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Schizophrenic behavior of 2,3-oxidosqualene sterol cyclase from pig liver towards 2,3-oxidosqualene analogs

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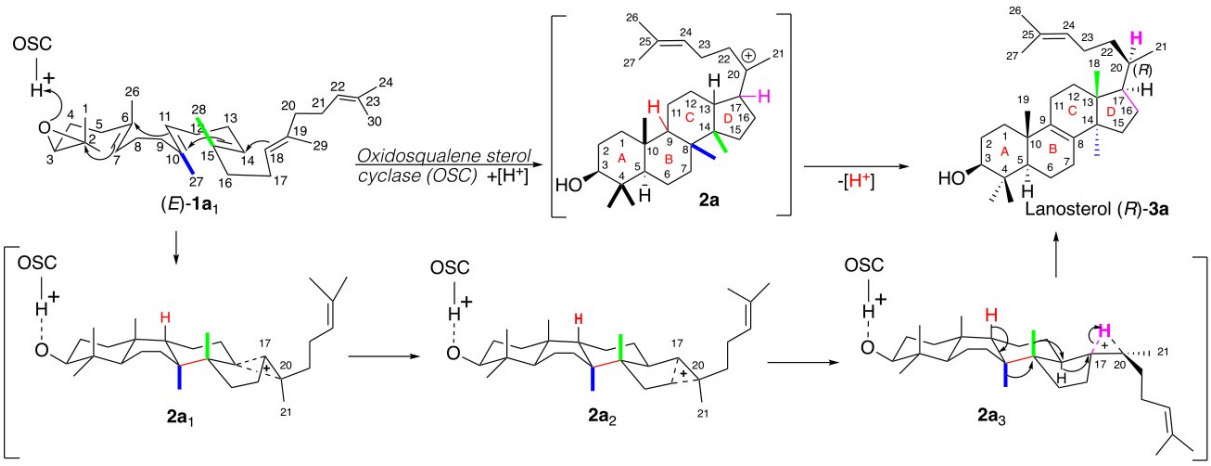
Abstract

We report the unusual behavior of oxidosqualene sterol cyclase from pig liver towards 2,3-oxidosqualene analogs bearing two alkyl groups different from a methyl, at their $\Delta^{18\text{-}19}$ double bond: unambiguous structure determinations of the products and tentative rational for their formation are described.

Keywords: Biosynthesis, multistep hemi-synthesis of natural product analogs, performance liquid chromatography, structure determination

INTRODUCTION

The transformation of linear squalene to (20*R*)-lanosterol (*R*)-**3a** possessing four cycles and seven chiral centers, by membrane-bound oxidosqualene sterol cyclase (OSC; lanosterol synthetase), has been the subject of constant interest for more than 70 years[1-5]. Woodward and Bloch[6] first proposed that lanosterol is an intermediate in the transformation of squalene to cholesterol, and soon after Stork[7,8] and the Zurich School[9,10] independently proposed a detailed mechanism for the construction of the steroid scaffold [Scheme 1].



Scheme 1. Description of the Zurich School mechanism from all (*E*)2,3-oxidosqualenes to lanosterol.

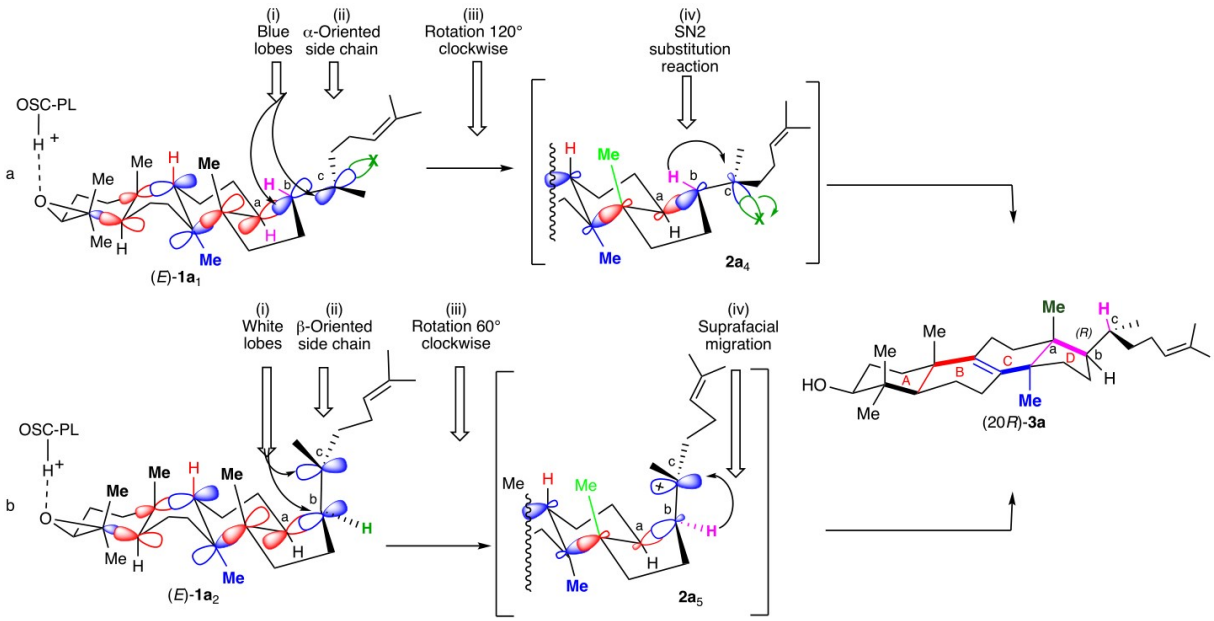
The original description of this mechanism [Scheme 1], adapted to the discovery by Corey *et al.*[11] and Van Tamelen *et al.*[12] that 2,3-oxidosqualene (*E*)-**1a** is intermediate in this transformation, involves: (i) a polycyclization, implying its $\Delta^{6\text{-}7}$, $\Delta^{9\text{-}10}$, $\Delta^{13\text{-}14}$, and $\Delta^{18\text{-}19}$ double bonds, initiated by an acid catalyst part of the enzyme and prefolded by the enzyme in a chair-boat-chair-**boat** conformation; (ii) this leads to the formation of the elusive intermediate **2a** named "protosterol", possessing a 6,6,6,5-membered polycyclic scaffold (A/B/C/D) and bearing a tertiary carbocation at C-20 (steroid nomenclature) on the side chain lying in α-position; and (iii) a series of 1,2-shifts on the latter, ending by the loss of the (H_9) proton delivering lanosterol (*R*)-**3a** possessing a $\Delta^{8\text{-}9}$ double bond (steroid nomenclature, Scheme 1).

The proposed mechanism is based on two stereochemical assumptions supported by strong experimental evidence: (i) antiperiplanar addition across each C=C double bond; and (ii) suprafacial 1,2-shifts driving each migration (H_{17}, H_{13}, Me_{14}, and Me_8).

However, this proposal leads to a protosterol with an alpha-oriented side chain whose stereochemistry at C-20 formerly requires a 120° clockwise rotation around the C_{17}-C_{20} bond to initiate the series of migrations leading to the lanosterol possessing the natural (20*R*)-stereochemistry. This is sketched[9,10] in the original proposal by a series of non-classical ion rearrangements (**2a**$_1$ → **2a**$_2$ → **2a**$_3$, Scheme 1).

