J. Med. Chem. **1998,** *41,* 540−554

29-Methylidene-2,3-oxidosqualene Derivatives as Stereospecific Mechanism-Based Inhibitors of Liver and Yeast Oxidosqualene Cyclase

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Received August 13, 1997

Two pairs of isomers (18*Z*)- (**8**), (18*E*)-29-methylidene-2,3-oxidohexanorsqualene (**21**), and (18*Z*)- (**31**), (18*E*)-29-methylidene-2,3-oxidosqualene (**34**), have been obtained in a fully stereospecific manner, as inhibitors of rat and yeast oxidosqualene cyclase. A new method for the synthesis of C_{22} squalene aldehyde 2,3-epoxide is reported, as well as that of other 19-modified 2,3-oxidosqualene analogues.We found that the activity is the opposite in the two series: the (*E*)-hexanormethylidene **21** and the (*Z*)-methylidene **31** are potent and irreversible inhibitors of oxidosqualene cyclase, while (*Z*)-hexanormethylidene **8** and (*E*)-methylidene **34** are almost completely inactive. Reduction of the 18,19-double bond, such as in **39**, eliminates the activity, while removal of both of the 19-linked groups such as in heptanor derivative **40** greatly reduces inhibition of the enzyme. (*E*)-Hexanormethylidene **21** results the first irreversible inhibitor of the series toward the yeast enzyme.

Oxidosqualene cyclase (OSC) (EC 5.4.99.7) is a widely distributed enzyme that catalyses the cyclization of (3*S*)-2,3-oxidosqualene (OS) to lanosterol in vertebrates and fungi and to cycloartenol or to a variety of tetracyclic and pentacyclic triterpenes in higher plants.[1,2] Several oxidosqualene cyclases have been purified to homogeneity from vertebrates,[3] plants,[4] and yeast.[5] Recently, several OSCs have been cloned and sequenced from different sources.[6] The predicted molecular masses ranged from 80 to 90 kDa, and the deduced amino acid sequence showed significant homology between rat, yeast, and plant enzyme. In addition, sequence comparison of OSCs with bacterial squalene cyclase[7] has shown 17−26% homogeneity and revealed the existence of a highly conserved repetitive motif (the QW motif) rich in aromatic amino acids.[8]

We are particularly interested in the study of oxidosqualene:lanosterol synthase inhibitors, which are potentially useful as hypocholesterolemic, antifungal, or phytotoxic drugs.[9] The enzymatic cyclization of 2,3-oxidosqualene **1** to lanosterol **7** involves the formation of the protosterol cation **5** and its backbone rearrangement to lanosterol (Scheme 1). The process is initiated by the protonation of the epoxide ring by a suitable electrophilic residue present in the enzyme, which triggers the cyclization of ring A and the formation of a series of rigidly held carbocationic intermediates.

Corey[10] recently reported chemical and molecular biology findings that suggest that the oxirane cleavage and the cyclization to form the ring A is a concerted process initiated by an highly conserved D456 aspartic acid residue of the cyclase. The formation of the discrete cationic intermediates going from C-8 bicyclic **2**, C-13 tricyclic **3** to the C-20 tetracyclic protosterol intermediate **5** has been suggested by different authors.[11] Johnson hypothesized that a stereocontrolled delivery of point charge nucleophiles by the enzyme could stabilize some cationic intermediates, such as C-8, C-13, and C-20.[12] To explain the apparent anti-Markovnikov closure of ring C, Corey suggested[13] that the formation of C-20 ion **5** could proceed through a normal Markovnikov type process to give first the tricyclic cation **3** followed by a ring expansion to form a tricyclic cyclohexyl carbocation **4**; by further cyclization, **4** annellated to the protosteryl ion bearing the side chain with a β configuration.

Our research group has demonstrated the existence of the transient carbocationic intermediates C-8 and C-20 indirectly by the specific inhibitory activity to OSCs shown by a series of azasqualenes, such as 2,3-epoxy-10-aza-10,11-dihydrosqualene (**10**) and 2,3-epoxy-19-aza-18,19,22,23-tetrahydrosqualene (**11**) (Figure 1) designed by replacing a positive-charged carbocation in the intermediates C-8 and C-20, respectively, with a nitrogen, protonated at physiological pH.[14,15] These two azasqualene derivatives have also been found to be time-dependent inhibitors of purified pig liver enzyme.

