Internal Oxidosqualenes: Determination of Absolute Configuration and Activity as Inhibitors of Purified Pig Liver Squalene Epoxidase†

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The preparation and characterization of oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and **4-(10*S*,11*S*)** is reported. Squalenediol **6** was converted into the corresponding mixture of (*R*)-Mosher esters **8** and **9**, which were separated by semipreparative HPLC. Esters **8** and **9** were reduced to the chiral diols **6-(6*R*,7*S*)** and **6-(6*S*,7*R*)**, respectively, which were finally converted into the corresponding epoxides **3-(6*R*,7*R*)** and **3-(6*S*,7*S*)**. A similar procedure was used for the preparation of chiral epoxy derivatives **4-(10*R*,11*R*)** and **4-(10*S*,11*S*)** from esters **10** and **11**, respectively. The determination of the absolute configuration of these epoxides was carried out by using the method reported by Ohtani et al. (*J. Am. Chem. Soc.* **1991**, *113*, 4092), which was adapted to the case of racemic mixtures from synthetic origin. For this purpose, the (*R*)-Mosher esters derived from the enantiomers of squalenediols **6** or **7** were used. The validity of this approach was confirmed by the absolute configuration found for the *threo* squalenediols **6-(6*R*,7*R*)**, **6-(6*S*,7*S*)**, **7-(10*R*,11*R*)**, and **7-(10*S*,11*S*)** formed in the Sharpless asymmetric dihydroxylation of squalene (Crispino, G. A.; Sharpless, K. B. *Tetrahedron Lett.* **1992**, *33*, 4273). Results on the inhibitory activity of oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and **4-(10*S*,11*S*)** using purified squalene epoxidase (SE) from pig liver showed that epoxide **3-(6*S*,7*S*)** was the best inhibitor within the compounds assayed (IC_{50} = 6.7 μM), although oxidosqualene **4-(10*R*,11*R*)** also exhibited a moderate inhibitory activity (IC_{50} = 25 μM). The inhibition elicited by the epoxy derivative **3-(6*S*,7*S*)** was competitive with respect to squalene (K_i = 2.7 μM). This activity is comparable to that reported for the most potent competitive SE inhibitors described so far. Finally, incubation of oxidosqualene **3-(6*S*,7*S*)** with purified SE led to the formation of dioxidosqualene **22-(3*S*,6*S*,7*S*)**, whereas its regioisomer **23-(3*S*,18*S*,19*S*)** was not detected. In contrast, incubation of epoxide **3-(6*R*,7*R*)** under the same conditions afforded a mixture of dioxides **22-(3*S*,6*R*,7*R*)** and **23-(3*S*,18*R*,19*R*)** in a 5:12 molar ratio. The fact that oxidosqualenes **3** and **4** have been found in nature, and our previous results showing that racemic dioxide **23** is a potent inhibitor of oxidosqualene–lanosterol cyclase in rat liver microsomes (Abad, J. L.; et al. *J. Org. Chem.* **1993**, *58*, 3991), confers a potential physiological relevance to the results reported herein.

Introduction

The epoxidation of squalene to (3*S*)-2,3-oxidosqualene promoted by squalene epoxidase (SE, EC 1.14.99.7) and the subsequent cyclization of this epoxide catalyzed by oxidosqualene–lanosterol cyclase (OSLC, EC 5.4.99.7) to give lanosterol are key steps in cholesterol biosynthesis.[1,2] This epoxide could also undergo enzyme-mediated cyclizations in plants and fungi.[3] Therefore, a search for good inhibitors of SE activity has been undertaken by groups working on hypocholesteremic drugs, as well as those interested in compounds with herbicidal or antifungal activity. Among the SE inhibitors reported to date,[4] a group of allylamine derivatives, which was developed initially for obtaining antifungal agents,[5] has given rise more recently to very potent mammalian SE inhibitors, such as NB-598.[6] These compounds show no obvious structural resemblance to squalene, which suggests that they might not act as substrate analogs. On the other hand, a number of squalene analogs containing different functionalities at the terminal ends of the squalene chain have also shown SE inhibitory activity. In addition to high-selectivity tight-binding compounds for vertebrate SE,[7] potent competitive[8] and mechanism-based inhibitors[9] have been described.

Another possibility for finding SE and OSLC inhibitors that has been less explored pertains to squalene derivatives potentially occurring as endogenous compounds. Although (3*S*)-2,3-oxidosqualene is the epoxy derivative involved in the above biosynthetic pathways, the occurrence or putative formation of internal oxidosqualene derivatives **3** and **4** (Scheme 1) has also been investigated. Thus, oxidosqualenes **3-(6*S*,7*S*)** and **4-(10*S*,11*S*)** had been isolated from the green algae *Caulerpa prolifera*,[10] whereas the enantiomer of the latter epoxide, i.e.,

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† Dedicated to the memory of Professor Felix Serratosa.

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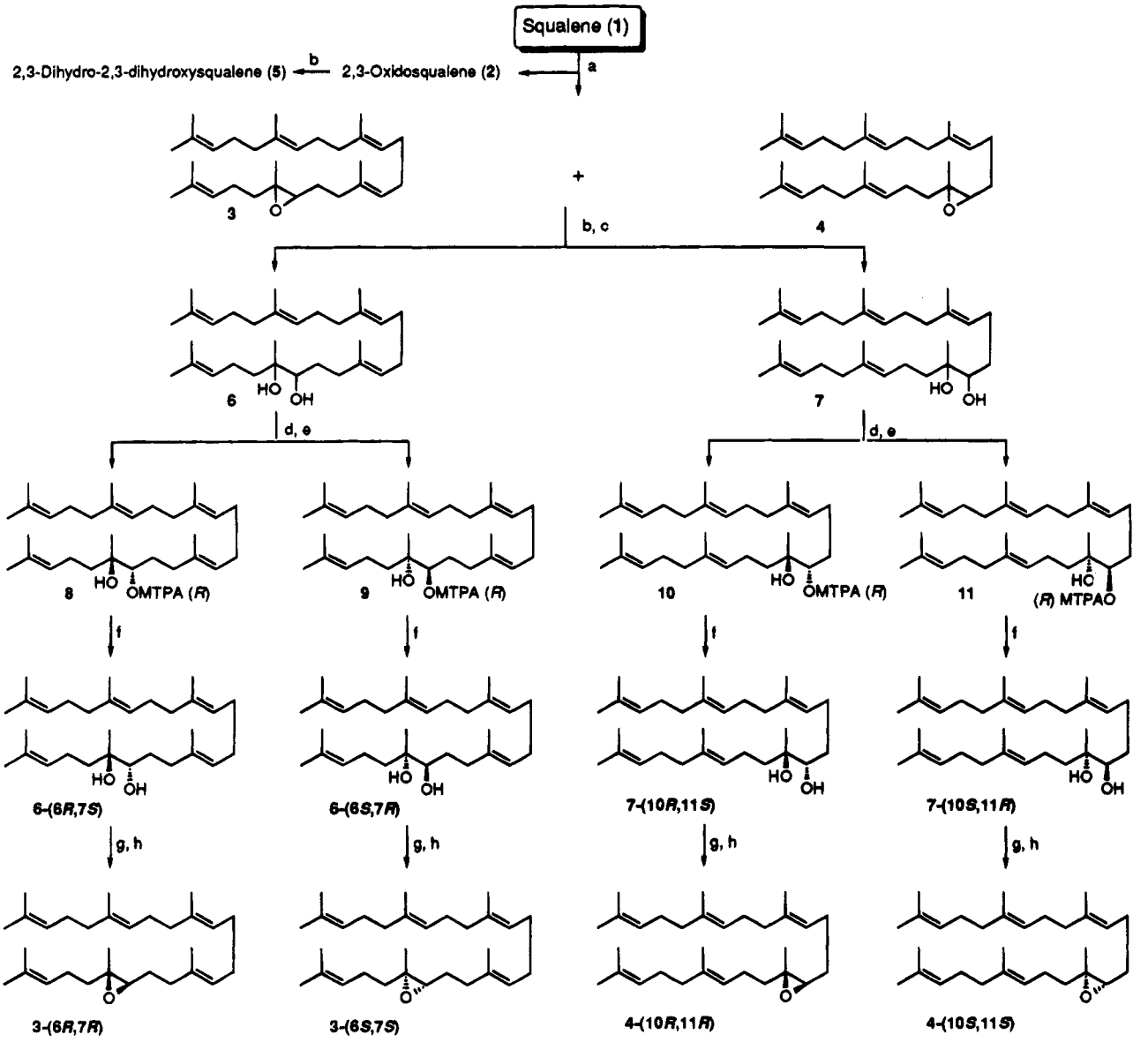
Scheme 1[a]



[a] (a) MCPBA, CH_2Cl_2; (b) $HClO_4$, THF, H_2O; (c) flash chromatography, SiO_2, 6% $AgNO_3$; (d) (*S*)-MTPA Cl, Et_3N, DMAP, CH_2Cl_2; (e) semipreparative reversed-phase HPLC; (f) $LiAlH_4$, Et_2O; (g) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (h) NaH, THF.

