2,3,18,19-Dioxidosqualene Stereoisomers: Characterization and Activity as Inhibitors of Purified Pig Liver 2,3-Oxidosqualene−Lanosterol Cyclase

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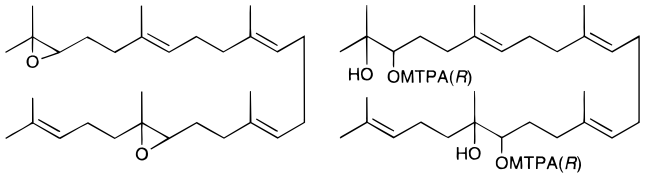
Introduction

2,3-Oxidosqualene−lanosterol cyclase (OSLC, EC 5.4.99.7) is the key enzyme of the ring cyclization reaction suffered by (3*S*)-2,3-oxidosqualene to give lanosterol as final steroid precursor in fungi and mammalian systems.[1] The mechanism of this cyclization is still a subject of debate, although current hypotheses are in favor of a stepwise process, which proceeds through a serie of conformationally rigid, partially cyclized carbocationic intermediates. These studies have been supported by the recent advances in the characterization and purification of OSLC. Thus, several cyclases have been purified to homogeneity from different sources[2−6] and OSLC from *Candida albicans* has been cloned and sequenced.[7,8] More recently, the cloning of rat[9,10] and human liver[11] OSLC cDNA has been reported.

The availability of potent inhibitors of OSLC constitutes also a valuable tool for unraveling specific features of the cyclization mechanism. Another objective of the search of efficient OSLC inhibitors seeks the development of potential hypocholesteremic agents.[12] As it occurs with other enzyme systems related to the squalene biosynthesis pathway, the fact that the OSLC step is beyond the synthesis of other physiologically relevant terpenoid compounds, such as farnesyl derivatives, dolichol and ubiquinones, makes this strategy therapeutically attractive. On the other hand, it has been recently pointed out that OSLC inhibitors can modulate sterol biosynthesis by acting on HMG-CoA reductase by a feedback mechanism involving the participation of oxysterols.[13]

Numerous OSLC inhibitors have elicited potent activity.[14] Among them 29-methylidene-2,3-oxidosqualene was reported as the first mechanism-based irreversible inhibitor of this enzyme.[15] Recently, improved substrate and intermediate mimic inhibitors containing either nitrogen[16,17] or sulfur[18] moieties have been described. From these compounds, the analog bearing a sulfur atom at C-19 in the squalene skeleton has resulted in the most powerful inhibitor to date for rat liver cyclase ($IC_{50} = 0.8$ nM).[19] In this context, our approach was to explore the OSLC potential inhibitory activity of substrate mimics containing the epoxy moiety. We reasoned that this class of compounds might be formed endogenously under stress or unregulated oxidation conditions. Accordingly, the preparation of internal oxidosqualenes and all possible dioxidosqualenes as racemates and their evaluation as OSLC inhibitors in rat liver microsomes were carried out. We found that 2,3,18,19-dioxidosqualene (**1**) elicited the highest activity as OSLC inhibitor ($IC_{50} = 0.11$ μM).[20,21]

Scheme 1



1-(3*R*,18*S*,19*S*) **2-(3*R*,18*S*,19*R*)**
1-(3*R*,18*R*,19*R*) **2-(3*R*,18*R*,19*S*)**
1-(3*S*,18*S*,19*S*) **2-(3*S*,18*S*,19*R*)**
1-(3*S*,18*R*,19*R*) **2-(3*S*,18*R*,19*S*)**

More recently, we reported that (6*S*,7*S*)-oxidosqualene is a strong competitive inhibitor of purified pig liver squalene epoxidase (SE, EC 1.14.99.7) ($IC_{50} = 6.7$ μM; $K_i = 2.7$ μM). Furthermore, incubation of the enantiomer of the above epoxy derivative, *i.e.*, (6*R*,7*R*)-oxidosqualene with purified SE led to the formation of dioxide **1-(3*S*,18*R*,19*R*)** (Scheme 1) as major compound.[22] In view of these results, it was then interesting to identify the specific stereoisomers of dioxidosqualene **1** which were contributing to the OSLC inhibitory activity exhibited by the racemic mixture.

To this aim, the present paper reports the preparation and determination of the absolute configuration of the different stereoisomers of dioxidosqualene **1**, *i.e.*, **1-(3*S*,18*S*,19*S*)**, **1-(3*S*,18*R*,19*R*)**, **1-(3*R*,18*S*,19*S*)**, and **1-(3*R*,18*R*,19*R*)** (Scheme 1), and their activities as inhibitors of OSLC purified from pig liver. Results on the determination of the kinetic parameters for the two most active compounds and on their possible irreversible interaction with OSLC are also reported.