It was then rationalized by Cornforth[13], who hypothesized that the protosterol should resemble **2a**$_4$ bearing an exogeneous X group at C-20 on the side chain lying in α-position. It results from: (i) the folding of the polyene originally in a chair-boat-chair-boat conformation (*E*)-**1a**$_1$ as previously reported by the Zurich School (Scheme 2, entry a); (ii) a series of concerted anti-addition of the different [C=C] double bonds ending by the addition on the [C_b=C_c] double bond of [C_a] (from the *re* face) on [C_b] and an exogeneous X group at [C_c] (from the *re* face) that produces concomitantly the D ring; and (iii) a 120° clockwise rotation of the side chain, located in α-position, around the [C_b-C_c] single bond leading to **2a**$_4$, in which the C_b-X bond is aligned with the C_b-H bond in an antiperiplanar conformation that initiates the departure of the X group and the series of migrations leading to the (20*R*)-lanosterol (20*R*)-**3a** (Scheme 2, **entry a**).

The Corey proposal (Scheme 2, **entry b**)[14,15] avoids the 120° rotation implied in each of the previous mechanisms by involving the intermediate formation of a protosterol **2a**$_5$ resulting from a chair-boat-chair-**chair** folding of the 2,3-oxidopolyene (*E*)-**1a**$_2$, different from the above, and bearing its side chain in β-



Scheme 2. Cornforth (entry a) and Corey (entry b) mechanisms accounting for the (*R*)-stereochemistry of lanosterol **3a** at C-20.

position. It accordingly results from the attack of C_a by the *si* face of C_b (Scheme 2, **entry b**). A rotation of the chain of only 60° clockwise around the C_b-C_c allows the alignment of the C_b-H bond with the π-bond of the carbocation at C_c and initiates the series of migrations leading to the (20*R*)-lanosterol (20*R*)-**3a** (Scheme 2, **entry b**)[1-5,14,15].

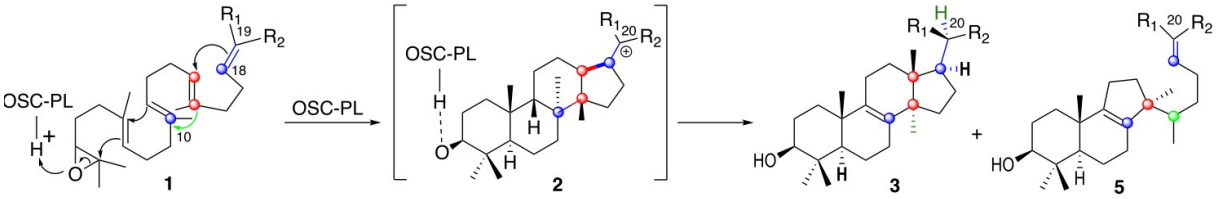
The latter mechanism, experimentally supported[14,15] by isolation of a stable protosterol analog in which the carbocation at C-20 is trapped by water from a suitable oxidosqualene analog, is now generally admitted[1-5].

Since the polycyclization process ends and the series of migration starts at the C-20 carbon, we expected to obtain precious information on the whole process by looking at what happens there. We therefore initiated, a long ago, a research program[16-18] aimed at gathering the behavior of oxidopolyenes analogs that differ from the original by the nature of the substituents at C-19 and the stereochemistry of their $\Delta^{18\text{-}19}$ double bond towards oxidosqualene sterol cyclases extracted from mammals (pig liver, OSC-PL) or yeast (*Saccharomyces cerevisiae*, OSC-SC).

RESULTS AND DISCUSSION

We report the first example of a transformation of an oxidosqualene analog by OSC-PL that leads to a mixture of lanosterol analog epimers at C-20 whose formation relates to carbocationic protosterols in which the side chain lies for the major one in the β-position, as disclosed in the Corey mechanism (Scheme 2, **entry b**), and for the minor one in the α-position, as originally proposed in the Zurich-Stork mechanism (detailed in part in Scheme 2, **entry a**). These results contrast with previous results from our laboratory involving related oxidosqualene analogs that exhibit complete stereocontrol.

We previously reported that oxidosqualene analogs (*E*)-**1b** and (*Z*)-**1b** possessing a methyl and an ethyl group at C-19 with the natural (*E*)- and the unnatural (*Z*)-stereochemistry at their $\Delta^{18\text{-}19}$ double bond are mainly cyclized by OSC-PL to lanosterol analogs **3b** that only differ from their stereochemistry at C-20, along with a few percent of the tricyclic compounds **5b** resulting from a partial polycyclization (Scheme 3, **compare entries a and b**)[16,17].



		R_1	R_2
a	(*E*)-**1b**	Et	Me
b	(*Z*)-**1b**	Me	Et
c	**1c**	Et	Et
d	**1d**	Pr	Pr
e	**1e**	CH_2-$(CH_2)_2$-CH_2	
f	**1f**	CH_2-$(CH_2)_3$-CH_2	

(*R*)-**3b**	74 %	**5b**$_E$	8 %
(*S*)-**3b**	46 %	**5b**$_Z$	12 %
3c	57 %	**5c**	3 %
3d	00 %	**5d**	00 %
3e	65 %	**5e**	3 %
3f	61 %	**5f**	7 %

Scheme 3. Results concerning the transformation of truncated 2,3-oxidosqualene analogs by OSC-PL.

However, the reaction of OSC-PL[19] with the 2,3-oxidosqualene analog (*Z*)-**1a** possessing the same hydrocarbon framework as the natural product (*E*)-**1a** but inverted stereochemistry at C-19 does not produce the 6.6.6.5 tetracyclic lanosterol epimer at C-20 but delivers the tricyclic 6.6.5 derivatives (*Z*)-**6a** and (*Z*)-**7a**. It results from partial polycyclizations that do not involve the participation of the $\Delta^{18\text{-}19}$ double bond [Scheme 4][18]. This is a behavior shared with the 18,19-dihydrosqualene 2,3-oxide missing the $\Delta^{18\text{-}19}$ double bond[20].

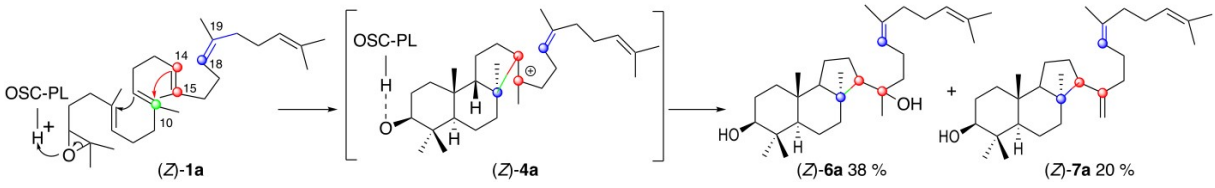
Since then, a few other oxidopolyenes have been reacted with OSC-PL, and it was observed that the amount of tetracyclic compounds over the tricyclic ones decreases dramatically by increasing the length of the side chain attached at C-19 in the $\Delta^{18\text{-}19}$ unnatural (*Z*)-series, whereas the reverse was found in the (*E*)-series[17,21].

We then became aware that changing the stereochemistry of the oxidopolyene has a dual impact on its interactions with the enzyme: It increases the interaction on the one side (*trans* to the hydrogen at C-18), but, at the same time, it decreases the interactions with the other side (*cis* to the hydrogen at C-18). We therefore decided to study the behavior, towards pig liver OSC-PL[19], of oxidosqualene analog **1** bearing at C-19 two alkyl groups different from a methyl group.