4-(10*R*,11*R*), was isolated from the red algae *Sclerotinia fructicola* and its structure confirmed by asymmetric synthesis.[11,12] On the other hand, the implication of 2,3:22,23-dioxidosqualene in steroid biosynthesis and the biological significance of this finding have been studied in detail.[13]

In this context, we described the preparation of internal oxidosqualenes and all possible dioxidosqualenes as racemates and evaluated their activity as inhibitors of OSLC in rat liver microsomes. We found that 2,3:18,19-dioxidosqualene (**23**), a compound that could be formed from the epoxidation of 6,7-oxidosqualene, elicited a high activity as OSLC inhibitor (IC_{50} = 0.11 μM).[14,15] More recently, we reported that racemic 6,7-oxidosqualene (**3**) is a substrate of rat liver microsomal SE, leading to the epoxidation at both terminal double bonds of the squalene chain of this oxide.[16] However, the sterochemical requirements operating on the SE action on the different enantiomers of oxidosqualene **3** were still unknown. In addition, we were also interested in assessing the potential inhibitory activity that these stereoisomers could exert on the SE and in identifying the diastereomers of dioxidosqualenes **22** and **23** that could be produced. In this context, the recent availability of partially purified SE from pig liver[17] would faciitate this study.

In the present paper, we report on the preparation and determination of the absolute configuration of oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and

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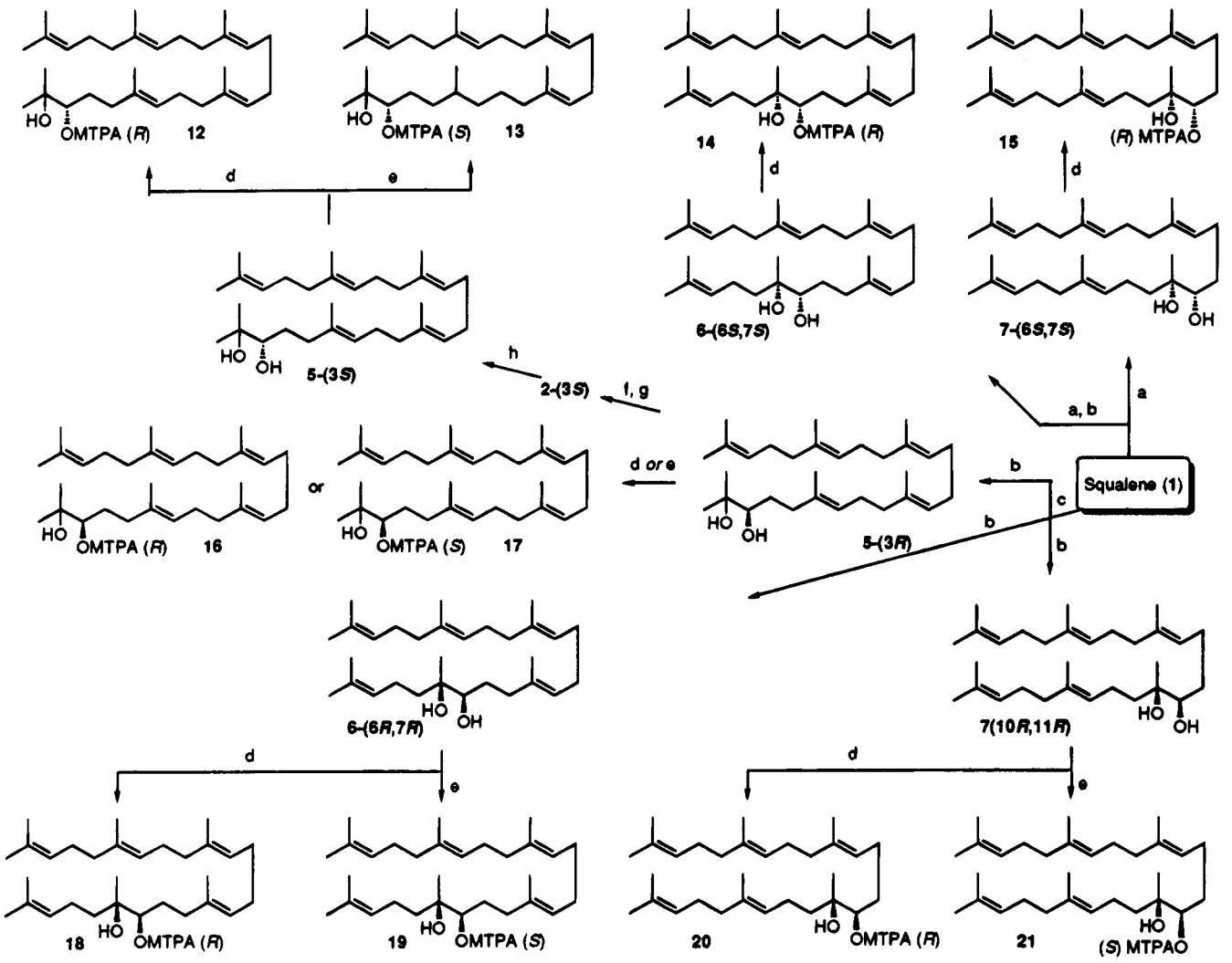
Scheme 2[a]



[a] (a) $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, K_2OsO_4, ^tBuOH, H_2O, 0 °C; this reaction leads to the formation of diol 5-(3*S*), which compound was obtained with better yields and purity by using the sequence involving steps f–h; (b) flash chromatography, SiO_2, 6% $AgNO_3$, (c) $(DHQD)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3, $CH_3SO_2NH_2$, K_2OsO_4, ^tBuOH, H_2O, 0 °C; (d) (*S*)-MTPA Cl, Et_3N, DMAP, CH_2Cl_2; (e) (*R*)-MTPA Cl, Et_3N, DMAP, CH_2Cl_2; (f) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (g) NaH, THF; (h) $HClO_4$, THF, H_2O.

4-(10*S*,11*S*) (Scheme 1). For this purpose, the Mosher (MTPA) esters of the squalenediols derived from the above epoxides have been prepared and their absolute configuration has been determined by ^1H NMR. A parallel study has been carried out with the MTPA esters derived from the *threo* squalenediols originated from the Sharpless asymmetric dihydroxylation of squalene (Scheme 2). In addition, results on the biological activity of the oxidosqualenes **3** and **4** as inhibitors of partially purified SE from pig liver are presented. These results are complemented by the identification of the dioxidosqualene derivatives formed after the incubation of the epoxides **3-(6*R*,7*R*)** and **3-(6*S*,7*S*)** with purified SE. Finally, the potential physiological significance of these findings is discussed.

Results and Discussion

Preparation and Determination of the Absolute Configuration of Internal Oxidosqualenes. Initially, the preparation of oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and **4-(10*S*,11*S*)** (Scheme 1) was assayed by a synthetic sequence involving the Sharpless asymmetric dihydroxylation of squalene, which was expected to provide the homochiral *threo* squalenediols **5**, **6**, and **7** with (*R*) or (*S*) configuration, depending upon the chiral ligand used.[18] However, all attempts to achieve the inversion of the secondary carbinol on the above *threo* diols by using the Mitsunobu approach were unsuccessful.[19] Nevertheless, these *threo* squalenediols (Scheme 2) were used as model compounds for the preliminary studies carried out to determine the absolute configuration of the above oxidosqualenes.