Results and Discussion

Preparation and Determination of the Absolute Configuration of the Four Stereoisomers of Di-

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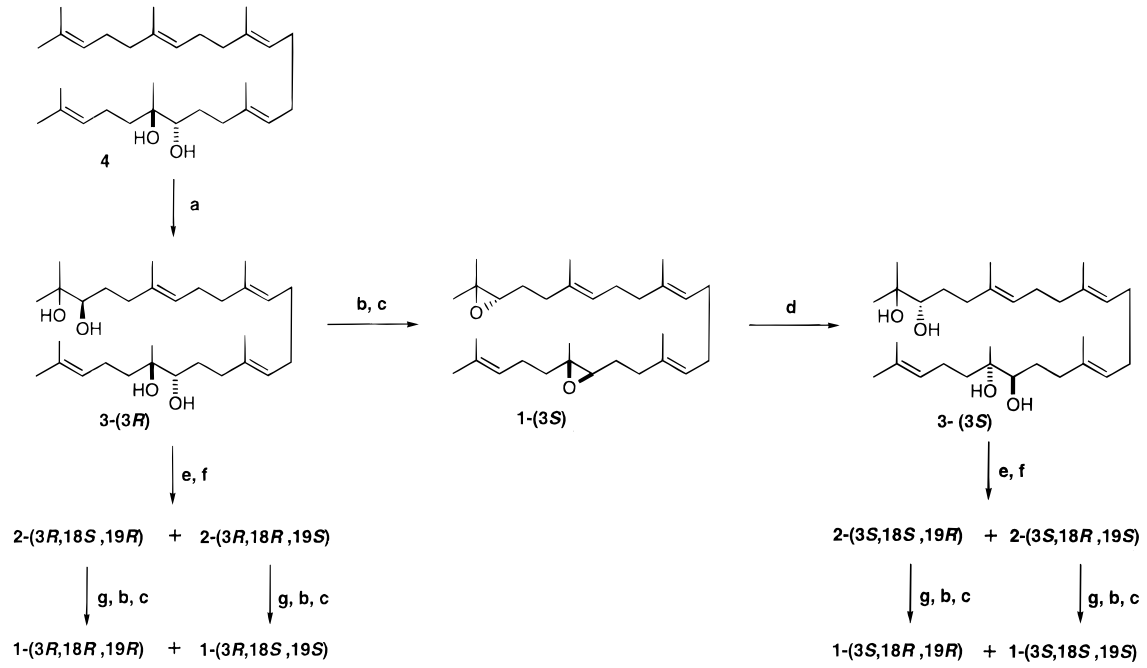
Scheme 2[a]



[a] (a) $(DHQD)_2$−PHAL/$K_3Fe(CN)_6/K_2CO_3/CH_3SO_2NH_2/K_2OsO_4$/^{t}BuOH/$H_2O$/0 °C; (b) $CH_3SO_2Cl/Et_3N/CH_2Cl_2$; (c) NaH/THF; (d) $HClO_4$/THF/H_2O; (e) (*S*)-MTPA-Cl/Et_3N/DMAP/CH_2Cl_2; (f) semipreparative reversed-phase HPLC; (g) $LiAlH_4$, Et_2O.

oxidosqualene 1. Our previous work on the preparation of the different internal oxidosqualene stereoisomers used a strategy involving the formation of the (*R*)-MTPA Mosher esters of the corresponding *erythro* internal squalenediol mixtures. These esters were employed for the chromatographic resolution of these mixtures and the further determination of the absolute configuration of the stereogenic centers present at the squalene skeleton for each diastereomer. For this latter purpose, the ^{1}H NMR procedure developed by the group of Kakisawa[23] was adapted to the case of enantiomer mixtures from synthetic origin. The final conversion of the above esters into the stereochemically related oxidosqualenes with high chemical and optical purities supported the validity of this approach.[22]

Consequently, we decided to use a similar strategy for synthesizing the four stereoisomers of dioxidosqualene **1**. In this case, however, the weak interactions expected between the stereocenter at C-3 and those at C-18 and C-19 would anticipate a more difficult chromatographic resolution of the diastereomeric mixtures of the corresponding Mosher diesters **2** (Scheme 1). Thus, acid hydrolysis of racemic dioxide **1** afforded the corresponding tetraol racemate **3** which was converted into the mixture of diastereomers **2** by treatment with (*S*)-MTPACl following the general procedure previously described.[22] Unfortunately, all assays carried out to resolve this mixture by reversed-phase HPLC led to the obtention of three peaks in a 1:2:1 ratio, which indicated the coelution of two diastereomers. Moreover, when these coeluted diastereomers were treated with $LiAlH_4$ and the resulting tetraols were rederivatized with (*R*)-MTPACl, again a diastereomeric mixture not separable by chromatographic means was obtained.