We now report that the 2,3-oxidosqualene analog **1c** bearing two ethyl at C-19 mainly produces the lanosterol analog **1c** bearing two ethyl groups at C-20 (Scheme 3, **entry c**), whereas its higher homolog **1d** bearing two propyl groups there is recovered unchanged under similar or even more drastic conditions (3 or 7 h reaction at 22 °C, Scheme 3, **entry d**), suggesting that it is not accepted by the enzyme. Interestingly, constraining the mobility of those two chains by incorporating them into a five-membered ring as in **1e** or a six-membered cycle as in **1f** is not deleterious for the formation of the lanosterol analogs **3e** and **3f** (Scheme 3, **entries e and f**).

Testing the behavior of 2,3-oxidosqualene analog **1g** bearing at C-19 an ethyl and a propyl group towards pig liver OSC-PL was obviously our next objective, hoping that at least one of the two stereoisomers at $\Delta^{18\text{-}19}$ would cyclize.

We laboriously prepared each of the two stereoisomers (*E*)-**1g** and (*Z*)-**1g** in pure form and unexpectedly found that each of them on incubation with pig liver OSC-PL delivers, in fair yields, lanosterol analog **3g**



Scheme 4. Results concerning the transformation of a stereoisomeric 2,3-oxidosqualene by OSC-PL.

possessing a propyl and an ethyl group at C-20 [Scheme 5]. Careful investigations and comparison of the biosynthetic compounds with authentic sample of (20*S*)-**3g** and (20*R*)-**3g** synthesized independently, as reported below, unambiguously shows that the oxidosqualene (*E*)-**1g** possessing a *E*-$\Delta^{18\text{-}19}$ C,C double bond produces the lanosterol analog (*R*)-**3g** possessing the (20*R*)-stereochemistry in 43% yield (Scheme 5, **entry a**), whereas its *Z*-$\Delta^{18\text{-}19}$ stereoisomer (*Z*)-**1g** delivers in 54% yield a 79/21 mixture of (*S*)-**3g** and (*R*)-**3g**, in which the former prevails (Scheme 5, **entry b**).

The results in Schemes 3-5 clearly show the importance of the length of the chains attached at C-19 on 2,3-oxidosqualene analogs towards OSC-PL. Comparing the results reported in Scheme 5 to those disclosed in Scheme 3, **entry d**, suggests the exceptional role of an "added carbon" in preventing the quite far removed oxido moiety from reaching the enzymic active site. Comparison of the behavior of **1g** to that of **1b** (Schemes 3-5, **entries a and b**) leads to a change in the status of the process from stereospecific to stereoselective.

The Corey model (Scheme 2, **entry b**)[14,15] fits well to rationalize the formation of (20*R*)-**3g** from the oxidosqualene analog (*E*)-**1g** (Scheme 6, **entry a**) as well as that of the major diastereoisomer of the sterol (20*S*)-**3g** obtained from the oxidosqualene analog (*Z*)-**1g**.

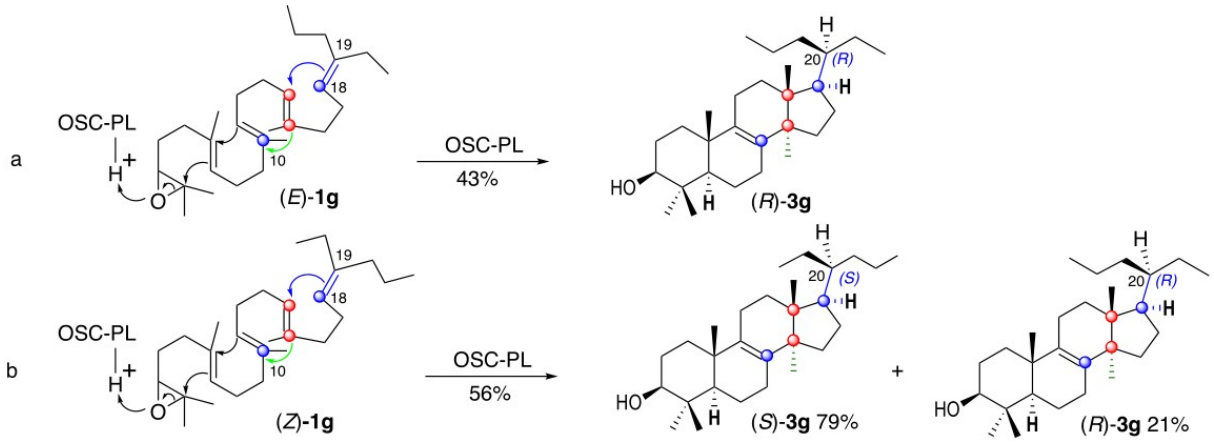
It is however less obvious to apply the Corey model to the formation of the minor isomer (20*R*)-**3g** from (*Z*)-**1g** (Scheme 6, **entry b**) since it requires an anticlockwise rotation of 120° of the side chain around the C_{17}-C_{20} bond before the series of Wagner-Meerwein backbone migrations takes place.

A related 120° rotation around the C_{17}-C_{20} bond in the model disclosed in Scheme 2, **entry a,** was previously rejected by Corey *et al.*[14] and is at the origin of its mechanistic model (Scheme 2, **entry b**)[14,15]. It is interesting to note that, although the Corey model could eventually be adapted, as discussed above, the Cornforth model[13] (Scheme 2, **entry a**) could not be applied to explain this experimental result.

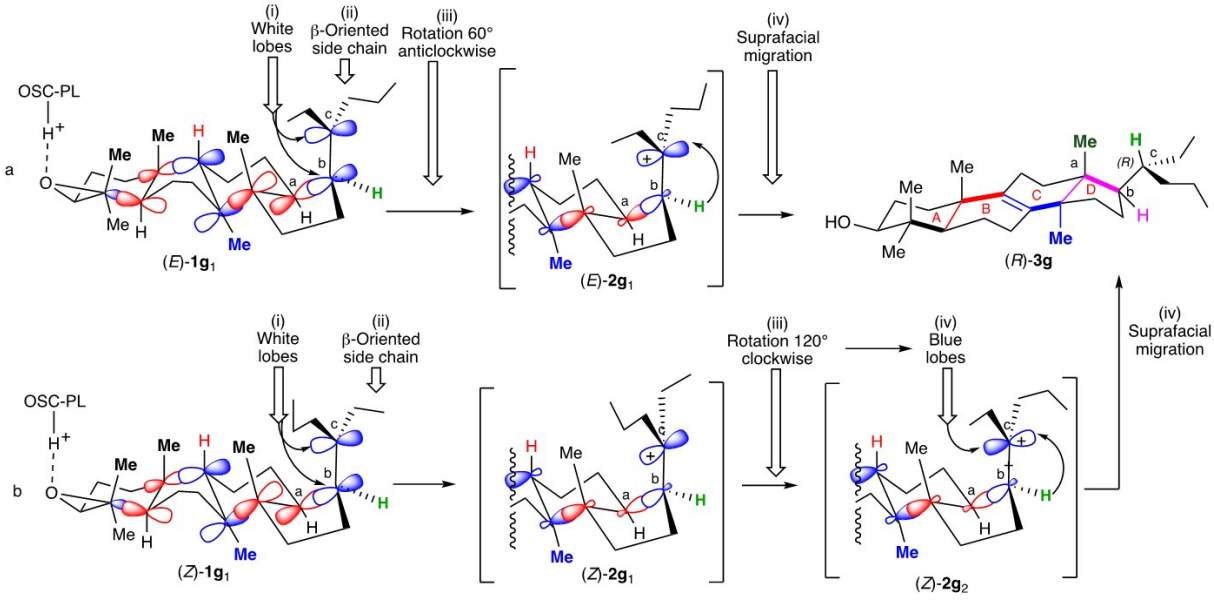
Could it be that the presence of the 3-isohexenyl group in such stereochemical arrangement favors, at least partly, a chair-boat-chair-**boat** conformation involving the (*Z*)-$\mathbf{1g}_2$ intermediate [Scheme 7] reminiscent of the original report of the Zurich School [Scheme 1][9,10] over the chair-boat-chair-**chair** conformation involving the (*Z*)-$\mathbf{1g}_1$ intermediate required by the Corey model [Scheme 6][14,15]?