The C-8 ion has also been mimicked by *N*-(1,5,9-trimethyldecyl)-4α,10-dimethyl-8-aza-*trans*-decal-3β-ol, an azadecalinol-type inhibitor bearing an isoprenoid side chain linked at the C-8,[16] as well as by some other related mono- and bicyclic aza analogues.[17] As mimicking inhibitors of OSCs, a series of squalene analogues containing a thioether at positions C-6, C-10, C-14, and C-19 has also been tested. Among these compounds, those containing a sulfur substitution at position C-19 were the most active inhibitors, especially with *C. albicans* cyclase.[18] A series of sulfur-containing oxidosqualene derivatives, in which the C-11, C-15, or C-18 was replaced by sulfur, were also similarly active with yeast and animal OSC.[19] Among these analogues, it has been shown that small changes in the location of sulfur (from C-19 to C-18) strongly affect the inhibitory activity, as only the *S*-18 analogue behaved as a mechanism-

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S0022-2623(97)00534-7 CCC: $15.00 © 1998 American Chemical Society
Published on Web 01/22/1998

Scheme 1. Mechanism of Cyclization of 2,3-Oxidosqualene (**1**) and (18*Z*)-29-Methylidene-2,3-oxidosqualene (**31**)

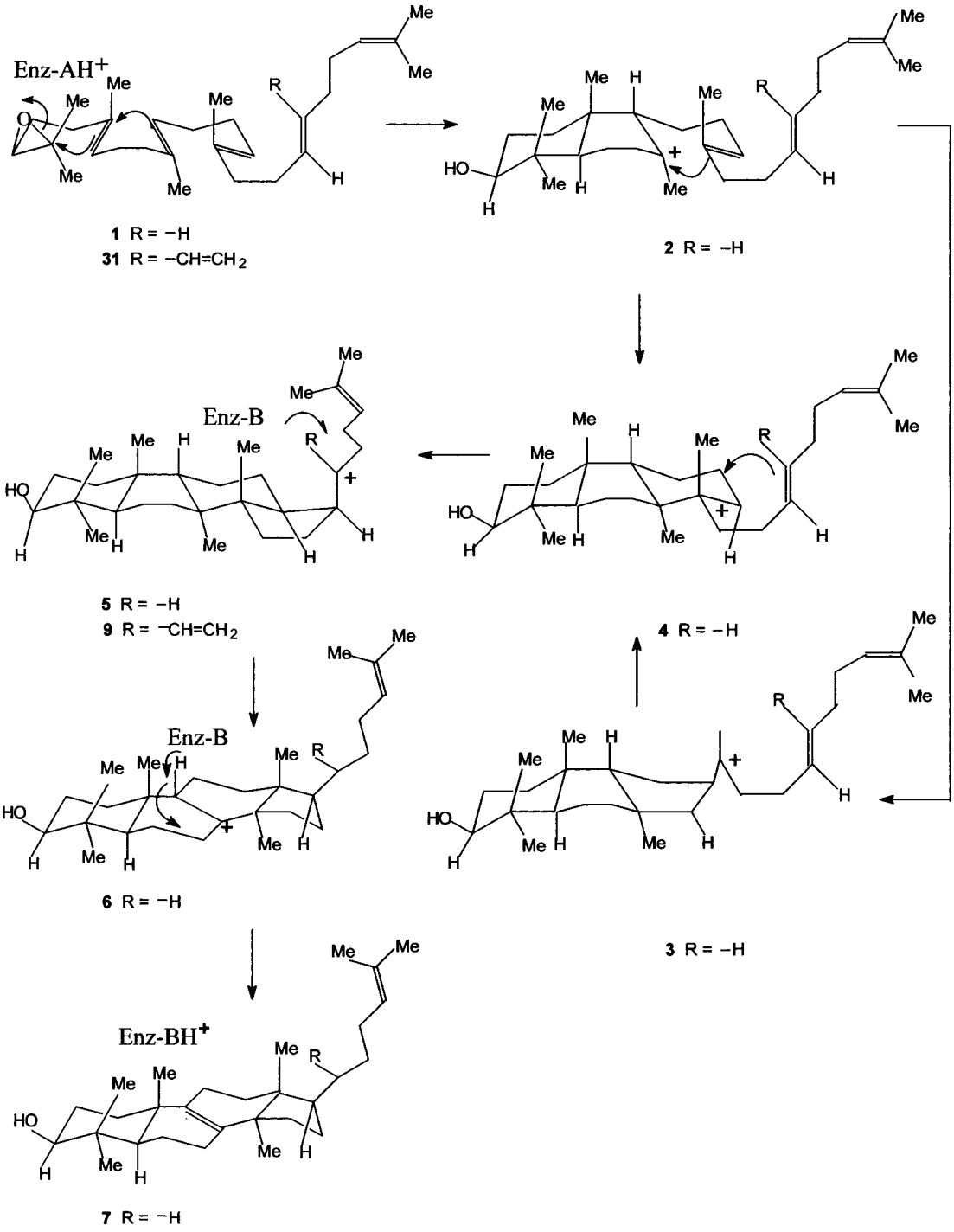


Figure 1. Structures of 10-aza- (**10**) and 19-azasqualene (**11**) derivatives.

based inhibitor for pig liver OSC, while the *S*-19 derivative acted as a competitive inhibitor.

So it has been widely demonstrated that the C-20 carbocation can interact with a very important part of the active site of the enzyme, which may help to lower the energy necessary to carry out the backbone rearrangement to lanosterol. Indeed, Xiao and Prestwich succeeded in preparing an irreversible site-directed inhibitor of vertebrate OSC, 29-methylidene-2,3-oxidosqualene (29-MOS), **31**.[20] The proposed mechanism of inhibition involves initial cyclization to the 21-methylidene-protosterol cation **9**, which instead of backbone rearrangement reacts with a nucleophilic site of the enzyme resulting in a irreversible inactivation of the enzyme (Scheme 1). The tritiated 29-MOS was used in affinity-labeling experiments with rat, pig, dog, and human cyclase to identify the 29-MOS binding site region of rat liver OSC which is the DCTAE motif, which