We then turned our attention to the strategy involving the resolution of the *erythro* squalenediols **6** and **7** by esterification with a chiral acid. This approach had been reported for epoxide **5** using a steroid as the chiral derivatization reagent.[20] In our case, it was anticipated that the use of Mosher acids as chiral reagents could be attractive for two reasons. First, MTPA esters are usually good substrates for resolution by chromatographic means; second, they had also been used by the group of Kakisawa for the determination by ^1H NMR of the absolute configuration of secondary carbinols present

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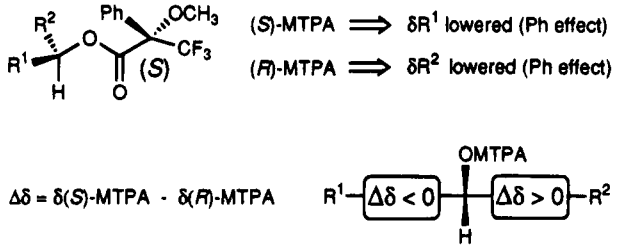
Scheme 3 Preferred Conformation for MTPA Esters Proposed by Mosher and a Model To Determine the Absolute Configuration of Secondary Alcohols According to the Kakisawa Rule[a]

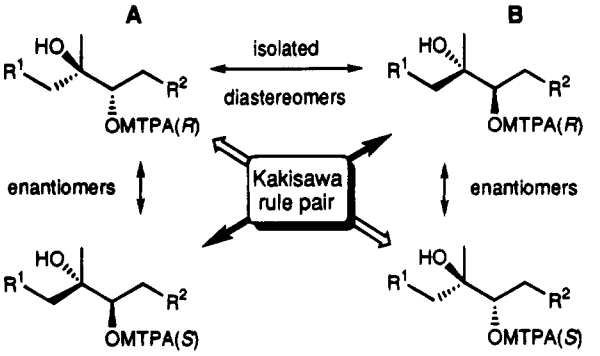


[a] cf. ref 21.

in natural products.[21] Thus, the preparation of oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and **4-(10*S*,11*S*)** was carried out by using this strategy (Scheme 1). The mixture of *erythro* squalenediols **6** was converted into the corresponding (*R*)-MTPA esters **8** and **9**, which were separated by semipreparative HPLC. Reduction of these esters led to the enantiomerically enriched diols **6-(6*R*,7*S*)** or **6-(6*S*,7*R*)**, which were converted into their respective oxidosqualenes **3-(6*R*,7*R*)** or **3-(6*S*,7*S*)** by mesylation and treatment with base. The same procedure was used for the preparation of the enantiomerically enriched epoxides **4-(10*R*,11*R*)** and **4-(10*S*,11*S*)** from the corresponding mixture of *erythro* squalenediols **7**. The ee values for MTPA esters **8**–**11** (92–94% for all cases) were determined by HPLC. The enantiomeric purity of the oxidosqualenes was determined by their conversion into the corresponding diols and subsequent formation of MTPA esters. A racemization lower than 5% was observed for the conversion of the chiral squalenediol into the epoxy derivative and vice versa.

To our knowledge, the Kakisawa rule for the determination of the absolute configuration of secondary carbinol stereogenic centers (Scheme 3) has been successfully applied to natural products[22–26] or to a single stereoisomer obtained by synthesis.[27] In these cases, the strategy involves the preparation of esters from both (*R*)- and (*S*)-Mosher acids. However, we were faced with another situation commonly encountered in compounds from synthetic origin, i.e., mixtures of enantiomers which can be resolved by means of a chiral auxiliary. Our strategy involved the formation of diastereomer esters from the (*R*)-Mosher acid and the *erythro* squalenediols **6** or **7** (Scheme 1). Thus, for each pair of resolved esters **8** and **9** and **10** and **11**, the stereochemical difference did not reside in the Mosher acyl moiety but in the carbinol center. However, since enantiomers must exhibit identical spectroscopic features, the NMR spectra of ester **8** should be identical to those of the ester derived from diol

Scheme 4



6-(6*S*,7*R*) and (*S*)-Mosher acid. A similar conclusion could be extended to the other MTPA esters **9**–**11**.

This formal equivalence would permit us to reformulate the Kakisawa rule in a useful way for preparative chemists. By this approach, the MTPA esters obtained from racemates containing secondary carbinol centers and a single enantiomer of Mosher acid, for instance the (*R*)-acid, could be used. Once resolved, the ^{1}H NMR spectra for both diastereomers could be registered, the chemical shifts for as many protons as possible with respect to each of the diastereomers could be assigned, and the chemical shift differences ($\Delta\delta = \delta_A - \delta_B$) could be obtained. In fact, chemical shifts δ_A are also the chemical shifts for the enantiomer of A, which has the same configuration as B at the carbinol center buthe opposite at the Mosher moiety (see Scheme 4). Therefore, the above $\Delta\delta$ values are identical to those that would have been obtained from the application of the Kakisawa procedure to compound B, which would allow for the determination of its absolute stereochemistry. Obviously, the absolute configuration of the other formally replaced diastereomer should be the opposite one.

Nevertheless, esters **8**–**11** contained structural features, i.e., they are long prenyl molecules with high conformational mobility,[28] that could lead to erratic chemical shift differences between diastereomers **8** and **9** or **10** and **11**, thus impeding the applicability of the above rule. For this reason, we decided to test the method by using the MTPA esters derived from the *threo* squalenediols **5-(3*R*)**, **5-(3*S*)**, **6-(6*R*,7*R*)**, **6-(6*S*,7*S*)**, **7-(10*R*,11*R*)**, and **7-(10*S*,11*S*)** (Scheme 2). Since these diols had been obtained by using the Sharpless asymmetric dihydroxylation, their absolute configuration was already known.

The results obtained from the ^{1}H NMR analysis of (*R*)- and (*S*)-MTPA esters **16**–**21** derived from homochiral squalenediols with (*R*) configuration are depicted in Scheme 5. As shown, a monotone regularity for the differences in chemical shifts between compounds with an (*S*) configuration at the Mosher acyl moiety with respect to those with an (*R*) configuration was obtained. In all cases, the application of the Kakisawa rule confirmed the expected (*R*) configuration for the carbinol stereogenic center. The same type of analysis was performed for the (*R*)-MTPA ester **12** and the (*S*)-MTPA ester **13**, derived from squalenediols with an (*S*) configuration. Again, the results obtained were in agreement with these assignments. Finally, the spectroscopic identity among pairs of enantiomers (i.e., **14** vs **19** and **15** vs

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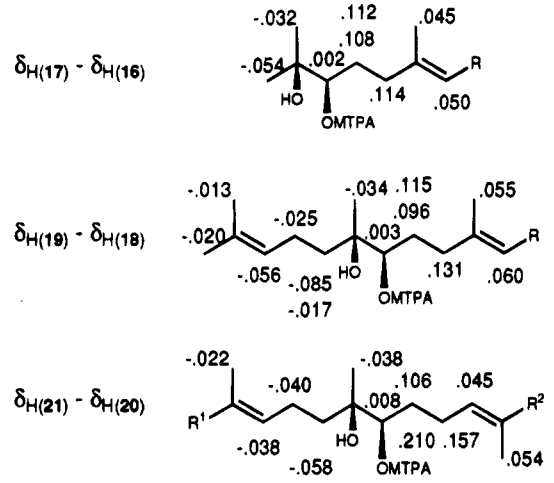
Scheme 5 ^{1}H NMR Chemical Shift Differences between (*S*)- and (*R*)-MTPA Esters 17 and 16, 19 and 18, and 21 and 20 Derived from Squalenediols 5-(3*R*), 6-(6*R*,7*R*), and 7-(10*R*,11*R*), Respectively, Expressed in ppm



21) led to the assignment of the (*S*) configuration to the carbinol centers present in esters **14** and **15**.

Once the method was tested with the esters derived from *threo* squalenediols, the ^{1}H NMR analysis of the (*R*)-MTPA esters **8**−**11** derived from the *erythro* squalenediols was carried out and the results obtained are shown in Scheme 6. In this case, we had two diastereomers with a fixed (*R*) configuration at the Mosher acyl moiety but with unknown (*R*) or (*S*) configurations at the stereogenic centers of the squalene framework. However, assuming the formal enantiomer change proposed above, the absolute configuration of these stereogenic centers for the pairs of esters **8** and **9** and **10** and **11** was determined unambiguously.

From these assignments, the corresponding correlation with their structurally related *erythro* squalenediols and oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and **4-(10*S*,11*S*)** could be carried out (cf. Scheme 1). On the other hand, the reversed phase HPLC analysis of the above MTPA esters revealed that, when using the (*R*)-Mosher acid, compounds with an (*S*) configuration at the secondary carbinol center, i.e., **8**, **10**, and **12**, eluted earlier than their corresponding diastereomers, **9**, **11**, and **16**, respectively. The same elution order was observed for the (*R*)-MTPA esters derived from the *threo* squalenediols.