Then we envisaged another approach involving the asymmetric dihydroxylation of diol **4** with the Sharpless reagent $(DHQD)_2$−PHAL (Scheme 2). This reaction led to the diastereomer mixture of tetraols **3** with (3*R*) configuration. The derivatization of these tetraols with (*S*)-MTPACl afforded the corresponding Mosher diesters **2** (one of the constituents of the second peak and the third peak in the above HPLC profile). These diesters were separated by semipreparative HPLC with good recoveries. Conversely, the low reaction rates and enantiomeric excesses usually obtained with the $(DHQ)_2$−PHAL reagent led us to use an alternative procedure for the preparation of the two remaining tetraol stereoisomers **3** with (3*S*) configuration. Thus, the diastereomeric mixture of tetraols **3-(3*R*)** was converted into the corresponding dioxidosqualenes **1-(3*S*)** by mesylation and treatment with base in 78% overall yield. The acid hydrolysis of this mixture afforded the pair of tetraols **3** with (3*S*) configuration. The Mosher diesters **2** derived from these tetraols were prepared and separated as described above, although with poorer recoveries in this case. Finally, the different dioxidosqualenes **1** were obtained from the respective diester **2** in 55−60% overall yields by treatment with $LiAlH_4$ to give the tetraol, followed by mesylation and ring closure. The stereochemical purity of these dioxidosqualenes was determined by the conversion into their corresponding tetraols **3** followed by formation of the MTPA esters and analysis of these derivatives by HPLC, ^{1}H NMR, or ^{19}F NMR. A racemization lower than 5% was observed for the conversion of the chiral squalenetetraol into the bisepoxy derivative and vice versa.

The absolute configuration of dioxidosqualenes **1** was determined by adapting the method reported by the group of Kakisawa to the case of stereoisomers mixtures from synthetic origin.[22] For this purpose, the ^{1}H NMR spectra for the four different Mosher diesters **2** were registered, and the chemical shifts for all protons with respect to each diastereomer were assigned. The chemical shifts differences ($\Delta\delta$) between pairs of diastereomer diesters from the same synthetic origin were not signifi-

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Scheme 3. ^{1}H NMR Chemical Shift Differences (in ppm) for All Positions of the Squalene Skeleton between the Four Mosher Diesters 2, Precursors of Dioxidosqualene Stereoisomers 1

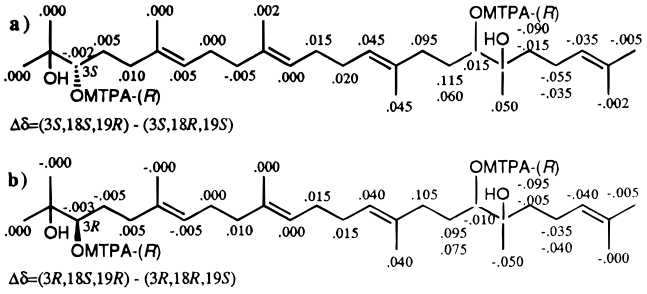

Table 1. IC_{50} and K_i Values of Purified Pig Liver 2,3-Oxidosqualene−Lanosterol Cyclase Inhibition for 2,3,18,19-Dioxidosqualene Stereoisomers 1[a]

compd[b]	IC_{50} (μM)	K_i (μM)
rac-**1**	0.12	n.d.
1-(3*R*,18*S*,19*S*)	0.37	n.d.
1-(3*R*,18*R*,19*R*)	0.22	n.d.
1-(3*S*,18*S*,19*S*)	0.071 (0.062)	0.076
1-(3*S*,18*R*,19*R*)	0.037 (0.027)	0.021

[a] For assay details and concentrations of inhibitors used, see supporting information. Substrate concentration was 20 μM; values in parentheses were determined by using 5 μM. [b] The enantiomeric excess determined for diepoxides **1-(3*R*,18*S*,19*S*)**, **1-(3*R*,18*R*,19*R*)**, **1-(3*S*,18*S*,19*S*)**, and **1-(3*S*,18*R*,19*R*)** were 90%, 94%, 95%, and 95%, respectively.

cant for hydrogen atoms in the proximity of C-3 (Scheme 3). Conversely, the $\Delta\delta$ values obtained for the hydrogen atoms in the vicinity of C-18 and C-19 made possible the assignment of the absolute configuration for these stereocenters (Scheme 3, cf. a and b). On the other hand, in addition to the stereochemical reference derived from the $(DHQD)_2$−PHAL-promoted asymmetric dihydroxylation of the terminal double bond mentioned above (cf. Scheme 2), the absolute configuration of the stereocenters at C-3 was confirmed by comparing the $\Delta\delta$ values from Mosher diesters with the same configuration at C-18 and C-19. Once the four Mosher diesters were fully characterized, the absolute configuration of the respective dioxidosqualenes **1** could be unequivocally inferred.

Inhibition of OSLC Activity. OSLC assays with dioxidosqualenes **1-(3*S*,18*S*,19*S*)**, **1-(3*S*,18*R*,19*R*)**, **1-(3*R*,18*S*,19*S*)**, and **1-(3*R*,18*R*,19*R*)** were carried out employing the partially purified enzyme from pig liver and following the procedure described by Duriatti et al.[24] Previously, the inhibitory activity due to (3*R*)-2,3-oxidosqualene was tested and found negligible, which permitted the use of 2,3-oxidosqualene as substrate for the OSLC activity assays. In this sense, internal oxides (6*S*,7*S*)- and (6*R*,7*R*)-oxidosqualenes[22] exhibited weak inhibitory activities (IC_{50} = 81 and 190 μM, respectively, using 20 μM of substrate), which discarded that the high activities shown below were due only to the presence of the internal epoxy moiety.

