyl, triethylsilyl, *tert*-butyldimethylsilyl or *tert*-butyldiphenylsilyl;

20

wherein X, Y and Z are independently same or different and selected from:

25

-H, -O-R, -S-R, -NHR, hal including I, Br, F;

wherein R is selected from; H, alkyl or substituted alkyl (C_1 - C_{18}), benzyl, $-C(=O)-R''$, $-SiR'_2R'''$;

30

and wherein R', R'' and R''' are independently same or different and are selected from alkyl, substituted alkyl aryl or substituted aryl;

where alkyl is any alkyl having C₁-C₁₈ straight or branched carbon skeleton, such as
 5 e.g. methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *i*-pentyl, *sec*-pentyl, neopentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, etc., which may be substituted by any halogen such as fluoro, chloro, bromo or iodo, C₁₋₆ alkyl, C₃₋₆ cycloalkyl, hydroxy, C₁₋₆ alkoxy, cyano, amino, nitro, C₁₋₆ alkylamino, C₂₋₆ alkenylamino, di-C₁₋₆ alkylamino, C₁₋₆ acylamino, di-
 10 C₁₋₆ acylamino, C₁₋₆ aryl, C₁₋₆ arylamino, C₁₋₆ aroylamino, benzylamino, C₁₋₆ arylamido, carboxy, C₁₋₆ alkoxy carbonyl or (C₁₋₆ aryl)(C₁₋₁₀ alkoxy)carbonyl, carbamoyl, mono-C₁₋₆ carbamoyl, di-C₁₋₆ carbamoyl or any of the above in which a hydrocarbyl moiety is itself substituted by halo, cyano, hydroxy, C₁₋₂ alkoxy, amino, nitro, carbamoyl, carboxy or C₁₋₂ alkoxy carbonyl. In groups containing an oxygen atom such as hydroxy and alkoxy,
 15 the oxygen atom can be replaced with sulfur to make groups such as thio (SH) and thioalkyl (S-alkyl). Optional substituents therefore include groups such as S-methyl. In thioalkyl groups, the sulfur atom may be further oxidized to make a sulfoxide or sulfone, and thus optional substituents therefore includes groups such as S(O)-alkyl and S(O)₂-alkyl. Alkyl may also be any straight or branched C₁-C₁₈ alkyl having one or more dou-
 20 ble and/or triple bonds e.g. 5-octenyl, 3-butenyl, 7-dodecenyl etc.;

where aryl is e.g. phenyl, substituted phenyl, benzyl, substituted benzyl, etc.

Substitution may take the form of double bonds, and may include heteroatoms. Thus
 25 an alkyl group with a carbonyl (C=O) instead of a CH₂ can be considered a substituted alkyl group.

Substituted groups thus include for example CFH₂, CF₂H, CF₃, CH₂NH₂, CH₂OH, CH₂CN, CH₂SCH₃, CH₂OCH₃, OCH₃, OCH₂CH₃, CH₃, CH₂CH₃, -OCH₂O-, CO₂CH₃,
 30 COCH₃, *i*-Pr, SCF₃, SO₂CH₃, N(CH₃)₂, CONH₂, CON(CH₃)₂, etc. In the case of aryl groups, the substitutions may be in the form of rings from adjacent carbon atoms in the aryl ring, for example cyclic acetals such as OCH₂O.

The invention relates to a compound of Formula (I), wherein A is -H and there is no
 35 double bond on the oxygen atom connected to A and wherein Z is butanoyloxy or 2-

methylbutanoyloxy and wherein X is -H, -OAc (acetyl), -OHex (hexanoyl), -OOct (octanoyl) or Hal, and Y is acetyl.

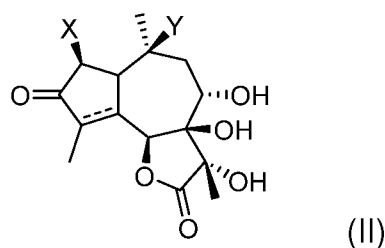
The invention also relates to a compound of Formula (I), wherein A is absent and
 5 wherein the optional double bond is present to form a carbonyl functional group and
 wherein Z is *n*-butanoyloxy, 2-methylbutanoyloxy or formyloxy, acetyloxy, propionyloxy,
 or an α,β -unsaturated acyloxy group which may be further optionally substituted such
 as angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-
 methylbut-2-enoyloxy or a Boc protected ω -aminoacyloxy like Boc-protected 12-
 10 aminododecanoyloxy, and wherein X is -H and Y is acetyloxy or X is acetyloxy and Y
 is acetyloxy.

The invention relates to a compound of Formula (I), wherein A is absent and wherein
 the optional double bond is present to form a carbonyl functional group and wherein Z
 15 is butanoyloxy or 2-methylbutanoyloxy and wherein X is -H, -OAc (acetyl), -OHex
 (hexanoyl), -OOct (octanoyl) or Hal, and Y is acetyl. X may be -H or -OOct.

The invention also relates to a compound according to Formula (I), wherein X is -OR,
 Y is -OR, and Z is -OR, wherein R is the same or different in X, Y and Z, and R is -
 20 C(=O)R". R" may be e.g. -H, -Me, -Et, -Pr, *i*-Pr, *n*-Bu, *n*-Hex or *n*-Oct. X may be -OOct,
 Y may be OAc and Z may be OBu. -OA may be -OH or =O.

The invention also relates to a compound of formula (II) or a pharmaceutically accepta-
 ble salt thereof

25



where the dotted bond denotes an optional bond and wherein X and Y are inde-
 30 pendently same or different and selected from:

-H, -O-R, -S-R, -NHR, -hal including I, Br, F;

wherein R is selected from; H, alkyl or substituted alkyl (C₁-C₁₈), benzyl, -C(=O)-R'', -SiR'₂R''';

5

and wherein R', R'' and R''' are independently same or different and are selected from alkyl or substituted alkyl;

where alkyl is any alkyl having C₁-C₁₈ straight or branched carbon skeleton such as
 10 methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *i*-pentyl, *sec*-pentyl, neopentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, etc., which may be substituted by halo (fluoro, chloro, bromo or iodo), C₁₋₆ alkyl, C₃₋₆ cycloalkyl, hydroxy, C₁₋₆ alkoxy, cyano, amino, nitro, C₁₋₆ alkylamino, C₂₋₆ alkenylamino, di-C₁₋₆ alkylamino, C₁₋₆ acylamino, di-C₁₋₆ acylamino, C₁₋₆ aryl,
 15 C₁₋₆ arylamino, C₁₋₆ aroylamino, benzylamino, C₁₋₆ arylamido, carboxy, C₁₋₆ alkoxy carbonyl or (C₁₋₆ aryl)(C₁₋₁₀ alkoxy)carbonyl, carbamoyl, mono-C₁₋₆ carbamoyl, di-C₁₋₆ carbamoyl or any of the above in which a hydrocarbonyl moiety is itself substituted by halo, cyano, hydroxy, C₁₋₂ alkoxy, amino, nitro, carbamoyl, carboxy or C₁₋₂ alkoxy carbonyl.

20 In groups containing an oxygen atom such as hydroxy and alkoxy, the oxygen atom can be replaced with sulfur to make groups such as thio (SH) and thioalkyl (S-alkyl). Optional substituents therefore include groups such as S-methyl. In thioalkyl groups, the sulfur atom may be further oxidized to make a sulfoxide or sulfone, and thus optional substituents therefore include groups such as S(O)-alkyl and S(O)₂-alkyl.

25

Alkyl may also be any straight or branched C₁-C₁₈ alkyl having one or more double and/or triple bonds e.g. 5-octenyl, 3-butenyl, 7-dodecenyl, etc.

Aryl is e.g. phenyl, substituted phenyl, benzyl, substituted benzyl, etc.

30

Substitution may take the form of double bonds, and may include heteroatoms. Thus an alkyl group with a carbonyl (C=O) instead of a CH₂ can be considered a substituted alkyl group.

35

Substituted groups thus include for example CFH₂, CF₂H, CF₃, CH₂NH₂, CH₂OH, CH₂CN, CH₂SCH₃, CH₂OCH₃, OCH₃, OCH₂CH₃, CH₃, CH₂CH₃, -OCH₂O-, CO₂CH₃,

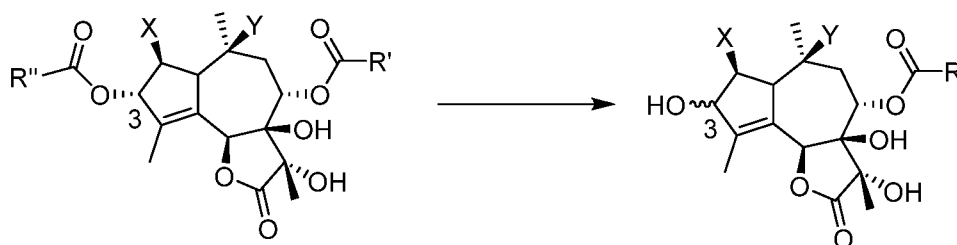
COCH₃, *i*-Pr, SCF₃, SO₂CH₃, N(CH₃)₂, CONH₂, CON(CH₃)₂, etc. In the case of aryl groups, the substitutions may be in the form of rings from adjacent carbon atoms in the aryl ring, for example cyclic acetals such as OCH₂O.

- 5 The invention also relates to a compound according to Formula (II), wherein X is -OR and Y is -OR, and wherein R is the same or different in X and Y, and R is -C(=O)R". R" may be e.g. -H, -Me, -Et, -Pr, *i*-Pr, *n*-Bu, *n*-Hex or *n*-Oct. R" may be specifically -Me.
- 10 Specifically X and Y are OAc, or X is OHex or OOct and Y is OAc.

Present invention also relates to a method of preparing compound according to Formula (I) or (II). The method of preparing a compound according to formula (I) or (II) may comprise a reaction to substitute the group in the 3-position with a hydroxyl group or

15 any group that might be converted into a hydroxyl group such as e.g. acyloxy or alkoxymethoxy in which the alkoxy group may contain from one to six carbon atoms. The reaction conditions may include the use of acid reagents such as hydrogen fluoride or other acids with similar pKa values or other acids *p*-TsOH in a mixture of water and an appropriate aprotic solvent like acetonitrile, tetrahydrofuran, dioxolane, dimethyl-

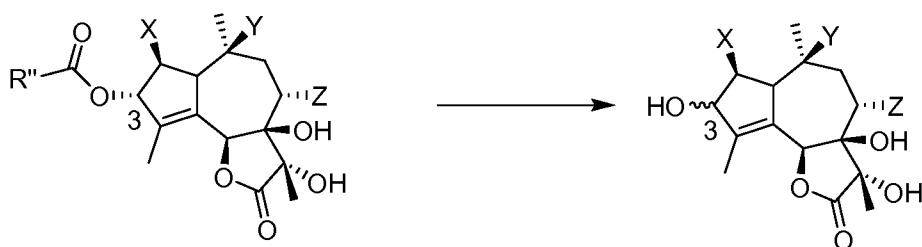
20 sulfoxide or *N,N*-dimethylformamide. The conditions may furthermore include heating of the reaction mixture to an appropriate temperature of 60°C. In the figure below, the reaction is exemplified by an acyloxy group in the 3-position:



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- wherein the -OC(=O)R' and -OC(=O)R" may be independently e.g. *n*-butanoyloxy, 2-methylbutanoyloxy- or formyloxy, acetyloxy, propionyloxy, or an α,β -unsaturated acyloxy group which may be further optionally substituted e.g. angeloyloxy, crotonyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-eneoyloxy or a
- 30 Boc protected ω -aminoacyloxy like Boc-protected 12-aminododecanoyloxy or a *N*-Boc-protected ω -aminoacyloxy having 6-28 carbon atoms.

A more general presentation is



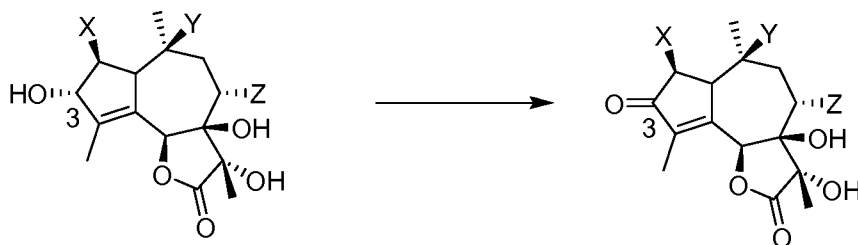
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wherein X, Y, Z and R'' are as defined herein-

Without wishing to be bound by any specific theory, it is believed that the reaction proceeds via an S_N1 reaction mechanism or a combination of an S_N1 and S_N2 mechanism.

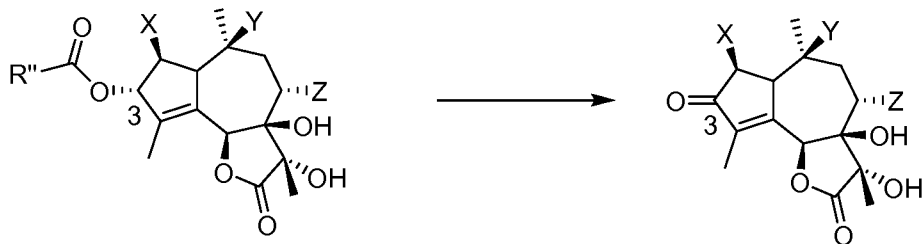
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The alcohol above may be oxidized to the corresponding ketone, or in a variant of the method (variant II) the alcohol is not isolated, but immediately oxidized in a one-pot two-step procedure as described herein.



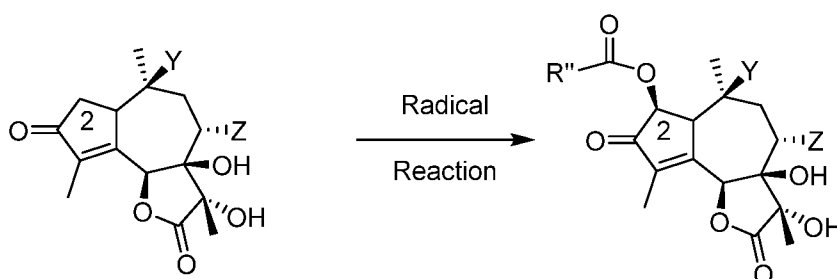
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or alternatively:

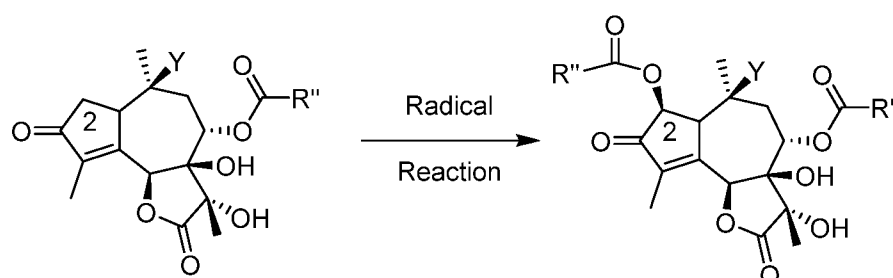


20 The method of preparing compounds according to Formula (I) or (II) (wherein X=H) may comprise a reaction step wherein the 2-position is *stereoselectively* substituted with an alkanoyloxy group by a radical reaction such as

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or exemplified with Z being $-\text{O}-\text{C}(=\text{O})\text{R}''$



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wherein $-\text{O}-\text{C}(=\text{O})\text{R}''$ may be e.g. *n*-butanoyloxy, 2-methylbutanoyloxy or formyloxy, acetyloxy, propionyloxy, or an α,β -unsaturated acyloxy group which may be further optionally substituted such as e.g. angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-

10 dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-enoyloxy or a Boc protected ω -aminoacyloxy like Boc-protected 12-aminododecanoyloxy, or a *N*-Boc-protected ω -aminoacyloxy having 6-28 carbon atoms;

wherein R'' may be e.g. methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, 6-

15 methylheptyl, octyl, 6-methyloctyl, nonyl or decyl.

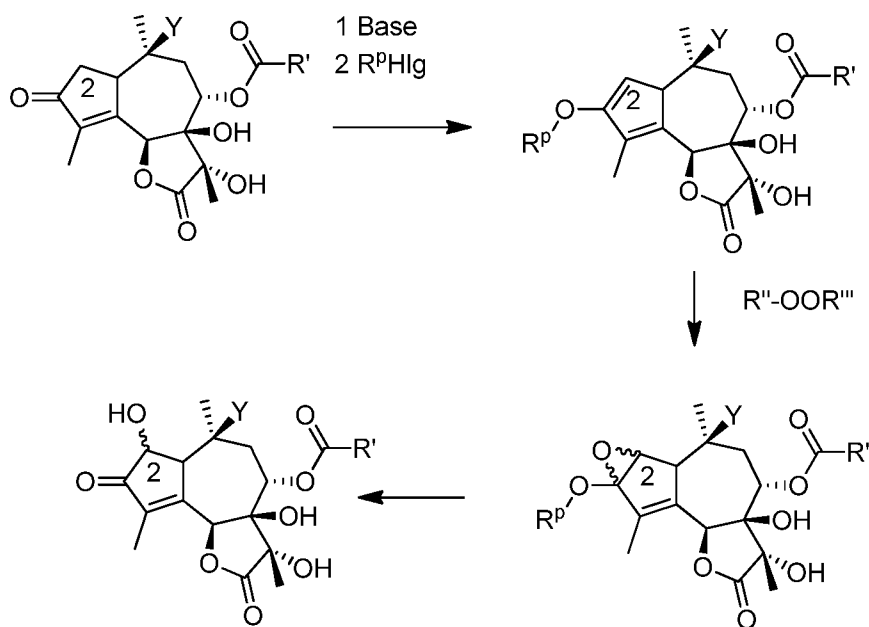
The reaction conditions may involve $\text{Mn}(\text{OCOR}'')_3$ as an oxidation reagent and may further include a mixture of a carboxylic acid and benzene as solvent, wherein R'' may be e.g. methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl or decyl or substituted

20 alkanoyl groups with amino groups such as omega-aminoalkanoyl groups..

Present invention also relates to a method of preparing compound according to Formula (II). The method for preparation may comprise epoxidation of the enol of compound I by reacting with a peroxy derivative:

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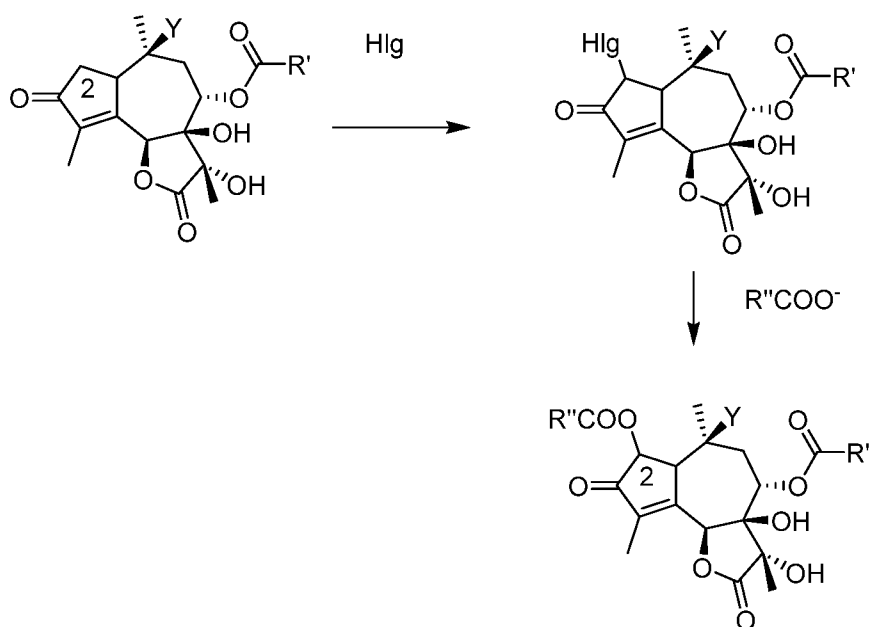
In following general reaction schemes as the above, Z may be exemplified as $-\text{O}-\text{C}(=\text{O})-\text{R}'$, but this is for illustrative purposes and not intended to limit Z specifically to $-\text{O}-\text{C}(=\text{O})-\text{R}'$.

In which R' might be an alkyl group and the base may be a lithium salt of a secondary amine such as a lithium amide which may e.g. be lithiumdiisopropyl amide (LDA), 2,2,6,6-tetramethylpiperidine (LiTMP), or the likes or a tertiary amine e.g. triethylamine, 1,8-diazabicycloundec-7-ene (DBU), etc.; Hlg might be Cl, Br, I or another good leaving group and R^p might be a protecting group like trimethylsilyl, triethylsilyl, *tert*-butyldimethylsilyl or *tert*-butyldiphenylsilyl.

The peroxide reagent $\text{R}''\text{-O-O-R}'''$ may be any standard peroxide reagent such as e.g. hydrogen peroxide, benzoyl peroxide, dimethyldioxirane (DMDO), *tert*-butyl hydroperoxide, 3-chloroperoxybenzoic acid (mCPBA), peroxyacetic acid but may also be other oxidizing reagents such as e.g. sodium or potassium persulfate.

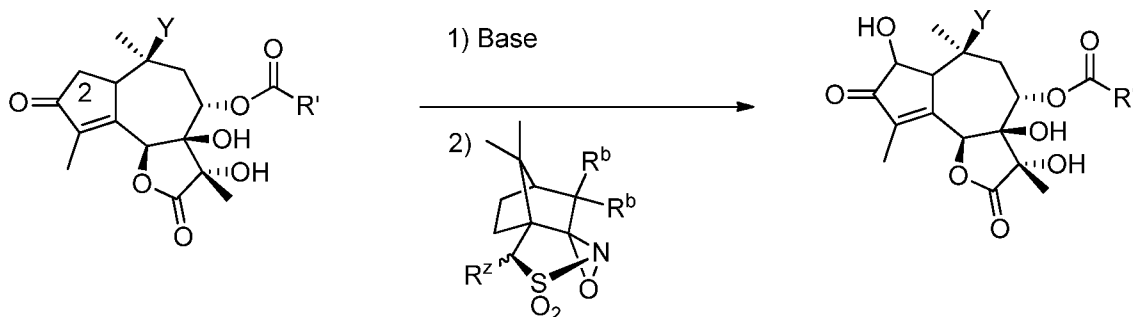
Present invention also relates to the preparation of compound II by introduction of a good leaving group in the 2-position followed by substitution with an alkanoyloxy group

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Hlg might be Br_2 or *N*-bromosuccinimide or *N*-iodosuccinimide, etc.

- 5 Present invention also relates to the preparation of compound according to Formula (II). The method for preparing compound according to Formula (II) may comprise hydroxylation of enolates by reacting with a camphor-based oxaziridine:



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in which the base can be a lithium salt of a secondary amine such as e.g. a lithium amide which may e.g. be lithiumdiisopropyl amide (LDA), 2,2,6,6-tetramethylpiperidine (LiTMP), or the likes. R^b can be H, OCH_3 or Cl and R^z can be H, OCH_3 , *p*- CH_3OBn or *p*- CF_3Bn .

15

Present invention also relates to the use of a compound according to Formula (I) as an intermediate in further synthesis towards pharmaceutically active compounds such as thapsigargin analogues and any prodrugs thereof.

Specifically, the use of a compound according to Formula (I) as an intermediate in further synthesis towards pharmaceutically active compounds such as thapsigargin analogues and any prodrugs thereof, is wherein A is –H and there is no double bond on the oxygen atom connected to A and wherein Z is butyloyl and wherein X is –H and Y is acetyl.

Moreover, the use of a compound according to Formula (I) as an intermediate in further synthesis towards pharmaceutically active compounds such as thapsigargin analogues and any prodrugs thereof, is wherein A is absent and wherein the optional double bond is present to form a carbonyl functional group and wherein Z is butanoyloxy or 2-methylbutanoyloxy and wherein X is –H and Y is ethanoyloxy, or X is ethanoyloxy and Y is ethanoyloxy.

Present invention also relates to a compound according to Formula (I) for use in medicine.

Present invention furthermore relates to a compound of Formula (I) or (II) for use in treatment of cancer such as prostate cancer, benign prostatic hyperplasia (BEH), glioblastoma multiforme, lung cancer, liver cancer, renal cell cancer, ovarian cancer, melanoma, bladder cancer, or breast cancer.

Present invention also relates to a pharmaceutical formulation comprising a compound of Formula (I) or (II). A suitable pharmaceutical formulation may be e.g. a composition for local administration wherein the composition releases the compound according to the invention locally in an extended release fashion. A suitable formulation may e.g. be a tablet, capsule, etc.

The Inventors of present invention have found that the use of another naturally occurring product can be used as a starting material in a shortened synthesis towards thapsigargin analogues. Trilobolide can be extracted from *Laser trilobum*, Apiaceae which is a plant growing in central Europe from France to Hungary and fruits of this plant are commercially available. It is therefore envisaged that since *L. trilobum* has a wider geographic distribution, the availability of suitable starting material is larger and will effectively decrease cost of preparation of thapsigargin analogues and ensure a sustainable supply.

Definitions

As used herein the term “alkyl” refers to any straight or branched chain composed of only sp³ carbon atoms, fully saturated with hydrogen atoms e.g. $-C_nH_{2n+1}$ for straight chain alkyls, wherein n can be in the range of 1 and 18 such as methyl, ethyl, propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, neopentyl, *i*-pentyl, 2,2,2-trimethylethyl, *n*-hexyl, *i*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl or *n*-decyl. The alkyl as used herein may be further substituted.

As used herein the term “alkanoyl” refers to any straight or branched chain composed of chains consisting of carbon atoms either sp³, sp² or sp hybridized. The chain is terminated with a carbonyl group. Examples of this term may be e.g. formyl, acetyl (ethanoyl), propionyl, butanoyl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, dodecanoyl, etc. Examples of alkanoyl may be, but not limited to e.g. *n*-butanoyl, 2-methylbutanoyl or formyl, acetyl, propionyl, or an α,β -unsaturated acyl group which may be further optionally substituted such as angeloyl, crotonoyl, acryloyl, 3,3-dimethylacryloyl, (*Z*) or (*E*)-2-methylbut-2-enoyl or a *N*-Boc protected ω -aminoacyl like *N*-Boc-protected 12-aminododecanoyloxy, or a *N*-Boc-protected ω -aminoacyl having 6-28 carbon atoms.