In such case, the whole sequence of antiperiplanar additions on (*Z*)-$\mathbf{1g}_2$ would lead to the protosterol (*Z*)-$\mathbf{2g}_3$ that only requires a 60° clockwise rotation to initiate the series of migrations leading to (20*R*)-**3g** (Scheme 7; compare to Scheme 6, **entry b**).

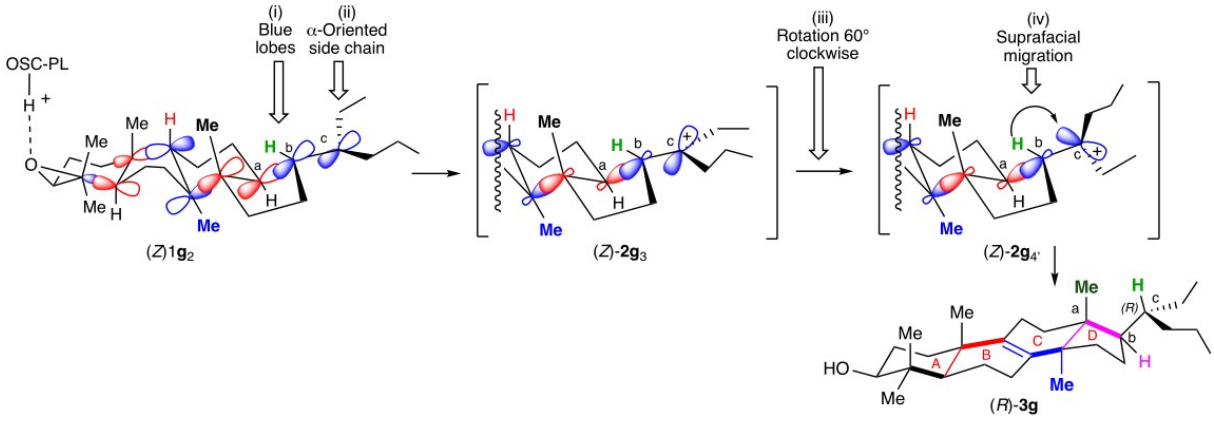
The series of results reported here seems to allow for some fine tuning regarding the interactions between the oxidosqualenes analog **1** and the enzymic environment of OSC-PL. We should refrain from the use of generalizations to deduce the intimate mechanism of lanosterol biosynthesis derived from specific



Scheme 5. OSC-PL behavior towards 2,3-oxidosqualene analogs bearing (*E*)- and (*Z*)-stereochemistry at $\Delta^{18\text{-}19}$.



Scheme 6. Rationalizing with the use of the Corey mechanism the formation of (*R*)-**3g** from (*E*)-**1g** and (*Z*)-**1g**.



Scheme 7. Rationalizing with the use of the adapted Zurich School mechanism the formation of (*R*)-**3g** from (*Z*)-**1g**.

experiments as it has been very often done.

Apparently, OSC-PL possesses an exceptional propensity to adapt its behavior to the substrate, a behavior it does not share for example with its distant ancestor cyclase, OSC-SC, which does not accept at all the $\Delta^{17\text{-}18}$ (Z)-analogs of 2,3-oxidosqualene tested[14,17].

EXPERIMENTAL

This study was carried out on C-14 radiolabeled racemic mixtures of 2,3-oxidopolyene **1** (epimeric at C-3) bearing either (*E*)- or (Z)-stereochemistry at $\Delta^{18\text{-}19}$ and therefore the yields were adjusted to the fact that only one enantiomer, the one possessing the (*S*)-stereochemistry at C-3, is biotransformed. The syntheses reported below and "classical" purifications provided at best samples of **1g** with a content of 10% of the other diastereoisomer. Isolation of each pure stereoisomer from each **1g** mixture (HPLC; Hypercarb 100 mm × 4.6 mm × 5 mm using a gradient from hexane 100% to hexane/MTBE 80%/20%) was carried out in partnership with the "Research Institute For Chromatography" (RIC, Kortrijk, Belgium) headed by Prof. Sandra with whom we have collaborated on many occasions[16,22]. RIC also carried out the comparisons of the products resulting from biosynthetic experiments on (*E*)-**1g** and (*Z*)-**1g** with those of authentic samples of (*S*)-**3g** and (*R*)-**3g** prepared as described below [(GC; Column HP-5MS (5% phenyl, 95% methyl polysiloxane), 30 m × 0.25 mm × 0.25 mm, 100 °C; 1 min then increase 10 °C/min until 320 °C, 15 min; (*S*)-**3g**: rt 33.23 min, (*R*)-**3g**: rt 33.53 min].

Synthesis of 2,3-oxidopolyenes

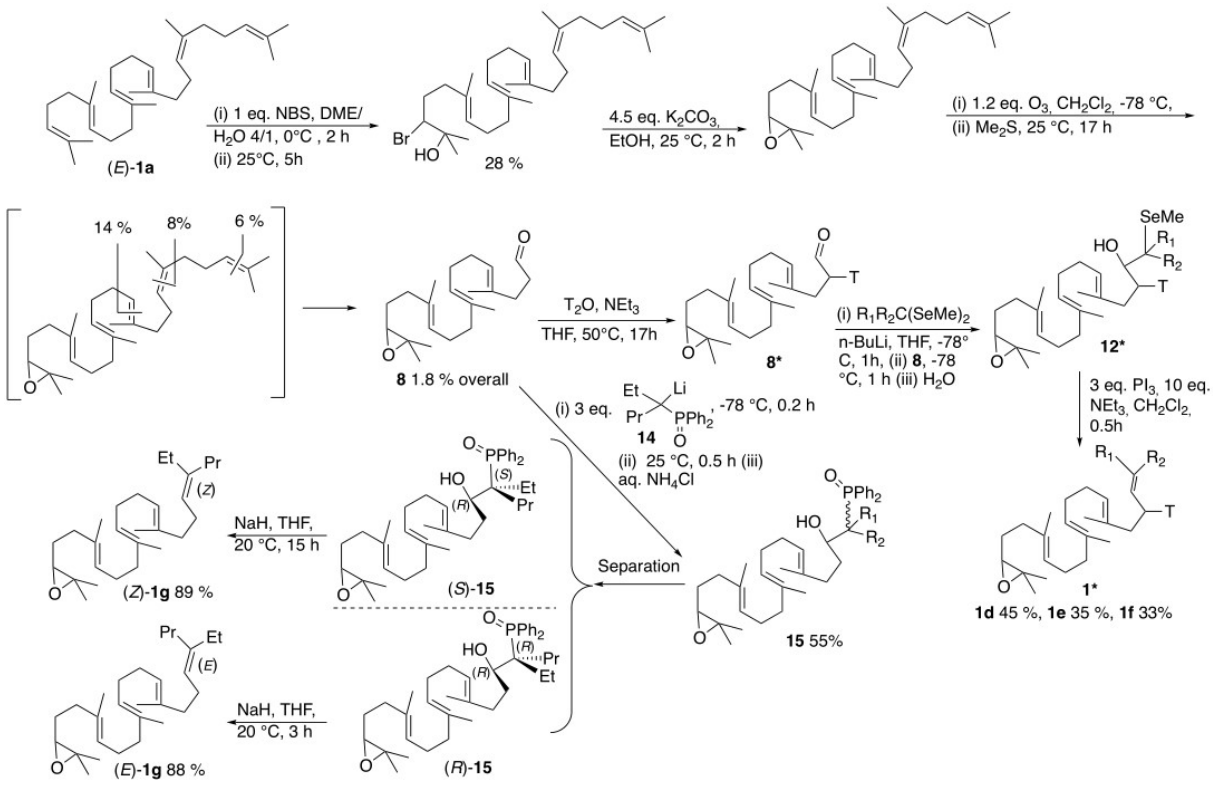
The synthesis of the 2,3-oxidopolyenes **1d-f** was achieved [Scheme 8] from the 2,3-oxidopolyenic aldehyde[17,23] **8** [Scheme 8] and methylselenoalkyllithium **11**[24] derived from 2-pentanone **9d**, cyclopentanone **9e**, and cyclohexanone **9f** [sequential reaction of selenoacetals **10** with: (i) 1 eq. *n*-BuLi, THF, -78 °C, 1 h; (ii) **8** in THF -78 °C, 1 h; and (iii) H_2O at -10 °C][24] followed by treatment of the resulting β-hydroxyalkylselenide **12** with phosphorus triiodide (3 eq. PI_3, 10 eq. NEt_3, CH_2Cl_2, 22 °C, 0.5 h) according to a protocol described in our laboratory[25] (**1d** 45%, **1e** 35%, and **1f** 33% yields over two steps). This choice was dictated since the Wittig reaction involving cyclohexylidene triphenylphosphorane provides extremely poor yield of **1f** (10% at best).