Conversely, dioxidosqualenes **1** elicited potent inhibitory activities on OSLC (Table 1). As expected, those stereoisomers with (3*S*) configuration were more active, approximately one order of magnitude, than those with (3*R*) configuration. From the pair of dioxides **1** with (3*S*) configuration, the most active compound was dioxide **1-(3*S*,18*R*,19*R*)** (IC_{50} = 27 nM using 5 μM of substrate). This compound is within the best OSLC inhibitors described to date.[12,19] Dioxide **1-(3*S*,18*S*,19*S*)** displayed also a high inhibitory activity (IC_{50} = 62 nM using 5 μM of substrate). From the kinetic data obtained for this pair of stereoisomers, dioxide **1-(3*S*,18*R*,19*R*)** elicited a noncompetitive inhibition (K_i = 21 nM), whereas dioxide **1-(3*S*,18*S*,19*S*)** exhibited a linear-mixed pattern inhibition (K_i = 76 nM) (Table 1, Figure 1). On the other hand, these results support the inhibition data previously found for the racemic mixture of dioxide **1** in rat liver microsomes, where it can be assumed that stereoisomers with (3*S*) configuration contributed predominantly to the inhibitory potency obtained in that case.[21] Finally, assays carried out as described by Bai and Prestwich[25] showed that the OSLC inhibition caused by dioxidosqualenes **1-(3*S*,18*R*,19*R*)** and **1-(3*S*,18*S*,19*S*)** was reversible.

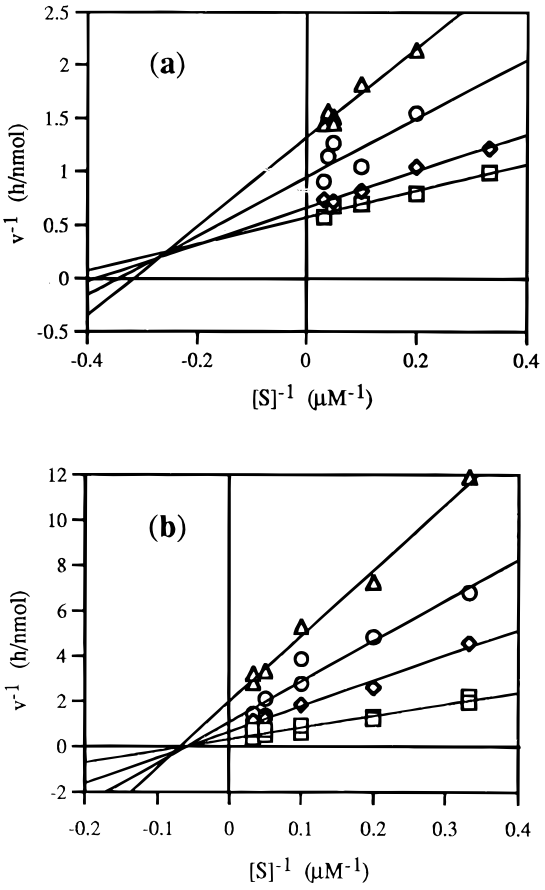

Figure 1. Lineweaver−Burk plots of the inhibition of purified pig liver OSLC by (a) **1-(3*S*,18*S*,19*S*)** and (b) **1-(3*S*,18*R*,19*R*)**. The concentrations of inhibitors used were 0 (squares), 0.05 (diamonds), 0.1 (circles), and 0.2 (triangles) μM, and 0 (squares), 0.025 (diamonds), 0.05 (circles), and 0.1 (triangles) μM, respectively.

The results shown above deserve some additional comments. First, the comparison from the activities of internal oxidosqualenes with those of dioxides **1** indicates that both epoxy moieties are needed for eliciting a potent OSLC inhibition, which confirmed our previous results on rat liver microsomes.[21] In addition, an exam of the numerous OSLC inhibitors described to date points out

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the importance of structural modifications in the vicinity of the C18−C19 position of the squalene skeleton. Thus, the most potent OSLC inhibitor reported so far bears a sulfur atom at C-19,[19] and the first mechanism-based OSLC irreversible inhibitor presents a methylidene moiety at C-29, which is the methyl group linked to C-19.[15] The case of dioxides **1-(3*S*,18*R*,19*R*)** and **1-(3*S*,18*S*,19*S*)** corroborates the above observation. Regarding the stereochemical features at the C18−C19 region, our results suggest that both epoxide configurations seem to interact with the enzyme, although the effect reached by the (*R*,*R*) stereoisomer leads to the higher inhibitory activity. This observation may serve for the future design of improved substrate-mimic inhibitors. On the other hand, the fact that dioxides with (3*R*) configuration showed inhibitory activities in the nanomolar range could be partially due to the increased polarity of these compounds in comparison with oxidosqualenes, which would facilitate the interaction with the enzyme, and to the presence of minor amounts of stereoisomers with (3*S*) configuration (cf. footnotes in Table 1).

The negative results shown by the irreversibility assays performed on these dioxides suggest that no covalent interaction with the enzyme occurs. In this sense, it is conceivable that the presence of moieties more reactive that these trisubstituted epoxides might facilitate the establishment of stronger interactions with OSLC.