As used herein the term “alkanoyloxy” refers to any straight or branched chain composed of chains consisting sp³ carbon atoms, fully saturated with hydrogen atoms e.g. $-C_nH_{2n+1}$ for straight chain alkyls, wherein n can be in the range of 1 and 18 such as methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, neopentyl, *i*-pentyl, 2,2,2-trimethylethyl, *n*-hexyl, *i*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl or *n*-decyl. The chain is terminated by a carbonyloxy group such that $-C(=O)-O-$. Examples of alkanoyloxy may be, but not limited to e.g. *n*-butanoyloxy, 2-methylbutanoyloxy or formyloxy, acetyloxy (ethanoyloxy), propionyloxy, or an α,β -unsaturated acyloxy group which may be further optionally substituted such as angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-enoyloxy or a Boc protected ω -aminoacyloxy like Boc-protected 12-aminododecanoyloxy, or a *N*-Boc-protected ω -aminoacyloxy having 6-28 carbon atoms.

As used herein the term “cycloalkyl” refers to a cyclic/ring structured carbon chains having the general formula of $-C_nH_{2n-1}$ where n is between 3-10 e.g. cyclopropyl, cyclo-

butyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl, bicycle[3.2.1]octyl, spiro[4,5]decyl, norpinyl, norbonyl, norcapryl, adamantyl and the likes.

As used herein, the term "alkene" refers to a straight or branched chain composed of carbon and hydrogen atoms wherein at least two carbon atoms are connected by a double bond e.g. C₂₋₁₀ alkenyl unsaturated hydrocarbon chain having two to ten carbon atoms and at least one double bond. C₂₋₆ alkenyl groups include, but are not limited to, vinyl, 1-propenyl, allyl, *i*-propenyl, *n*-butenyl, *n*-pentenyl, *n*-hexenyl and the likes.

The term "C₁₋₁₀ alkoxy" in the present context designates a group -O-C₁₋₆ alkyl used alone or in combination, wherein C₁₋₁₀ alkyl is as defined above. Examples of linear alkoxy groups are methoxy, ethoxy, propoxy, butoxy, pentoxy and hexoxy. Examples of branched alkoxy are *i*-propoxy, *sec*-butoxy, *tert*-butoxy, *i*-pentoxy and *i*-hexoxy. Examples of cyclic alkoxy are cyclopropyloxy, cyclobutyloxy, cyclopentyloxy and cyclohexyloxy.

The term "C₃₋₇ heterocycloalkyl" as used herein denotes a radical of a totally saturated heterocycle like a cyclic hydrocarbon containing one or more heteroatoms selected from nitrogen, oxygen and sulfur independently in the cycle. Examples of heterocycles include, but are not limited to, pyrrolidine (1-pyrrolidine, 2-pyrrolidine, 3-pyrrolidine, 4-pyrrolidine, 5-pyrrolidine), pyrazolidine (1-pyrazolidine, 2-pyrazolidine, 3-pyrazolidine, 4-pyrazolidine, 5-pyrazolidine), imidazolidine (1-imidazolidine, 2-imidazolidine, 3-imidazolidine, 4-imidazolidine, 5-imidazolidine), thiazolidine (2-thiazolidine, 3-thiazolidine, 4-thiazolidine, 5-thiazolidine), piperidine (1-piperidine, 2-piperidine, 3-piperidine, 4-piperidine, 5-piperidine, 6-piperidine), piperazine (1-piperazine, 2-piperazine, 3-piperazine, 4-piperazine, 5-piperazine, 6-piperazine), morpholine (2-morpholine, 3-morpholine, 4-morpholine, 5-morpholine, 6-morpholine), thiomorpholine (2-thiomorpholine, 3-thiomorpholine, 4-thiomorpholine, 5-thiomorpholine, 6-thiomorpholine), 1,2-oxathiolane (3-(1,2-oxathiolane), 4-(1,2-oxathiolane), 5-(1,2-oxathiolane)), 1,3-dioxolane (2-(1,3-dioxolane), 3-(1,3-dioxolane), 4-(1,3-dioxolane)), tetrahydropyrane (2-tetrahydropyrane, 3-tetrahydropyrane, 4-tetrahydropyrane, 5-tetrahydropyrane, 6-tetrahydropyrane), hexahydropyridazine, (1-(hexahydropyridazine), 2-(hexahydropyridazine), 3-(hexahydropyridazine), 4-(hexahydropyridazine), 5-(hexahydropyridazine), 6-(hexahydropyridazine)).

The term " C_{1-10} alkyl- C_{3-10} cycloalkyl" as used herein refers to a cycloalkyl group as defined above attached through an alkyl group as defined above having the indicated number of carbon atoms.

- 5 The term " C_{1-10} alkyl- C_{3-7} heterocycloalkyl" as used herein refers to a heterocycloalkyl group as defined above attached through an alkyl group as defined above having the indicated number of carbon atoms.

The term "aryl" as used herein is intended to include carbocyclic aromatic ring systems.

- 10 Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems enumerated below.

The term "heteroaryl" as used herein includes heterocyclic unsaturated ring systems containing one or more heteroatoms selected among nitrogen, oxygen and sulfur, such

- 15 as furyl, thienyl, pyrrolyl, and is also intended to include the partially hydrogenated derivatives of the heterocyclic systems enumerated below.

The terms "aryl" and "heteroaryl" as used herein refers to an aryl, which can be optionally unsubstituted or mono-, di- or trisubstituted, or a heteroaryl, which can be optional-

- 20 ly unsubstituted or mono-, di- or trisubstituted. Examples of "aryl" and "heteroaryl" include, but are not limited to, phenyl, biphenyl, indenyl, naphthyl (1-naphthyl, 2-naphthyl), *N*-hydroxytetrazolyl, *N*-hydroxytriazolyl, *N*-hydroxyimidazolyl, anthracenyl (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), phenanthrenyl, fluorenyl, pentalenyl, azulenyl, biphenylenyl, thiophenyl (1-thienyl, 2-thienyl), furyl (1-furyl, 2-furyl), furanyl, thiophenyl, isoxazolyl, isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, pyranyl, pyridazinyl, pyrazinyl, 1,2,3-triazinyl, 1,2,4-triazinyl, 1,3,5-triazinyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, tetrazolyl, thiadiazinyl, indolyl, isoindolyl, benzofuranyl, benzothiophenyl (thianaphthenyl), indolyl, oxadiazolyl, isoxazolyl, quinazolinyl, fluorenyl, xanthenyl, isoindanyl, benzhydryl, acridinyl, benzisoxazolyl, purinyl, quinazolinyl, quinoliziny, quinolinyl, isoquinolinyl, quinoxaliny, naphthyridinyl, phteridinyl, azepinyl, diazepinyl, pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), 5-thiophene-2-yl-2H-pyrazol-3-yl, imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl, 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-
- 35

pyrimidinyl), pyrazinyl, pyridazinyl (3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), iso-quinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), benzo[*b*]furanyl (2-benzo[*b*]furanyl, 3-benzo[*b*]furanyl, 4-benzo[*b*]furanyl, 5-benzo[*b*]furanyl, 6-benzo[*b*]furanyl, 7-benzo[*b*]furanyl), 2,3-dihydro-benzo[*b*]furanyl (2-(2,3-dihydro-benzo[*b*]furanyl), 3-(2,3-dihydro-benzo[*b*]furanyl), 4-(2,3-dihydro-benzo[*b*]furanyl), 5-(2,3-dihydro-benzo[*b*]furanyl), 6-(2,3-dihydro-benzo[*b*]furanyl), 7-(2,3-dihydro-benzo[*b*]furanyl)), benzo[*b*]thiophenyl (2-benzo[*b*]thiophenyl, 3-benzo[*b*]thiophenyl, 4-benzo[*b*]thiophenyl, 5-benzo[*b*]thiophenyl, 6-benzo[*b*]thiophenyl, 7-benzo[*b*]thiophenyl), 2,3-dihydro-benzo[*b*]thiophenyl (2-(2,3-dihydro-benzo[*b*]thiophenyl), 3-(2,3-dihydro-benzo[*b*]thiophenyl), 4-(2,3-dihydro-benzo[*b*]thiophenyl), 5-(2,3-dihydro-benzo[*b*]thiophenyl), 6-(2,3-dihydro-benzo[*b*]thiophenyl), 7-(2,3-dihydro-benzo[*b*]thiophenyl)), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazolyl (1-indazolyl, 2-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl, (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl). Non-limiting examples of partially hydrogenated derivatives are 1,2,3,4-tetrahydronaphthyl, 1,4-dihydronaphthyl, pyrrolinyl, pyrazolinyl, indolinyl, oxazolidinyl, oxazolinyl, oxazepinyl and the likes.

As used herein the term “acyl” refers to a carbonyl group $-C(=O)R$ wherein the R group is any of the above defined groups. Specific examples are formyl, acetyl, propionyl, butyryl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, benzoyl and the likes.

“Optionally substituted” as applied to any group means that the said group may, if desired, be substituted with one or more substituents, which may be the same or different. ‘Optionally substituted alkyl’ includes both ‘alkyl’ and ‘substituted alkyl’.

Examples of suitable substituents for “substituted” and “optionally substituted” moieties include halo (fluoro, chloro, bromo or iodo), C_{1-6} alkyl, C_{3-6} cycloalkyl, hydroxy, C_{1-6} alkoxy, cyano, amino, nitro, C_{1-6} alkylamino, C_{2-6} alkenylamino, di- C_{1-6} alkylamino, C_{1-6} acylamino, di- C_{1-6} acylamino, C_{1-6} aryl, C_{1-6} arylamino, C_{1-6} arylamino, benzylamino, C_{1-6} arylamido, carboxy, C_{1-6} alkoxycarbonyl or (C_{1-6} aryl)(C_{1-10} alkoxy)carbonyl, car-

bamoyl, mono-C₁₋₆ carbamoyl, di-C₁₋₆ carbamoyl or any of the above in which a hydro-carbyl moiety is itself substituted by halo, cyano, hydroxy, C₁₋₂ alkoxy, amino, nitro, carbamoyl, carboxy or C₁₋₂ alkoxy-carbonyl. In groups containing an oxygen atom such as hydroxy and alkoxy, the oxygen atom can be replaced with sulfur to make groups
5 such as thio (SH) and thioalkyl (S-alkyl). Optional substituents therefore include groups such as S-methyl. In thioalkyl groups, the sulfur atom may be further oxidized to make a sulfoxide or sulfone, and thus optional substituents therefore include groups such as S(O)-alkyl and S(O)₂-alkyl.

10 Substitution may take the form of double bonds, and may include heteroatoms. Thus an alkyl group with a carbonyl (C=O) instead of a CH₂ can be considered a substituted alkyl group.

Substituted groups thus include for example CFH₂, CF₂H, CF₃, CH₂NH₂, CH₂OH,
15 CH₂CN, CH₂SCH₃, CH₂OCH₃, OCH₃, OCH₂CH₃, CH₃, CH₂CH₃, -OCH₂O-, CO₂CH₃, C(O)CH₃, *i*-Pr, SCF₃, SO₂CH₃, N(CH₃)₂, CONH₂, CON(CH₃)₂, etc. In the case of aryl groups, the substitutions may be in the form of rings from adjacent carbon atoms in the aryl ring, for example cyclic acetals such as O-CH₂-O.

20 The pharmaceutically acceptable salts of the compound of the invention include conventional salts formed from pharmaceutically acceptable inorganic or organic acids or bases as well as quaternary ammonium acid addition salts. More specific examples of suitable acid salts include hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, per-chloric, fumaric, acetic, propionic, succinic, glycolic, formic, lactic, maleic, tartaric, citric,
25 palmoic, malonic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, fumaric, toluenesulfonic, methanesulfonic, naphthalene-2-sulfonic, benzenesulfonic hydroxynaphthoic, hydroiodic, malic, steric, tannic and the likes. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compounds of the invention
30 and their pharmaceutically acceptable salts. More specific examples of suitable basic salts include sodium, lithium, potassium, magnesium, aluminium, calcium, zinc, *N,N*-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, *N*-methylglucamine and procaine salts.

35 **Description of Figures**

Fig. 1. Illustrates the cleavage of the thapsigargin prodrug G202 where the arrows indicate the sites in which the peptide moiety is cleaved by the PSMA enzyme, to liberate thapsigargin-12ADT-Asp. PSMA is only present in the blood vessel system of the tumor, and by cleaving off the peptide an activated form of a thapsigargin analogue emerges that proceeds into the tumor, blocking the SERCA in the walls of the blood vessels of the tumor and ultimately depriving the tumor of any possibilities for further growth resulting in cells and tumor death.

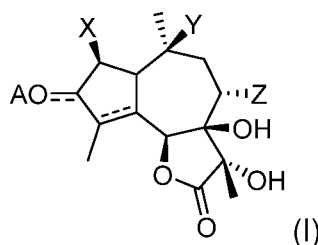
Fig. 2. Illustrates the cleavage of the thapsigargin prodrug G115 where the arrow indicates the sites in which the peptide moiety is cleaved by the PSA enzyme, to liberate thapsigargin-12ADT-Leu.

Fig. 3. Illustrates synthetic pathway from nortribolide to a thapsigargin derivative

Fig 4. Illustrates variant III of a method according to the invention. A one-pot two-step synthesis

Detailed description of the Invention

The invention relates to a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein Formula (I) is



wherein the dotted bond between the carbon and the oxygen represents an optional bond thus forming a carbonyl functional group and when the dotted bond is present, A is absent.

A is -H or alkyl e.g. methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, neopentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl or an acyl group such as formyl, acetyl, propionyl, *n*-butyryl or an α,β -unsaturated acyl group which may be further optionally substituted e.g. angeloyl,

crotonoyl, acryloyl, 3,3-dimethylacryloyl, (*Z*) or (*E*)-2-methylbut-2-enoyl, etc. A may also be a trialkylsilyl group or a diarylalkylsilyl group such as trimethylsilyl, triethylsilyl, *tert*-butyldimethylsilyl or *tert*-butyldiphenylsilyl;

5 wherein X, Y and Z are independently same or different and selected from:

-H, -O-R, -S-R, -NHR, hal including I, Br, F;

10 wherein R is selected from; H, alkyl or substituted alkyl (C₁-C₁₈), benzyl, -O-R, -C(=O)-R'', -SiR'₂R''';

and wherein R', R'' and R''' are independently same or different and are selected from alkyl or substituted alkyl, aryl or substituted aryl;

15 where alkyl is any alkyl having C₁-C₁₈ straight or branched carbon skeleton which may be substituted by halo (fluoro, chloro, bromo or iodo), C₁₋₆ alkyl, C₃₋₆ cycloalkyl, hydroxy, C₁₋₆ alkoxy, cyano, amino, nitro, C₁₋₆ alkylamino, C₂₋₆ alkenylamino, di-C₁₋₆ alkylamino, C₁₋₆ acylamino, di-C₁₋₆ acylamino, C₁₋₆ aryl, C₁₋₆ arylamino, C₁₋₆ aroylamino, benzylamino, C₁₋₆ arylamido, carboxy, C₁₋₆ alkoxycarbonyl or (C₁₋₆ aryl)(C₁₋₁₀ alkoxy)carbonyl,
20 carbamoyl, mono-C₁₋₆ carbamoyl, di-C₁₋₆ carbamoyl or any of the above in which a hydrocarbyl moiety is itself substituted by halo, cyano, hydroxy, C₁₋₂ alkoxy, amino, nitro, carbamoyl, carboxy or C₁₋₂ alkoxycarbonyl. In groups containing an oxygen atom such as hydroxy and alkoxy, the oxygen atom can be replaced with sulfur to make groups such as thio (SH) and thio-alkyl (S-alkyl);

25

Alkyl may also be any straight or branched C₁-C₁₈ alkyl having one or more double and/or triple bonds e.g. 5-octenyl, 3-butenyl, 7-dodecenyl etc.;

where aryl is e.g. phenyl, substituted phenyl, benzyl, substituted benzyl, etc.

30

Optional substituents therefore include groups such as S-methyl. In thioalkyl groups, the sulfur atom may be further oxidized to make a sulfoxide or sulfone, and thus optional substituents therefore include groups such as S(O)-alkyl and S(O)₂-alkyl.

Specific alkyls may be e.g. methyl, ethyl, *n*-propyl, *i*-propyl or *n*-butyl, *sec*-butyl, *i*-butyl, *tert*-butyl, *n*-pentyl, neopentyl, *i*-pentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, (*S*)-1-methylpropyl, (*R*)-1-methylpropyl, etc.

- 5 Substitution may take the form of double bonds and may include heteroatoms. Thus, an alkyl group with a carbonyl (C=O) instead of a CH₂ can be considered a substituted alkyl group.

- Substituted groups thus include for example CFH₂, CF₂H, CF₃, CH₂NH₂, CH₂OH,
 10 CH₂CN, CH₂SCH₃, CH₂OCH₃, OCH₃, OCH₂CH₃, CH₃, CH₂CH₃, -OCH₂O-, CO₂CH₃, C(O)CH₃, *i*-Pr, SCF₃, SO₂CH₃, N(CH₃)₂, CONH₂, CON(CH₃)₂, etc. In the case of aryl groups, the substitutions may be in the form of rings from adjacent carbon atoms in the aryl ring, for example cyclic acetals such as O-CH₂-O, or the substituents may be groups like -NH-CO-Q, where Q is O-alkyl.

15

The invention relates to a compound of Formula (I), wherein A is -H and there is no double bond on the oxygen atom connected to A and wherein Z is butanoyloxy or 2-methylbutanoyloxy and wherein X is -H, -OAc (acetyl), -OHex (hexanoyl), -OOct (octanoyl) or Hal, and Y is acetyl. X may be -H.

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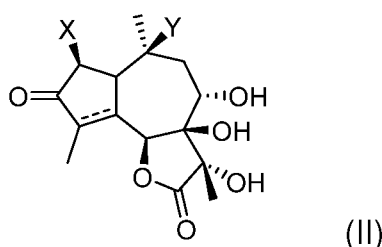
- The invention also relates to a compound of Formula (I), wherein A is absent and wherein the optional double bond is present to form a carbonyl functional group and wherein Z is *n*-butanoyloxy, 2-methylbutanoyloxy or formyloxy, acetyloxy, propionyloxy, or an α,β -unsaturated acyloxy group which may be further optionally substituted such
 25 as e.g. angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-enoyloxy or a Boc protected ω -aminoacyloxy like Boc-protected 12-aminododecanoyloxy, and wherein X is -H and Y is acetyloxy or X is acetyloxy and Y is acetyloxy.

- 30 The invention relates to a compound of Formula (I), wherein A is absent and wherein the optional double bond is present to form a carbonyl functional group and wherein Z is butanoyloxy or 2-methylbutanoyloxy and wherein X is -H, -OAc (acetyl), -OHex (hexanoyl), -OOct (octanoyl) or Hal, and Y is acetyl. X may be -H or -OOct.

- 35 The invention also relates to a compound according to Formula (I), wherein X is -OR, Y is -OR, and Z is -OR, wherein R is the same or different in X, Y and Z, and R is -

C(=O)R". R" may be e.g. -H, -Me, -Et, -Pr, *i*-Pr, *n*-Bu, *n*-Hex or *n*-Oct. X may be OOct, Y may be OAc and Z may be OBu. -OA may be -OH or =O.

The invention also relates to a compound of Formula (I) wherein the dotted bond is present to form a carbonyl functionality and wherein Z is -H to constitute Formula (II) or a pharmaceutically acceptable salt thereof, wherein consequently Formula (II) is



wherein X and Y are independently same or different and selected from:

-O-R, -S-R, -NHR, H, or hal including I, Br, F;

wherein R is selected from; H, alkyl, alkyl substituted with -NH-CO-Q, where Q is Oalkyl, or substituted alkyl (C₁-C₁₈), benzyl, -C(=O)-R", -SiR'₂R''';

and wherein R', R" and R''' are independently same or different and are selected from alkyl or substituted alkyl, aryl or substituted aryl;

where alkyl is any alkyl having C₁-C₁₈ straight or branched carbon skeleton which may be substituted by halo (fluoro, chloro, bromo or iodo), C₁₋₆ alkyl, C₃₋₆ cycloalkyl, hydroxy, C₁₋₆ alkoxy, cyano, amino, nitro, C₁₋₆ alkylamino, C₂₋₆ alkenylamino, di-C₁₋₆ alkylamino, C₁₋₆ acylamino, di-C₁₋₆ acylamino, C₁₋₆ aryl, C₁₋₆ arylamino, C₁₋₆ aroylamino, benzylamino, C₁₋₆ arylamido, carboxy, C₁₋₆ alkoxycarbonyl or (C₁₋₆ aryl)(C₁₋₁₀ alkoxy)carbonyl, carbamoyl, mono-C₁₋₆ carbamoyl, di-C₁₋₆ carbamoyl or any of the above in which a hydrocarbyl moiety is itself substituted by halo, cyano, hydroxy, C₁₋₂ alkoxy, amino, nitro, carbamoyl, carboxy or C₁₋₂ alkoxycarbonyl.

Specific alkyls may be e.g. methyl, ethyl, *n*-propyl, *i*-propyl or *n*-butyl, *sec*-butyl, *i*-butyl, *tert*-butyl, 2,2,2-trimethylethyl, *n*-pentyl, neopentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-

decyl, *n*-undecyl, amino-*n*-undecyl, 11-amino-*n*-undecyl, *n*-dodecyl, (*S*)-2-methylbutyl, (*R*)-1-methylbutyl, etc.

- In groups containing an oxygen atom such as hydroxy and alkoxy, the oxygen atom
 5 can be replaced with sulfur to make groups such as thio (SH) and thioalkyl (S-alkyl). Optional substituents therefore include groups such as S-methyl. In thioalkyl groups, the sulfur atom may be further oxidized to make a sulfoxide or sulfone, and thus optional substituents therefore include groups such as S(O)-alkyl and S(O)₂-alkyl.
- 10 Substitution may take the form of double bonds and may include heteroatoms. Thus, an alkyl group with a carbonyl (C=O) instead of a CH₂ can be considered a substituted alkyl group.

- Alkyl may also be any straight or branched C₁-C₁₈ alkyl having one or more double
 15 and/or triple bonds such as 5-octenyl, 3-butenyl, 7-dodecenyl etc.

Aryl is e.g. phenyl, substituted phenyl, benzyl, substituted benzyl etc.

- Substituted groups thus include for example CFH₂, CF₂H, CF₃, CH₂NH₂, CH₂OH,
 20 CH₂CN, CH₂SCH₃, CH₂OCH₃, OCH₃, OCH₂CH₃, CH₃, CH₂CH₃, -OCH₂O-, CO₂CH₃, C(O)CH₃, *i*-Pr, SCF₃, SO₂CH₃, N(CH₃)₂, CONH₂, CON(CH₃)₂, etc. In the case of aryl groups, the substitutions may be in the form of rings from adjacent carbon atoms in the aryl ring, for example cyclic acetals such as O-CH₂-O.

- 25 The invention also relates to a compound according to Formula (I) above, wherein X is -OR and Y is -OR, and R may be the same or different in X and Y, wherein R is -C(=O)R". R" may be e.g. -H, -methyl, -ethyl, *n*-propyl, *i*-propyl or *n*-butyl. R" may be specifically -Me.

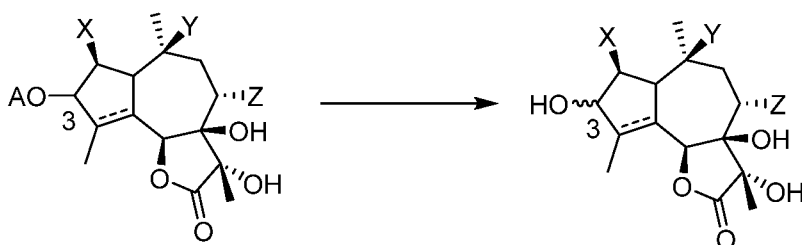
- 30 Specifically X and Y may be OAc, or X may be OHex or OOct and Y may be OAc.

- Present invention also relates to a method of preparing compound according to Formula (I). The method of preparing a compound according to formula (I) may comprise a substitution, in which an acyloxy group in the 3-position is substituted with a hydroxy
 35 group or a precursor to a hydroxy group e.g. an alkoxymethyl group. The reaction conditions may include the use of acid reagents e.g. hydrogen fluoride or *p*-TsOH in a mix-

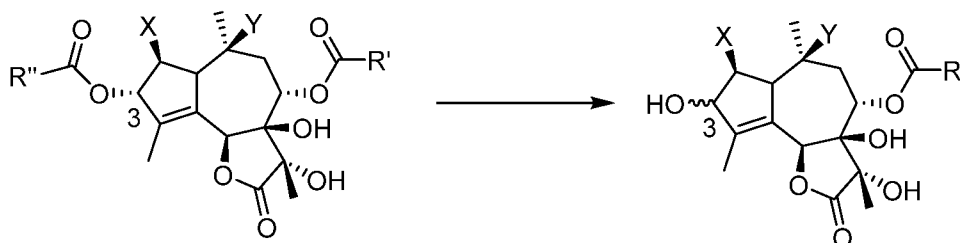
ture of water and an aprotic solvent such as acetonitrile dichloromethane, trichloromethane, dimethylsulfoxide, *N,N*-dimethylformamide, tetrahydrofuran, 1,4-dioxane, pyran, ethyl acetate, acetone, but not excluding other aprotic solvents. Other acidic compounds may e.g. be hydrogen chloride (HCl) or hydrogen fluoride (HF) or carboxylic acids such as acetic or formic acid or sulfonic acids such as methanesulfonic acid or trifluoromethanesulfonic acid, trifluoroacetic acid, sulfuric acid. The conditions may furthermore include slight heating of the reaction mixture to about 60°C, or e.g. about 40°C, about 50°C, about 70°C, about 80°C or about 90°C or reflux of the solvent. The conditions may furthermore include heating under microwave (MW) irradiations.