The 2,3-oxidopolyenic aldehyde **8** has been prepared (28% yield) from squalene and N-bromosuccinimide in H_2O/DME[23], followed by treatment with excess K_2CO_3 (4.5 eq.) in methanol and ozonolysis of the resulting epoxide (88% yield) with ozone [(i) 1.2 eq. CH_2Cl_2, (ii) excess Me_2S; yield in **8**: 8%].

The synthesis of **1g** was performed using Horner-Warren method[26,27] since we were unable to separate the related stereoisomeric mixture of β-hydroxyalkylselenide **12g** [Scheme 8]. Thus, the 2,3-oxidopolyenic aldehyde **8** was reacted with an excess of ylide **14** generated from 3-diphenylphosphinyloxyhexane **13** [(i) 3 eq. **13**, 6 eq. LDA, THF, 0 °C 0.2 h; (ii) -78 °C; (iii) 1eq. **8**, THF, -78 °C, 0.2 h; (iv) 20 °C, 0.5 h; and (v) aq. NH_4Cl], leading to a stereoisomeric mixture of β-hydroxyalkylphosphinyloxide **15** (55% yield), separated on a silica gel column (ether/pentane of 9/1, as two 90/10 mixtures after several subsequent separations). These were then transformed into (*E*)-**1g** and (*Z*)-**1g** (soiled with 10% each of the other stereoisomer) in good yields on reaction with NaH (4 eq., THF, 22 °C, yields: 88% and 89%, respectively). Each mixture (90/10) was further purified by HPLC, as disclosed above.

Synthesis of tritium radiolabeled 2,3-oxidopolyenes

Radiolabeled 2,3-oxidosqualene analogs **1d*** (967 dpm/nmol), **1e***(610 dpm/nmol), and **1f*** (547 dpm/nmol) were synthesized from the α-tritiated aldehyde **8*** using the protocol described above. The latter was



Scheme 8. Description of the synthetic schemes allowing the synthesis of 2,3-oxidosqualene analogs bearing different substituents and stereochemistry at Δ^{18-19}.

generated on heating **8** with T_2O in the presence of triethylamine (50 °C, 15 h) and directly used after usual workup.

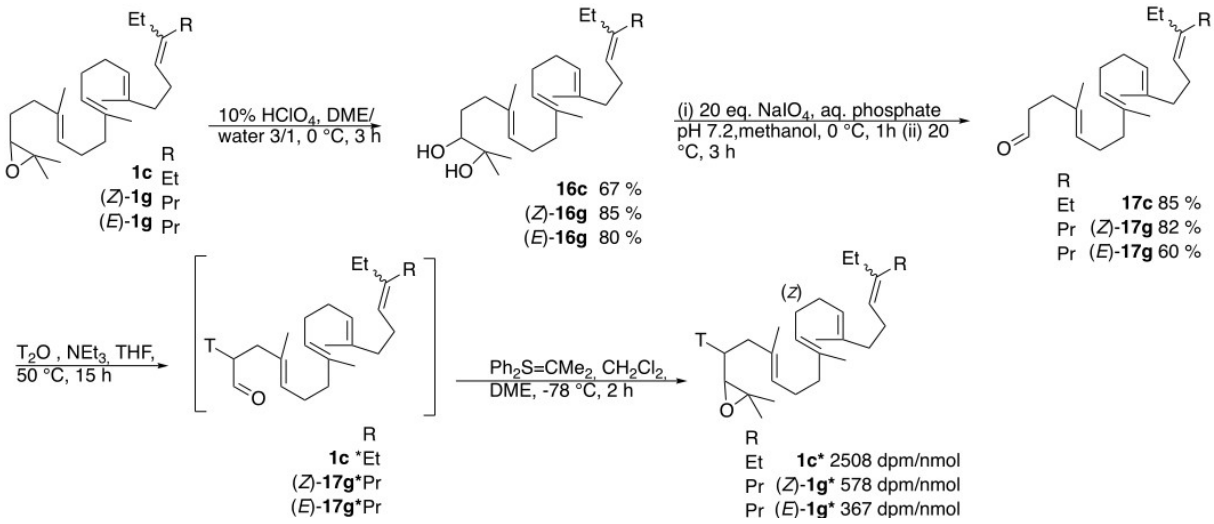
A different strategy was used for the synthesis of radiolabeled **1c*** (2508 dpm/nmol) as model and **1g** to avoid the manipulation of radiolabeled material during the tedious separation of the stereoisomers. It was achieved on each stereoisomer after their separation [Scheme 9].

Accordingly, **1c** and each **1g** stereoisomer were degraded to the corresponding aldehyde by acid catalyzed epoxide ring opening leading to the diol **16** (70% $HClO_4$, DME/water 3/1, 0 °C, 3 h) and then transformed to the aldehyde **17** (20 eq. $NaIO_4$, aqueous phosphate pH 7.2, methanol, 0 °C, 1 h, 20 °C, 3 h), tritiated as disclosed above for other aldehydes, and the resulting **17*** was reacted with an excess of isopropylidene triphenylsulfurane to deliver radiolabeled epoxide **1c**, (*Z*)-**1g**, and (*E*)-**1g** [(i) 35 eq. isopropyl diphenyl sulfonium tetrafluoroborate, 35 eq. CH_2Cl_2, DME, -78 °C, 0.5 h; (ii) 1 eq. 17*, DME, -78 °C, 2 h; and (iii) H_2O leading to **1c*** 48%, 2508 dpm/nmol; (*Z*)-**1g*** 44%, 578 dpm/nmol; and (*E*)-**1g*** 45% 367 dpm/nmol].

Reaction of 2,3-oxidopolyenes with OSC-PL

The oxidosqualene analogs were reacted with OSC from pig liver prepared according to Hogeboom[19]. The conditions used proved to efficiently cyclize 2,3-oxidosqualene (*E*)-**1a** to lanosterol (*R*)-**3a** in up 80% yield (40% since the reaction was carried out on epimeric mixture of (*E*)-**1a** at C-3).