Finally, the fact that internal oxidosqualenes have been described in nature,[26] and that (6*R*,7*R*)-oxidosqualene is substrate of purified SE from pig liver to give precisely dioxide **1-(3*S*,18*R*,19*R*)** as major product,[22] confers a particular interest to the potential role of this inhibitor in the steroidogenesis pathway. On the other hand, research is on progress in our laboratory to find out the presence or absence of OSLC-promoted cyclization products for the pair of dioxides **1** with (3*S*) configuration, which would provide important information on the inhibition mechanism of these compounds.

Experimental Section

Apparatus. The HPLC analysis was performed by using a Spherisorb ODS-2 (5 μm) column and eluting with CH_3CN-H_2O mixtures at 1 mL/min. The gas chromatography−mass spectrometry analysis was performed with positive chemical ionization (GC-MS-CI), using methane as ionization gas and a 30 m HP-5 bonded phase capillary column (0.25 mm i.d.). The liquid chromatography−thermospray-mass spectrometry analysis (HPLC-TSP-MS) was carried out with a quadrupole apparatus (direct flow injection with 50 mM HCO_2NH_4/CH_3CN (50:50) at 1 mL/min; positive mode; TSP tip, 180 °C; TSP stem, 96 °C; and TSP ion source, 250 °C). Optical rotations were determined at 25 °C in $CHCl_3$ solution at the specified concentration (expressed in g/L, 10 cm cell). The enantiomeric excess (ee) values were calculated by HPLC analysis of the corresponding (*R*)-MTPA diester derivatives.

NMR Spectra. The NMR spectra (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz; ^{19}F NMR, 282 MHz) were recorded with a 4 pretuned nucleus auto NMR probe. All spectra were performed in freshly neutralized $CDCl_3$ solutions, and chemical shifts are given in ppm downfield from $Si(CH_3)_4$ for ^{1}H, $CDCl_3$ for ^{13}C, and $CFCl_3$ (internal reference) for ^{19}F. The standard ^{1}H DQFCOSY spectra recorded for the determination of the absolute configuration of MTPA diesters **2** were obtained as previously described.[22]

Compounds. Unless otherwise stated, organic solutions obtained from the workup of crude reaction mixtures were dried over $MgSO_4$, and the purification procedures were carried out by flash chromatography on silica gel. In some cases, the silica gel was previously impregnated with Et_3N to minimize decomposition of squalene derivatives.

Asymmetric Dihydroxylation of 6,7-Dihydroxy-6,7-dihydrosqualene. Tetraol **3-(3*R*)** was prepared by using the procedure reported by Crispino and Sharpless (*Tetrahedron Lett.* **1992**, *33*, 4273−4274). Thus, treatment of 0.88 g (2 mmol) of **6(*R**),7(*S**)-dihydroxy-6,7-dihydrosqualene** (**4**)[22] with the mixture of reagents containing the ligand $(DHQD)_2-PHAL$ for 48 h at 0 °C led to a crude reaction mixture which was purified by chromatography to give, among other tetraol derivatives, 0.05 g (5% yield) of compound **3-(3*R*)**. **3(*R*),18(*R**),19(*S**)-2,3,18,19-tetrahydroxy-2,3,18,19-tetrahydrosqualene 3-(3*R*)**: IR: 3415 (OH); ^{1}H NMR: δ 5.30−5.02 (4 H), 3.36 (2 H), 2.34−1.88 (16 H (2 OH)),1.69 (3 H), 1.60 (br, 3 H), 1.80−1.05 (4 H), 1.20 (s, 3 H), 1.17 (s, 3 H), 1.15 (s, 3 H); ^{13}C NMR: δ 135.1, 134.8, 131.8, 125.1, 125.0, 124.6, 124.3, 78.3, 78.2, 74.6, 73.0, 39.6, 36.9, 36.8, 35.9, 29.6, 29.2, 28.1, 28.1, 26.3, 26.3, 25.7, 23.4, 23.3, 22.1, 17.7, 15.9, 15.9; MS (*m*/*z*) (relative intensity): 478 (M, 32), 460 (M − 18, 10), 443 (M + 1 − 36, 100), 425 (M + 1 − 48, 80). Anal. Calcd. for $C_{30}H_{54}O_4$: C; 75.26; H; 11.37. Found: C; 75.17; H; 11.48.

Conversion of Tetraol 3-(3*R*) into Tetraol 3-(3*S*). This conversion was carried out following the procedure described previously for the case of terminal squalenediols.[22] In this case, reaction of tetraol **3-(3*R*)** (48 mg, 0.1 mmol) with CH_3SO_3Cl (18 μL, 0.22 mmol) and Et_3N (60 μL, 0.40 mmol) in CH_2Cl_2 for 15 min at −10 °C afforded the expected bismesylate, which was allowed to react with NaH (24 mg, 1 mmol) in THF solution, to give after chromatography purification diepoxide **1-(3*S*)** (35 mg, 78% overall yield). Treatment of a solution of this diepoxide (35 mg, 0.08 mmol) in 5 mL of a 4:1 mixture of THF:H_2O with 10 μL of 60% $HClO_4$ for 24 h at 20 °C, followed by purification of the crude reaction mixture by chromatography, afforded tetraol **3-(3*S*)** (28 mg, 74% yield).