10

The reaction may be depicted as



15 A more specific example of this reaction may be e.g. when A and Z are acyl groups as seen below:



20

wherein the $-OC(=O)R'$ and $-OC(=O)R''$ may be independently e.g. *n*-butanoyloxy, 2-methylbutanoyloxy- or formyloxy, acetyloxy, propionyloxy, or an α,β -unsaturated acyloxy group which may be further optionally substituted such as e.g. angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-enoyloxy or
 25 a Boc protected ω -aminoacyloxy like Boc-protected 12-aminododecanoyloxy or a *N*-Boc-protected ω -aminoacyloxy having 6-28 carbon atoms. Benzene, dichloromethane,

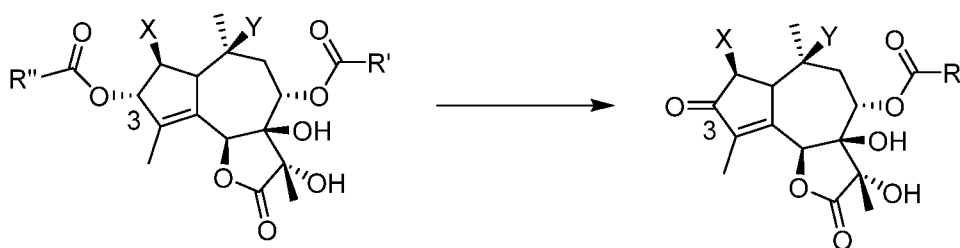
chloroform, toluene, tetrahydrofuran, hexane, heptane, *N,N*-dimethylformamide or similar solvents might be used.

The inventors of present invention discovered that during several attempts to synthesize the intermediate 2, standard procedure for saponification did not work and did not yield the desired product. Instead, after extensive experimentation the Inventors surprisingly found that an S_N1 reaction under acidic conditions in an aprotic solution with added appropriate amounts of water was successful in bringing about the desired intermediate. It should be mentioned that the reaction was regioselective in that no other functionality was altered or reacted during these conditions. This has the advantage of dispensing with the use of protective groups which would require further reaction steps in protecting and deprotecting functional groups in the molecule. It should also be noted that the yields of this reaction were in general high with yields of about 30-35% or higher.

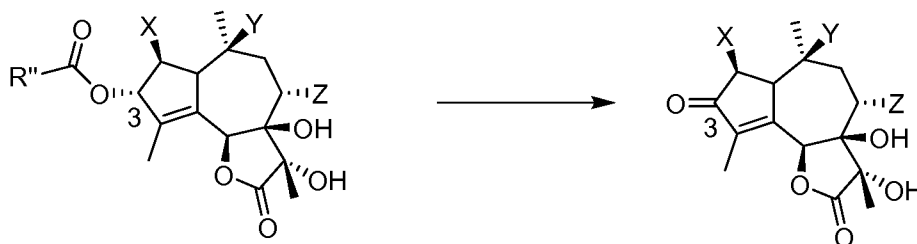
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A method of preparing compounds of Formula (I) may comprise a one-pot two-steps procedure which oxidize the alcohol as soon as it is formed during the cleaving of the ester at position 3 as seen below (the latent alcohol formed is seen from the formula above)

20



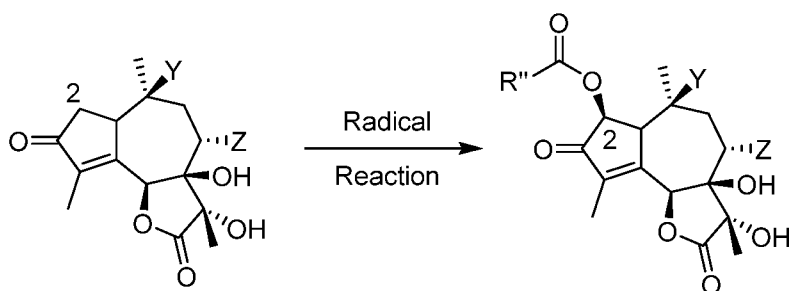
or in more general terms:



25

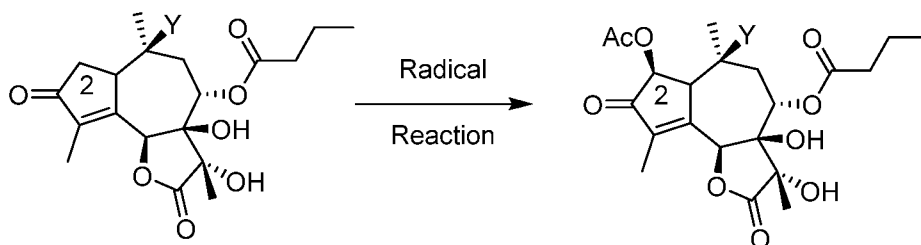
The method of preparing compounds according to Formula (I) (wherein X=H) may comprise a reaction step wherein the 2-position is *stereoselectively* oxygenated by introduction of an acyloxy group like acetoxy or any other alkanoyloxy group like hexanoyloxy, octanoyloxy, or protected aminoalkanoyloxy groups by a radical reaction such

5 that

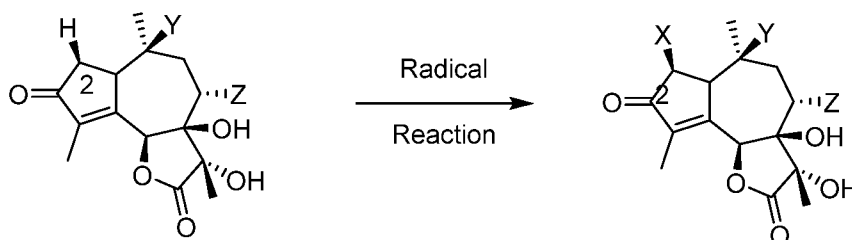


In which R'' is an alkyl group e.g. methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *sec*-butyl, *tert*-butyl, *i*-butyl, 2,2,2-trimethylethyl, *n*-pentyl, neopentyl, *i*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, amino-*n*-undecyl, 11-amino-*n*-undecyl, *n*-dodecyl, (*S*)-2-methylbutyl, (*R*)-1-methylbutyl, not substituted or substituted with amino groups masked as carbamoyl groups, etc.

15 One specific example of this reaction may be e.g.:



or in more general terms:



20

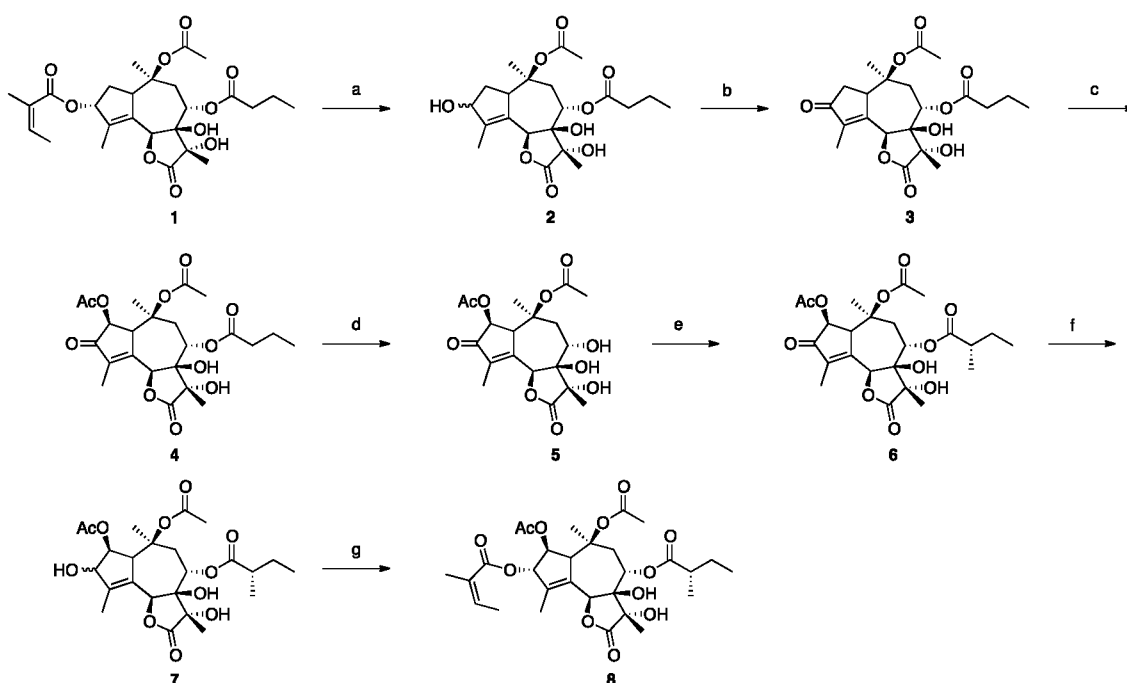
where X is OR, notably OAc, OHex or OOct or a masked amine. X may also be protected with a Boc or benzoyloxycarbonyl or another protecting group.

The reaction conditions may involve $\text{Mn}(\text{OAc})_3$ (or $\text{Mn}(\text{OR})_3$) as oxidizing reagent or another manganese(III) salt of an organic acid such as but not exclusively octanoic or hexanoic acid, acetic acid and a further organic solvent such as benzene, toluene, dichloromethane, tetrahydrofuran, chloroform, dimethylsulfoxide, *N,N*-dimethylformamide, hexane, 1,4-dioxane, diethyl ether, acetonitrile, ethyl acetate, acetone. What is important to understand in this respect is that the reaction is *stereoselective* and hence, yielding the desired stereoisomer of the desired isolated intermediate. This has the advantage of dispensing with any need for separation of mixes of enantiomers or diastereoisomers which would decrease the yield of the reaction.

Method variant I

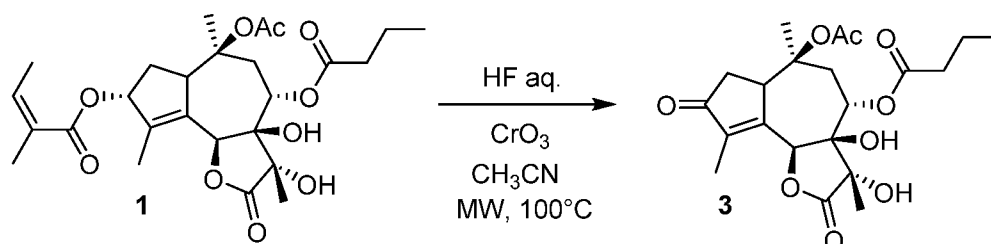
Consequently, in one instance a method of preparing a thapsigargin analogue may be according to Scheme 1a, which constitutes a proof-of-concept of the method allowing for functionalization of the trilobolide scaffold into the thapsigargin scaffold as starting materials for compounds possessing the skeleton of thapsigargin as seen in Scheme 1a, compound 8. Further details appear from Schemes 1b, 1c, 2a and 2b.

Scheme 1a



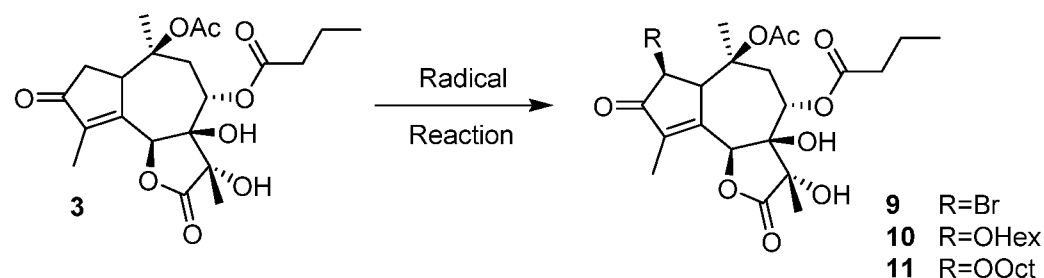
Reagents and conditions: (a) *p*-TsOH, MeCN:H₂O, 60 °C, 75%; (b) DMP, DCM, rt, 87%; (c) $\text{Mn}(\text{OAc})_3$ (H₂O)₂, benzene:AcOH, reflux; (d) Et₃N, MeOH, 60 °C, 57% over two steps; (e) (*S*)-(+)-2-Methylbutyric anhydride, DMAP, THF, rt, 78%; (f) $\text{Zn}(\text{BH}_4)_2$, THF, -30 °C to rt, 61%; (g) 2,4,6-trichlorobenzoyl chloride, Et₃N, angelic acid, toluene, 75 °C, 21%

Scheme 1b



5

Scheme 1c



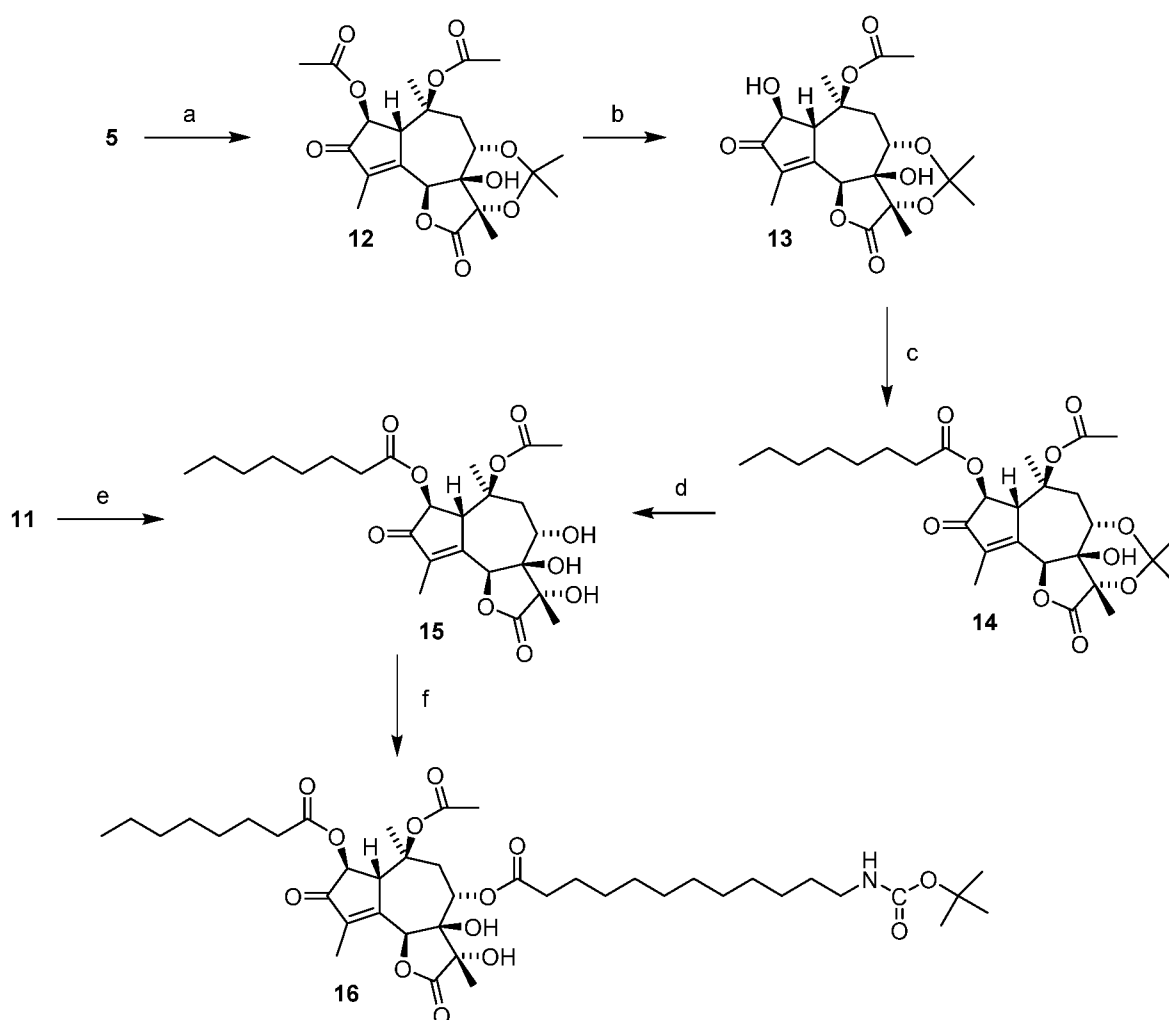
- 10 Additionally, it emerged the idea to potentially oxidize compound **2** as soon as it is formed during the cleaving of the ester in a “one-pot” two-steps procedure in order to obtain directly compound **3** from compound **1**. It quickly appeared that chromium trioxide could be the reagent of choice: it is aqueous soluble in acidic media, easy to handle and purify and noteworthy completely inert when added to a solution of compound **1**.
- 15 Thus, compound **3** has been obtained after treatment of compound **1** with an aqueous solution of hydrogen fluoride in acetonitrile in the presence of chromium trioxide under microwave (MW) irradiations at 100 °C as depicted in Scheme 1b.

- It is envisaged that intermediates **2**, **3**, **4**, **5**, **9**, **10** and **11** as seen in Schemes 1a, 1b and 1c will be useful key elements for further synthesis of thapsigargin analogues such as compound **8** as seen in Scheme 1a.

Scheme 2a

25

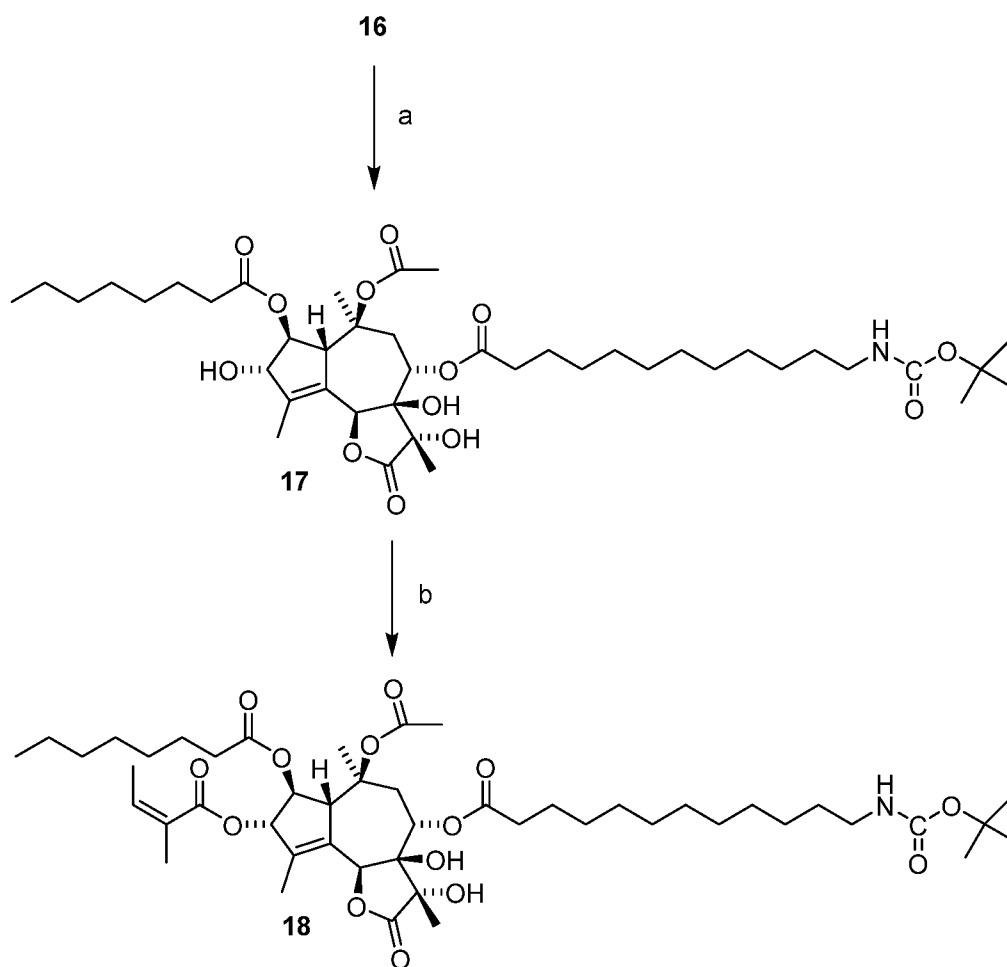
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a: 2,2-dimethoxypropane, *p*-TsOH; b: KOH, MeOH; c: octanoic anhydride, DMAP; d: MeOH, H₂O, H⁺; e: Et₃N, MeOH; f: *N*-Boc-12-amindodecanoic acid, DCC, DMAP.

Compounds **5** and **11** might also be used for preparation of starting material for e.g. G202 as seen in Schemes 2a and 2b (e.g. compound **18**).

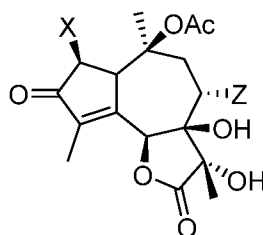
29



a: $\text{Zn}(\text{BH}_4)_2$; b: benzoyl chloride, angelic acid, Et_3N .

In particular, a compound of the according to the Formula below

5



is considered as a key intermediate for further derivatization to thapsigargin analogues, where X is $-\text{H}$ or $-\text{OAcyl}$, such as e.g. formyl, acetyl, propionyl, butanoyl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl and dodecanoyl and wherein Z is $-\text{OH}$ or $-\text{O}-\text{C}(=\text{O})\text{R}'$ group e.g. *n*-butanoyloxy, 2-methylbutanoyloxy- or formyloxy, acetyloxy, propionyloxy, or an α,β -unsaturated acyloxy group which may be

further optionally substituted such as angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-eneoyloxy or a *N*-Boc protected ω -aminoacyloxy like *N*-Boc-protected 12-aminododecanoyloxy, or a *N*-Boc-protected ω -aminoacyloxy having 6-28 carbon atoms.

5

Specific compounds are those, wherein X is -H, -OAc, -OHex, -OOct, or Br, and Z is -OH or -OC(=O)nPr such as compounds **3**, **4**, **5**, **9**, **10** or **11** in Scheme 1a-c above.

Method variant II

- 10 Further experiments have been performed and are reported in the following. In the following text references to schemes and compounds are to the schemes and compounds mentioned in the following paragraphs

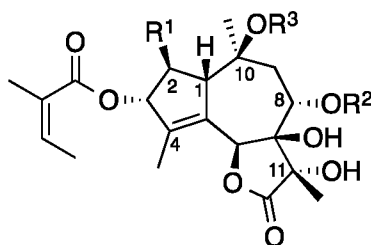
- As seen from the experimental section herein an alternative method is depicted in
15 Schemes 3-13, wherein the first step in Scheme 1a is combined with the second step, i.e. a one-pot synthesis with two steps. Thus, the alcohol **2** formed is immediately in one-pot oxidized to provide compound **3**. More details appear under the experimental section herein.
- 20 The reactivities of the hexaoxygenated thapsigargin and the pentaxygenated nortrilobolide are compared in order to develop a chemo- and regioselective method for the conversion of nortrilobolide into the natural product 2-acetoxytrilobolide.

- For the first time, the stereoselective synthesis of the 2-acetoxytrilobolide is described,
25 which involved two key reactions: the first chemical step is a one-pot substitution-oxidation reaction of an allylic ester into its corresponding α,β -unsaturated ketone. The second major process consists of the stereoselective α' -acetocylation of a key intermediate to afford its corresponding enantiopure acetoxyketone, which was easily converted into 2-acetoxytrilobolide within few steps.

30

- Hexaoxygenated guaianolides like thapsigargin **1** isolated from *Thapsia garganica* L. and pentaxygenated guaianolides like trilobolide **2** isolated from *Laser trilobum* (L.) Borkh are potent inhibitors of the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA). Both types of guaianolides have the same binding site in the SERCA pump, but trilobolide has a smaller affinity than thapsigargin. The sub-nanomolar affinity for SERCA has
35 made in particular thapsigargin a major tool for investigating of the Ca^{2+} homeostasis in

cells and as a potential drug for cancer treatment. Hexaoxygenated as well as penta-oxygenated guaianolides have only been found in umbelliferous plants (Apiaceae). Until recently hexaoxygenated guaianolides had only been found in the genus *Thapsia*, whereas pentaooxygenated guaianolides were known from *Thapsia* (nortrilobolide **3**) and *Laser*. Recently, two hexaoxygenated 2-acetoxytrilobolide **4** and 2-hydroxydeacetyltrilobolide **5** were isolated from *L. trilobum* revealing that both *L. trilobum* and *T. garganica* express the enzyme needed for producing hexaoxygenated guaianolides.



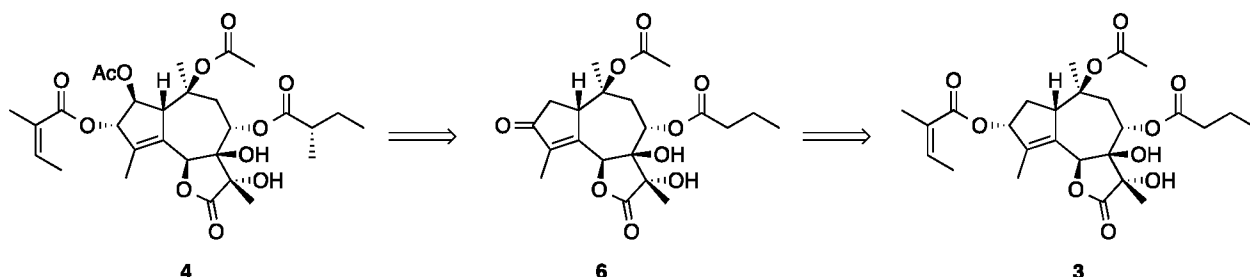
1	Thapsigargin	R ¹ = O-Oct	R ² = But	R ³ = Ac
2	Trilobolide	R ¹ = H	R ² = (<i>S</i>)-2-MeBut	R ³ = Ac
3	Nortrilobolide	R ¹ = H	R ² = But	R ³ = Ac
4	2-Acetoxy trilobolide	R ¹ = O-Ac	R ² = (<i>S</i>)-2-MeBut	R ³ = Ac
5	2-Hydroxy-10-deacetyltrilobolide	R ¹ = OH	R ² = (<i>S</i>)-2-MeBut	R ³ = OH

Abbreviations: Oct = Octanoyl, Ac = Acetyl, But = Butanoyl

Absolute configuration of a number of penta- and hexaoxygenated guaianolides

Even though the biological societies quickly acted on the unique properties of thapsigargin **1**, the chemical and biochemical societies have reacted more slowly. Consequently, the mechanism of guaianolide biosynthesis largely remains unknown. At the present kunzeaol is assumed to be the first monocyclic precursor for the biosynthesis of the gainolide backbone of thapsigargin⁹ but no knowledge of neither formation of the tricyclic guaianolide skeleton nor the introduction of the many oxygen atoms on the skeleton exists. Unfortunately, in the absence of this significant information of its biosynthesis, the possibility for genetically modifying other organisms to produce the highly oxygenated guaianolide does not exist. However, a possible pathway for accessing the hexaoxygenated guaianolides is to use the pentaooxygenated guaianolides as starting materials. Indeed, although oxygenation of C-2 in nortrilobolide **3** is not feasible since this methylene group is not activated, an appropriate transformation of C-3 into a

ketone as observed in derivative **6**, would allow a stereoselective oxidation of C-2 which should provide 2-acetoxytrilobolide **4** after few subsequent chemical modifications (Scheme 3).