Accordingly, the radiolabeled 2,3-oxidopolyene **1** (300-2000 nmol) mixed with Tween 80 [10% in acetone (10%) and then evaporation of acetone] and dissolved in bi-distilled water was stirred under argon for 3 h



Scheme 9. Transformation of 2,3-oxidosqualenes **1c** and **1g** into their tritium-labeled analogs at C-4.

(17 h in the large-scale experiment, 5 mg of **1**) with an aqueous solution of OSC-PL prepared as discussed above[19]. The mixture was then saponified on stirring with a 10% potassium hydroxide solution in ethanol and extracted with ether, leading to a recovery of compounds possessing 88%-95% of the radioactivity. The crude mixture was separated by preparative layer chromatography (SiO_2, toluene/ethyl acetate 95/05, and the following products were isolated: recovered oxidosqualene **1**, rf = 0.7-0.8; lanosterol, rf = 0.4-0.5; or analogs **3**, rf = 0.1-0.2). In the case of **1g**, the reactions were carried out at 1 µmol, and the purifications on SiO_2 (toluene/ethyl acetate 95/05) led to: from (*Z*)-**1g**, Zone A_Z, rf = 0.7-0.8 (61%, oxidosqualene); Zone B_Z, rf = 0.4-0.5 (54%, lanosteryl derivatives); and Zone C_Z, rf = 0.1-0.2 (11%, unknown); and, from (*E*)-**1g**, Zone A_Z, rf = 0.6-0.8 (53%, oxidosqualene), and Zone B_Z, rf = 0.4-0.45 (43%, lanosteryl derivatives).

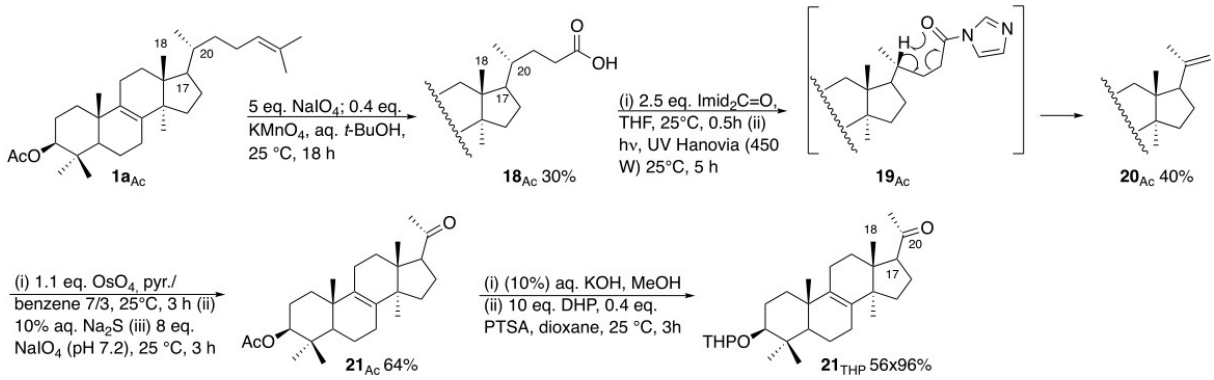
Hemi-synthesis of lanosteryl analogs (R)-3g and (R)-3g

To unambiguously prove the structure of the compounds resulting from the reaction of **1g** with 2,3-oxidosqualene sterol cyclase, we achieved the hemi-synthesis of the two epimeric (20*R*)-**3g** and (20*S*)-**3g** from lanosterol **1a** as a common intermediate using a methodology previously used in our laboratory[28,29].

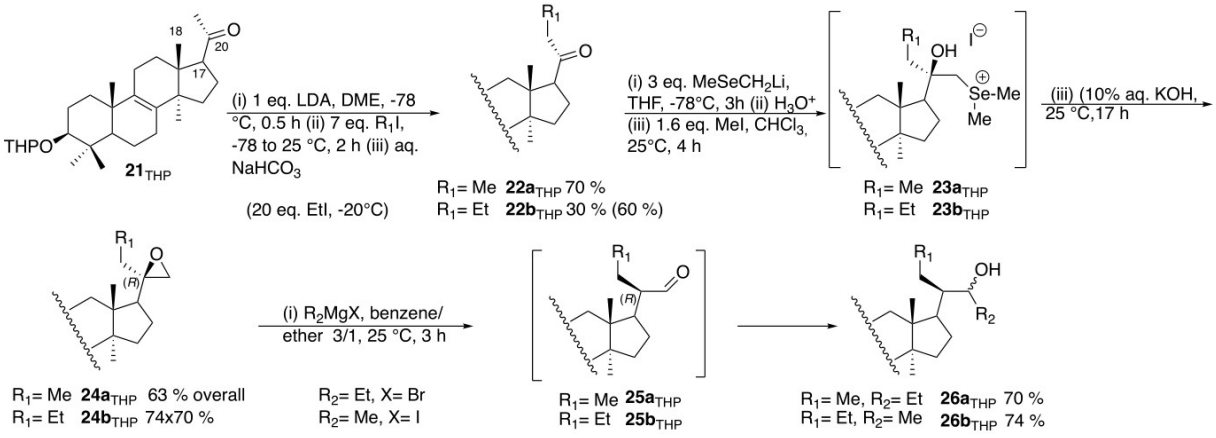
The synthetic strategy involves the following [Schemes 10 and 11]:

(i) Degrade the lanosteryl acetate $\mathbf{1a}_{Ac}$ side chain up to C-20, leading to the methyl ketone **21** in order to destroy the stereochemical information there keeping intact the structure and stereochemistry of the tetracyclic scaffold [Scheme 10][30].

(ii) Restore the chirality at C-20 by taking advantage of [Scheme 11]: (a) the face-selective addition of nucleophile on the (*Si*)-face of the carbonyl of the ketones **22** directed by the presence of the C-18 methyl group using a nucleophile bearing a leaving group or a potential leaving group able to produce the epoxides **24** [Scheme 9]; and (b) rearrangement of the epoxide **24** to the intermediate aldehyde **25** on reaction with Grignard reagents that involves the highly stereoselective migration of a hydrogen of the epoxide ring leading mainly to the (20*R*)-epimers of **25** (*R* = Me or Et) that are trapped in situ by the Grignard reagents.



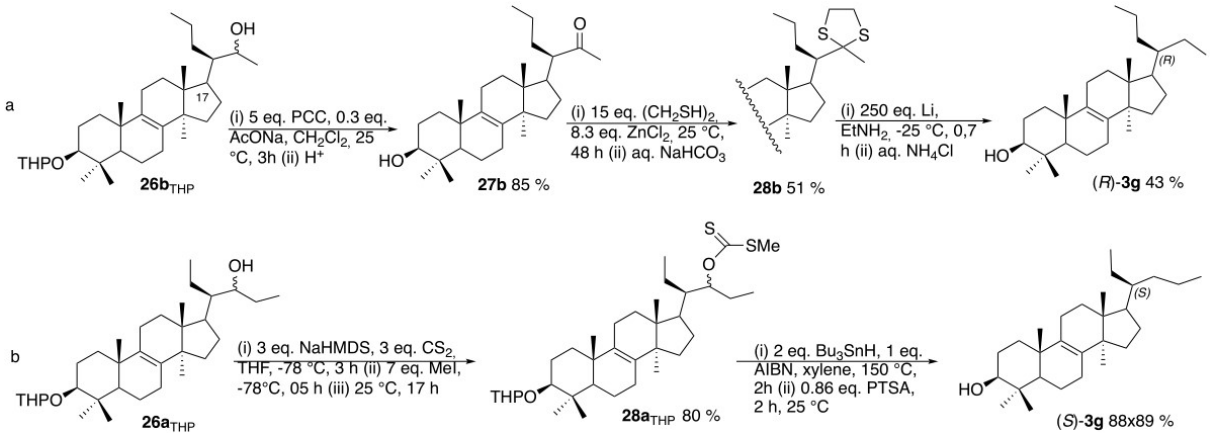
Scheme 10. Description of the synthetic scheme allowing the degradation of the side chain of lanosterol up to C-20.