Preparation of MTPA Diesters 2-(3*R*,18*S*,19*R*), 2-(3*R*,18*R*,19*S*), 2-(3*S*,18*S*,19*R*), and 2-(3*S*,18*R*,19*S*). These compounds were obtained by using the procedure described previously for the case of squalenediols.[22] Thus, starting from tetraol **3-(3*R*)** (30 mg, 0.06 mmol), the expected mixture of diesters **2-(3*R*,18*S*,19*R*)** and **2-(3*R*,18*R*,19*S*)** (49 mg, 90% yield) was isolated. This mixture was separated by semipreparative HPLC (15 × 1 cm ODS-2 column, 10 μm, eluting with 79:21 CH_3CN:H_2O at 3.6 mL/min), followed by chromatography of the collected compound, to give 18 mg of each diastereomer. **Diester 2-(3*R*,18*S*,19*R*)**: $[\alpha]_D$ = +18.0 (*c* = 1.5, 95% ee); ^{1}H NMR: δ 7.70−7.52 (4 H), 7.47−7.32 (6 H), 5.22−4.94 (6 H), 3.59 (q, 3 H, *J* = 1.5 Hz), 3.57 (q, 3 H, *J* = 1.5 Hz), 2.14−1.80 (14 H), 1.67 (s, 3 H), 1.59 (br, 6 H), 1.56 (d, 3 H, *J* = 1 Hz), 1.52 (s, 3 H), 1.80−1.10 (6 H), 1.22 (s, 3 H), 1.16 (s, 3 H), 1.12 (s, 3 H); ^{13}C NMR: δ 166.9, 166.2, 135.1, 133.8, 133.5, 132.3, 132.1, 129.6, 128.4, 127.6, 127.4, 125.3, 125.2, 124.2, 124.0, 123.4 (q, CF_3, J_{F-C} = 287 Hz), 84.4 and 84.4 (q, C, J_{F-C} = 28 Hz), 82.4, 82.0, 74.1, 72.7, 55.5, 39.6, 36.9, 36.3, 35.7, 28.7, 28.3, 28.3, 28.1, 27.0, 26.7, 25.7, 23.7, 23.2, 21.8, 17.6, 16.0, 15.9, 15.8; ^{19}F NMR: δ −71.23, −71.26; HPLC-TSP-MS: 928 (M + 18), 911 (M + 1), 893 (M − 18 + 1), 694 (M − 217 + 1). **Diester 2-(3*R*,18*R*,19*S*)**: $[\alpha]_D$ = +27.0 (*c* = 1.5, 95% ee); ^{1}H NMR: δ 7.70−7.54 (4 H), 7.47−7.32 (6 H), 5.22−4.94 (6 H), 3.58 (q, 3 H, *J* = 1 Hz), 3.55 (q, 3 H, *J* = 1 Hz), 2.20−1.80 (14 H), 1.68 (s, 3 H), 1.59 (br, 6 H), 1.52 (br, 6 H), 1.75−1.10 (6 H), 1.22 (s, 3 H), 1.17 (s, 3 H), 1.16 (s, 3 H); ^{13}C NMR: δ 166.9, 166.7, 135.1, 133.8, 133.5, 132.2, 132.1, 132.0, 129.6, 128.4, 127.7, 127.6, 125.2, 125.2, 124.2, 124.1, 123.4 (q, CF_3, J_{F-C} = 287 Hz), 84.7 (q, C, J_{F-C} = 28 Hz), 82.4, 82.2, 74.2, 72.7, 55.5, 55.4, 39.6, 36.3, 36.0, 35.8, 28.7, 28.3, 28.2, 28.1, 27.0, 26.6, 25.7, 23.9, 23.7, 21.7, 17.6, 16.0, 15.9, 15.8; ^{19}F NMR: δ −71.06, −71.23; HPLC-TSP-MS: 928 (M + 18), 911 (M + 1), 893 (M − 18 + 18), 694 (M − 217 + 1).

By a similar procedure, tetraol **3-(3*S*)** (28 mg, 0.58 mmol) was converted into the mixture of diesters **2-(3*S*,18*S*,19*R*)** and **2-(3*S*,18*R*,19*S*)** (49 mg, 90% yield). This mixture was separated by semipreparative HPLC and each collected compound purified as indicated above to give 20 mg of diester **2-(3*S*,18*S*,19*R*)** and 14 mg of diester **2-(3*S*,18*R*,19*S*)**. Since these compounds are enantiomers of the diesters described above, *i.e.*, **2-(3*R*,18*R*,19*S*)** and **(3*R*,18*S*,19*R*)**, respectively, only their ee and $[\alpha]_D$ values are given. **2-(3*S*,18*S*,19*R*)**: $[\alpha]_D$ = +11.9 (*c* = 1.5, 94% ee). **2-(3*S*,18*R*,19*S*)**: $[\alpha]_D$ = +15.3 (*c* = 1, 90% ee).

(26) De Napoli, L.; Fattorusso, E.; Magno, S.; Mayol, L. *Phytochemistry* **1982**, *21*, 782−784.