5 **Scheme 3.** Retrosynthesis of 2-acetoxytrilobolide **4** from nortrilobolide **3**

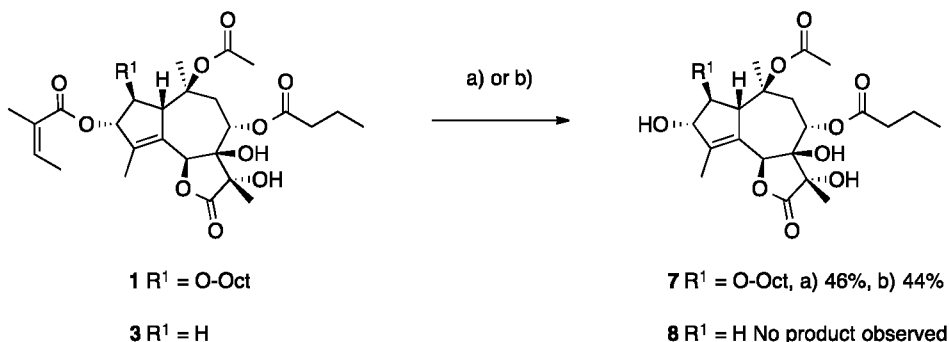
Since angelic acid is an α,β -unsaturated acid this ester group is the most resistant against saponification among the other esters in thapsigargin **1** and trilobolide **2**. However, taking advantage of the double bond of the angelate moiety several methods have been developed for selective cleavage of the ester either by potassium permanganate or osmium tetroxide-periodate oxidation in order to get the corresponding pyruvate ester which could selectively be cleaved by solvolysis in the presence of pyridine in methanol to form the expected 3-alcohol. The 3-alcohol can easily be oxidized into the corresponding 3-ketone.

A number of procedures for α -oxygenation of a carbonyl group are described including oxidation with manganese(III) pieces or other heavy metals, sigmatropic rearrangement of the corresponding acyloxyenamines, or hypiodite catalyzed α -oxyacylation. In addition, α -halogenation of a ketone followed by substitution with a carboxylate group might be a possibility. Ley et al. had stereoselectively α -hydroxylated similar complex molecules by epoxidation of the corresponding trimethylsilyl enol ethers. Herein, we wish to report for the first time the synthesis of 2-acetoxytrilobolide from nortrilobolide whereas the procedure remarkably highlights a one-pot cleavage of an angelate ester and the oxidation of the alcohol intermediate into its corresponding ketone as well as a stereoselective α' -acetoxylation.

Results and Discussion – regarding Variant II

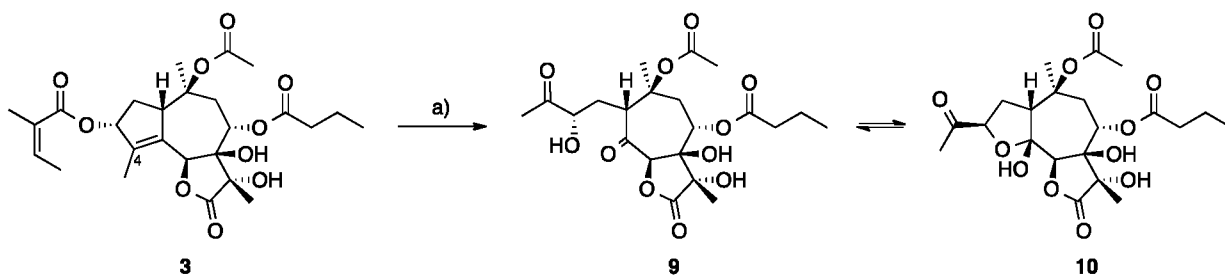
Attempts to selectively remove the angelate ester of nortrilobolide **3** employing either the potassium permanganate (KMnO_4) or osmium tetroxide (OsO_4) procedures, which

were successful for thapsigargin **1**, failed most likely due to oxidation of C-4 double bond in nortrilobolide **3** (Scheme 4).



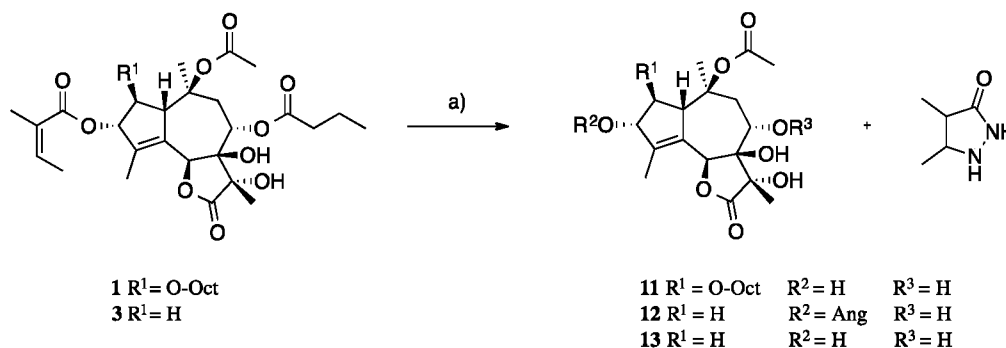
Scheme 4. Selective angelate cleavage. Reaction conditions: a) 1) KMnO_4 (4 equiv.), BnEt_3NCl (0.08 equiv.), PhMe , H_2O , 7 h, rt; 2) MeOH , pyridine, H_2O , reflux conditions, 7 h; b) 1) OsO_4 (0.015 equiv.), NMO (1.15 equiv.), acetone, H_2O , 4 h, rt; 2) NaIO_4 (3 equiv.), 16 h, rt; 3) MeOH , pyridine, H_2O , reflux conditions, 16h

Milder procedure for the removal of angelate ester via ozonolysis was attempted. Ozonolysis of angelate ester of thapsigargin **1** proceeded fairly selective to afford the corresponding pyruvate ester, which was solvolysed to give the 3-alcohol. However, performing this procedure on nortrilobolide **3** resulted in the ring-opening of the five-membered ring to afford diketone **9** and acetal **10** (Scheme 5). The lower reactivity of the Δ^4 double bond in thapsigargin **1** is probably caused by steric hindrance of the octanoyl group in the 2-position.



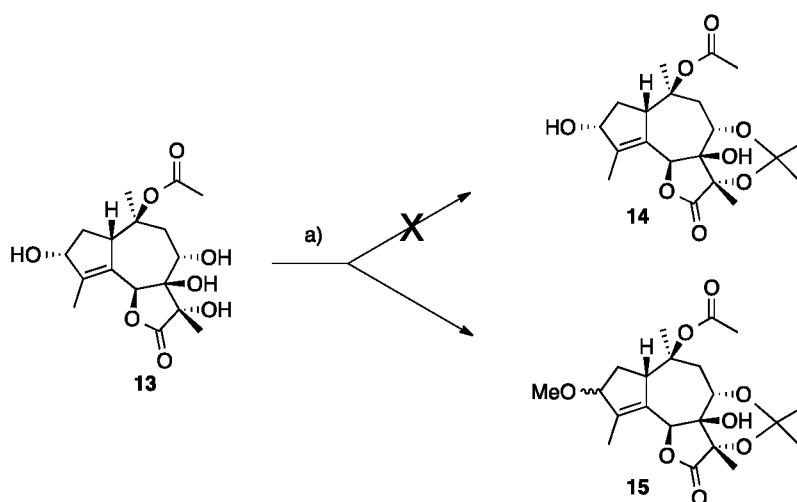
Scheme 5. Ozonolysis of nortrilobolide **3** yielding compounds **9** and **10**. Reaction conditions: a) 1) O_3 , CH_2Cl_2 , -78°C , 10 min then PPh_3 (7 equiv.), rt, 16 h; 2) MeOH , pyridine, H_2O , reflux conditions, 6 h, 33%; the stereochemistry at C5 may be different from that shown above.

Hydrazinolysis for converting the angelate ester into 3-hydrazinopropionate derivatives, which spontaneously cyclizes to afford the free alcohol and the corresponding pyrazolidin-3-ones has successfully been applied to selectively cleave off esters in thymidine. In the case of thapsigargin **1** hydrazinolysis afforded the expected deacetylated compound **11** (Scheme 6). In contrast hydrazinolysis of nortrilobolide **3** only afforded the tetraol **13** in poor yield.



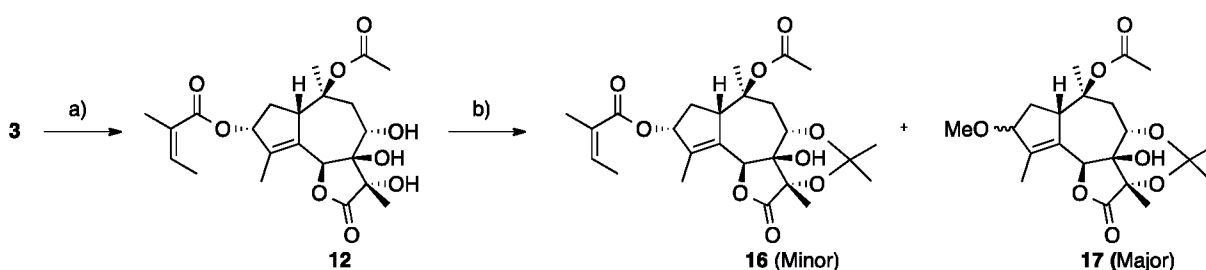
Scheme 6. Hydrazinolysis of thapsigargin **1** and nortrilobolide **3**. Reaction conditions: $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (1.26 equiv.), EtOH, reflux conditions, 16 h.

- 10 In order to see if the tetraol **13** could be used as starting material in an alternative synthetic route to **4**, the 8-hydroxy group had to be protected to enable selective oxidation of the 2-hydroxy group. In the case of debutanoylthapsigargin **11** treatment with dimethoxypropane in acetone afforded the corresponding acetonide in good yield. Unfortunately, attempts to convert tetraol **13** into acetonide **14** by treatment with dimethoxypropane under acidic conditions were not successful as only the methoxy ketal **15** was isolated in 43% yield (Scheme 7)
- 15



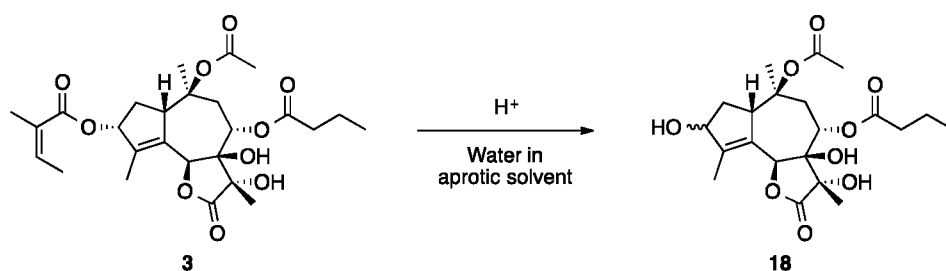
Scheme 7. Acetalation of **13**. Reaction conditions: a) 2,2-dimethoxypropane (125 equiv.), *p*-TsOH (cat.), acetone, 50 °C, 16 h, 43% dr 1:1

- 5 Analogously, **12** formed by deacetylation of **3** by treatment with trimethylamine in methanol was converted into a mixture of a minor amount of the 3-angelate ester **16** and a major amount of the 3-methyl ether **17** by treatment with dimethoxypropane and acetone under acidic conditions. Most likely the substitution at C-3 occurs by an S_N1
- 10 reaction for debutanoylthapsigargin runs in excellent yield to give the angeloyl acetal.



Scheme 8. Acetalation of **10**. Reaction conditions: a) Et₃N (20 equiv.), MeOH, rt, 16 h; b) 2,2-dimethoxypropane (82 equiv.), *p*-TsOH (cat.), acetone, 50 °C, 16 h.

- 15 These findings suggested that the angelate ester of nortrilobolide **3** might be prone to substitution with a hydroxyl group in an acidic mixture of water and an organic aprotic solvent. As predicted treatment of nortrilobolide **3** with an acidic mixture of water and acetonitrile did afford the desired 3-alcohol **18** as shown in Table 1.

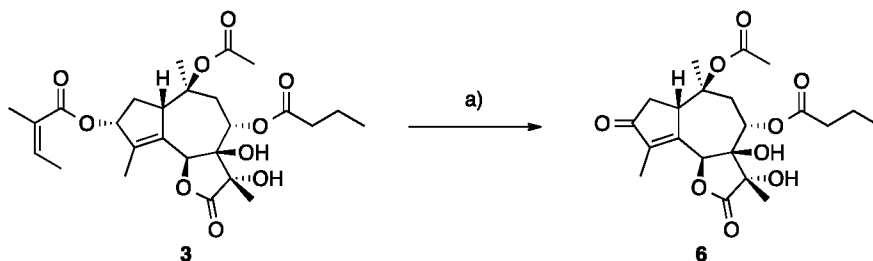
Table 1. Substitution of angelate ester of nortrilobolide **3** under acidic aqueous conditions

Entry	Acid (equiv.)	Temperature (°C)	Reaction time (h)	Solvent	Conversion (%)
1	<i>p</i> -TsOH (0.2)	RT	72	CH ₃ CN/H ₂ O ^c	~ 10
2	<i>p</i> -TsOH (2)	42	18	CH ₃ CN/H ₂ O ^d	~ 50
3	<i>p</i> -TsOH (2)	60	6	CH ₃ CN/H ₂ O ^c	~ 90
4	<i>p</i> -TsOH (2)	100	1	CH ₃ CN/H ₂ O ^c	100
5	AcOH (3)	60	18	CH ₃ CN/H ₂ O ^d	~ 10
6	AcOH (12)	60	2	CH ₃ CN	0
7	TFA (5)	80 ^b	1	CH ₃ CN/H ₂ O ^d	100
8	TFA (0.5)	80	4	CH ₃ CN/H ₂ O ^d	~ 90
9	HF (0.5) ^a	60	16	CH ₃ CN	~ 60
10	HF (3) ^a	85 ^b	1	CH ₃ CN	~ 95

^a: 1 M aqueous solution; ^b: under MW irradiations; ^c: ratio CH₃CN/H₂O was 5:1; ^d: ratio
 5 CH₃CN/H₂O was 9:1 ^e: Determined by NMR analysis.

During the optimization of the reaction conditions for the conversion of nortrilobolide **3** into the corresponding 3-alcohol **18**, it was found that a weak acid (pKa 4-5) required prolonged reaction time, while a stronger acid (pKa -2 - 0.5) caused decomposition of the allylic alcohol **18**. The reaction was accelerated by high temperature obtained either by heating or by the use of microwave irradiations. At elevated temperatures only a catalytic amount of acid was needed to achieve high conversion of nortrilobolide **3** into product. However, the allylic alcohol **18** appeared to be unstable in an acidic medium explaining the observed poor yields of the desired allylic alcohol **18** even though the starting material was fully converted. The reactions were performed on 0.1 -1 mmol scale resulted in yields ranging mostly from 30 to 35%. Only once a 75% yield was obtained.

With the aim to limit the degradation of the allylic alcohol intermediate **18** during the angelate cleavage in the acidic media a one-pot procedure was developed in order to oxidize *in situ* the formed allylic alcohol into the corresponding ketone **6**. Thus, treatment of allylic alcohol **18** with chromium trioxide in hydrofluoric acid under microwave conditions afforded the desired ketone in 68% yield (Scheme 9)

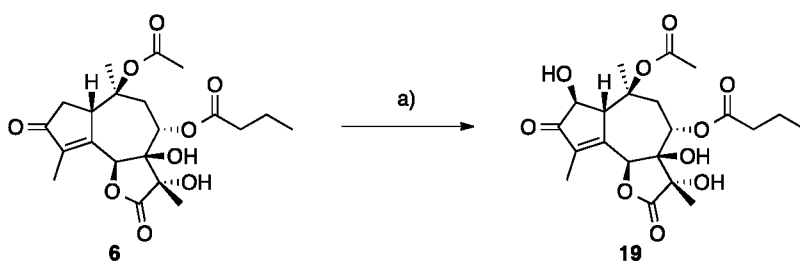


Scheme 9. One-pot two-steps substitution/oxidation of **3** into ketone **6**. Reaction conditions: a) CrO₃ (2.5 equiv.), 1M aq. HF (5 equiv.), MW irradiations, 100 °C, 35 min, 68%.

This is an unprecedented procedure for converting an allylic ester into an α,β-unsaturated ketone.

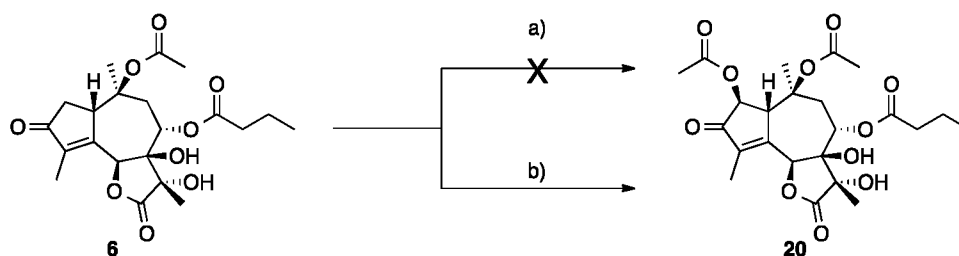
With the easy access to the α,β-unsaturated ketone **6** the next challenge was to introduce an acetoxy group in the α'-position. Preliminary attempts of α'-oxidation of ketone **6** to afford α'-hydroxyketone **19** were performed using the camphor-based oxaziridine method developed by Davis (Scheme 10).

38



Scheme 10. α' -Hydroxylation of **6**. Reaction conditions: LDA (3 equiv.), dry THF, -78°C , 3 h, then (1*R*)-(-)-(10-camphorsulfonyl)-oxaziridine (2 equiv.), -78°C to -30°C , 3 h.

- 5 Treatment of ketone **6** with LDA (3 equiv.) followed by the addition of camphorsulfonyl oxaziridine (2 equiv.) did not give the expected results, but it may be obtained by slight variation in the reaction conditions. At the present reaction conditions lead to formation of the α' -hydroxyketone **19**. Probably, the two hydroxyl groups at the 7- and 11-positions prevent the lithiation of the α' -position. Introduction of an acetoxy group in
- 10 the 2-position using a recently described procedure for similar complex guaianolide failed (Scheme 11). The major difference between **6** and the model compounds in the mentioned study is the presence of an ester in the 8-position.



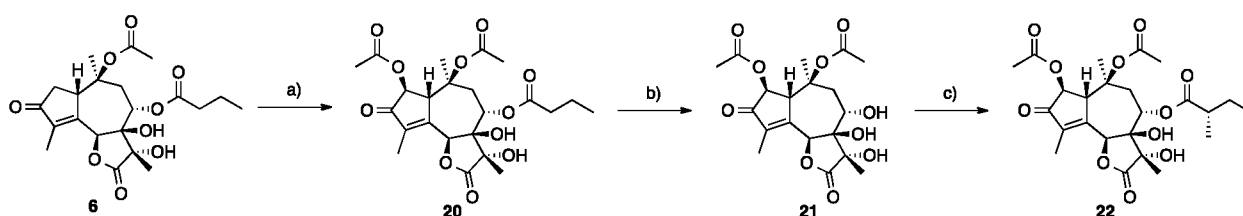
- Scheme 11.** α' -Acetoxycylation of **6**. Reaction conditions: a) KMnO_4 (2.10 equiv.), AcOH (35 equiv.), Ac_2O (9.5 equiv.), PhH, 85°C , 16 h²⁰; b) $\text{Mn}(\text{OAc})_3 \cdot 2\text{H}_2\text{O}$ (2.15), AcOH, PhH, Dean-Stark, reflux, 6 h.

Selective α' -acetoxycylation of α,β -unsaturated ketones using manganese(III) acetate with Dean-Stark trap had previously been reported by Demir *et al.* Herein, it was found that using acetic anhydride as a co-solvent instead of acetic acid did enhance the conversion rates; however, in our particular case acetic acid was much more effective.

20 Thus, treatment of **6** with manganese(III) acetate in a mixture of benzene and glacial acetic acid using a Dean-Stark trap to remove the water formed during the reaction resulted in the α' -acetoxycetone **20** with the desired stereochemistry at C-2 which was

confirmed by NMR analysis. Indeed, ROESY experiments showed a fine correlation between H-2 and H-14.

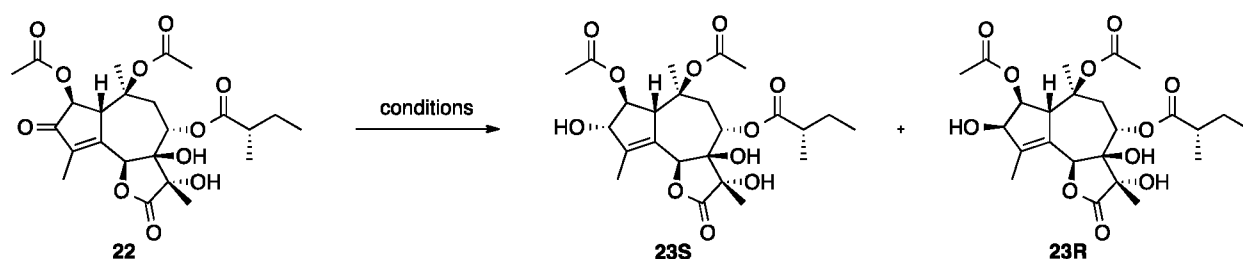
Methanolysis of α' -acetoxyketone **20** using triethylamine in methanol was easily achieved to afford the desired O-8-debutanoyl hydroxyketone **21**, which was successfully esterified to give (*S*)-methyl butanoate **22** at O-8 in 78% yield as seen in Scheme 12.



Scheme 12. Formation of **22** from **6**. Reaction conditions: a) $\text{Mn}(\text{OAc})_3 \cdot 2\text{H}_2\text{O}$ (2.15), AcOH, PhH, Dean-Stark, reflux, 6 h; b) Et_3N (26.5 equiv.), MeOH, 60 °C, 30 min, 57% over two steps from **6**; c) (*S*)-2-methylbutyric anhydride (4 equiv.), DMAP (cat.), THF, rt, 1 h, 78%.

Stereoselective reduction of similar ketones to give the α -alcohols has previously been performed using zinc borohydride. However, when this procedure was applied to acetoxy ketone **22** a selectivity of approximately 2:1 towards the undesired *syn*-diastereomer **23R** (3β -alcohol) was obtained (Table 2).

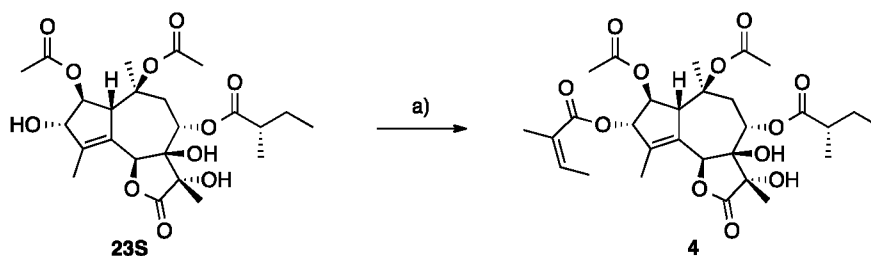
Table 2. Reduction of acetoxyketone **22**



Reaction conditions	dr (23S / 23R)	Yield (%)
NaBH_4 , MeOH, 0 °C	1:2	37
$\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, MeOH then NaBH_4 , -60 °C	1:8	60
$\text{Zn}(\text{BH}_4)$, THF, -30 °C $\rightarrow$ 10 °C, 16 h (EDTA work-up)	1:1.95	61

A chelation of zinc to both C-3 ketone and the carbonyl group of the acetyl group might explain the observed outcome of this reduction. Due to the flexibility of the acetyl group at C-2 the hydride might preferentially approach from the α -face resulting in the undesired *syn*-product **23R** (3 β - alcohol) as the major product. The same unfavourable ratio of isomers was observed when sodium borohydride was used. Premixing the ketone **22** with cerium(III) chloride prior to the addition of sodium borohydride did not improve the selectivity in favour of the desired **23S** derivative, on contrary the selectivity was enhanced towards the undesired **23R** derivative. In spite of this unwanted selectivity of the reaction the procedure is still attractive since the unwanted alcohol **23R** might be oxidized to the ketone **22**, which then can be recycled for preparation of the 3 α - alcohol **23S**.

Angeloylation of alcohol **23S** under Yamaguchi conditions afforded the desired 2-acetoxytrilobolide **4** in 49 % yield.



Scheme 13. Angeloylation of **23S** to afford 2-acetoxytrilobolide **4**. Reaction conditions: a) 2,4,6-trichlorobenzoyl chloride (2 equiv.), Et₃N (2 equiv.), angelic acid (2 equiv.), PhMe, 75 °C, 48 h, 49%.

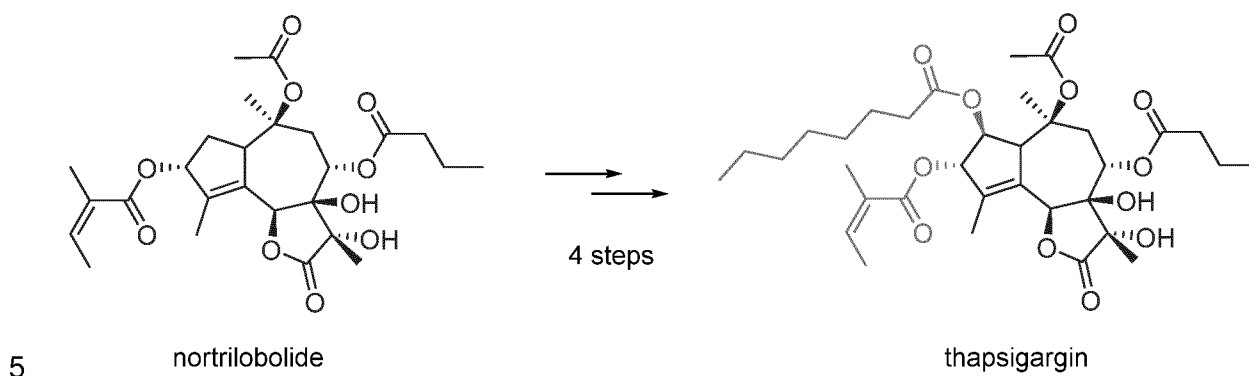
Comparison of the synthesized 2-acetoxytrilobolide **4** with the reported isolated sample of the natural product confirmed the correct stereochemistry at C-2 and C-3.