Scheme 11. Stereoselective introduction of asymmetric C-20 carbon precursors of lanosteryl analogs (*R*)-**3g** and (*S*)-**3g**.

(iii) Synthesize both epimeric derivatives (20*R*)-**3g** and (20*S*)-**3g** that involve the electrophilic alkylation (R_1-X, X = halogen, R_1 = Me, Et)[31] of the methyl ketone **21** and later the nucleophilic alkylation (R_2-MgX, R_2 = Et, Me) of the aldehyde **25** using the same series of reactions but inverting the sequential introduction of methyl and ethyl groups to access either to (20*R*)-**3g** (R_1 = Me, R_2 = Et) through (**22a** and **26a**, Scheme 11) or to (20*S*)-**3g** (R_1 = Et, R_2 = Me) through (**22b** and **26b**, Scheme 11).

A transformation related to that of **26** to **3g** was originally performed in our laboratory[28], and is disclosed in Scheme 12, **entry a**. It allows the synthesis of (*R*)-**3g** through the oxidation of $\mathbf{26b}_{THP}$ and then acid catalyzed deprotection leading to**27b**. The former reaction was readily achieved by using excess of PCC in the presence of sodium acetate (5 eq. PCC, 0.3 eq. AcONa, CH2Cl2, 25 °C, 3 h)[32]. However, we experienced unexpected drawbacks in the synthesis of the thioacetal **28b** from **27b**. We could not extend to **27b** the method used (HS-CH_2-CH_2-SH, BF_3.Et_2O, AcOH, 25 °C) on a closely related ketone[30]. Neither alternative methods disclosed by Evans *et al.*[33] such as (Me_3Si-S-CH_2-CH_2-S-$SiMe_3$, ZnI_2, Et_2O, 24 h) or the more conventional ones [HS-CH_2-CH_2-SH (15 eq.), $ZnCl_2$ (8 eq.), CH_2Cl_2, 25 °C, 8 h] proved to be successful to reach our goal. The latter reaction however, when carried out for longer time (48 h instead of 8 h), delivered the thioacetal **28b** in modest yield (51%, Scheme 9). Reduction of the latter with lithium in ethylamine [(i) 250 eq. Li, $EtNH_2$, -25 °C, 0.7 h; and (ii) aq. NH_4Cl] affords (*R*)-**3g** in 43% yield along with (*S*)-**3g** (15%) and one unidentified product.



Scheme 12. Final steps towards the synthesis of lanosteryl analogs (*R*)-**3g** and (*S*)-**3g**.

Due to the difficulty encountered in the transformation disclosed above, we decided to adopt a different strategy to transform **26a** into (*S*)-**3g** (Scheme 12, **entry b**) that involves the formal reduction of the alcohol **26a**$_{THP}$. It was efficiently achieved by modification of the well-known Barton-McCombie method[34] that uses the reduction of the related xanthate.

The synthesis of the xanthate **28a**$_{THP}$ was readily achieved [(i) 3 eq. NaHMDS, 3 eq. CS_2, THF, -78 °C, 3 h; (ii) 7 eq. MeI, -78 °C, 05 h; and (iii) 25 °C, 17 h, 80%], but neither its reduction with tin hydride in the presence of azo-bis-isobutyronitrile (AIBN) as a radical initiator (1.5 eq. Bu_3SnH, xylene, reflux, 15 h) according to the original procedure[34] nor those involving 2 eq. Bu_3SnH and 1 eq. AIBN in toluene (110 °C, 2 h, 10% yield) worked properly. However, we found that performing the reaction at high temperature but for a short time to avoid the degradation of the product proved to be an excellent alternative (2 eq. Bu_3SnH, 1 eq. AIBN, xylene, 150 °C, 2 h, 88%).

Both alcohols (20*R*)-**3g** and (20*S*)-**3g** were acetylated (Ac_2O, pyridine, 25 °C, 17 h), delivering (20*R*)-**3g**$_{Ac}$ and (20*S*)-**3g**$_{Ac}$ in 84% yield. Those acetates proved to contain 10% of the other diastereoisomer (15% and 10%, respectively), probably arising from the rearrangement of the epoxide with the Grignard reagent step that is less stereoselective than expected[28,29].

CONCLUSIONS

We report the original behavior of OSC-PL towards oxidosqualene analogs bearing two alkyl groups at C_{19} by the incremental addition of a methyl group from: (a) Me,Et (tetracyclic, stereospecific)[16,17]; (b) Et,Et (tetracyclic); (c) Et,Pr (tetracyclic, non-stereospecific); and (d) Pr;Pr (**no cyclization**). It adds to the previous work that includes: (e) Me, isohexenyl (tetracyclic/tricylic non-chemospecific)[18]; (f) saturated C_{18}-C_{19} bond (tricyclic)[20]; and (g) Me, isohexadienyl (tetracyclic, protosterol)[14]. We observed the exceptional ability of this enzyme to respond in a different manner to different substrate analogs, providing each time products possessing a different type of structure.

We also propose an original hypothesis to rationalize the formation for the first time of a lanosterol analog bearing the natural stereochemistry at C-20 from an oxidosqualene analog bearing a non natural stereochemistry at $\Delta^{18\text{-}19}$. A possible support for this hypothesis could arise from reacting OSC-PL with an oxidosqualene analog bearing one [Figure 1, (*Z*)-**1h** or (*Z*)-**1i**] or two unsaturations [Figure 1, (*Z*)-**1j**] on the side chain hoping that protosterols bearing the side chain lying in alpha-position would be isolated using a

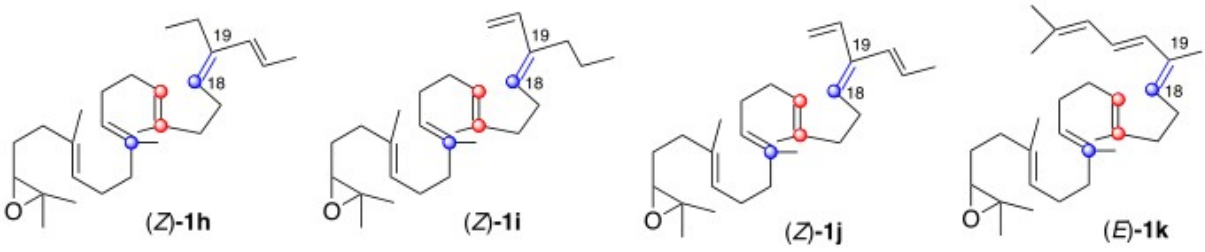


Figure 1. Potential precursors of protesterols bearing a side-chain lying in alpha-position.

strategy similar to the one developed by Corey *et al.*[14] using (*Z*)-**1k** [Figure 1] to support his proposal reported in Scheme 2, **entry b**[14,15]. Modeling *in silico* the process is another alternative that we are pursuing using the detailed X-ray structure of human sterolcyclase reported by Thoma *et al.*[5].