is a well-conserved region in all the known OSCs.[21] In this case, the initially formed 21-methylideneprotosterol cation **9** is trapped by the nucleophilic attack of the carboxyl group of aspartate residue D456. In contrast, the 29-MOS acts as a slow tight-binding inhibitor to yeast and plant OSC enzymes, since it has been found that each of these enzymes was not labeled by the radioactive inhibitor even under forcing conditions.

Some years ago, in an attempt to achieve mechanism-based inhibitors able to trap the C-20 carbocation, our laboratory synthesized the 20-oxa-22,23-dihydro-2,3-oxidosqualene.[22] Subsequently, Corey synthesized,[23] using the same procedure, the 20-oxa-2,3-oxidosqualene as a tool to provide information on the 17β-configuration of protosterol ion side chain and also to ascertain the intermediancy of the tricyclic carbocation.[13] Very recently, Corey[24] found that the 20-oxa-2,3-oxidosqualene was an irreversible inhibitor of both yeast and rat OSC. Moreover, this compound, as well as various truncated squalene-1,3-dienes (as a mixture $E + Z$ isomers) after incubation with yeast lanosterol synthase attached covalently to the histidine H234, which by site-directed mutagenesis experiments, appeared essential for enzyme function. By indirect site directed mutagenesis experiments, Corey[10] also hypotized that aspartate D456, present in the sequence DCTAE common in yeast and vertebrate OSC,[21] may be the "electrophilic residue" of the enzyme responsible of the opening of the oxirane ring. Working contemporary, Madden and Prestwich[25] synthesized a series of irreversible inhibitors of rat liver OSC and suggested that each of these inactivates the enzyme through the same cyclization-induced alkylation of aspartate D456 previously found for 29-MOS, whereas the truncated hexanorsqualene, isomer *Z* **8**, was not an irreversible inhibitor of rat liver enzyme.