Conclusion regarding Variant II

A new procedure for selective substitution of the angeoyloxy group of nortrilobolide **3** and subsequently oxidize the corresponding allylic alcohol **18** into key-intermediate **6** in one-pot using chromium trioxide as the oxidizing agent has been developed. Combined with a stereoselective α' -acetoxylation of **6** a semi-synthesis of 2-acetoxytrilobolide **4** has been successfully completed in 6 steps from nortrilobolide **3**.

4-step synthesis method – Method variant III

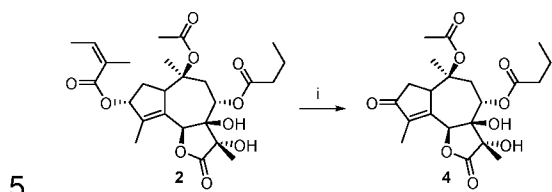
A third and only 4-step synthesis has also been developed with a relatively high overall yield of above 20%. The first step in this method comprises the step from **1** to **3** as described above



This method provides an expedient semisynthetic protocol for the preparation of compound **1** (thapsigargin) from natural product **2** (nortrilobolide) in only 4 steps in a 21.3% overall yield. This concise synthesis highlights two key transformations: a one-pot
 10 cleavage of an angelate ester and the oxidation of the alcohol intermediate into its corresponding ketone as well as a stereoselective α' -acyloxylation.

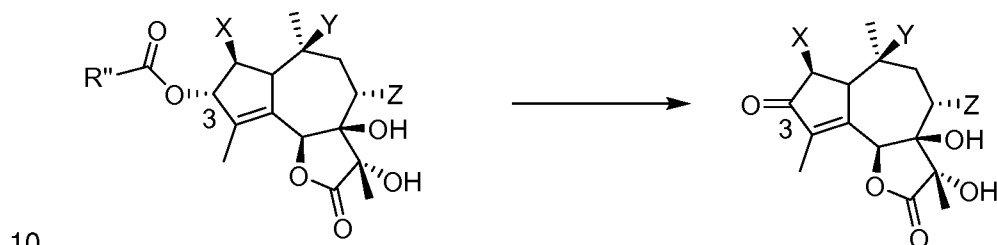
First, the angelate moiety was cleaved upon treatment of nortrilobolide (**2**) with an acid (AcOH, HF, TFA, *p*-TsOH·H₂O) in the presence of water and acetonitrile to afford the
 15 corresponding epimeric alcohols **3**. However, it appeared that these allylic alcohols were extremely sensitive substrates and had the tendency to decompose and/or quickly dehydrate forming several by-products. This explained the observed high conversion of the starting material but with a relatively low yield. It seemed crucial to trap the intermediate **3** during the cleaving of the angelic ester in order to avoid decomposition
 20 and/or formation of by-products. It emerged the idea to potentially oxidize the alcohol as soon as it is formed during the cleaving of the ester in a one-pot two-steps procedure as seen in Scheme 14. We envisioned that chromium(VI) oxide could be the reagent of choice: it is aqueous soluble in acidic media, easy to handle and purify and noteworthy inert when mixed with nortrilobolide (**2**). Aqueous hydrogen fluoride (HF)
 25 was chosen as acidic medium for the reaction. Thus, preliminary attempts showed that the use of a catalytic amount of CrO₃ (0.3 equiv) was not sufficient to fully convert the starting material (Table 3, entry 1). To our delight, increasing up to 2.5 equivalents of chromium(VI) oxide provided the desired ketone **4** in 60% after 6.5 h at 85°C (Table 3, entry 2). The reaction was enhanced by the use of microwave (MW) irradiations (Table

3, entries 3, 4 and 5) leading to the ketone in yields ranging from 61–68%. Finally, the use of only 1.4 equivalent of the oxidizing reagent under MW conditions at 95 °C for 1.5 h in the presence of 2 equivalent of HF gave compound **4** with 74% yield (Table 3, entry 7). Remarkably, this reaction was easily performed on a gram scale synthesis.



Scheme 14. Reagents and conditions: (i) HF (2 equiv), CrO₃ (1.4 equiv), CH₃CN, MW, 95 °C, 2 h, 74%.

Presented in a more general manner:



wherein X, Y, Z and R'' are as defined herein before.

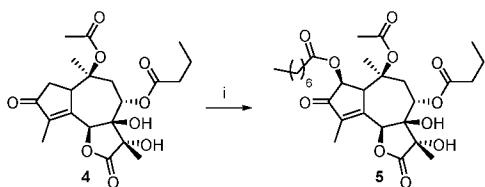
Table 3. Synthesis of ketone **4** from nortrilobolide (**2**).

Entry	HF (equiv)	CrO ₃ (equiv)	Temp. (°C)	Time	Yield (%)
1	5	0.3	60 ^a	16 h	20 ^c
2	5	2.5	85 ^a	6.5 h	60 ^d
3	5	2.5	80 ^b	1.5 h	61
4	5	2.5	85 ^b	100 min	67
5	5	2.5	100 ^b	35 min	68
6	2.5	1.25	100 ^b	70 min	68
7	2	1.4	95 ^b	2 h	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.

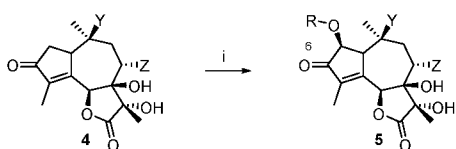
The next challenge was the stereoselective introduction of the octanoyl backbone on the C-2 position of the ketone intermediate **4** via an α' -acyloxylation. Several methods for selective α -oxygenation of carbony groups have been largely studied including for

example oxidation with heavy metals, hypiodite catalyzed α -oxyacylation, sigmatropic rearrangement acyloxyenamines. We recently obtained successful results by using manganese(III) acetate and decided to pursue with this reagent. Thus, ketone **4** was heated for 7 h at 120 °C in a mixture dry benzene–caprylic acid (5:1) in the presence of 2.5 equivalents of $\text{Mn}(\text{OAc})_3 \cdot 2\text{H}_2\text{O}$ using a Dean-Stark apparatus. Gratefully, α' -acylated ketone **5** was obtained in 51% as shown in Scheme 15. Notably, this procedure resulted in the formation of **5** with the desired stereochemistry at C-2 as confirmed by ^1H NMR comparison with a pure isolated sample.



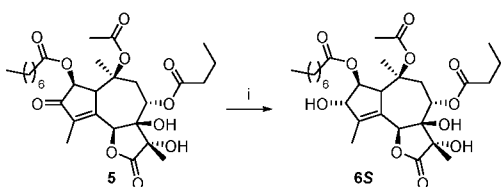
Scheme 15. Reagents and conditions: (i) $\text{Mn}(\text{OAc})_3 \cdot 2\text{H}_2\text{O}$ (2.5 equiv), dry benzene–caprylic acid (5:1), 120 °C, Dean-Stark, 7 h, 51%.

Presented in a more general manner:



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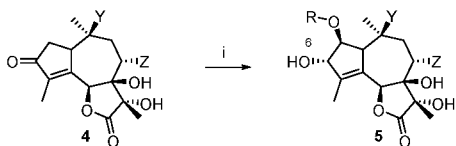
Stereoselective reductions of similar ketones to give the α -alcohols have previously been performed using zinc borohydride as the reducing reagent. In our studied case, treatment of acylated ketone **5** with $\text{Zn}(\text{BH}_4)_2$ provided after subsequent treatment with disodium dihydrate EDTA the two epimeric alcohols **6S** and **6R** in a 4:3 ratio when the reaction was performed in THF. To our delight, the target alcohol **6S** was isolated in 87% yield when the reaction was performed in dry diethyl ether with only trace amounts of **6R**, as seen in Scheme 16.



25

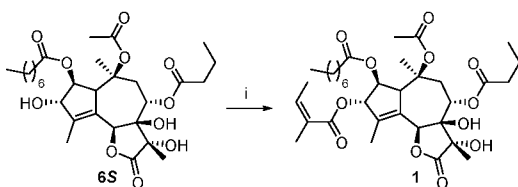
Scheme 16. Reagents and conditions: (i) $\text{Zn}(\text{BH}_4)_2$ (11.6 equiv), dry Et_2O , -20 °C, 3.5 h, 87%.

Presented in a more general manner:



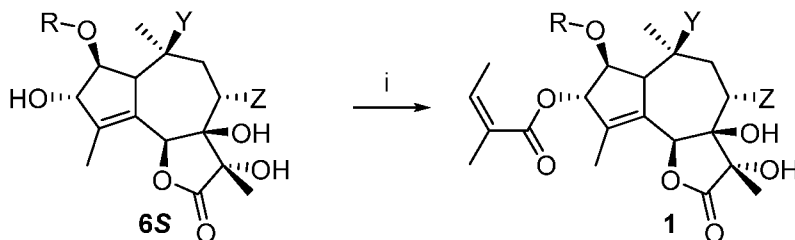
5

As depicted in Scheme 17, final angeloylation of alcohol **6S** furnished thapsigargin (**1**) in 65% yield.



- 10 **Scheme 17.** 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%.

Presented in a more general manner:



15

wherein R'', Y and Z is as defined herein before. The angelate moiety may be replaced with other acid moieties.

- 20 In conclusion, an expedient synthesis of thapsigargin (**1**) has been performed within only 4 steps starting from natural product nortrilobolide (**2**). Noteworthy, the strategy does not involve any protection/deprotection steps.

- 25 **Use of compounds according to the invention or obtainable according to a method of the invention**

Consequently, present invention also relates to the use of a compound according to Formula (I) as an intermediate in further synthesis towards thapsigargin analogues and any prodrugs thereof.

- 5 Present invention also relates to a compound according to Formula (I) or (II) for use in medicine.

Present invention furthermore relates to a compound of Formula (I) or (II) for use in treatment of cancer such as e.g. prostate cancer, benign prostatic hyperplasia (BEH),
10 glioblastoma multiforme, lung cancer, liver cancer, renal cell cancer, ovarian cancer, melanoma, bladder cancer, or breast cancer.

Present invention also relates to a pharmaceutical formulation comprising a compound of Formula (I) or (II). A suitable pharmaceutical formulation may be e.g. a composition
15 for local administration wherein the composition releases the compounds according to the invention locally in an extended release fashion. A suitable formulation may e.g. be a tablet, capsule, etc.

Present invention also relates to oxidation of the 2 position of Formula I by other meth-
20 ods such as halogenation of the 2 position followed by substitution with a hydroxy group or a masked hydroxyl group, or epoxidation of the enol form of Formula I with peroxyreagents followed by hydrolysis of the epoxide as previously described herein.

Experimental Section relating to Schemes 1a, 1b, 1c, 2a and 2b

25 General

The crude product of nortrilobolide was received from GenSpera and purified by dry column vacuum chromatography prior to use (silica gel, DCM-EtOAc 5:1, R_f 0.23). All solvents and reagents were obtained from commercial suppliers and used without further purification except otherwise stated. All air- and moisture- sensitive reactions were
30 conducted under nitrogen or argon atmosphere using oven- or flame-dried glassware and in dried solvents according to standard procedures. Dichloromethane and tetrahydrofuran were obtained by using a Solvent Purification System. Acetone, toluene and methanol were dried over molecular sieves 3Å. Solvents were removed on an evaporator under reduced pressure and at a temperature around 40°C.

Reactions were followed by thin-layer chromatography (TLC) using precoated aluminium plates (Merck, Silica Gel 60, F₂₅₄) and visualized by using vanillin stain (15g vanillin, 250 mL ethanol and 2.5 mL sulfuric acid). The TLC-plates were heated until visual spots appeared. Flash column chromatography was performed with Fisher Scientific silica gel 60 (35-75 µm). Dry column vacuum chromatography was carried out with Fisher Scientific silica gel 60 (20-45 µm).

Synthesis

With reference to Schemes 1a, 1b and 1c, the compounds numbered therein were synthesized according to the following procedures.

Alcohol mixture **2**: To a solution of nortrilobolide **1** (100 mg, 0.2 mmol) in MeCN–H₂O (6 mL, 5:1) was added *p*-TsOH hydrate (60 mg, 0.32 mmol) at room temperature. The reaction mixture was stirred at 60 °C. After for 6 h the mixture was cooled to room temperature, diluted with EtOAc (30 mL) and washed with water (until pH 6). The organic phase was then washed with brine (30 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, toluene–EtOAc 4:1) to afford a 1.25:1 (R/S) mixture of the two epimeric alcohols **2** (63 mg, 75%) as a pale yellow solid.

Ketone **3**: Dess-Martin periodinane (180 mg, 0.42 mmol) was added portionwise to a solution of alcohol mixture **2** (120 mg, 0.28 mmol) in dry DCM (10 mL) and pyridine (0.2 mL, 2.5 mmol) at room temperature. The reaction mixture turned immediately dark brown. The mixture was stirred for 2 h at room temperature. The resulting yellow solution was quenched by addition of a saturated aqueous solution of Na₂S₂O₃ (5 mL) followed by a saturated aqueous solution of NaHCO₃ (5 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (30 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography (silica gel, toluene–EtOAc 2:1) to furnish the ketone **3** (104.3 mg, 87%) as a white solid.

Ketone **3** could also be obtained as followed: To a microwave (MW) vial containing a solution of nortrilobolide **1** (1.05 g, 2.07 mmol) in acetonitrile (12 mL) was successively added a 1 M aqueous solution of hydrogen fluoride (4.1 mL, 4.10 mmol) and chromium(VI) oxide (290 mg, 2.89 mmol) at room temperature. The MW vial was sealed and

heated under MW irradiations for 120 min at 95 °C. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (40 mL). The separated organic phase was successively washed with water, a 2 M aqueous solution of NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting off-white solid was purified by flash column chromatography (silica gel, EtOAc–heptane 1:1) to give ketone **3** (651 mg, 74 %) as a white solid.

2-Acetoxy ketone **4**: A solution of ketone **3** (230 mg, 0.54 mmol) and manganese(III) triacetate dihydrate (310 mg, 1.16 mmol) in dry benzene–glacial acetic acid (45 mL, 5:1) was stirred under reflux using a Dean-Stark apparatus. After 6 h the dark color of manganese triacetate disappeared and the reaction mixture was diluted with EtOAc (30 mL) then the organic phase was washed with brine (30 mL), dried over MgSO₄, filtered and concentrated under reduced pressure to afford the crude 2-acetoxy ketone **4** as a yellow solid which was used without further purification in the next step.

Triol **5**: Triethylamine (2 mL, 14.3 mmol) was added to a solution of 2-acetoxy ketone **4** (222 mg, 0.54 mmol) in dry MeOH (20 mL) at room temperature under an argon atmosphere. The reaction mixture was stirred at 60 °C for 30 min then concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, toluene–EtOAc 2:1) to provide the triol **5** (63 mg, 57 % over two steps) as a white solid.

Ketone **6**: To a solution of triol **5** (70 mg, 0.17 mmol) in dry THF (1 mL) were successively added (*S*)-(+)-2-methylbutyric anhydride (130 mg, 0.7 mmol) in dry THF (0.5 mL) and DMAP (2 mg, 0.016 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred for 1 h at this temperature then diluted with EtOAc (10 mL). The organic phase was successively washed with a 2 M aqueous solution of H₂SO₄ (5 mL), a saturated aqueous solution of NaHCO₃ (10 mL) and brine (10 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography (silica gel, toluene–EtOAc 2:1) to afford the ketone **6** (65.5 mg, 78%) as a white solid.

Epimeric alcohols **7**: To a solution of ketone **6** (24 mg, 0.048 mmol) in freshly distilled THF (2 mL) was added a pre-cooled solution of zinc borohydride (3.5 mL of a 0.5 M solution in Et₂O, 1.75 mmol) at -30 °C under an argon atmosphere. After 2 h at this temperature, an additional quantity of zinc borohydride (1 mL of a 0.5 M solution in Et₂O,

0.5 mmol) solution was added. The reaction mixture was allowed to warm up to 10 °C and stirred overnight. The reaction mixture was diluted with EtOAc (30 mL) and quenched by the slowly addition of an aqueous solution of EDTA (30 mL, 30 % w/w). The biphasic system was vigorously stirred at room temperature for 2 h. The aqueous
5 phase was extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with brine (50 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography (silica gel, toluene-EtOAc 2:1) to give a 1:1.87 3-(*S/R*) mixture of epimeric alcohols **7** (14.6 mg, 61%) as a white solid.

10

2-Acetoxytrilobolide **8**: 2,4,6-Trichlorobenzoyl chloride (9.4 µL, 0.06 mmol) and triethylamine (8.4 µL, 0.06 mmol) were successively added to a solution of angelic acid (6 mg, 0.06 mmol) in dry toluene (100 µL) at room temperature under an argon atmosphere. The resulting mixture was stirred at this temperature for 2 h and was subsequently
15 treated with a solution of 3-(*S*)-alcohol **7** (15 mg, 0.03 mmol) in dry toluene (100 µL). The reaction mixture was stirred at 75 °C for 2 days. The resulting mixture was cooled to room temperature and quenched by the addition of an aqueous saturated ammonium chloride solution (3 mL). The separated aqueous phase was extracted with EtOAc (2 x 5 mL) and the combined organic phases were dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography (silica gel, toluene-EtOAc 3:1) to provide the target 2-acetoxytrilobolide **8** (8.5 mg, 49%) as a colorless oil.

20

2-Bromo ketone **9**: To a solution of ketone **3** (20 mg, 0.05 mmol) in acetonitrile (1 mL)
25 were successively added *N*-bromosuccinimide (14 mg, 0.08 mmol) and ammonium acetate (0.6 mg, 20 mol%) at room temperature. The reaction mixture was heated at 65 °C for 1 h then stirred at room temperature for 20 h. After addition of EtOAc, the organic layer was washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude material was purified by flash column chromatography (silica gel, EtOAc-heptane 1:1) to provide the bromo ketone **9** (11.3 mg, 48%) as a white
30 solid. Starting material **3** (7.7 mg, 38%) was also recovered.

30

2-Hexanoyloxy ketone **10**: A solution of ketone **3** (50 mg, 0.12 mmol), hexanoic anhydride (0.41 mL, 1.77 mmol) and manganese(III) triacetate dihydrate (70 mg, 0.26
35 mmol) in dry benzene-hexanoic acid (5 mL, 4:1) was stirred at 75 °C for 16 h then cooled to room temperature. The reaction mixture was diluted with EtOAc (20 mL) then

the organic phase was successively washed with water (20 mL), a saturated aqueous solution of NaHCO_3 (2 x 20 mL), brine (20 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude material was first filtered through a pad of Celite containing some alumina then purified by flash column chromatography (silica gel, EtOAc-heptane 1:2) to provide the 2-hexanoyloxy ketone **10** (15 mg, 24%) as a pale yellow semi-solid. Starting material **3** (10 mg, 20%) was also recovered.

2-Octanoyloxy ketone **11**: To a solution of ketone **3** (424 mg, 1.0 mmol) in dry benzene-octanoic acid (90 mL, 5:1) at 60 °C was added manganese(III) triacetate dihydrate (670 mg, 2.5 mmol). The reaction mixture was heated under reflux conditions using a Dean-Stark apparatus for 7 h then cooled to room temperature. The reaction mixture was diluted with EtOAc (50 mL) then the organic phase was successively washed with water (50 mL), a 2 M aqueous solution of Na_2CO_3 (50 mL), brine (50 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. The crude material was purified by flash column chromatography (silica gel, EtOAc-heptane 1:1) to provide the 2-octanoyloxy ketone **11** (290 mg, 51%) as a pale orange-yellow solid. A mixture of starting material **3** and 2-acetoxy ketone **4** (36 mg) in a 1.2:1 ratio was also recovered

Experimental details regarding Schemes 3-13

General Experimental Procedures.

A crude extract containing nortrilobolide (**3**) was received from GenSpera (San Antonio, TX, USA) and purified by dry column vacuum chromatography on silica gel using DCM-EtOAc (5:1) as eluent ($R_f = 0.23$) prior to use. All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. All air- and moisture-sensitive reactions were conducted under argon using oven or flame-dried glassware and in dried solvents according to standard procedures. Reactions were followed by thin-layer chromatography (TLC) using precoated aluminum plates and visualized using vanillin reagent (15 g of vanillin, 250 mL of EtOH, and 2.5 mL of conc H_2SO_4). Flash column chromatography was performed with silica gel (35–75 μm). Dry column vacuum chromatography was carried out with silica gel (20–45 μm).

Optical rotations were measured as $[\alpha]_D$ values (c in g/100 mL). Yields refer to isolated compounds estimated to be >95% pure as determined by ^1H NMR spectroscopy. NMR spectra were recorded on 400 and 600 MHz instruments. The chemical shifts (δ) are given in parts per million (ppm) relative to residual signals of the solvent (CDCl_3 and CD_3OD). Coupling constants (J values) are given in hertz (Hz). Multiplicities of ^1H NMR signals are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublets of doublets; dtt, doublet of triplets of triplets; t, triplet; m: multiplet; q, quartet; dq, doublet of quartets; qq, quartet of quartets; b, broad signal.

10

Assignments of the NMR signals were made using 1D (^1H , ^{13}C , DEPTQ) and 2D (COSY, HSQC, HMBC, ROESY) spectra.

Microwave-assisted synthesis was carried out in a Biotage Initiator apparatus operating in single mode; the microwave cavity produced controlled irradiation at 2.45 GHz. The reactions were run in sealed vessels. These experiments were performed by employing magnetic stirring and a fixed hold time using variable power to reach the desired temperature (for 1–2 min) and then maintained at the desired temperature in the vessel for the programmed time period. The temperature was monitored by an IR sensor focused on a point on the reactor vial glass. The IR sensor was calibrated to internal solution reaction temperature by the manufacturer. HRMS data were recorded on a micrOTOF-Q instrument using electrospray (ESI) as ionization method.

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Ozonolysis of Nortrilobolide (3). Ozone was bubbled through a solution of nortrilobolide (3) (0.30 g, 0.59 mmol) in dry DCM (100 mL) at -78°C for 10 min until the solution turned pale blue. The solution was first flushed with oxygen for 10 min, then flushed with nitrogen for 10 min before Ph₃P (1.10 g, 4.19 mmol) was added to the solution and stirred at room temperature overnight. The solution was concentrated under reduced pressure, and the crude product was purified through a short plug of silica gel using toluene–EtOAc (3:1 to 2:1) as eluent before it was dissolved in dry MeOH (50 mL) and treated with pyridine (5 mL) and H₂O (5 mL). The reaction mixture was stirred under reflux for 6 h, cooled to room temperature, and quenched by the addition of a saturated aqueous NH₄Cl solution (100 mL). The aqueous phase was extracted with DCM (3 × 50 mL), and the combined organic phases were dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by dry vacuum column chromatography on silica gel using toluene–EtOAc (3:1 to 2:1) as eluent to afford diketone **9** and acetal **10** (0.09 g, 33%) in a 1:1 ratio as a pale yellow oil. An analytically pure sample of compound **9** was obtained as a pale yellow oil: ¹H NMR (600 MHz, CDCl₃) δ 5.45 (1H, d, *J* = 4.8 Hz, H-8), 5.06 (1H, s, OH), 4.96 (1H, s, H-6), 4.15 (1H, d, *J* = 11.2 Hz, H-1), 4.08 (1H, dt, *J* = 8.3, 4.1 Hz, H-3), 3.94 (1H, s, OH), 3.82 (1H, s, OH), 2.97 (1H, d, *J* = 15.1 Hz, H-9a), 2.67 (1H, dd, *J* = 15.4, 5.9 Hz, H-9b), 2.61 (1H, ddd, *J* = 13.4, 11.3, 4.4 Hz, H-2a), 2.23 (3H, s, H-15), 2.18–2.04 (2H, m, butanoyl H-2), 2.00 (3H, s, acetyl CH₃), 1.68 (1H, ddd, *J* = 13.6, 8.1, 2.1 Hz, H-2b), 1.56 (3H, s, H-13), 1.54–1.48 (2H, m, butanoyl H-3), 1.29 (3H, s, H-14), 0.87 (3H, t, *J* = 7.4 Hz, butanoyl H-4); ¹³C NMR (101 MHz, CDCl₃) δ 209.8 (C-4), 200.5 (C-5), 176.1 (C-12), 172.4 (butanoyl C=O), 170.7 (acetyl C=O), 85.8 (C-6), 82.1 (C-10), 78.8 (C-11), 76.3 (C-7), 74.7 (C-3), 70.3 (C-8), 50.1 (C-1), 36.5 (C-9), 36.1 (butanoyl C-2), 29.5 (C-2), 25.4 (C-15), 22.6 (acetyl CH₃), 22.5 (C-14), 21.9 (C-13), 17.7 (butanoyl C-3), 13.7 (butanoyl C-4); HRMS *m/z* 499.1794 [M + Na + H₂O]⁺, calcd for C₂₁H₃₂O₁₂Na 499.1791.

An analytically pure sample of compound **10** was obtained as a pale yellow oil: ¹H NMR (400 MHz, CDCl₃) δ 5.57 (1H, t, J = 3.8 Hz, H- 8), 5.23 (1H, s, H-6), 4.60 (1H, t, J = 8.7 Hz, H-3), 4.50 (1H, s, OH), 4.15 (1H, s, OH), 3.40 (1H, t, J = 5.4 Hz, H-1), 3.04 (1H, s), 2.86 (1H, dd, J = 14.9, 3.3 Hz, H-9a), 2.52–2.39 (3H, m, H-2, H-9b), 2.30–2.22 (5H, m, H-15, butanoyl H-2), 1.99 (3H, s, acetyl CH₃), 1.70–1.55 (2H, m, butanoyl H-3), 1.52 (3H, s, H-14), 1.47 (3H, s, H- 13), 0.93 (3H, t, J = 7.4 Hz, butanoyl H-4); ¹³C NMR (101 MHz, CDCl₃) δ 209.4 (C-4), 175.6 (C-12), 172.4 (butanoyl C=O), 170.1 acetyl C=O), 104.9 (C-5), 84.2 (C-10), 84.0 (C-3), 80.4 (C-6), 79.5 (C-11), 76.6 (C-7), 66.6 (C-8), 56.9 (C-1), 38.8 (C-9), 36.7 (butanoyl C-2), 28.6 (C-2), 26.2 (C-15), 22.7 (acetyl CH₃), 22.1 (C-14), 18.1 (butanoyl C-3), 16.56 (C-13), 13.9 (butanoyl C-4); HRMS m/z 499.1781 [M + Na + H₂O]⁺, calcd for C₂₁H₃₂O₁₂Na 499.1791.