Inhibition of SE Activity. SE assays with oxidosqualenes **3-(6*R*,7*R*)**, **3-(6*S*,7*S*)**, **4-(10*R*,11*R*)**, and **4-(10*S*,11*S*)** were carried out using the partially purified enzyme from pig liver and the procedure described by Bai and Prestwich.[17] This procedure is based on the amount of radioactive 2,3-squalene oxide formed by incubation of [1,25-^{14}C]squalene in the presence of the added effector. Results obtained on the inhibition of SE activity are shown in Table 1. Among the pair of 6,7-oxidosqualenes **3**, **3-(6*S*,7*S*)** elicited a high inhibitory activity (IC_{50} = 6.7 μM, which is approximately the same concentration of substrate used in the assay). Conversely, a 15% inhibition percentage was found for **3-(6*R*,7*R*)** at the highest concentration tested (28 μM). Likewise, only one of the corresponding pair of the 10,11-oxidosqualenes, i.e., **4-(10*R*,11*R*)**, elicited SE inhibitory activity (IC_{50} = 25 μM). Its enantiomer **4-(10*S*,11*S*)** showed a 20% inhibition at the highest concentration tested (56 μM).

A survey of the different SE inhibitors reported to date that are squalene analogs[4] reveals that they contain structural modifications at the terminal prenyl units of the chain, with the exception of the 26-functionalized derivatives reported by Bai et al.[8] In this case, the modification is at the CH_3 group located on the second prenyl moiety. The activities elicited by oxidosqualenes **3-(6*S*,7*S*)** and **4-(10*R*,11*R*)** show that functionalization on the carbon framework of either the second or the third prenyl unit of squalene can cause inhibition responses. On the other hand, it should be remarked that the highest SE inhibitory activities were elicited by two internal oxidosqualene stereoisomers that are also present in living organisms, i.e., **3-(6*S*,7*S*)**, isolated from green algae,[10] and **4-(10*R*,11*R*)**, obtained from red algae.[11,12]

From the kinetic data obtained for the most potent inhibitory squalene oxide assayed, i.e., **3-(6*S*,7*S*)**, it was shown that the inhibition elicited was competitive with respect to squalene (K_i = 2.7 μM, Figure 1). This activity is comparable to that reported for the more potent competitive SE inhibitors described so far,[17,29] although it is lower than that found for the best SE inhibitors, which belong mainly to the noncompetitive class.[6,7,9,17,30,31]

Finally, we investigated the putative formation of dioxidosqualenes[15] in the incubation of epoxides **3-(6*S*,7*S*)** or **3-(6*R*,7*R*)** with purified SE (Scheme 7). Dioxide **22** (26 nmol) was detected as a major compound from the incubation mixture containing oxidosqualene **3-(6*S*,7*S*)**. The (3*S*,6*S*,7*S*) configuration was assigned to this dioxide assuming the accepted stereochemical course for SE epoxidations at the terminal double bond of the squalene skeleton. This assumption had been confirmed in our preliminary incubation experiments carried out with racemic oxidosqualene **3**.[16] On the other hand, the presence of dioxide **23**, which would result from the epoxidation of epoxide **3-(6*S*,7*S*)** at the distal prenyl moiety, was not detected. It should be remarked that dioxides **23** are more stable than their regioisomers **22**;[15] thus, the nondetection of **23** in the above incubation was not due to the decomposition of this compound under the assay conditions. These results indicate that a highly regiochemical epoxidation had occurred in this instance. Conversely, incubation of epoxy derivative **3-(6*R*,7*R*)** led to the formation of 17 nmol of the mixture of dioxides **22-(3*S*,6*R*,7*R*)** and **23-(3*S*,18*R*,19*R*)** in a 5:12 molecular ratio. From the comparison of both incubations, it was observed that the epoxidation of oxidosqualene **3-(6*S*,7*S*)** took place at a higher rate than that of its enantiomer. Therefore, it is presumable that the amount of epoxy derivative **3-(6*S*,7*S*)** present in the incubated sample of **3-(6*R*,7*R*)**, which had a 92% ee, could account for a nonnegligible percentage of the dioxide **22** detected in this assay.

The only case reported in the literature of a compound being a strong competitive inhibitor and a substrate for SE is the 26-hydroxysqualene mentioned above. This alcohol showed an IC_{50} of 10 μM (K_i = 4 μM) for pig liver SE.[8] These values are in the same order as those found for oxidosqualene **3-(6*S*,7*S*)**. In addition, incubation of this 26-hydroxysqualene derivative with purified pig liver SE afforded a 3:1 mixture of the proximal 2,3- and distal

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Scheme 6 ^{1}H NMR Chemical Shifts of the *Erythro* (*R*)-MTPA Esters 8–11 Expressed in ppm

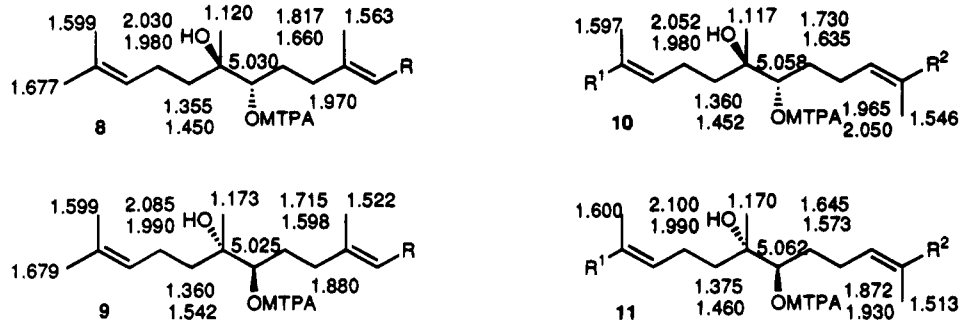

Table 1. IC_{50} and K_i Values of Squalene Epoxidase Inhibition for Internal Oxidosqualenes[a]

compound	IC_{50} (μM)	K_i (μM)
3-(6*R*,7*R*)	>28	
3-(6*S*,7*S*)	6.7	2.7
4-(10*R*,11*R*)	25	
4-(10*S*,11*S*)	>56	

[a] For assay details and concentrations of inhibitors used, see the Experimental Section.

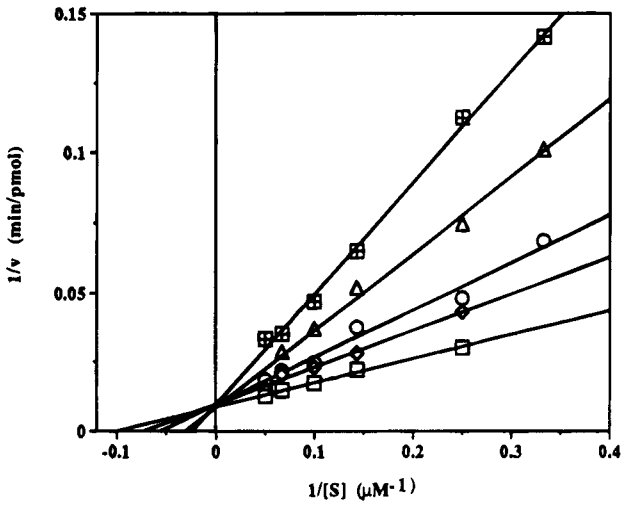

Figure 1. Lineweaver–Burk plot of the inhibition of squalene epoxidase by 6(*S*),7(*S*)-oxidosqualene. The concentrations of inhibitor used were 0 (squares), 1.75 (diamonds), 3.5 (circles), 7 (triangles), and 10.5 (grilled squares) μM.

22,23-epoxy derivatives, respectively.[8] The preference for epoxidizing the proximal end was also shown by oxidosqualene **3-(6*S*,7*S*)**, which suggests that a similar interaction with the enzyme could be occurring for both inhibitors. Conversely, the epoxidation at the distal prenyl unit was favored in the incubation using the weaker SE inhibitor **3-(6*R*,7*R*)**. This result might also be of biological relevance since it shows that, in in vitro conditions, definite stereoisomers of dioxide **23**, a compound that as a racemate had elicited a potent OSLC inhibitory activity, can be formed.[15] The identification of the precise enantiomers of **23** which are responsible for this inhibition would help to clarify the importance of the above findings.

In conclusion, the preparation and determination of the absolute configuration for the internal oxidosqualene stereoisomers have been carried out. For this latter purpose, we have adapted the ^{1}H NMR procedure developed by the group of Kakisawa[21] to the case of enantiomer mixtures from synthetic origin. Thus, the formation of (*R*)-MTPA esters of the diastereomeric mixtures of *erythro* internal squalenediols allowed 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. To our knowledge, this is the first report of such a strategy for acyclic compounds with high conformational mobility. On the other hand, the availability of these diol and epoxy derivatives brings up valuable tools for exploring the potential roles of internal oxidosqualenes on the two enzymatic systems involved in the transformation of squalene into a steroid. In this context, the results obtained from the incubations of internal oxidosqualenes **3** and **4** with purified pig liver SE have shown the remarkable competitive inhibitory activity elicited by oxidosqualene **3-(6*S*,7*S*)**. In addition, it has been observed that both oxidosqualenes are substrates for SE activity, although the epoxidation takes place with different regioselectivity with respect to the proximal or distal terminal prenyl moiety. The fact that epoxides **3** and **4** have been found in nature confirms the possibility that they could be formed under certain physiological conditions and confers a potential relevance to the results reported herein.