Preparation of Chiral Dioxidosqualenes 1. These compounds were obtained from the respective MTPA diesters **2** by using the procedure described previously for the preparation of chiral oxidosqualenes.[22] Thus, treatment of diester **2** with $LiAlH_4$ in Et_2O followed by purification of the crude reaction mixture by chromatography eluting with 3:1 hexane:AcOEt afforded the corresponding tetraol **3**. A sample of tetraol **3** was isolated in 75% yield from the acid hydrolysis ($HClO_4/H_2O$/THF) of racemic dioxidosqualene **1**. **3**: MS *m/z* (relative intensity) 478 (M, 3), 461 (M − 18 + 1,10), 443 (M + 1 − 36, base peak). Anal. Calcd for $C_{30}H_{54}O_4$: C, 75.26; H, 11.37. Found: C, 75.17; H, 11.48.

(3*R*,18*R*,19*S*)-2,3,18,19-Tetrahydroxy-2,3,18,19-tetrahydrosqualene. This tetraol was isolated (12 mg, 84% yield) starting from 27 mg of diester **2-(3*R*,18*R*,19*S*)**. **(3-(3*R*,18*R*, 19*S*)**: $[\alpha]_D = +17.9$ ($c = 1$, 95% ee); ^{1}H NMR: δ 5.30−5.02 (4 H), 3.37 (dd, 1 H, $J_1 = 11$ Hz, $J_2 = 1$ Hz), 3.36 (dd, 1 H, $J_1 = 10.5$ Hz, $J_2 = 2$ Hz), 2.34−1.94 (14 H), 1.69 (s, 3 H), 1.62 (br, 9 H), 1.60 (s, 3 H), 1.90−1.10 (6 H), 1.20 (s, 3 H), 1.17 (s, 3 H), 1.15 (s, 3 H); ^{13}C NMR: δ 135.1, 135.1, 134.8, 131.9, 125.1, 125.1, 124.6, 124.4, 78.3, 78.3, 74.7, 73.0, 39.6, 37.0, 36.8, 35.8, 29.6, 29.2, 28.2, 28.1, 26.4, 26.3, 25.7, 23.4, 23.3, 22.1, 17.7, 16.0, 15.9.

(3*R*,18*S*,19*R*)-2,3,18,19-Tetrahydroxy-2,3,18,19-tetrahydrosqualene. This tetraol was isolated (12 mg, 81% yield) starting from 29 mg of diester **2-(3*R*,18*S*,19*R*)**. **3-(3*R*,18*S*, 19*R*)**: $[\alpha]_D = +2.0$ ($c = 1$, 95% ee).

(3*S*,18*R*,19*S*)-2,3,18,19-Tetrahydroxy-2,3,18,19-tetrahydrosqualene. This tetraol was isolated (8 mg, 80% yield) starting from 19 mg of diester **2-(3*S*,18*R*,19*S*)**. **3-(3*S*,18*R*, 19*S*)**: $[\alpha]_D = +17.9$ ($c = 1$, 95% ee); ^{1}H NMR: δ 5.30−5.02 (4 H), 3.37 (dd, 1 H, $J_1 = 11$ Hz, $J_2 = 1$ Hz), 3.36 (dd, 1 H, $J_1 = 10.5$ Hz, $J_2 = 2$ Hz), 2.34−1.94 (14 H), 1.69 (d, 3 H, $J = 1$ Hz), 1.62 (br, 9 H), 1.60 (d, 3 H, $J = 1$ Hz), 1.90−1.10 (6 H), 1.20 (s, 3 H), 1.17 (s, 3 H), 1.15 (s, 3 H); ^{13}C NMR: δ 135.1, 135.1, 134.8, 131.9, 125.1, 125.1, 124.6, 124.4 , 78.3, 78.2, 74.7, 73.0, 39.6, 37.0, 36,8, 35.8, 29.6, 29.2, 28.2, 28.1, 26.4, 26.3, 25.7, 23.4, 23.3, 22.1, 17.7, 16.0, 15.9, 15.9.

(3*S*,18*S*,19*R*)-2,3,18,19-Tetrahydroxy-2,3,18,19-tetrahydrosqualene. This tetraol was isolated (6 mg, 83% yield) starting from 14 mg of diester **2-(3*S*,18*S*,19*R*)**. **3-(3*S*,18*S*, 19*R*)**: $[\alpha]_D = -17.8$ ($c = 1$, 94% ee).

Conversion of each tetraol into the respective dioxidosqualene stereoisomer was carried out following the general procedure described previously.[22] For the spectroscopic and analytical characterization of **1** see ref 21.

(3*S*,18*S*,19*S*)-2,3:18,19-Dioxidosqualene. This compound was isolated (8 mg, 74% yield) starting from 12 mg of tetraol **3-(3*R*,18*R*,19*S*)**. **1-(3*S*,18*S*,19*S*)**: $[\alpha]_D = -3.9$ ($c = 0.5$, 95% ee).

(3*S*,18*R*,19*R*)-2,3:18,19-Dioxidosqualene. This compound was isolated (8 mg, 78% yield) starting from 11 mg of tetraol **3-(3*R*,18*S*,19*R*)**. **1-(3*S*,18*R*,19*R*)**: $[\alpha]_D = 0.6$ ($c = 0.5$, 95% ee).