Hydrazinolysis of Nortrilobolide (3). To a solution of nortrilobolide (**3**) (1.00 g, 1.97 mmol) in absolute EtOH (30 mL) was added hydrazine hydrate (0.12 mL, 2.47 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred under reflux for 16 h, then cooled to room temperature. The reaction was concentrated, and the resulting crude material was purified by flash column chromatography on silica gel using gradient elution (toluene–EtOAc, 2:1 to 1:2) to furnish triol **12**⁵ as a pale yellow solid (0.31 g, 35%), tetraol **13** as a pale yellow solid (0.06 g, 9%), and relactonized tetraol **14** (0.01 g, 1%) as a pale yellow solid.

Compound **13**: ¹H NMR (600 MHz, CD₃OD) δ 5.83–5.80 (1H, m, H-6), 4.53–4.46 (1H, m, H-3), 4.33 (1H, t, J = 3.6 Hz, H-8), 4.21– 4.16 (1H, m, H-1), 2.93 (1H, dd, J = 14.0, 3.5 Hz, H-9a), 2.36–2.28 (2H, m, H-9b, H-2a), 1.99 (3H, s, acetyl CH₃), 1.93 (3H, s, H-15), 1.58 (1H, ddd, J = 13.4, 8.0, 6.9 Hz, H-2b), 1.41 (3H, s, H-14), 1.40 (3H, s, H-13); ¹³C NMR (151 MHz, CD₃OD) δ 177.6 (C-12), 172.12 (acetyl C=O), 145.5 (C-5), 130.7 (C-4), 88.0 (C-10), 80.43 (C-7/C- 11), 80.37 (C-7/C-11), 79.4 (C-6), 78.2 (C-3), 70.2 (C-8), 51.5 (C- 1), 41.1 (C-9), 35.8 (C-2), 22.45 (bs, acetyl CH₃, C-14), 16.4 (C-13), 12.8 (C-15); HRMS m/z 379.1366 [M + Na]⁺, calcd for C₁₇H₂₄O₈Na

379.1363. Compound **14**: ¹H NMR (600 MHz, CD₃OD) δ 5.06 (1H, dd, J = 13.4, 3.3 Hz, H-8), 4.68 (1H, s, H-6), 4.42 (1H, t, J = 6.9 Hz, H-3), 3.56 (1H, t, J = 6.9 Hz, H-1), 2.62 (1H, dd, J = 15.5, 3.3 Hz, H- 9a), 2.29 (1H, dt, J = 13.2, 7.7 Hz, H-2a), 2.05–2.01 (1H, m, H-9b), 2.03 (3H, s, acetyl CH₃), 1.95 (3H, s, H-15), 1.58 (3H, s, H-13), 1.50 (1H, ddd, J = 13.4, 7.5, 6.2 Hz, H-2b), 1.44 (3H, s, H-14); ¹³C NMR (151 MHz, CD₃OD) δ 180.0 (C-12), 172.3 (acetyl C=O), 143.9 (C- 5), 135.5 (C-4), 84.59 (C-8), 83.8 (C-10), 80.1 (C-7/C-11), 78.6 (C- 3), 77.2 (C-7/C-11), 70.8 (C-6), 49.2 (C-1), 40.4 (C-9), 35.5 (C-2), 24.4 (C-14), 23.6 (C-13), 22.2 (acetyl CH₃), 12.7 (C-15); HRMS m/z

379.1361 [M + Na]⁺, calcd for C₁₇H₂₄O₈Na 379.1363.

Methoxy Ketal 16. To a solution of tetraol 13 (40 mg, 0.11 mmol) in dry acetone (2 mL) were added 2,2-dimethoxypropane (1.7 mL, 13.8 mmol) and p-TsOH (2 mg, 2 mol %)
5 % at room temperature under an argon atmosphere. The reaction was stirred at 50 °C overnight, cooled to room temperature, and quenched by the addition of a saturated aqueous NaHCO₃ solution (20 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organic
phases were dried over MgSO₄, filtered, and concentrated under reduced pressure.
10 The crude material was purified by dry vacuum column chromatography on silica gel using toluene–EtOAc (5:1) as eluent to lead to a mixture of epimeric methyl ethers **16** (20 mg, 43%) in a 1:1 (R/S) ratio as a pale yellow solid. An analytically pure sample of compound **16R** was obtained as a pale yellow solid: ¹H NMR (600 MHz, CDCl₃) δ 5.82 (1H, s, H-6), 4.27 (1H, dd, J = 4.7, 2.9 Hz, H-8),
15 4.13 (1H, dt, J = 8.9, 4.5 Hz, H-3), 3.88 (1H, dtt, J = 9.3, 4.7, 2.3 Hz, H-1), 3.38 (3H, s, OCH₃), 2.99 (1H, dd, J = 14.8, 4.5 Hz, H-9a), 2.35–2.31 (1H, m, H-9b), 2.31–2.25 (1H, m, H-2a), 1.98 (3H, s, acetyl CH₃), 1.95 (3H, s, H-15), 1.55–1.53 (7H, m, H-2a, H-13, C(CH₃)-(CH₃)), 1.41 (3H, s, C(CH₃)-(CH₃)), 1.35 (3H, s, H-14); ¹³C NMR (151 MHz, CDCl₃) δ 173.2 (C-12), 170.8 (acetyl-C=O), 143.9 (C-5), 127.4 (C-4), 101.1 (C(CH₃)-(CH₃)), 86.3 (C-10), 86.1 (C-3), 79.4 (C-7/C-11), 78.8 (C-6), 76.3 (C-7/C-11), 66.3 (C-8), 56.9 (OCH₃), 50.8 (C-1), 38.5 (C-9), 32.3 (C-2), 30.7 (C(CH₃)-(CH₃)), 23.8 (C(CH₃)-(CH₃)), 22.7 (CH₃CO), 21.2 (C-14), 16.1 (C-13), 12.7 (C-15); HRMS m/z 433.1826 [M + Na]⁺, calcd for C₂₁H₃₀O₈Na 433.1833.

An analytically pure sample of compound **16S** has been obtained as a pale yellow solid: ¹H NMR (600 MHz, CDCl₃) δ 5.77 (1H, s, H-6), 4.24 (1H, dd, J = 4.6, 2.9 Hz, H-8),
25 4.13–4.05 (2H, m, H-3, H-1), 3.31 (3H, s, OCH₃), 2.79 (1H, dt, J = 14.7, 3.8 Hz, H-9a), 2.52 (1H, dt, J = 14.6, 2.1 Hz, H-9b), 1.99 (6H, s, H-15, acetyl CH₃), 1.98–1.94 (1H, m, H-2a), 1.85–1.76 (1H, 1H, H-2b), 1.53 (3H, s, H-13), 1.52 (3H, s, C(CH₃)-(CH₃)), 1.40 (3H, s, C(CH₃)-(CH₃)), 1.30 (3H, s, H-14); ¹³C NMR (151 MHz, CDCl₃) δ 173.3 (C-12), 170.5 (acetyl-C=O), 142.5 (C-5),
30 130.9 (C-4), 100.9 (C(CH₃)-(CH₃)), 88.9 (C-3), 85.8 (C-10), 79.6 (C-7/C-11), 78.7 (C-6), 76.2 (C-7/C-11), 66.2 (C-8), 56.5 (OCH₃), 53.8 (C-1), 39.1 (C-9), 31.1 (C-2), 30.7 (C(CH₃)-(CH₃)), 23.8 (C(CH₃)-(CH₃)),

22.6 (CH₃CO), 20.3 (C-14), 16.2 (C-13), 14.5 (C-15); HRMS m/z 433.1830 [M + Na]⁺, calcd for C₂₁H₃₀O₈Na 433.1833.

Acetonide 18. To a solution of nortrilobolide (3) (1.00 g, 1.97 mmol) in dry MeOH (100 mL) was added TEA (5.5 mL, 39.5 mmol) at room temperature under an atmosphere of argon. The reaction was stirred at room temperature for 4 h before it was quenched by the addition of an aqueous saturated NH₄Cl solution (100 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic phases were washed with brine (150 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the crude triol **17**, which was used in the next reaction without further purification. An analytically pure sample of compound **17**⁵ was obtained as a white solid: HRMS m/z 461.1767 [M + Na]⁺, calcd for C₂₂H₃₀O₉Na 461.1782. To a solution of crude **17** (1.97 mmol) in dry acetone (20 mL) were added 2,2-dimethoxypropane (20 mL, 163 mmol) and p-TsOH (cat.) at room temperature under an argon atmosphere. The reaction was stirred at 50 °C overnight, cooled to room temperature, and quenched by the addition of a saturated aqueous NaHCO₃ solution (50 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic phases were washed with brine (100 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography on silica gel using toluene–EtOAc (5:1) as eluent to provide a mixture of **16** and acetonide **18** (0.33 g, 35%) in a 9:1 ratio as a pale yellow solid. An analytically pure sample of compound **18** has been obtained as a pale yellow solid: ¹H NMR (600 MHz, CDCl₃) δ 6.11 (1H, q, J = 7.3 Hz, angeoyl H-3), 5.84 (1H, s, H-6), 5.57–5.51 (1H, m, H-3), 4.31–4.26 (1H, m, H-8), 4.03–3.96 (1H, m, H-1), 3.19 (1H, s, OH), 2.94 (1H, dd, J = 14.6, 4.7 Hz, H-9a), 2.54 (1H, dt, J = 13.5, 7.7 Hz, H-2a), 2.42 (1H, dd, J = 14.7, 2.9 Hz, H-9b), 2.03–1.99 (3H, m, angeoyl H-4), 1.95 (3H, s, angeoyl 2-CH₃), 1.93–1.89 (6H, m, acetyl CH₃, H-15), 1.61–1.56 (1H, m, H-2b), 1.55 (3H, s, H-13), 1.53 (3H, s, C(CH₃)-(CH₃)), 1.42 (3H, s, C(CH₃)-(CH₃)), 1.36 (3H, s, H-14); ¹³C NMR (151 MHz, CDCl₃) δ 173.3 (C-12), 170.8 (angeoyl C=O), 168.0 (acetyl C=O), 140.9 (C-4), 138.8 (angeoyl C-3), 129.9 (C-5), 127.9 (angeoyl C-4), 101.1 (C(CH₃)-(CH₃), 85.7 (C-10), 79.9 (C-3), 79.5, 78.8 (C-6), 76.2, 66.2 (C-8), 51.6 (C-1), 38.4 (C-9), 33.3 (C-2), 30.7 (C(CH₃)-(CH₃)), 23.8 (C(CH₃)-(CH₃)), 22.6 (angeoyl 2-CH₃), 20.9 (C-14), 20.8 (acetyl CH₃), 16.1 (C-13), 12.8 (C-15); HRMS m/z 501.2101 [M + Na]⁺, calcd for C₂₅H₃₄O₉Na 501.2095.

Allylic Alcohol 19. To a solution of nortrilobolide (3) (100 mg, 0.2 mmol) in a mixture of MeCN–H₂O (6 mL, 5:1) was added p-TsOH (60 mg, 0.32 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 6 h, cooled to room temperature, diluted with EtOAc (30 mL), and washed with water (until pH ~6). The organic phase was then washed with brine (30 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using toluene–EtOAc (4:1) as eluent to afford a mixture of epimeric alcohols **19** (63 mg, 75%) in a 1.25:1 (R/S) ratio as a pale yellow solid.

An analytically pure sample of compound **19R** was obtained as a pale yellow solid: ¹H NMR (600 MHz, CDCl₃) δ 5.69 (1H, s, H-6), 5.61 (1H, t, J = 3.6 Hz, H-8), 4.59 (1H, t, J = 6.8 Hz, H-3), 4.16 (1H, t, J = 7.1 Hz, H-1), 3.08 (1H, dd, J = 15.0, 3.5 Hz, H-9a), 2.52 (1H, s, OH), 2.40 (1H, dt, J = 13.5, 8.2 Hz, H-2a), 2.27 (3H, t, J = 6.8 Hz, butanoyl H-2), 2.22 (1H, dd, J = 14.8, 3.9 Hz, H-9b), 1.97 (3H, s, acetyl CH₃), 1.95 (3H, s, H-15), 1.79 (1H, s), 1.69–1.53 (3H, m, H-2b, butanoyl H-3), 1.49 (3H, s, H-13), 1.34 (3H, s, H-14), 0.95 (3H, t, J = 7.4 Hz, butanoyl H-4); ¹³C NMR (151 MHz, CDCl₃) δ 176.2 (C-12), 172.9 (butanoyl C=O), 171.3 (acetyl C=O), 146.4 (C-5), 129.1 (C-4), 86.3 (C-10), 78.9 (C-7/C-11), 78.2 (C-6), 77.7 (C-3), 66.7 (C-8), 50.1 (C-1), 38.8 (C-9), 36.8 (butanoyl C-2), 34.7 (C-2), 22.6 (acetyl CH₃), 22.5 (C-14), 18.1 (butanoyl C-3), 16.3 (C-13), 13.9 (butanoyl C-4), 12.9 (C-15); HRMS m/z 449.1767 [M + Na]⁺, calcd for C₂₁H₃₀O₉Na 449.1782. An analytically pure sample of compound **19S** was obtained as a pale yellow solid: ¹H NMR (600 MHz, CDCl₃) δ 5.61 (1H, s, H-6), 5.59 (1H, t, J = 4.0 Hz, H-8), 4.56 (1H, d, J = 7.5 Hz, H-1), 4.51 (1H, bs, H-3), 3.48 (1H, s, OH), 3.09 (1H, dd, J = 14.8, 3.6 Hz, H-9a), 2.42 (1H, s, OH), 2.25 (2H, t, J = 7.6 Hz, butanoyl H-2), 2.19–2.09 (2H, m, H-9b, H-2a), 1.97 (6H, s, H-15, acetyl CH₃), 1.81 (1H, ddd, J = 14.7, 8.1, 2.5 Hz, H-2b), 1.67–1.58 (2H, m, butanoyl H-3), 1.50 (3H, s, H-13), 1.21 (3H, s, H-14), 0.94 (3H, t, J = 7.4 Hz, butanoyl H-4); ¹³C NMR (151 MHz, CDCl₃) δ 176.4 (C-12), 172.9 (butanoyl C=O), 171.3 (acetyl C=O), 147.0 (C-5), 130.8 (C-4), 86.5 (C-10), 80.1 (C-3), 79.0 (C-7/C-11), 78.8 (C-7/C-11), 78.2 (C-6), 66.7 (C-8), 51.7 (C-1), 39.1 (C-9), 36.8 (butanoyl C-2), 35.1 (C-2), 22.5 (acetyl CH₃), 21.9 (C-14), 18.1 (butanoyl C-3), 16.3 (C-13), 14.1 (butanoyl C-4), 13.9 (C-15); HRMS m/z 449.1784 [M + Na]⁺, calcd for C₂₁H₃₀O₉Na 449.1782.

Ketone 6. *Procedure A.* Dess–Martin periodinane (180 mg, 0.42 mmol) was added portionwise to a solution of allylic alcohol **19** (120 mg, 0.28 mmol) in dry DCM (10 mL) and pyridine (0.2 mL, 2.5

mmol) at room temperature under an argon atmosphere. The reaction mixture, which immediately turned dark brown, was stirred for 2 h at room temperature. The resulting yellow solution was quenched by addition of a saturated aqueous Na₂S₂O₃ solution (5 mL) and a saturated aqueous NaHCO₃ solution (5 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organic phases were washed with brine (30 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude material was purified by dry vacuum column chromatography on silica gel using toluene–EtOAc (2:1) as eluent to furnish ketone **6** (104 mg, 87%) as a white solid: [α]_D²⁵ –4 (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.81 (1H, s, H-6), 5.71 (1H, t, J = 3.7 Hz, H-8), 4.76 (1H, bs, H-1), 4.13 (1H, s, OH), 3.32 (1H, dd, J = 14.8, 3.7 Hz, H-9a), 3.13 (1H, s, OH), 2.43 (1H, dd, J = 19.5, 6.3 Hz, H-2a), 2.34 (1H, dd, J = 19.1, 2.8 Hz, H-2b), 2.27 (2H, t, J = 7.5 Hz, butanoyl H-2), 2.09 (1H, dd, J = 14.7, 3.8 Hz, H-9b), 1.98 (3H, s, acetyl C=O), 1.92 (3H, dd, J = 2.2, 1.3 Hz, H-15), 1.69–1.54 (2H, m, butanoyl H-3), 1.50 (3H, s, H-13), 1.20 (3H, s, H-14), 0.93 (3H, t, J = 7.4 Hz, butanoyl H-4); ¹³C NMR (151 MHz, CDCl₃) δ 207.3 (C-3), 174.8 (C-12), 172.7 (butanoyl C=O), 171.3 (acetyl C=O), 159.4 (C-5), 145.0 (C-4), 85.3 (C-10), 79.3 (C-7/C-11), 78.8 (C-7/C-11), 77.8 (C-6), 66.4 (C-8), 46.2 (C-1), 39.1 (C-9), 36.74 (C-2/butanoyl C-2), 36.69 (C-2/butanoyl C-2), 22.5 (acetyl CH₃), 22.1 (H-14), 18.1 (butanoyl C-3), 16.3 (C-13), 13.8 (butanoyl C-4), 9.9 (C-15); HRMS m/z 425.1834 [M + H]⁺, calcd for C₂₁H₂₉O₉ 425.1806.

Procedure B. To a MW vial containing a solution of nortrilobolide (**3**) (1.05 g, 2.07 mmol) was successively added a 1 M aqueous solution of hydrogen fluoride (4.14 mL, 4.14 mmol) and chromium-(VI) oxide (290 mg, 2.89 mmol) at room temperature. The MW vial was sealed and heated under MW irradiation for 2 h at 95 °C. After cooling to room temperature, the reaction mixture was diluted with water (70 mL) and extracted with EtOAc (60 mL). The organic layer was successively washed with water, a 2 M aqueous solution of NaHCO₃, and brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The resulting off-white solid was purified by column chromatography on silica gel using EtOAc–heptane (1:1) as eluent to afford ketone **6** (651 mg, 74%) as a white solid with spectroscopic data in accordance with previous characterizations.

O-8-Debutanoyl 2-Acetoxyketone (22). A solution of ketone **6** (230 mg, 0.54 mmol) and manganese triacetate dihydrate (310 mg, 1.16 mmol) in dry benzene–glacial acetic acid (45 mL, 5:1) was stirred under reflux using a Dean–Stark apparatus. After 6 h, the dark color of the solution disappeared and the reaction mixture was diluted with EtOAc (30 mL) and washed with brine (30 mL). The separated organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the crude 2-acetoxyketone **21** as a yellow solid, which was used in the subsequent reaction without any further purification. An analytically pure sample of compound **21** was obtained as a pale yellow solid: $[\alpha]_{22D} - 91.4$ (c 0.35, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.82 (1H, 1H, H-6), 5.68 (1H, t, J = 3.8 Hz, H-8), 5.18 (1H, d, J = 3.6 Hz, H-2), 4.60–4.56 (1H, m, H-1), 4.09 (1H, s, OH), 3.23 (1H, dd, J = 14.9, 3.7 Hz, H-9a), 3.12 (1H, s, OH), 2.27 (2H, t, J = 7.3 Hz, butanoyl H-2), 2.23 (1H, dd, J = 14.7, 3.9 Hz, H-9b), 2.09 (3H, s, acetylC-2 CH₃), 2.01–1.97 (3H, m, H-15), 1.94 (3H, s, acetylC-10 CH₃), 1.66–1.58 (2H, m, butanoyl H-3), 1.47 (3H, s, H-13), 1.38 (3H, s, H-14), 0.94 (3H, t, J = 7.4 Hz, butanoyl H-4); ¹³C NMR (151 MHz, CDCl₃) δ 201.5 (C-3), 175.0 (C-12), 172.8 (butanoyl C=O), 171.2 (acetylC-10 C=O), 170.2 (acetylC-2 C=O), 156.7 (C-5), 142.1 (C-4), 84.1 (C-10), 79.1 (C-7/C-11), 78.7 (C-7/C-11), 78.0 (C-6), 73.5 (C-2), 66.2 (C-8), 51.8 (C-1), 38.8 (C-9), 36.7 (butanoyl C-2), 23.0 (C-14), 22.6 (acetylC-10 CH₃), 20.7 (acetylC-2 CH₃), 18.1 (butanoyl C-3), 16.2 (C-13), 13.8 (butanoyl C-4), 10.4 (C-15); HRMS m/z 505.1682 [M + Na]⁺, calcd for C₂₃H₃₀O₁₁Na 505.1680. To a solution of crude 2-acetoxyketone **21** (0.54 mmol) in dry MeOH (20 mL) was added TEA (2 mL, 14.3 mmol) at room temperature under an atmosphere of argon. The reaction mixture was stirred at 60 °C for 30 min before it was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using toluene–EtOAc (2:1) to afford the O-8-debutanoyl 2-acetoxyketone (**22**) (63 mg, 57% over two steps from **6**) as a white solid: ¹H NMR (600 MHz, CD₃OD) δ 6.07 (1H, s, H-6), 5.22 (1H, d, J = 3.5 Hz, H-2), 4.67–4.64 (1H, m, H-1), 4.38 (1H, t, J = 3.6 Hz, H-8), 3.13 (1H, dd, J = 14.3, 3.7 Hz, H-9a), 2.36 (1H, dd, J = 14.3, 3.5 Hz, H-9b), 2.11 (3H, s, acetylC-2 CH₃), 1.99–1.98 (3H, m, H-15), 1.97 (3H, s, acetylC-10 CH₃), 1.47 (3H, s, H-14), 1.44 (3H, s, H-13); ¹³C NMR (151 MHz, CD₃OD) δ 203.8 (C-3), 176.5 (C-12), 172.0 (acetylC-10 C=O), 171.4 (acetylC-2 C=O), 160.6 (C-5), 141.1 (C-4), 85.9 (C-10), 80.8 (C-7/C-11), 80.2 (C-7/C-11), 79.1 (C-6), 75.2 (C-2), 69.8 (C-8), 53.0 (C-1), 40.8 (C-9), 23.1 (C-14), 22.6 (acetylC-10 CH₃), 20.6 (acetylC-2 CH₃), 16.3 (C-13), 10.1 (C-15); HRMS m/z 435.1264 [M + Na]⁺, calcd for C₁₉H₂₄O₁₀Na 435.1262.

(S)-Methylbutanoate (23). To a solution of O-8-debutanoyl 2-acetoxyketone (22) (70 mg, 0.17 mmol) in dry THF (1 mL) were added successively (S)-(+)-2-methylbutyric anhydride (130 mg, 0.7 mmol) in dry THF (0.5 mL) and DMAP (2 mg, 0.016 mmol), at
5 room temperature under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature, then diluted with EtOAc (10 mL) and washed successively with a 2 M aqueous H₂SO₄ solution (5 mL), a saturated aqueous NaHCO₃ solution (10 mL), and brine (10 mL). The organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by dry vacuum column
10 chromatography on silica gel using toluene–EtOAc (2:1) as eluent to furnish the (S)-methylbutanoate (**23**) (65.5 mg, 78%) as a white solid: $[\alpha]_{22D}^{26} -40$ (c 0.3, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 5.80 (1H, s, H-6), 5.69 (1H, t, J = 3.7 Hz, H-8), 5.18 (1H, d, J = 3.4 Hz, H-2), 4.64–4.60 (1H, m, H-1), 4.26 (1H, s, OH), 3.27 (1H, dd, J = 14.8, 3.7 Hz, H-9a), 3.24 (1H, s, OH), 2.33 (1H, q, J = 6.9 Hz, 2-methyl butanoyl H-2), 2.18
15 (1H, dd, J = 14.7, 3.7 Hz, H-9b), 2.09 (3H, s, acetylC-2 CH₃), 1.98 (3H, s, H-15), 1.94 (3H, s, acetylC-10 CH₃), 1.73–1.64 (1H, m, 2-methyl butanoyl H-3a), 1.47 (3H, s, H-13), 1.46–1.40 (1H, m, 2-methyl butanoyl H-3b), 1.38 (3H, s, H-14), 1.13 (3H, d, J = 7.0 Hz, 2-methyl butanoyl 2-CH₃), 0.90 (3H, t, J = 7.5 Hz, 2-methyl butanoyl H-4); ¹³C NMR (151 MHz, CDCl₃) δ 201.5 (C-3), 175.6 (2-methyl butanoyl C=O), 175.1 (C-12),
20 171.2 (acetylC-10 C=O), 170.2 (acetylC-2 C=O), 156.9 (C-5), 142.1 (C-4), 84.2 (C-10), 79.1 (C-7/C-11), 78.6 (C-7/C-11), 78.0 (C-6), 73.4 (C-2), 66.2 (C-8), 51.7 (C-1), 41.6 (2-methyl butanoyl C-2), 38.8 (C-9), 26.3 (2-methyl butanoyl C-3), 23.2 (C-14), 22.6 (acetylC-10 CH₃), 20.7 (acetylC-2 CH₃), 16.4 (2-methyl butanoyl 2-CH₃), 16.2 (C-13), 11.8 (2-methyl butanoyl C-4), 10.4 (C-15); HRMS *m/z* 519.1843 [M + Na]⁺,
25 calcd for C₂₄H₃₂O₁₁Na 519.1837.