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 the ionization gas and a 30 m HP-5 bonded phase capillary column (0.25 mm id). The liquid chromatography–thermospray–mass spectrometry analyses (HPLC–TSP–MS) were performed 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 grams per liter, 10 cm cell). The enantiomeric excess (ee) values were calculated by HPLC analysis of the corresponding (*R*)- or (*S*)-MTPA ester derivatives. The elemental analyses were performed at the Microanalysis Service, CID.

NMR Spectra. The NMR spectra (^{1}H, 300 MHz; ^{13}C, 75 MHz; ^{19}F, 282 MHz) were recorded with a four-pretuned nucleus auto NMR probe. All spectra were recorded 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[32] spectra recorded for the determination of the absolute configuration of MTPA esters **8**–**21** were obtained at 25 °C, with an acquisition resolution of 3 Hz (0.333 s acquisition time) in the first dimension and the sufficient number of increments to render a resolution of 4 Hz in the second dimension, using a

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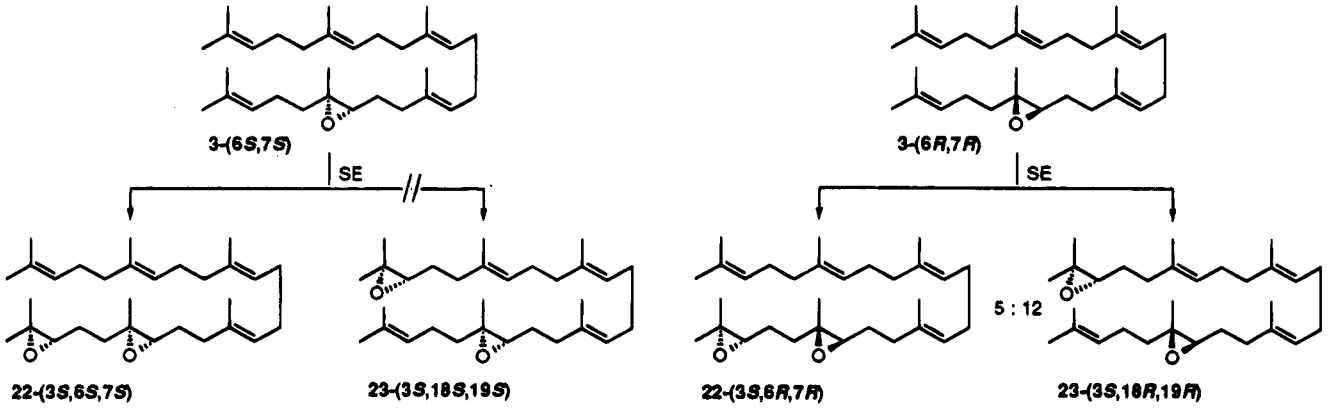
Scheme 7 Dioxidosqualenes Detected in the Incubation of Oxidosqualenes 3-(6S,7S) or 3-(6R,7R) with Purified Pig Liver Squalene Epoxidase



5 ppm spectral window (from olefin to methyl resonance range). In all cases, data matrices were zero-filled until 2 × 2 K points and transformed using a sinebell window function. XH-CORFE spectra[33] were obtained in a similar way by using a window for ^{13}C that includes only the sp^3 carbons and the above-described window for ^1H. Acquisition resolutions were adjusted to 4 and 7 Hz, respectively, and the experiment was optimized to a $^1J_{H,C}$ = 120 Hz. The Fourier transforms were performed over a matrix of 4 × 2 K data points and using a sinebell window function. The ^{19}F NMR spectra were obtained with the standard spectral window and with a 1 s acquisition time.

Compounds. Unless otherwise stated, organic solutions obtained from 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 different percentages of $AgNO_3$ to improve the resolution or Et_3N to minimize decomposition. An indication before the composition of the eluent mixture used denotes the cases where these procedures were utilized. 2,3-Oxidosqualene (**2**)[34] was prepared by treatment of squalene with NBS in THF/H_2O[35] followed by reaction of the purified bromohydrin with NaH in THF. Racemic 6,7-oxidosqualene (**3**) and 10,11-oxidosqualene (**4**) were prepared by epoxidation of squalene with *m*-CPBA as described elsewhere.[15]

6(*R),7(*S**)-Dihydroxy-6,7-dihydrosqualene (6) and 10(*R**),11(*S**)-Dihydroxy-10,11-dihydrosqualene (7).** These compounds were prepared from the above mixture of oxidosqualene (1 g, 2.4 mmol) by using the procedure reported by Abdallah and Shah.[36] Purification of the crude reaction mixture (elution with 10:1 hexane/EtOAc) afforded 0.93 g (86%) of the mixture of diols **6** and **7**. A 0.40 g aliquot of this mixture was repurified (6% $AgNO_3$, elution with 9:1 hexane/EtOAc) to give 0.19 g of diol **6** and 0.17 g of diol **7** as pure products. **6(*R**),7(*S**)-Dihydroxy-6,7-dihydrosqualene (6):** ^1H NMR δ 5.28–5.02 (5 H), 3.38 (br, 1 H), 2.4–1.9 (16 H), 1.68 (s, 6 H), 1.63 (s, 3 H), 1.62 (d, 3 H, *J* = 1 Hz), 1.60 (s, 9 H), 1.8–1.2 (4 H), 1.17 (s, 3 H); ^{13}C NMR δ 135.3, 135.1, 135.0, 131.9, 131.3, 125.1, 124.6, 124.3, 124.1, 124.1, 78.3, 74.6, 39.7, 39.7, 37.0, 35.8, 29.3, 28.2, 28.2, 26.7, 26.6, 25.7, 25.7, 23.4, 22.1, 17.7, 16.0, 16.0, 15.9; MS *m/z* 445 (M + 1), 427 (M − 18 + 1), 137 (base peak). **10(*R**),11(*S**)-Dihydroxy-10,11-dihydrosqualene (7):** ^1H NMR δ 5.28–4.98 (5 H), 3.38 (ddd, 1 H, J_1 = 11.5 Hz, J_2 = 4.5 Hz, J_3 = 2 Hz), 2.4–1.9 (16 H), 1.68 (s, 6 H), 1.64 (s, 3 H), 1.63 (s, 3 H), 1.60 (s, 9 H), 1.9–1.26 (4 H), 1.17 (s, 3 H); ^{13}C NMR δ 136.2, 135.5, 135.1, 131.4, 131.3, 124.4, 124.3, 124.2, 124.0, 123.8, 78.3, 74.7, 39.7, 39.7, 35.7, 31.2, 26.7, 26.6, 26.6, 25.7, 25.7, 25.2, 23.4, 22.0, 17.7, 17.7, 16.1, 16.0, 16.0; MS *m/z* 445 (M + 1), 427 (M − 18 + 1), 137 (base peak).

Asymmetric Dihydroxylation of Squalene. Preparation of Diols 5-(3*R*), 6-(6*R*,7*R*), and 7-(10*R*,11*R*). These compounds were prepared by using the procedure reported by Crispino and Sharpless.[18] Thus, treatment of 4.1 g (10 mmol) of **1** with the mixture of reagents containing the ligand $(DHQD)_2$-PHAL for 4 days at 0 °C led to a crude reaction mixture which was purified as described above to give 0.35 g (8% yield) of diol **5-(3*R*)**, 0.25 g of diol **6-(6*R*,7*R*)**, and 0.11 g of diol **7-(10*R*,11*R*)**. **2,3(*R*)-Dihydroxy-6,7-dihydrosqualene:**[37] $[\alpha]_D$ +10.8 (*c* = 1.5, 90% ee). **6(*R*),7(*R*)-Dihydroxy-6,7-dihydrosqualene:** ^1H NMR δ 5.30–5.01 (5 H), 3.42 (d, 1 H, *J* = 10 Hz), 2.35–1.86 (16 H), 1.68 (s, 6 H), 1.62 (s, 6 H), 1.60 (s, 9 H), 1.80–1.10 (4 H), 1.12 (s, 3 H); ^{13}C NMR δ 135.3, 135.0, 134.9, 132.0, 131.2, 125.1, 124.4, 124.3, 124.2, 124.1, 76.9, 74.9, 39.7, 38.7, 36.9, 29.5, 28.3, 28.2, 26.8, 26.6, 25.7, 22.0, 21.0, 17.7, 16.0, 16.0; MS *m/z* 445 (M + 1), 427 (M − 18 + 1), 137 (base peak); $[\alpha]_D$ +11.8 (*c* = 0.5, 90% ee, $CHCl_3$). **10(*R*),11(*R*)-Dihydroxy-10,11-dihydrosqualene:** ^1H NMR δ 5.24–5.02 (5 H), 3.45 (ddd, 1 H, J_1 = 10 Hz, J_2 = 4 Hz, J_3 = 2.5 Hz), 2.35–1.88 (16 H), 1.68 (s, 6 H), 1.64 (s, 3 H), 1.62 (s, 3 H), 1.60 (s, 9 H), 1.75–1.15 (4 H), 1.11 (s, 3 H); ^{13}C NMR δ 136.2, 135.7, 135.1, 131.5, 131.3, 124.3, 124.2, 124.1, 123.8, 76.8, 75.0, 39.7, 39.7, 38.8, 31.6, 31.4, 26.8, 26.6, 25.7, 25.1, 22.7, 21.9, 20.9, 17.7, 16.1, 15.9; MS *m/z* (relative intensity) 445 (M + 1, 2), 427 (M − 18 + 1), 137 (base peak); $[\alpha]_D$ +11.0 (*c* = 1, 88% ee).