DECLARATIONS

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Authors' contributions

Made similar contributions to conception and design of the study and performed data analysis and interpretation: Krief A, Sable R, Ronvaux A, Dumont W, Sandra P, David F

Availability of data and materials

Data are deposited at the Université of Namur in the PhD theses of Dr. Alain Ronvaux (1998) and Dr. Romuald Sable (2007) and are accessible on request.

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Conflicts of interest

Krief A is the Honorary Editor-in-Chief of *Chemical Synthesis*, while the other authors have declared that they have no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Written informed consent for publication was obtained for all the authors and from Dr. Willy Dumont wife (Mrs Josiane Gerlache) and one of his sons (Professor Patrick Dumont, UCL, Louvain le Neuve, Belgium).

REFERENCES

1. Barrett A, Ma T, Mies T. Recent developments in polyene cyclizations and their applications in natural product synthesis. *Synthesis* 2018;51:67-82. DOI
2. Yoder RA, Johnston JN. A case study in biomimetic total synthesis: polyolefin carbocyclizations to terpenes and steroids. *Chem Rev* 2005;105:4730-56. DOI PubMed PMC
3. Huff MW, Telford DE. Lord of the rings - the mechanism for oxidosqualene:lanosterol cyclase becomes crystal clear. *Trends Pharmacol Sci* 2005;26:335-40. DOI PubMed
4. Harrison DM. The biosynthesis of triterpenoids and steroids. *Nat Prod Rep* 1985;2:525-60. DOI PubMed
5. Thoma R, Schulz-Gasch T, D'Arcy B, et al. Insight into steroid scaffold formation from the structure of human oxidosqualene cyclase. *Nature* 2004;432:118-22. DOI PubMed
6. Woodward RB, Bloch K. The cyclization of squalene in cholesterol synthesis. *J Am Chem Soc* 1953;75:2023-4. DOI
7. Stork G. The stereochemistry of polyene cyclization. Harvard University Organic colloquium Abstract. 1950. Burgstahler AW, PhD. Thesis. Harvard University: 1952.
8. Stork G, Burgstahler AW. The stereochemistry of polyene cyclization. *J Am Chem Soc* 1955;77:5068-77. DOI
9. Eschenmoser A, Ruzicka L, Jeger O, Arigoni D. Zur Kenntnis der Triterpene. 190. Mitteilung. Eine stereochemische Interpretation der biogenetischen Isoprenregel bei den Triterpenen. *Helv Chim Acta* 1955;38:1890-904. DOI
10. Eschenmoser A, Arigoni D. Revisited after 50 years: the “stereochemical interpretation of the biogenetic isoprene rule for the triterpenes”. *HCA* 2005;88:3011-50. DOI
11. Corey EJ, Russey WE, Ortiz de Montellano PR. 2,3-Oxidosqualene, an intermediate in the biological synthesis of sterols from squalene. *J Am Chem Soc* 1966;88:4750-1. DOI PubMed
12. Tamelen EE, Willett JD, Clayton RB, Lord KE. Enzymic conversion of squalene 2,3-oxide to lanosterol and cholesterol. *J Am Chem Soc* 1966;88:4752-4. DOI PubMed
13. Cornforth JW. Olefin alkylation in biosynthesis. *Angew Chem Int Ed Engl* 1968;7:903-11. DOI PubMed
14. Corey EJ, Virgil SC, Sarshar S. New mechanistic and stereochemical insights on the biosynthesis of sterols from 2,3-oxidosqualene. *J Am Chem Soc* 1991;113:8171-2. DOI
15. Wendt KU, Schulz GE, Corey EJ, Liu DR. Enzyme mechanisms for polycyclic triterpene formation. *Angew. Chem. Int. Edn Engl* 2000; 39:2812-33. DOI
16. Hérin M, Sandra P, Krief A. Stereospecific enzymic cyclization of a synthetic 2,3-oxidosqualene analogue bearing an 18Z carbon-carbon double bond. *Tetrahedron Letters* 1979;20:3103-6. DOI
17. Krief A, Pasau P, Guittet E, Shan YY, Hérin M. Novel results on the biocyclisation of 2,3-oxidosqualene analogs by sterol cyclase. *Bioorganic & Medicinal Chemistry Letters* 1993;3:365-8. DOI
18. Krief A, Schauder JR, Guittet E, Herve du Penhoat C, Lallemand JY. About the mechanism of sterol biosynthesis. *J Am Chem Soc* 1987;109:7910-1. DOI
19. Hogeboom GH. Methods enzymol. Fractionation of cell components of animal tissues. Elsevier; 1955. p. 16-9. DOI
20. Tamelen EE, Sharpless KB, Hanzlik R, Clayton RB, Burlingame AL, Wszolek PC. Enzymic cyclization of trans,trans,trans-18,19-dihydrosqualene 2,3-oxide. *J Am Chem Soc* 1967;89:7150-1. DOI PubMed
21. Krief A, Pasau P, Quéré L. Comparison of the behavior of oxidosqualene cyclases from pig liver and yeast toward epoxy-squalene analogues possessing a $\Delta^{18\text{-}19}$ Z or E (C, C) double bond. *Bioorganic & Medicinal Chemistry Letters* 1991;1:365-8. DOI
22. Krief A, Dunkle M, Bahar M, Bultinck P, Herrebout W, Sandra P. Elucidation of the absolute configuration of rhizopine by chiral supercritical fluid chromatography and vibrational circular dichroism. *J Sep Sci* 2015;38:2545-50. DOI PubMed
23. Tamelen E, Sharpless KB. Positional selectivity during controlled oxidation of polyolefins. *Tetrahedron Letters* 1967;8:2655-9. DOI
24. Krief A, Dumont W, Clarembeau M, Bernard G, Badaoui E. Synthesis of α-selenoalkyllithium compounds. *Tetrahedron* 1989;45:2005-22. DOI
25. Labar D, Krief A. Connective (C-C) route to hindered epoxides and olefins from hindered ketones. *J Chem Soc, Chem Commun* 1982;10:564-6. DOI
26. Horner L, Hoffmann H, Wippel HG, Klahre G. Phosphororganische Verbindungen, XX. Phosphinoxyde als Olefinierungsreagenzien. *Chem Ber* 1959;92:2499-505. DOI
27. Buss AD, Warren S. The Wittig-Horner route to tri-substituted alkanes: synthesis of -α-bisabolene. *Tetrahedron Letters* 1983;24:111-4. DOI
28. Schauder J, Krief A. Regio and stereochemically controlled ring opening of epoxides with Grignard reagents. Stereocontrolled synthesis of the steroid side chains. first stereoselective hemisynthesis of 20s isolanosterol. *Tetrahedron Letters* 1982;23:4389-92. DOI
29. Koreeda M, Koizumi N. Stereochemically controlled synthesis of 20-isocholesterol. *Tetrahedron Letters* 1978;19:1641-4. DOI
30. Pasau P. PhD thesis, Facultés Universitaires Notre-Dame de la Paix (FUNDP). Namur; 1992.
31. House HO, Gall M, Olmstead HD. Chemistry of carbanions. XIX. Alkylation of enolates from unsymmetrical ketones. *J Org Chem* 1971;36:2361-71. DOI PubMed
32. Corey EJ, Suggs JW. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl

compounds. *Tetrahedron Letters* 1975;16:2647-50. DOI

33. Evans DA, Truesdale LK, Grimm KG, Nesbitt SL. Thiosilanes, a promising class of reagents for selective carbonyl protection. *J Am Chem Soc* 1977;99:5009-17. DOI

34. Barton DHR, Mccombie SW. A new method for the deoxygenation of secondary alcohols. *J Chem Soc, Perkin Trans 1* 1975. DOI