Alcohol 24S. To a solution of (S)-methylbutanoate (23) (24 mg, 0.048 mmol) in freshly distilled THF (2 mL) was cannulated a precooled solution of zinc borohydride (3.5 mL of a 0.5 M solution in

Et₂O, 1.75 mmol) at –30 °C. After stirring the solution for a further 2 h at –30 °C, an additional quantity of zinc borohydride (1 mL of a 0.5 M solution in Et₂O, 0.5 mmol) solution was added. The reaction mixture was allowed to warm to 10 °C and stirred overnight. The reaction mixture was diluted with EtOAc (30 mL) and quenched by the slow addition of an aqueous EDTA solution (30 mL, 30% w/w). The biphasic system was vigorously stirred at room temperature for 2 h. The separated aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with brine (50 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography on silica gel using toluene–EtOAc (2:1) as eluent to afford a mixture of epimeric alcohols **24** (14.6 mg, 61%) in a 1.85:1 (R/S) ratio as a white solid. An analytically pure sample of compound **24S** was obtained as a white solid: ¹H NMR (600 MHz, CDCl₃) δ 5.67–5.61 (2H, m, H-6, H-8), 4.92 (1H, dd, J = 4.8, 3.3 Hz, H-2), 4.46–4.44 (1H, m, H-3), 4.34–4.32 (1H, m, H-1), 3.44 (1H, s, OH), 3.21 (1H, dd, J = 14.8, 3.6 Hz, H-9a), 2.70 (1H, s, OH), 2.39–2.29 (2H, m, 2-methyl butanoyl H-2, OH), 2.24 (1H, dd, J = 14.8, 3.9 Hz, H-9b), 2.10 (3H, s, acetyl CH₃), 1.96–1.94 (3H, m, H-15), 1.93 (3H, s, acetyl CH₃), 1.75–1.64 (1H, m, 2-methyl butanoyl H-3a), 1.50 (3H, s, H-13), 1.46–1.41 (1H, m, 2-methyl butanoyl H-3b), 1.40 (3H, s, H-14), 1.14 (3H, d, J = 7.0 Hz, 2-methyl butanoyl 2-CH₃), 0.91 (3H, t, J = 7.5 Hz, 2-methyl butanoyl H-4); ¹³C NMR (151 MHz, CDCl₃) δ 175.5 (2-methyl butanoyl C=O), 175.1 (C-12), 172.9 (acetyl C=O), 170.3 (acetyl C=O), 144.8 (C-5), 126.6 (C-4), 84.9 (C-10), 84.6 (C-2), 83.7 (C-3), 79.0 (C-7/C-11), 78.7 (C-7/C-11), 77.1 (C-6), 66.4 (C-8), 55.6 (C-1), 41.6 (2-methyl butanoyl C-2), 38.5 (C-9), 26.3 (2-methyl butanoyl C-3), 23.6 (C-14), 22.6 (acetyl CH₃), 21.1 (acetyl CH₃), 16.5 (C-13), 16.3 (2-methyl butanoyl 2-CH₃), 13.1 (C-15), 11.8 (2-methyl butanoyl C-4); HRMS m/z 521.1996 [M + Na]⁺, calcd for C₂₄H₃₄O₁₁Na 521.1993.

2-Acetoxytrilobolide (4). 9 2,4,6-Trichlorobenzoyl chloride (9.4 μ L, 0.06 mmol) and TEA (8.4 μ L, 0.06 mmol) were added successively to a solution of angelic acid (6 mg, 0.06 mmol) in dry toluene (100 μ L) at room temperature under an argon atmosphere. The resulting mixture was stirred for 2 h and then treated with alcohol **24S** (15 mg, 0.03 mmol). The reaction mixture was stirred at 75 °C for 2 days, then cooled to room temperature and quenched by the addition of a saturated aqueous NH₄Cl solution (3 mL). The aqueous phase was extracted with EtOAc (2 $\times$ 5 mL), and the combined organic phases were dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by dry vacuum column chromatography on silica gel using toluene–EtOAc (3:1) as eluent to lead to the desired natural product **4** (8.5 mg, 49%) as a colorless oil: ¹H NMR (600 MHz, CD₃OD) δ 6.19 (1H, qq, J = 7.2, 1.5 Hz, angeoyl H-3), 5.72–5.69 (2H, m, H-3, H-6), 5.62 (1H, t, J = 3.7 Hz, H-8), 5.51 (1H, dd, J = 4.1, 2.9 Hz, H-2), 4.40–4.36 (1H, m, H-1), 3.01 (1H, dd, J = 14.6, 3.7 Hz, H-9a), 2.36 (1H, q, J = 6.9 Hz, 2-methyl butanoyl H-2), 2.31 (1H, dd, J = 14.6, 3.9 Hz, H-9a), 2.09 (3H, s, acetyl CH₃), 2.01 (3H, dq, J = 7.2, 1.5 Hz, angeoyl H-4), 1.94 (3H, p, J = 1.5 Hz, angeoyl 2-CH₃), 1.91 (3H, s, acetyl CH₃), 1.88–1.86 (3H, m, H-15), 1.76–1.71 (1H, m, 2-methyl butanoyl H-3a), 1.52–1.46 (1H, m, 2-methyl butanoyl H-3b), 1.45 (3H, s, H-14), 1.38 (3H, s, H-13), 1.17 (3H, d, J = 7.1 Hz, 2-methyl butanoyl 2-CH₃), 0.95 (3H, t, J = 7.5 Hz, 2-methyl butanoyl H-4); ¹³C NMR (151 MHz, CD₃OD) δ 178.1 (C-12), 176.2 (2-methyl butanoyl C=O), 171.9 (acetyl C=O), 171.7 (acetyl C=O), 168.7 (angeoyl C=O), 141.0 (C-5), 139.4 (angeoyl C-3), 133.3 (C-4), 128.8 (angeoyl C-2), 85.9 (C-10), 85.6 (C-3), 79.6 (C-2), 79.5 (C-11), 79.4 (C-7), 78.0 (C-6), 67.4 (C-8), 58.9 (C-1), 42.7 (2-methyl butanoyl C-2), 39.5 (C-9), 27.3 (2-methyl butanoyl C-3), 23.5 (C-14), 22.6 (acetyl CH₃), 21.1 (acetyl CH₃), 20.7 (angeoyl 2-CH₃), 16.7 (2-methyl butanoyl 2-CH₃), 16.0 (angeoyl C-4), 15.9 (C-13), 13.0 (C-15), 12.0 (2-methyl butanoyl C-4); HRMS m/z 603.2394 [M + Na]⁺ calcd for C₂₉H₄₀O₁₂Na 603.2412.

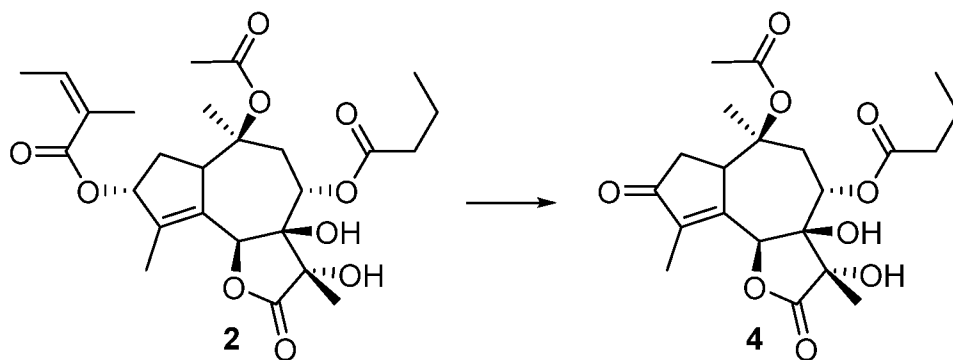
Experimental details regarding Schemes 14-17

Materials and Methods

A crude extract containing nortrilobolide (**2**) was received from GenSpera (San Antonio, TX, USA) and purified by dry column vacuum chromatography on silica gel using CH₂Cl₂–EtOAc (5:1) as eluent (*R_f* = 0.23 in CH₂Cl₂–EtOAc, 5:1) prior to use. Solvents and reagents were obtained from commercial suppliers and used without further purifications. Syringes which were used to transfer anhydrous solvents or reagents were purged with nitrogen prior to use. Other solvents were analytical or HPLC grade and were used as received. Yields refer to isolated compounds estimated to be > 95 % pure as determined by ¹H NMR (25 °C). Thin-layer chromatography (TLC) was carried out on silica gel 60 F₂₅₄ plates. Visualization was accomplished by UV lamp (254 nm).

Flash column chromatography was performed on chromatography grade, silica 60Å particle size 35–70 micron using the solvent system as stated. Dry column vacuum chromatography was carried out with silica gel (20–45 µm). Reactions were followed by TLC and visualized using vanillin reagent (15 g of vanillin, 250 mL of ethanol, and 2.5 mL of concentrated sulfuric acid). ¹H spectra were recorded on Bruker (400 and 600 MHz instruments) using CDCl₃ as deuterated solvent and with the residual solvent as the internal reference. For all NMR experiences the deuterated solvent signal was used as the internal lock. Coupling constants (*J* values) are given in Hertz (Hz). Multiplicities of ¹H NMR signals are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dq, doublet of quartets; qq, quartet of quartets; m: multiplet; br, broad signal. Microwave-assisted (MW) synthesis was carried out in a Biotage Initiator apparatus operating in single mode; the microwave cavity producing controlled irradiation at 2.45 GHz. The reactions were run in sealed vessels. These experiments were performed by employing magnetic stirring and a fixed hold time using variable power to reach the desired temperature (during 1-2 min) and then maintained at the desired temperature in the vessel for the programmed time period. The temperature was monitored by an IR sensor focused on a point on the reactor vial glass. The IR sensor was calibrated to internal solution reaction temperature by the manufacturer. Zn(BH₄)₂ was prepared according to known procedure.¹ The following abbreviations are used: MW: microwave; EtOAc: ethyl acetate; EDTA: ethylenediaminetetraacetic acid; TEA: triethylamine.

¹ Andrews, S. P.; Ball, M.; Wierschem, F.; Cleator, E.; Oliver, S.; Hoegenauer, K.; Simic, O.; Antonello, A.; Huenger, U.; Smith, M. D.; Ley, S. V. *Chem. – Eur. J.* **2007**, *13*, 5688–5712.

Ketone 4²

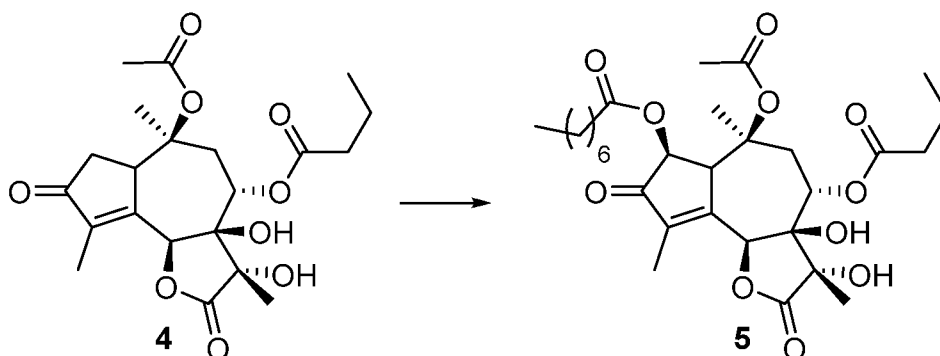
To a MW vial containing a solution of nortrilobolide (**2**) (1.05 g, 2.07 mmol) in acetonitrile (12 mL) was successively added a 1 M aqueous solution of hydrogen fluoride (4.14 mL, 4.14 mmol) and chromium(VI) oxide (290 mg, 2.89 mmol) at room temperature. The MW vial was sealed and heated under MW irradiations for 2 h at 95 °C. After cooling to room temperature, the reaction mixture was diluted with water (70 mL) and extracted with EtOAc (60 mL). The organic layer was successively washed with water, a 2 M aqueous solution of NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting off-white solid was purified by flash column chromatography on silica gel using EtOAc–heptane (1:1) as eluent to afford ketone **4** as a white solid (651 mg, 74 %). *R*_f = 0.30 (EtOAc–heptane, 1:1); ¹H NMR (400 MHz) δ 5.81 (s, 1H), 5.71 (t, *J* = 3.7 Hz, 1H), 4.76 (br s, 1H), 4.13 (s, 1H), 3.32 (dd, *J* = 14.8 and 3.7 Hz, 1H), 3.13 (s, 1H), 2.43 (dd, *J* = 19.5 and 6.3 Hz, 1H), 2.34 (dd, *J* = 19.1 and 2.8 Hz, 1H), 2.27 (t, *J* = 7.5 Hz, 2H), 2.09 (dd, *J* = 14.7 and 3.8 Hz, 1H), 1.98 (s, 3H), 1.92 (dd, *J* = 2.2, 1.3 Hz), 1.54–1.69 (m, 2H), 1.50 (s, 3H), 1.20 (s, 3H), 0.93 (t, *J* = 7.4 Hz, 3H). The other analytical data perfectly matched those reported in the literature.³

α'-Acylated ketone 5³

² Doan, N. T. Q.; Crestey, F.; Olsen, C. E.; Christensen, S. B. *J. Nat. Prod.* **2015**, DOI: 10.1021/acs.jnatprod.5b00333.

³ Andersen, A.; Cornett, C.; Lauridsen, A.; Olsen, C. E.; Christensen, S. B. *Acta Chem. Scand.* **1994**, *48*, 340–346.

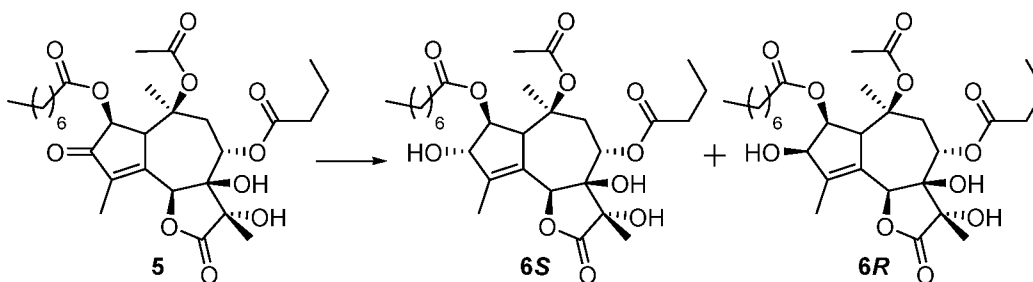
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To a solution of ketone **4** (424 mg, 1.0 mmol) in dry benzene–octanoic acid (90 mL, 5:1) at 60 °C was added manganese triacetate dihydrate (670 mg, 2.5 mmol). The resulting mixture was stirred under reflux conditions (120 °C) using a Dean-Stark apparatus for 7 h then cooled to room temperature. Volatiles were removed under reduced pressure then the crude material was taken up in EtOAc, washed successively with water, a 2 M aqueous solution of Na₂CO₃ and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting orange solid was purified by flash column chromatography on silica gel using EtOAc–heptane (1:1) as eluent to afford α' -acylated ketone **5** as an off-white solid (290 mg, 51 %). R_f = 0.54 (EtOAc–heptane, 1:1); ¹H NMR (600 MHz) δ 5.82 (s, 1H), 5.68 (t, J = 3.6 Hz, 1H), 5.20–5.23 (m, 1H), 4.53–4.57 (m, 1H), 3.70 (br s, 1H), 3.22 (dd, J = 15.0 and 3.6 Hz, 1H), 2.90 (br s, 1H), 2.22–2.38 (m, 5H), 1.99 (s, 3H), 1.93 (s, 3H), 1.57–1.66 (m, 4H), 1.47 (s, 3H), 1.38 (s, 3H), 1.23–1.36 (m, 8H), 0.94 (t, J = 7.8 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H). The other analytical data perfectly matched those reported in the literature.^{2,4}

A mixture (36 mg) of starting material **4** (4.5 %) and a 2-acetoxy ketone derivative³ (3.6 %) was recovered in a 1:0.8 ratio as determined by ¹H NMR.

20 Epimeric alcohol **6S**⁴ and **6R**⁴

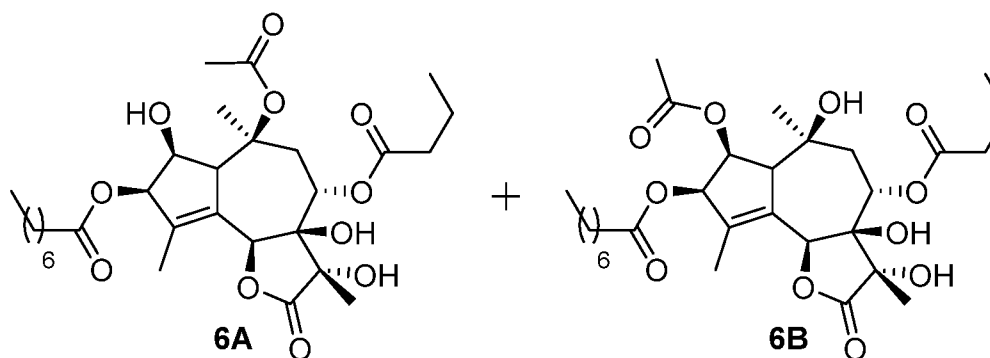
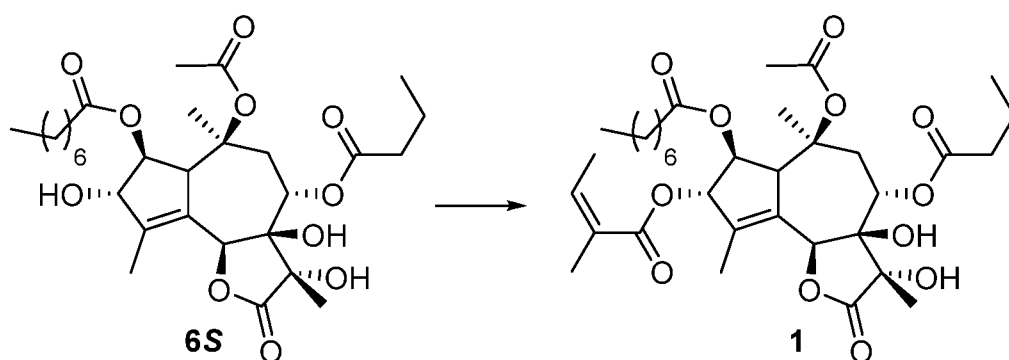


Procedure with diethyl ether as solvent. To a solution of α' -acylated ketone **5** (77 mg, 0.14 mmol) in dry Et₂O (5 mL) was added a cooled solution of Zn(BH₄)₂ (0.5 M in dry Et₂O, 3.3 mL, 1.63 mmol) dropwise at -20 °C under nitrogen atmosphere. The resulting mixture was stirred for 3.5 h at this temperature prior to the slow addition of cold water.

- 5 The reaction mixture was diluted with EtOAc and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The resulting crude material was purified by flash column chromatography on silica gel using EtOAc–heptane (1:1) as eluent to provide alcohol **6S** (67 mg, 87%) as a colorless gummy solid. R_f = 0.50 (EtOAc–heptane, 1:1); ¹H NMR (600 MHz) δ 5.62–5.67 (m, 2H), 4.89 (t, J = 3.8 Hz, 1H), 4.41 (br s, 1H), 4.36 (br s, 1H), 3.33 (br s, 1H), 3.24 (dd, J = 14.5 and 3.5 Hz, 1H), 2.90 (br s, 1H), 2.27–2.37 (m, 2H), 2.26 (t, J = 7.2 Hz, 2H), 2.18 (dd, J = 14.5 and 3.8 Hz, 1H) 1.93 (br s, 3H), 1.90 (s, 3H), 1.58–1.65 (m, 4H), 1.46 (s, 3H), 1.37 (s, 3H), 1.23–1.33 (m, 8H), 0.93 (t, J = 7.4 Hz, 3H), 0.85–0.89 (m, 3H). Other data perfectly matched those reported in the literature.^{2,4} Only trace amounts of **6R** was detected by TLC.
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- Procedure with THF as solvent. To a solution of α' -acylated ketone **5** (163 mg, 0.29 mmol) in dry THF (7 mL) was added a cooled solution of Zn(BH₄)₂ (0.5 M in dry Et₂O, 6.9 mL, 3.45 mmol) dropwise at -30 °C under nitrogen atmosphere. The resulting mixture was stirred at this temperature for 2 h prior to add an additional amount of Zn(BH₄)₂ (2.4 mL, 1.2 mmol). The resulting mixture was stirred at this temperature for 1.5 h prior to add an additional amount of Zn(BH₄)₂ (1.0 mL, 0.5 mmol) then the mixture was slowly allowed to warm up to 15 °C within 19.5 h, diluted with EtOAc and quenched by the slow addition of a 1 M aqueous solution of disodium dihydrate EDTA. After stirring vigorously for 2 h the aqueous layer was extracted twice with EtOAc then the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting off-white solid was purified by flash column chromatography on silica gel using a gradient elution (EtOAc–heptane, 1:1 to 2:1) to lead to a mixture of the two epimeric alcohols **6S** (colorless gummy solid, 39 mg, 24%) and **6R** (colorless gummy solid, 29 mg, 18%, R_f = 0.45 in EtOAc–heptane, 1:1) in a 4:3 ratio. In addition, two other by-products were obtained resulting from the single migration of the octanoyl moiety (compound **6A**, 19 mg, 12%, R_f = 0.40 in EtOAc–heptane, 1:1) and from the double migration of the octanoyl and the acetyl moieties (compound **6B**, 16 mg, 10%, R_f = 0.16 in EtOAc–heptane, 1:1) as depicted in the scheme below. See Andersen and co-workers, *Acta Chem. Scand.* **1994**, *48*, 340–346 for more details about those by-products.
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**Thapsigargin (1)⁴**

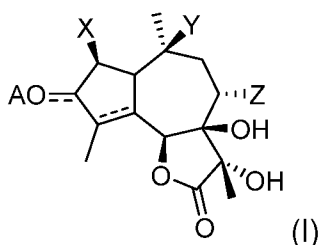
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To a solution of angelic acid (11.1 mg, 0.11 mmol) in dry toluene (100 mL) at room temperature under nitrogen were successively added benzoyl chloride (15.6 mg, 0.11 mmol) and TEA (15.4 mL, 0.11 mmol). The reaction mixture was heated at 40 °C for 2 h then a solution of alcohol **6S** (17.9 mg, 0.03 mmol) in dry toluene (300 mL) was added and the reaction mixture was heated at 90 °C for 72 h then cooled to room temperature. Volatiles were removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica gel using a gradient elution (EtOAc–heptane, 1:2 to 2:3) to lead to thapsigargin (**1**) as a white solid (13.4 mg, 65 %). R_f = 0.23 (EtOAc–heptane, 1:2); ^1H NMR (400 MHz) δ 6.10 (qq, J = 7.0 and 1.4 Hz, 1H), 5.68 (br s, 1H), 5.65 (br s, 1H), 5.62 (t, J = 3.6 Hz, 1H), 5.47–5.50 (m, 1H), 4.26–4.29 (m, 1H), 3.36 (br s, 1H), 3.02 (dd, J = 14.8 and 3.4 Hz, 1H), 2.77 (br s, 1H), 2.23–2.38 (m, 5H), 1.99 (dq, J = 7.0 and 1.4 Hz, 3H), 1.91–1.93 (m, 3H), 1.89 (s, 3H), 1.86 (s, 3H), 1.55–1.67 (m, 4H), 1.48 (s, 3H), 1.40 (s, 3H), 1.24–1.34 (m, 8H), 0.93 (t, J = 7.2 Hz, 3H), 0.84–0.89 (m, 3H). The other analytical data perfectly matched those reported in the literature. Starting material **6S** (2.1 mg, 12 %) was also recovered.

Claims

1. A compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein Formula (I) is

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- 10 wherein a dotted bond denotes an optional bond that may or may not be present and the dotted bond between the carbon and the oxygen represents an optional bond thus forming a carbonyl functional group and when the dotted bond is present, A is absent and,
- 15 wherein A is $-H$ or alkyl e.g. methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, *i*-pentyl, neopentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl or an acyl group such as formyl, acetyl, propionyl, *n*-butyryl or an α,β -unsaturated acyl group which may be further optionally substituted such as e.g. angeloyl, crotonoyl, acryloyl, 3,3-dimethylacryloyl, (*Z*) or (*E*)-2-methylbut-
- 20 2-eneoyl etc. or a *N*-Boc protected ω -aminoacyloxy like *N*-Boc-protected 12-aminododecanoyloxy, A may also be a trialkylsilyl group or a diarylalkyl group such as e.g. trimethylsilyl, triethylsilyl, *tert*-butyldimethylsilyl or *tert*-butyldiphenylsilyl;

wherein X, Y and Z are independently same or different and selected from:

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$-H$, $-O-R$, $-S-R$, $-NHR$, hal including I, Br, F;

wherein R is independently selected from; H, C_1-C_{18} alkyl or C_1-C_{18} substituted alkyl, benzyl, $-O-R$, $-C(=O)-R''$, $-SiR'_2R'''$;

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and wherein R, R', R'' and R''' are independently same or different and are selected from alkyl or substituted alkyl, aryl or substituted aryl;

where alkyl is any alkyl having C₁-C₁₈ straight or branched carbon skeleton which may
 5 be substituted by halo (fluoro, chloro, bromo or iodo), C₁₋₁₈ alkyl, C₃₋₆ cycloalkyl, hydroxy, C₁₋₆ alkoxy, cyano, amino, nitro, C₁₋₆ alkylamino, C₂₋₆ alkenylamino, di-C₁₋₆ alkylamino, C₁₋₆ acylamino, di-C₁₋₆ acylamino, C₁₋₆ aryl, C₁₋₆ arylamino, C₁₋₆ aroylamino, benzylamino, C₁₋₆ arylamido, carboxy, C₁₋₆ alkoxycarbonyl or (C₁₋₆ aryl)(C₁₋₁₀
 alkoxy)carbonyl, carbamoyl, mono-C₁₋₆ carbamoyl, di-C₁₋₆ carbamoyl or any of the
 10 above in which a hydrocarbyl moiety is itself substituted by halo, cyano, hydroxy, C₁₋₂ alkoxy, amino, nitro, carbamoyl, carboxy or C₁₋₂ alkoxycarbonyl, or any straight or branched C₁-C₁₈ alkyl having one or more double and/or triple bonds such as e.g. 5-octenyl, 3-butenyl, 7-dodecenyl, etc. and wherein aryl is e.g. phenyl, substituted phenyl, benzyl, substituted benzyl, etc., or alkyl substituted with an amine protected as
 15 carbamate such as Boc, benzoyloxycarbonyl or other carbamates,

and wherein e.g. the -O-C(=O)R'' group is e.g. formyl, acetyl, propionyl, *n*-butyryl or an α,β -unsaturated acyl group which may be further optionally substituted such as e.g. angeloyl, crotonoyl, acryloyl, 3,3-dimethylacryloyl, (*Z*) or (*E*)-2-methylbut-2-enoyl, 2-
 20 methylbutanoyl, etc.