Preparation of Diols 6-(6*S*,7*S*) and 7-(10*S*,11*S*). These compounds were obtained from squalene (1.64 g, 4 mmol) by using the ligand $(DHQ)_2$-PHAL (10 days at 0 °C). In this case, purification of the crude reaction mixture containing the internal diols (120 mg) was carried out as described above for the corresponding enantiomers to render **6-(6*S*,7*S*)** in 65% ee and **7-(10*S*,11*S*)** in 60% ee.[18] All these ee values were calculated by conversion to the corresponding MTPA ester derivatives.

2,3(*S*)-Dihydroxy-6,7-dihydrosqualene [5-(3*S*)]. A solution of diol **5-(3*R*)** (70 mg, 160 μmol) and Et_3N (65 μL, 170 μmol) in CH_2Cl_2 (2 mL) was treated with CH_3SO_3Cl (13 μL, 170 μmol), and the mixture was stirred under argon for 1 h at −10 °C. Then, the solvent and the excess of reagents were removed under N_2, and the residue was redissolved in hexane and concentrated. The new residue was redissolved in THF (5 mL) and treated with excess NaH (5 molar equiv) for 16 h at 20 °C (TLC monitoring). The crude reaction mixture was filtered over Celite and concentrated to give a residue which after purification (2.5% Et_3N, elution with 98:2 hexane/EtOAc) afforded 55 mg (80% yield) of **2,3(*S*)-squalene [2-(3*S*)]**: $[\alpha]_D$ −1.6 (*c* = 1, 84% ee).[37] A solution of this epoxide (45 mg) in 5

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(35) Nadeau, R. G.; Hanzlik, R. P. In *Methods in Enzymology*; Clayton, R. B., Ed.; Academic Press: New York, 1969; Vol. 15, pp 346–351.
(36) Abdallah, M. A.; Shah, J. N. *J. Chem. Soc., Perkin Trans. 1* **1975**, 888–894.
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mL of a 4:1 mixture of THF/H_2O was treated with 10 μL of 60% $HClO_4$ for 2 h at 20 °C. The purification of the crude reaction mixture (elution with 85:15 hexane/EtOAc) afforded 37 mg (80% yield) of diol **5-(3S)** (82% ee).[37]

Preparation of MTPA Esters 8–21. General Procedure.[38] The corresponding Mosher acid chloride (0.6 mmol) was added, at 20 °C and under argon, to a solution of the appropriate squalenediol (22 mg, 50 μmol), Et_3N (20 μL, 150 μmol), and DMAP (6 mg, 20 μmol) in CH_2Cl_2 (2 mL). The mixture was stirred until the reaction was complete (TLC monitoring). Then, the solvent was evaporated and the residue purified (elution with 96:4 hexane/EtOAc) to give the expected esters in 90–95% yields. In the case of pairs of enantiomers, full spectroscopic data are reported for the first enantiomer described, whereas only the ee and the $[\alpha]_D$ values are given for the second.

Esters 8 and 9. This diastereomeric mixture was obtained from diol **6** and (*S*)-MTPA chloride and purified by semipreparative HPLC (15 × 1 cm ODS-2 column, 5 μm, eluting with 87:13 CH_3CN/H_2O at 3.2 mL/min), followed by flash chromatography of each collected diastereomer: IR (film, diastereomeric mixture) 3515, 2965, 2925, 2855, 1745, 1450, 1375, 1265, 1185, 1170, 1125, 1020 cm^{-1}. **Ester 8** (lower retention time in HPLC): $[\alpha]$ +10.6 (*c* = 1, (99% ee); ^1H NMR δ 7.66–7.58 (2 H), 7.44–7.36 (3 H), 5.20–5.00 (5 H), 5.02 (dd, 1 H, J_1 = 9.5 Hz, J_2 = 2 Hz), 3.59 (s, 3 H), 2.14–1.90 (16 H), 1.90–1.15 (4 H), 1.68 (s, 6 H), 1.60 (s, 12 H), 1.56 (d, 3 H, *J* = 1 Hz), 1.12 (s, 3 H); ^{13}C NMR δ 166.2, 135.2, 134.9, 133.8, 132.2, 132.1, 131.2, 129.6, 128.4, 127.4, 125.3, 124.4, 124.2, 124.1, 124.0, 123.5 (q, CF_3, J_{C-F} = 288 Hz), 84.4 (q, C, J_{C-F} = 28 Hz), 82.0, 74.1, 55.5, 39.7, 36.9, 36.3, 28.4, 28.3, 28.1, 26.9, 26.7, 26.6, 25.7, 23.2, 21.7, 17.6, 17.6, 16.0, 16.0, 15.9; ^{19}F NMR δ −71.26; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 [M − 217 (Mosher acyl group) + 1]. **Ester 9** (higher retention time in HPLC): $[\alpha]_D$ +16.0 (*c* = 1, 98% ee); ^1H NMR δ 7.68–7.58 (2 H), 7.46–7.35 (3 H), 5.20–5.02 (6 H), 5.02 (dd, 1 H, J_1 = 10 Hz, J_2 = 1 Hz), 3.55 (s, 3 H), 2.16–1.92 (14 H), 1.88 (t, 2 H, *J* = 8 Hz), 1.68 (s, 6 H), 1.60 (s, 9 H), 1.52 (s, 3 H), 1.80–1.2 (4 H), 1.17 (s, 3 H); ^{13}C NMR δ 166.7, 135.2, 134.9, 133.8, 132.2, 132.1, 131.2, 129.6, 128.4, 127.7, 125.2, 124.4, 124.2, 124.1, 123.5 (q, CF_3, J_{C-F} = 288 Hz), 84.7 (q, C, J_{C-F} = 28 Hz), 82.2, 74.3, 55.4, 39.7, 36.3, 36.0, 28.3, 28.3, 28.1, 26.8, 26.7, 25.7, 23.9, 21.7, 17.7, 17.6, 16.0, 16.0, 15.9; ^{19}F NMR δ −71.08; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1). Anal. Calcd for $C_{40}H_{59}F_3O_4$ (as a mixture of diastereomers **8** and **9**): C, 72.69; H, 9.00. Found: C, 72.75; H, 9.09.