(3*R*,18*S*,19*S*)-2,3:18,19-Dioxidosqualene. This compound was isolated (4 mg, 72% yield) starting from 6 mg of tetraol **3-(3*S*,18*R*,19*S*)**. **1-(3*R*,18*S*,19*S*)**: $[\alpha]_D = -0.4$ ($c = 0.4$, 90% ee).

(3*R*,18*R*,19*R*)-2,3:18,19-Dioxidosqualene. This compound was isolated (5 mg, 74% yield) starting from 7.5 mg of tetraol **3-(3*S*,18*S*,19*R*)**. **1-(3*R*,18*R*,19*R*)**: $[\alpha]_D = 4.0$ ($c = 0.5$, 94% ee).

Assay Method for OSLC. Partially purified OSLC was obtained from pig liver following the procedures described by Abe et al.[3] The specific activity of the purified enzyme was 2059 nmol/h mg protein. The procedure used for the OSLC assays was based on that reported by Duriatti et al.[24] with minor modifications.[21] Briefly, isopropyl alcohol solutions of the substrate and inhibitors were added to the test tubes (the alcohol contents did not exceed 0.4% v/v of the overall test mixture), followed by addition of 1 mL of 0.1 M Tris-HCl buffer (pH = 7.4) containing 30 μL of pig liver OSLC solution, 1 mM EDTA, and Triton X-100 (final concentration 0.05% w/v). For determination of IC_{50} values final concentrations of substrate (*R*,*S*)-2,3-oxidosqualene were 40 or 10 μM as indicated for each case. The mixture (final volume 1 mL) was incubated for 60 min at 37 °C. The enzymatic reaction was quenched by treatment with 6% KOH in methanol (1 mL) for 60 min at 37 °C. Then, 24,25-dihydrolanosterol was added as internal standard, and the mixture was extracted with hexane (3 × 2 mL). The combined extracts were evaporated to dryness, redissolved in 10:1 hexane/MTBE mixture (3 × 50 μL), loaded onto a column fitted with silica gel (0.75 g, 35−70 μm) containing 5% Et_3N, freshly prepared, and eluted with the same solvent mixture. After discarding the first 8 mL, lanosterol and internal standard were collected in a 5 mL fraction. The eluates were evaporated to dryness, redissolved in hexane, and injected onto a GC system (for conditions cf. ref 21). Incubations were performed by duplicate, and a minimum of two experiments per point were carried out. The IC_{50} values were determined by interpolation from the respective plot of percent inhibition vs log [I]. The concentration ranges used were: 0.005−1 μM for **1-(3*S*,18*S*, 19*S*)**, 0.01−0.1 μM for **1-(3*S*,18*R*,19*R*)**, 0.005−5 μM for **1-(3*R*,18*S*,19*S*)** and 0.005−5 μM for **1-(3*R*,18*R*,19*R*)**. The K_i values for dioxidosqualenes **1-(3*S*,18*S*,19*S*)** and **1-(3*S*,18*R*,19*R*)** were determined from replots of slopes and intercepts of Lineweaver−Burk double-reciprocal plot vs the inhibitor concentration. In these determinations the substrate concentrations were 3, 5, 10, 20, 25, and 30 μM, whereas those of inhibitors were 0, 0.05, 0.1 and 0.2 μM for **1-(3*S*,18*S*,19*S*)** and 0, 0.025, 0.05 and 0.1 μM for **1-(3*S*,18*R*,19*R*)**.

Irreversibility assays were carried out by adapting the procedure reported by Bai and Prestwich[25] to our case. Thus, 100 μL of pig liver OSLC solution were incubated with the corresponding inhibitor, *i.e.*, 1 μM **1-(3*S*,18*S*,19*S*)** or 0.5 μM **1-(3*S*,18*R*,19*R*)**, in buffer A (0.1 M Tris-HCl, 1 mM EDTA, 1 mM DTT and 0.2% Triton X, final volume 1 mL), in absence of organic solvents for 1 h at 37 °C. The inhibitor was removed by adsorption of the OSLC to a DEAE-Sepharose column preequilibrated with buffer A. Elution with buffer A containing 25 mM KCl (5 mL) followed by 200 mM KCl in buffer A (5 mL) permitted the collection of a 1.5 mL fraction containing the OSLC activity after discarding 6.5 mL of eluate. The effect of buffer A containing 200 mM KCl on OSLC activity was previously checked. All this chromatography process was carried out at 4 °C. The fraction containing the residual OSLC activity was then incubated in the presence of (*R*,*S*)-2,3-oxidosqualene (40 μM) and 0.4% (v/v) isopropyl alcohol for 1 h at 37 °C. The quenching of the enzymatic reaction, extraction, purification, and quantification of formed lanosterol was carried out as described above. In the control assays performed with no separation of enzyme and inhibitor, the crude preparation obtained after the preincubation was incubated in the presence of (*R*,*S*)-2,3-oxidosqualene (40 μM) and 0.4% (v/v) isopropyl alcohol (final volume 1.5 mL buffer A) for 1 h at 37 °C.

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Supporting Information Available: Experimental procedures, including synthesis and characterization data of all new compounds, ^{1}H and ^{13}C NMR chemical shifts assignment for diesters **2** and OSLC inhibition plots (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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