2. A compound according to claim 1, wherein alkyl is e.g. methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *sec*-butyl, *i*-butyl, *tert*-butyl, *n*-pentyl, *i*-pentyl, neopentyl, 2,2,2-trimethylethyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, 11-
 25 amino-*n*-undecyl, *N*-Boc protected ω -aminoacyloxy like *N*-Boc-protected 11-aminododecanoyl, (*S*)-1-methylpropyl, (*R*)-1-methylpropyl, etc.

3. A compound according to any of the preceding claims, wherein A is -H and there is no double bond on the oxygen atom connected to A.

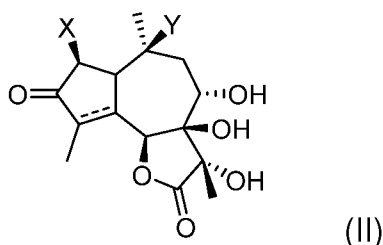
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4. A compound according to any of the preceding claims wherein Z is formyloxy, acetyloxy, propionyloxy, *n*-butanoyloxy or an α,β -unsaturated acyl group which may be further optionally substituted such as e.g. angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-enoyloxy, 2-methylbutanoyloxy, etc. or a
 35 *N*-Boc protected ω -aminoacyloxy like *N*-Boc-protected 12-aminododecanoyloxy, or a

trialkylsilyl group or a diarylalkyl group such as e.g. trimethylsilyloxy, triethylsilyloxy, tert-butyldimethylsilyloxy or tert-butyldiphenylsilyloxy.

5 5. A compound according to any of the preceding claims, wherein A is $-H$ and there is no double bond on the oxygen atom connected to A, and wherein Z is butanoyloxy or 2-methylbutanoyloxy, X is $-H$, $-OAc$, $-OHex$, $-OOct$ or Hal , and Y is $-OAc$.

6. A compound according to claim 1 or 2, wherein the dotted bond is present to form a carbonyl functionality and wherein Z is $-OH$ to constitute Formula (II) or a pharmaceu-
10 tically acceptable salt thereof, wherein consequently Formula (II) is



15 wherein X and Y are independently the same or different and selected from;

$-O-R$, $-S-R$, $-NHR$, $-H$, hal including I, Br, F;

wherein R is selected from; H, alkyl or substituted alkyl (C_1-C_{18}), benzyl, $-C(=O)-R''$, $-SiR'_2R'''$;
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and wherein R, R', R'' and R''' are independently same or different and are selected from alkyl or substituted alkyl,

25 -wherein alkyl is any alkyl having C_1-C_{18} straight or branched carbon skeleton which may be substituted by halo (fluoro, chloro, bromo or iodo), C_{1-18} alkyl, C_{3-6} cycloalkyl, hydroxy, C_{1-6} alkoxy, cyano, amino, nitro, C_{1-6} alkylamino, C_{2-6} alkenylamino, di- C_{1-6} alkylamino, C_{1-6} acylamino, di- C_{1-6} acylamino, C_{1-6} aryl, C_{1-6} arylamino, C_{1-6} aroylamino, benzylamino, C_{1-6} arylamino, carboxy, C_{1-6} alkoxycarbonyl or $(C_{1-6} aryl)(C_{1-10}$
30 alkoxy)carbonyl, carbamoyl, mono- C_{1-6} carbamoyl, di- C_{1-6} carbamoyl or any of the above in which a hydrocarbonyl moiety is itself substituted by halo, cyano, hydroxy, C_{1-2} alkoxy, amino, nitro, carbamoyl, carboxy or C_{1-2} alkoxycarbonyl.

7. A compound according to any of claims 1-2, 6, wherein A is absent and wherein the optional double bond is present to form a carbonyl functional group and wherein Z is *n*-butanoyloxy or 2-methylbutanoyloxy, X is -H, -OAc, -OHex, -OOct or Hal, and Y is -OAc.

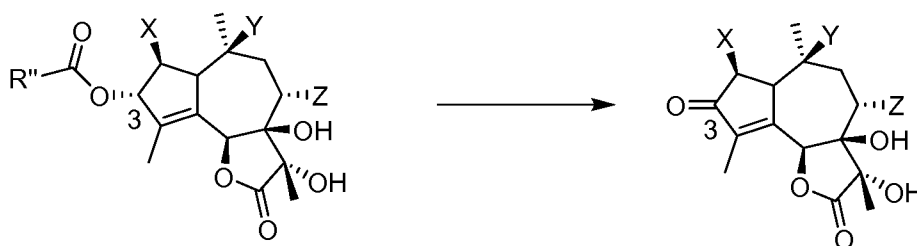
8. A compound according to any of claims 1-2, 6-7, wherein A is absent and wherein the optional double bond is present to form a carbonyl functional group and wherein Z is *n*-butanoyloxy and wherein X is -H or acetoxy and Y is acetoxy.

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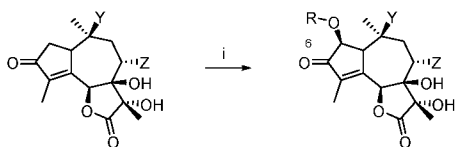
9. A compound according to any of claims 1, 2, 6-8, wherein X and Y are -OAc.

10. A method for preparing a compound according to any of claims 1-5, the method comprises

15 i) cleavage of the ester group at position 3 followed by oxidation :

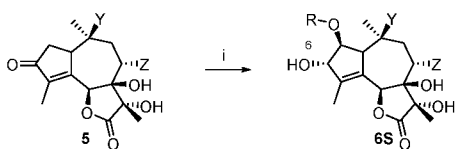


ii) stereoselective introduction of $-O(C=O)R''$ on the on the C-2 position of the ketone intermediate:



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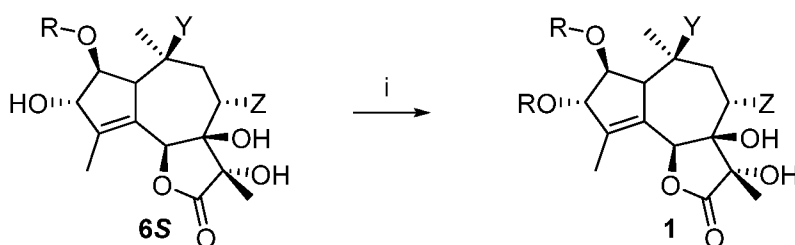
iii) stereoselective reductions of the obtained ketone to give the α -alcohol:



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iv) esterification of alcohol 6S:

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wherein X, Y, Z and R are as defined herein before, and –OR in position 2 or 3 is the
 5 same or different and –OR in position 3 may be angelate moiety.

11. A method according to claim 10, wherein the oxidation in step i) is performed using chromium (VI) oxide.

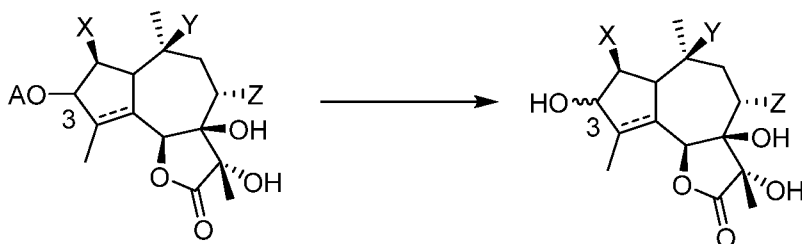
10 12. A method according to claim 10 or 11, wherein the stereoselective introduction of –OR on the C-2 position ii) is performed involving: oxidation with heavy metals, hypiodite catalyzed α -oxidation, sigmatropic rearrangement acyloxyenamines or using manganese(III)-OR.

15 13. A method according to any of claims 10-12, wherein the stereoselective reduction iii) is performed using zinc borohydride as reducing agent.

14. A method according to any of claims 10-13, wherein esterification of the –OH group at position C-3 is performed using benzoyl chloride.

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15. A method of preparing a compound according to claim 3, wherein the method comprises a reaction step wherein the oxygen functionality in the 3-position is reacted to produce an alcohol functionality by a substitution reaction and where the reaction is



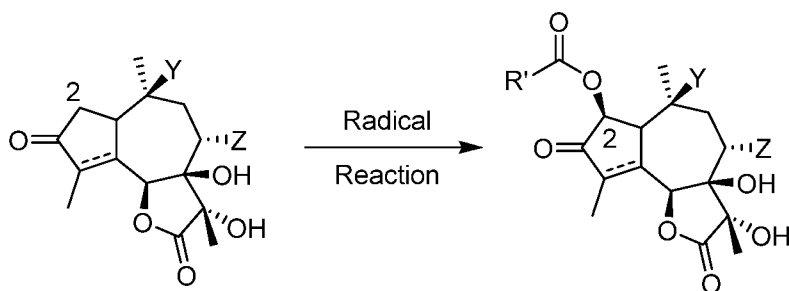
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16. A method according to claim 15, wherein A is e.g. formyl, acetyl, propionyl or an α,β -unsaturated acyl group e.g. angeloyl, crotonoyl, acryloyl, 3,3-dimethylacryloyl, (*Z*) or (*E*)-2-methylbut-2-enoyl, etc and wherein Z is an O-Acyl, i.e. $-\text{OC}(=\text{O})\text{R}'$ group such as e.g. formyloxy, acetyloxy, propionyloxy n-butanoyloxy or an α,β -unsaturated acyl group which may be further optionally substituted such as angeloyloxy, crotonoyloxy, acryloyloxy, 3,3-dimethylacryloyloxy, (*Z*) or (*E*)-2-methylbut-2-enoyloxy, 2-methylbutanoyloxy etc. or a *N*-Boc protected ω -aminoacyloxy like *N*-Boc-protected 12-aminododecanoyloxy, or a trialkylsilyl group or a diarylalkyl group such as trimethylsilyloxy, triethylsilyloxy, *tert*-butyldimethylsilyloxy or *tert*-butyldiphenylsilyloxy, or e a trialkylsilyl group or a diarylalkylsilyl group such as trimethylsilyl, triethylsilyl, *tert*-butyldimethylsilyl or *tert*-butyldiphenylsilyl.

17. A method according to any of claims 15-16, wherein X is $-\text{H}$ and Y is $-\text{OAc}$.

18. A method according to any of claims 5-17, wherein the reaction is accomplished under acidic conditions e.g. *p*-TsOH, sulfuric acid, methanesulfonic acid, triflic acid, hydrogen fluoride, hydrogen chloride, trifluoroacetic acid, etc. in an aqueous solution with water and acetonitrile dichloromethane, trichloromethane, dimethylsulfoxide, tetrahydrofuran, 1,4-dioxane, pyrane, ethyl acetate, acetone, benzene, toluene, hexane, *N,N*-dimethylformamide, etc. at about 60°C, about 40°C, about 50°C, about 70°C, about 80°C or about 90°C or reflux of the solvent.

19. A method of preparing a compound according to any of claims 1-2, 6-9, wherein X is an acyloxy group as defined in claim 1 and wherein the method comprises a reaction step where by oxygenation of the 2-position of Formula (I) is accomplished stereoselectively and proceeds by a radical reaction and the reaction is



20. A method according to claim 19, wherein Z is an –OAcyl group e.g. butanoyloxy and R' is an alkyl group including alkyl group such as e.g. methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *sec*-butyl, *i*-butyl, 2,2,2-trimethylethyl, *n*-pentyl, neopentyl, *i*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, amino-*n*-undecyl, 11-amino-*n*-undecyl, *n*-dodecyl, (*S*)-2-methyl-butyl, (*R*)-1-methyl-butyl, 2-methylbutanoyloxy etc.

21. A method according to any of claims 19-20, wherein the reaction is accomplished by using a salt of manganese(III) with any carboxylic acid e.g. octanoic acid, hexanoic acid or acetic acid and wherein the solvent used in the reaction is e.g. benzene, toluene, dichloromethane, tetrahydrofuran, chloroform, dimethylsulfoxide, *N,N*-dimethylformamide, hexane, 1,4-dioxane, diethyl ether, acetonitrile, ethyl acetate or acetone.

22. Use of a compound according to any of claims 1-9, for the preparation of a thapsigargin analogue.

23. Use according to claim 22, wherein the compounds are one or more of the compounds according to claims 4, 5, 7-9, respectively.

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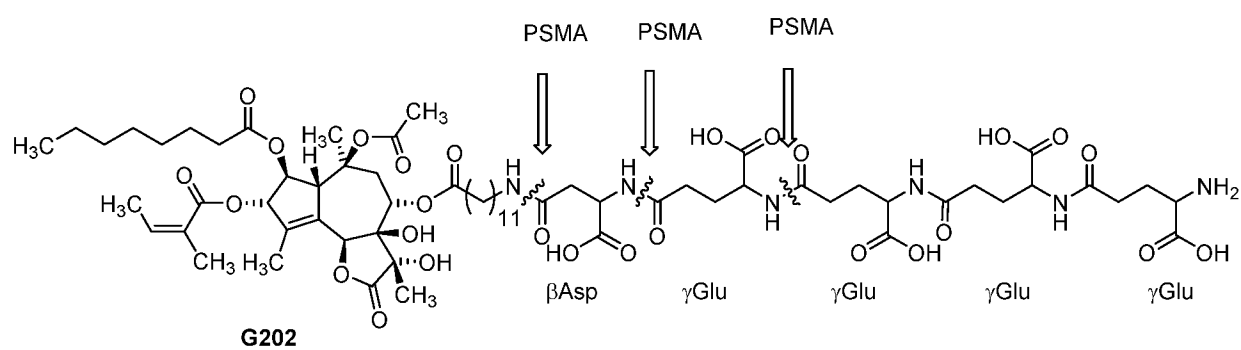


Fig. 1

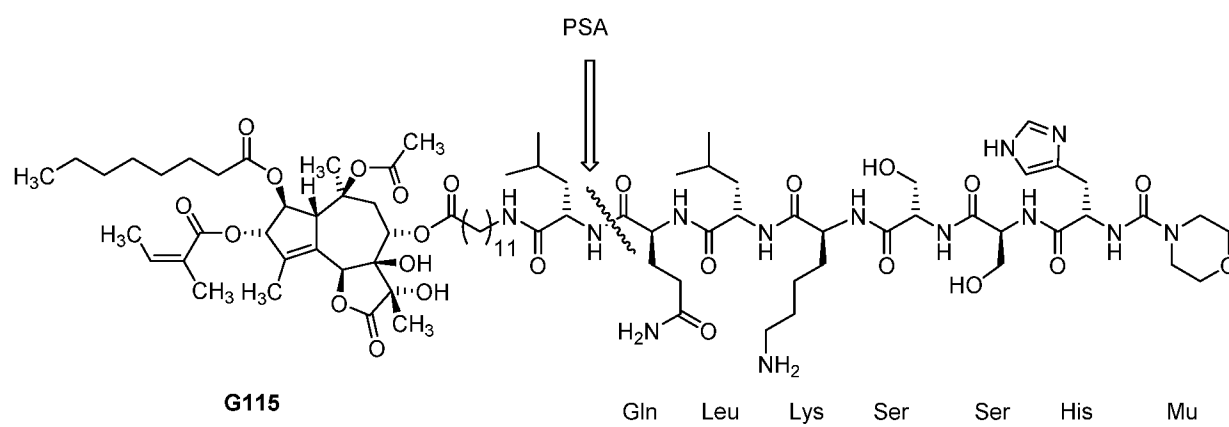


Fig. 2

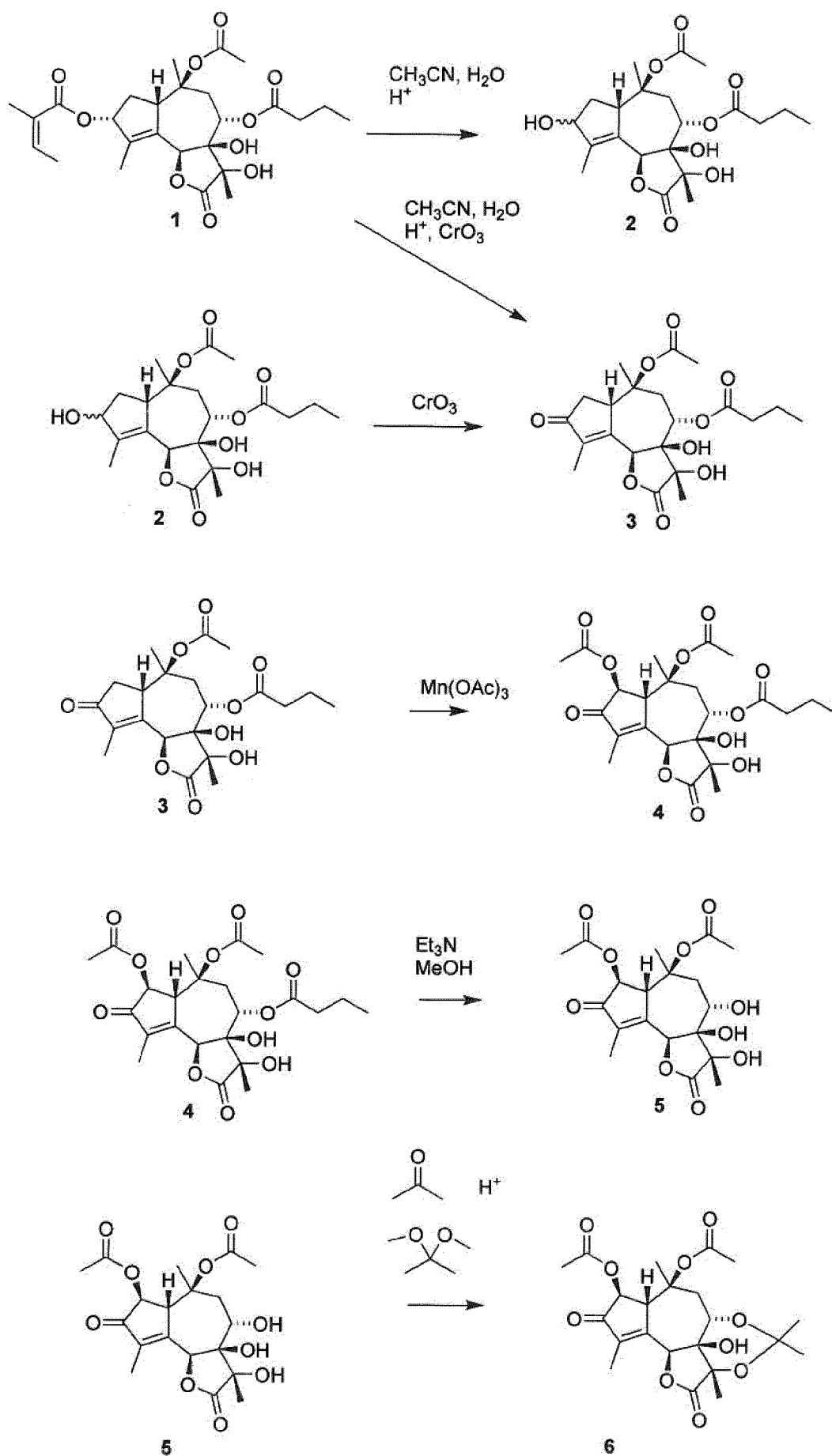


Fig. 3a

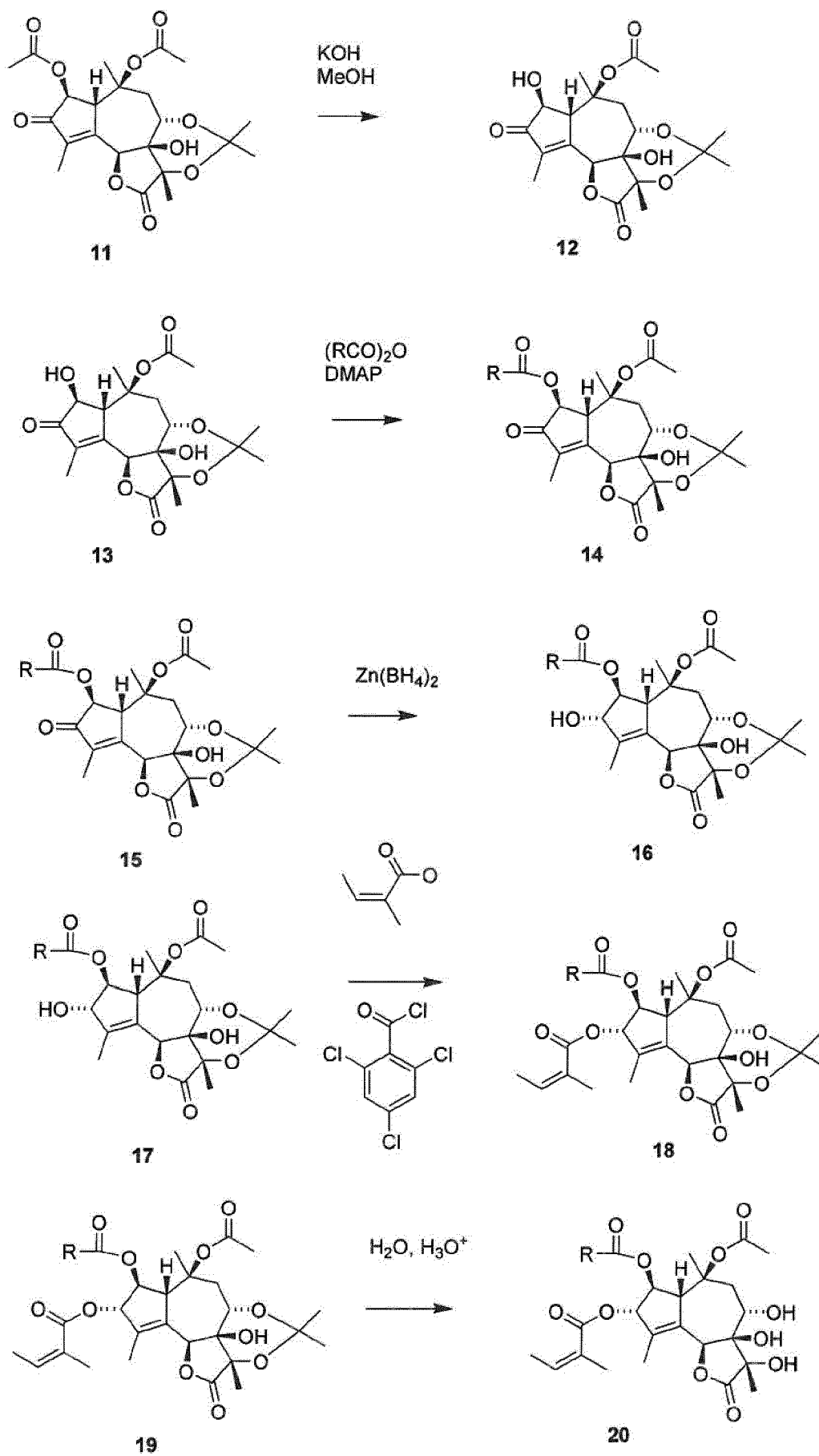


Fig. 3b

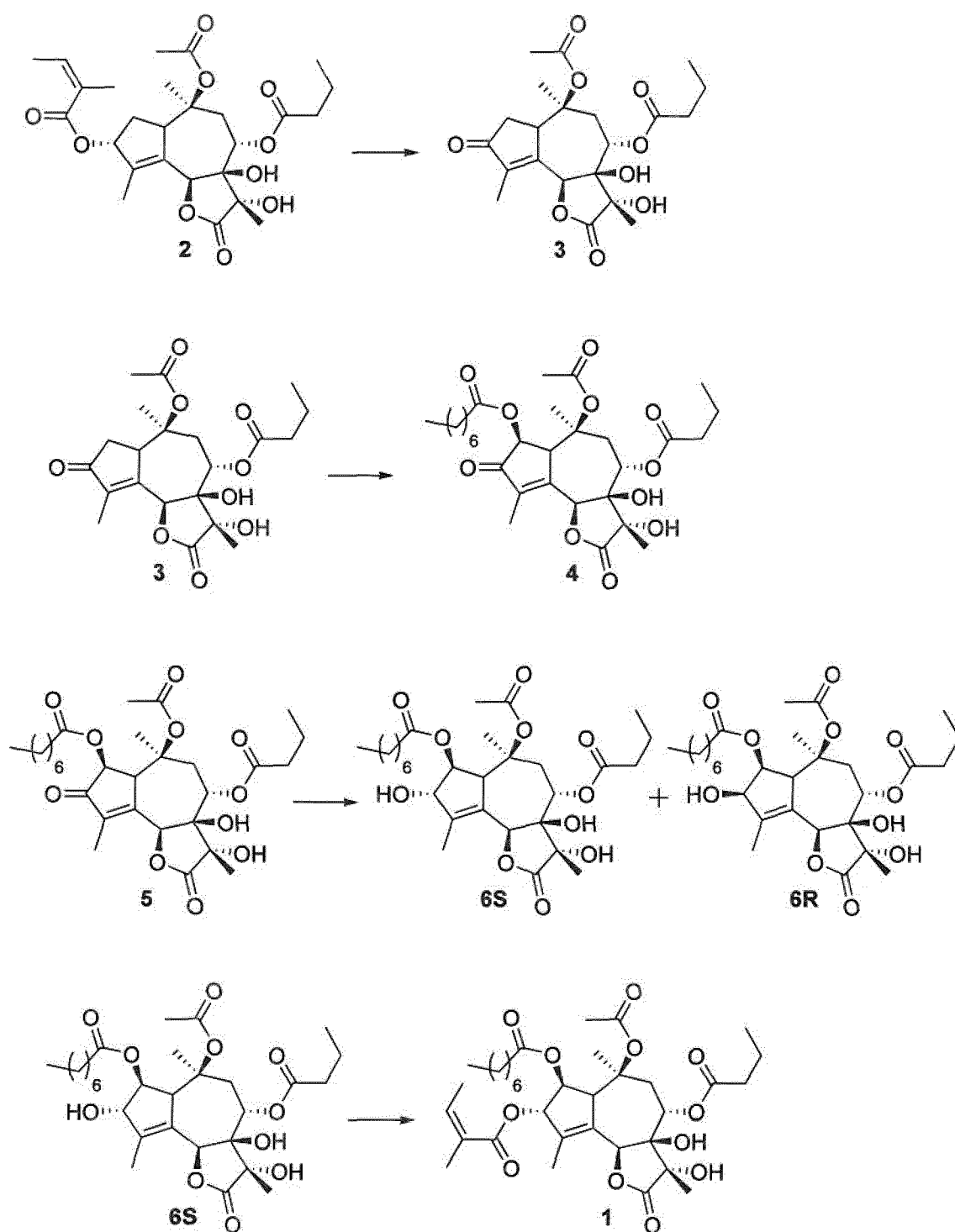


Fig 4