Esters 10 and 11. This diastereomeric mixture was obtained from diol **7** and (*S*)-MTPA chloride and purified by semipreparative HPLC and flash chromatography as described above: IR (film, diastereomeric mixture) 3500, 2965, 2925, 2855, 1745, 1450, 1375, 1265, 1185, 1170, 1125, 1020 cm^{-1}. **Ester 10** (lower retention time in HPLC): $[\alpha]_D$ +9.3 (*c* = 1, 98% ee); ^1H NMR δ 7.68–7.55 (2 H), 7.45–7.33 (3 H), 5.18–4.98 (6 H), 3.59 (s, 3 H), 2.16–1.88 (16 H), 1.82–1.22 (4 H), 1.68 (d, 6 H, *J* = 1 Hz), 1.60 (s, 12 H), 1.55 (d, 3 H, *J* = 1 Hz), 1.12 (s, 3 H); ^{13}C NMR δ 166.3, 136.5, 135.9, 135.1, 132.2, 131.5, 131.3, 129.6, 128.4, 127.4, 124.3, 124.2, 124.1, 123.9, 123.4 (q, CF_3, J_{C-F} = 288 Hz), 122.8, 84.5 (q, C, J_{C-F} = 28 Hz), 82.1, 74.2, 55.5, 39.7, 36.9, 29.9, 26.8, 26.6, 25.7, 24.9, 23.3, 21.7, 17.7, 16.0; ^{19}F NMR δ −71.42; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1). **Ester 11** (higher retention time in HPLC): $[\alpha]_D$ +21.8 (*c* = 1, 98% ee); ^1H NMR δ 7.68–7.55 (2 H), 7.45–7.33 (3 H), 5.18–5.00 (6 H), 3.55 (s, 3 H), 2.20–1.90 (14 H), 1.87 (m, 2 H), 1.68 (s, 6 H), 1.60 (s, 12 H), 1.56 (s, 1 H, OH), 1.51 (d, 3 H, *J* = 1 Hz), 1.73–1.2 (4 H), 1.17 (s, 3 H); ^{13}C NMR δ 166.7, 136.4, 135.8, 135.0, 132.1, 131.5, 131.3, 129.6, 128.4, 127.7, 124.4, 124.2, 124.1, 124.0, 123.5 (q, CF_3, J_{C-F} = 288 Hz), 122.8, 84.8 (q, C, J_{C-F} = 28 Hz), 82.3, 74.3, 55.4, 39.7, 39.7, 39.7, 36.3, 29.9, 26.8, 26.6, 25.7, 24.6, 23.9, 21.7, 17.7, 16.0, 16.0, 15.9; ^{19}F NMR δ −71.16; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1). Anal. Calcd for $C_{40}H_{59}F_3O_4$ (as a mixture of diastereomers **8** and **9**): C, 72.69; H, 9.00. Found: C, 72.78; H, 9.01.

Ester 12. This compound was obtained from diol **5-(3S)** and (*S*)-MTPA chloride. **12**: $[\alpha]_D$ +9.5 (*c* = 1, 80% ee); ^1H NMR δ 7.68–7.56 (2 H), 7.46–7.34 (3 H), 5.22–5.02 (5 H), 4.99 (dd, 1 H, J_1 = 9.5 Hz, J_2 = 2.5 Hz), 3.58 (s, 3 H), 2.15–1.92 (18 H), 1.68 (d, 3 H, *J* = 1 Hz), 1.60 (s, 12 H), 1.56 (s, 3 H), 1.70–1.0 (2 H), 1.17 (s, 3 H), 1.13 (s, 3 H); ^{13}C NMR δ 166.4, 135.1, 135.0, 134.9, 133.5, 132.1, 131.2, 129.7, 128.4, 127.4, 127.4, 125.4, 124.4, 124.2, 123.5 (q, CF_3, J_{C-F} = 288 Hz), 84.5 (q, C, J_{C-F} = 28 Hz), 82.7, 72.5, 55.5, 39.7, 39.6, 36.1, 28.7, 28.3, 26.8, 26.7, 26.6, 26.3, 25.7, 24.4, 17.7, 16.0, 15.9; ^{19}F NMR δ −71.25; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1).

Ester 17. This compound was obtained from diol **5-(3*R*)** and (*R*)-MTPA chloride. **17** (*ent*-**12**): $[\alpha]_D$ −7.8 (*c* = 1.5, 80% ee).

Ester 16. This compound was obtained from diol **5-(3*R*)** and (*S*)-MTPA chloride. **16**: $[\alpha]_D$ +21.1 (*c* = 1, 88% ee); ^1H NMR δ 7.70–7.56 (2 H), 7.46–7.34 (3 H), 5.26–5.04 (5 H), 4.99 (dd, 1 H, J_1 = 10 Hz, J_2 = 2.5 Hz), 3.58 (s, 3 H), 2.15–1.9 (16 H), 1.86 (t, 2 H, *J* = 11.5 Hz), 1.68 (d, 3 H, *J* = 1 Hz), 1.60 (s, 12 H), 1.52 (s, 3 H), 1.80–1.30 (2 H), 1.23 (s, 3 H), 1.16 (s, 3 H); ^{13}C NMR δ 166.9, 135.1, 135.0, 134.9, 133.5, 132.2, 131.2, 129.6, 128.4, 127.7, 127.7, 125.3, 124.4, 124.3, 124.2, 123.6 (q, CF_3, J_{C-F} = 288 Hz), 84.5 (q, C, J_{C-F} = 28 Hz), 82.4, 72.7, 55.5, 39.7, 39.7, 39.6, 35.8, 28.7, 28.3, 27.0, 26.8, 26.7, 25.7, 23.7, 17.7, 16.0, 15.9; ^{19}F NMR δ −71.24; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1).

Ester 13. This compound was obtained from diol **5-(3*S*)** and (*R*)-MTPA chloride. **13** (*ent*-**16**): $[\alpha]_D$ −18.8 (*c* = 2, 80% ee).

Ester 18. This compound was obtained from diol **6-(6*R*,7*R*)** and (*S*)-MTPA chloride. **18**: $[\alpha]_D$ +20.3 (*c* = 1, 90% ee); IR (film) 3535, 2965, 2925, 2855, 1745, 1450, 1375, 1265, 1185, 1120, 1170, 1120, 1020, 715 cm^{-1}; ^1H NMR δ 7.70–7.58 (2 H), 7.46–7.34 (3 H), 5.20–5.00 (6 H), 3.59 (s, 3 H), 2.16–1.92 (16 H), 1.85 (t, 2 H, *J* = 7.5 Hz), 1.75–1.15 (4 H), 1.70 (d, 3 H, *J* = 1 Hz), 1.68 (d, 3 H, *J* = 1 Hz), 1.63 (s, 3 H), 1.60 (s, 9 H), 1.51 (s, 3 H), 1.70–1.0 (4 H), 1.12 (s, 3 H); ^{13}C NMR δ 167.1, 135.3, 134.9, 133.7, 132.5, 132.3, 131.3, 129.6, 128.4, 127.7, 125.3, 124.4, 124.2, 124.1, 123.7, 123.5 (q, CF_3, J_{C-F} = 289 Hz), 84.8 (q, C, J_{C-F} = 28 Hz), 81.5, 74.8, 55.6, 39.7, 39.4, 35.8, 28.7, 28.3, 28.1, 26.8, 26.7, 25.7, 25.7, 21.7, 21.2, 17.7, 16.0, 16.0, 15.9; ^{19}F NMR δ −71.21; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1).

Ester 14. This compound was obtained from diol **6-(6*S*,7*S*)** and (*S*)-MTPA chloride. **14**: $[\alpha]_D$ +13.6 (*c* = 1, 94% ee); ^1H NMR δ 7.67–7.57 (2 H), 7.44–7.36 (3 H), 5.20–5.00 (6 H), 3.59 (s, 3 H), 2.14–1.90 (16 H), 1.82–1.35 (4 H), 1.68 (s, 6 H), 1.61 (s, 3 H), 1.60 (s, 9 H), 1.57 (d, 3 H, *J* = 1 Hz), 1.70–1.10 (4 H), 1.09 (s, 3 H); ^{13}C NMR δ 166.5, 135.3, 134.9, 133.7, 132.2, 131.2, 129.6, 128.4, 127.5, 125.4, 124.4, 124.2, 124.1, 123.8, 123.5 (q, CF_3, J_{C-F} = 288 Hz), 84.5 (q, C, J_{C-F} = 28 Hz), 81.8, 74.5, 55.5, 39.7, 38.8, 36.2, 28.7, 28.3, 28.1, 26.8, 26.7, 25.7, 21.8, 21.6, 17.7, 16.0, 16.0; ^{19}F NMR δ −71.36; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1).

Ester 19. This compound was obtained from diol **6-(6*R*,7*R*)** and (*RS*)-MTPA chloride. **19** (*ent*-**14**): $[\alpha]_D$ −11.4 (*c* = 1, 98% ee).

Ester 20. This compound was obtained from diol **7-(10*R*,11*R*)** and (*S*)-MTPA chloride. **20**: $[\alpha]_D$ +26.9 (*c* = 1, 90% ee); IR (film) 3540, 2965, 2925, 2855, 1745, 1450, 1375, 1260, 1185, 1170, 1120, 1020, 715 cm^{-1}; ^1H NMR δ 7.68–7.60 (2 H), 7.45–7.33 (3 H), 5.20–5.00 (6 H), 3.58 (s, 3 H), 2.18–1.80 (16 H), 1.68 (d, 6 H, *J* = 1 Hz), 1.63 (d, 3 H, *J* = 0.5 Hz), 1.61 (s, 3 H), 1.60 (s, 6 H), 1.50 (s, 3 H), 1.76–1.2 (4 H), 1.12 (s, 3 H); ^{13}C NMR δ 167.2, 136.5, 136.1, 135.0, 132.3, 131.5, 131.3, 129.6, 128.4, 127.7, 124.3, 124.1, 124.0, 123.5, 123.5 (q, CF_3, J_{C-F} = 288 Hz), 122.7, 84.8 (q, C, J_{C-F} = 28 Hz), 81.5, 74.8, 55.5, 39.7, 39.6, 39.4, 30.2, 26.7, 26.6, 26.6, 25.7, 24.4, 21.6, 21.2, 17.7, 16.0, 16.0, 15.9; ^{19}F NMR δ −71.27; HPLC–TSP–MS *m/z* 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1).

Ester 15. This compound was obtained from diol **7-(10*S*,11*S*)** and (*R*)-MTPA chloride. **15**: $[\alpha]_D$ +10.8 (*c* = 0.18,

(38) Ward, D. E.; Rhee, C. K. *Tetrahedron Lett.* **1991**, *32*, 7165–7166.

88% ee); IR (film) 3540, 2965, 2925, 2855, 1745, 1450, 1375, 1260, 1185, 1170, 1120, 1020, 715 cm^{-1}; ^1H NMR δ 7.67–7.57 (2 H), 7.44–7.36 (3 H), 5.20–5.00 (6 H), 3.59 (s, 3 H), 2.14–1.90 (16 H), 1.82–1.35 (4 H), 1.68 (d, 6 H, J = 1 Hz), 1.61 (d, 3 H, J = 1 Hz), 1.60 (s, 9 H), 1.55 (d, 3 H, J = 1 Hz), 1.08 (s, 3 H); ^{13}C NMR δ 166.6, 136.6, 135.9, 135.1, 132.2, 131.5, 131.3, 129.6, 128.4, 127.5, 124.3, 124.2, 124.0, 123.6, 123.4 (q, CF_3, J_{C-F} = 288 Hz), 84.6 (q, C, J_{C-F} = 28 Hz), 122.7, 81.8, 74.5, 55.5, 39.7, 39.7, 38.9, 30.2, 26.7, 26.6, 25.7, 24.8, 21.7, 21.6, 17.7, 16.0, 16.0; ^{19}F NMR δ −71.52; HPLC–TSP–MS m/z 678 (M + 18), 661 (M + 1), 444 (M − 217 + 1).

Ester 21. This compound was obtained from diol **7-(10*R*,11*R*)** and (*R*)-MTPA chloride. **21** (*ent*-**15**): $[\alpha]_D$ −8.5 (c = 1, 89% ee).

Preparation of Squalenediols by Reduction of MTPA Esters. General Procedure. A solution of the corresponding MTPA ester in Et_2O, maintained under argon and at 20 °C, was treated with 5 molar equiv of $LiAlH_4$, and the mixture was stirred until the reaction was completed (TLC monitoring). After the usual workup, the residue obtained was purified (elution with 96:4 hexane/EtOAc) to afford the expected pure diol.

6(*R*),7(*S*)-Dihydroxy-6,7-dihydrosqualene [6-(6*R*,7*S*)]. This diol was isolated (8 mg, 92% yield) starting from 13 mg of ester **8**: $[\alpha]_D$ −10.4 (c = 0.5), 96% ee).

6(*S*),7(*R*)-Dihydroxy-6,7-dihydrosqualene [6-(6*S*,7*R*)]. This diol was isolated (8 mg, 92% yield) starting from 13 mg of ester **9**: $[\alpha]_D$ +10.4 (c = 0.5, 96% ee).

10(*R*),11(*S*)-Dihydroxy-10,11-dihydrosqualene [7-(10*R*,11*S*)]. This diol was isolated (14 mg, 92% yield) starting from 23 mg of ester **10**: $[\alpha]_D$ −6.0 (c = 1, 96% ee).

10(*S*),11(*R*)-+Dihydroxy-10,11-dihydrosqualene [7-(10*S*,11*R*)]. This diol was isolated (13 mg, 93% yield) starting from 21 mg of ester **11**: $[\alpha]_D$ +6.4 (c = 1, 98% ee).

Preparation of Chiral Internal Oxidosqualenes. These compounds were obtained from the respective diols by using the procedure described above for the preparation of oxidosqualene **2-(3S)**.

6(*R*),7(*R*)-Oxidosqualene [3-(6*R*,7*R*)]. This compound was obtained from diol **6-(6*R*,7*S*)** in 78% yield: $[\alpha]_D$ +2.8 (c = 0.4, 92% ee).

6(*S*),7(*S*)-Oxidosqualene [3-(6*S*,7*S*)]. This compound was obtained from diol **6-(6*S*,7*R*)** in 86% yield: $[\alpha]_D$ −2.0 (c = 0.8, 92% ee).

10(*R*),11(*R*)-Oxidosqualene [4-(10*R*,11*R*)]. This compound was obtained from diol **7-(10*R*,11*S*)** in 64% yield: $[\alpha]_D$ +10 (c = 0.8, 92% ee).

10(*S*),11(*S*)-Oxidosqualene [4-(10*S*,11*S*)]. This compound was obtained from diol **7-(10*S*,11*R*)** in 78% yield: $[\alpha]_D$ −9.5 (c = 0.8, 94% ee).

Assay Method for SE. Partially purified SE and NADPH cytochrome P450 reductase were obtained from pig liver following the procedures described by Bai and Prestwich.[17] The specific activities of the purified enzymes were 280 pmol/min·mg for SE and 4.48 units/mg for the reductase. The procedure used for the SE assays was based on that reported by Bai and Prestwich[17] with minor modifications. Briefly, isopropyl alcohol solutions of squalene (7 μM, containing 15 000 dpm of [1,25-^{14}C]squalene, 1.18 mCi/mmol, kindly provided by Prof. G. Prestwich) and inhibitors were added to the test tubes (the alcohol contents did not exceed 1% of the overall test mixture), followed by addition of 60 μL of purified SE, 0.15 unit of NADPH/cytochrome P450 reductase, 0.05 mM FAD, and 0.8 mM NADPH in a total volume of 0.5 mL is 20 mM Tris-HCl buffer (pH 7.5). The incubation mixture was shaken for 45 min at 37 °C, and the reaction was quenched by treatment with 1 mL of 10% KOH in MeOH for 30 min at 37 °C. Then, the mixture was extracted three times with an equivalent volume of 4:1 hexane/EtOAc. The combined extracts were evaporated to dryness, redissolved in hexane (3 × 80 μL), spotted onto a silica gel TLC plate, and eluted with a 10:1 hexane/methyl *tert*-butyl ether mixture. Bands corresponding to squalene and 2,3-oxidosqualene were detected with an RITA TLC radioscanner (Isomess); cutoff and radioactivity was counted with a liquid scintillation counter (LKB 1217 Rackbeta). 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 versus log of inhibitor concentration. The plots were generated by using five different concentrations of inhibitor. The concentration ranges used were 1.75–28 μM for 6(*S*),7(*S*)-oxidosqualene [**3-(6*S*,7*S*)**] and 7–56 μM for 10(*R*),11(*R*)-oxidosqualene [**4-(10*R*,11*R*)**]. The K_i value for oxidosqualene **3-(6*S*,7*S*)** was determined from replots of slopes and intercepts of Lineweaver–Burk double reciprocal plot versus the inhibitor concentration. In this determination, the substrate concentrations were 3, 4, 7, 10, 15, and 20 μM, and those of inhibitor were 0, 1.75, 3.5, 7, and 10.5 μM. Protein contents were determined according to Bradford,[39] using BSA with the appropriate amount of Triton X-100 as standard for the calibration curve.

In the experiments carried out to identify the dioxidosqualenes produced, isopropyl alcohol solutions of the corresponding oxidosqualene (**3-(6*S*,7*S*)** or **3-(6*R*,7*R*)**, 20 μM) were incubated for 2 h as described above in a total volume of 5 mL of 0.1 M Tris-HCl buffer (pH 7.5). Dioxidosqualenes were purified by TLC on silica gel impregnated with Et_3N, eluting with 95:5 hexane/EtOAc, and identified by comparison with authentic standards from synthetic origin (TLC, GC, reversed phase HPLC).[15,16] Quantification of formed dioxidosqualenes was performed by HPLC (Merck-Lichrosorb ODS-2, 90:10 CH_3CN/H_2O at 1 mL/min, λ = 210 nm). Under these conditions, the retention time for 2,3:6,7-dioxidosqualene (**22**) and 2,3:18,19-dioxidosqualene (**23**) were 18.6 and 15.8 min, respectively. Calibration curves were established with known injected amounts of the corresponding dioxidosqualenes ranging from 50 to 500 pmol.

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Supplementary Material Available: NMR spectra of MTPA esters **8**, **9**, **10**, and **11** (20 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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