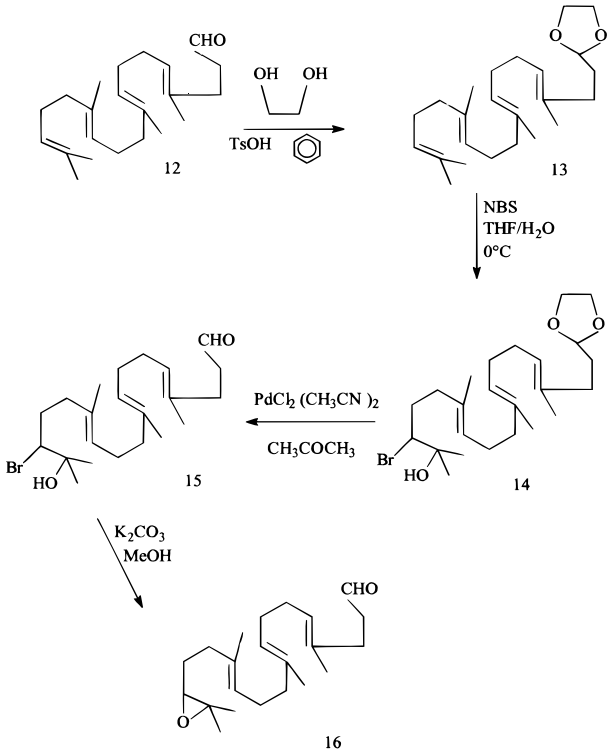
We believed that stereospecific syntheses of both the *Z* and *E* isomers of 29-methylidene-2,3-oxidohexanorsqualene **8** and **21**, and of 29-MOS **31** and **34**, together with other related compounds, such as **37**, **39**, and **40**, might give a better insight into the complex mechanism responsible for the enzymatic cyclization of 2,3-oxidosqualene.

Preliminary reports of the stereospecific syntheses and biological activities of the truncated methylidene derivatives **8** and **21** were object of some our communications in international meetings.[26,27] The new method of synthesis of C_{22} squalene aldehyde external bromohydrin **15** (Scheme 2) was previously reported by us.[27]

Chemistry

We have developed a general method for the protection of the "internal" double bonds of polyenic carbonyl compounds such as **12**, toward electrophilic reagents such as NBS (Scheme 2). Some months after our preliminary communications,[27] the Oehlschlager group[28] reported a similar method of protection of the aldehydic group, while deprotection was accomplished differently and in lower yields. We protected C_{22} squalene aldehyde **12** by treatment with 1,2-ethanediol in benzene, with *p*-toluenesulfonic acid as catalyst, forming the corresponding dioxolane **13**. Dioxolane **13** was treated with NBS in aqueous THF at 0 °C, affording dioxolane

Scheme 2. Synthesis of C_{22} Squalene Aldehyde External Epoxide **16**

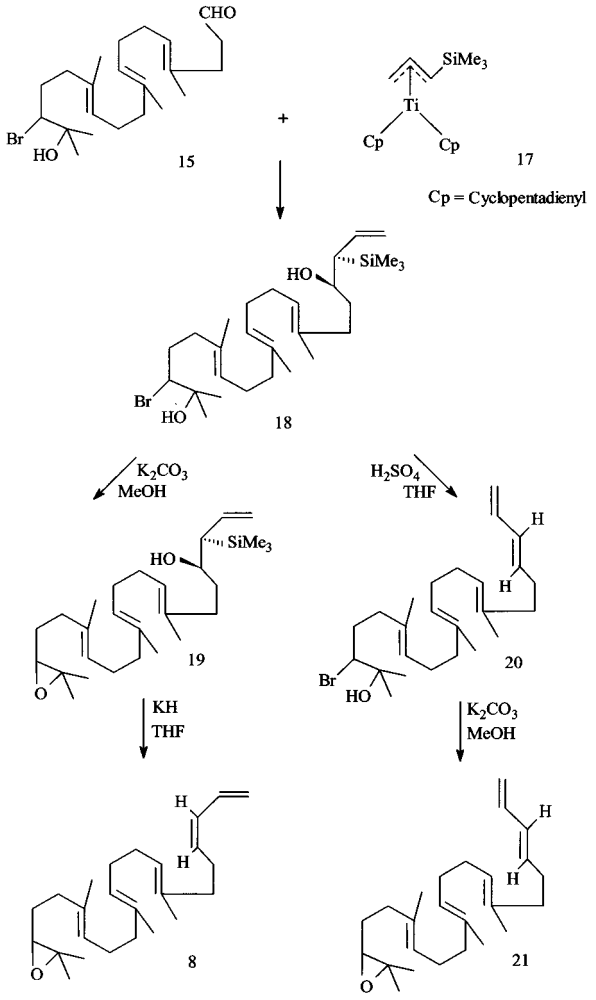


external bromohydrin **14**, which was deprotected to the free aldehyde bromohydrin **15** with bis(acetonitrile)-palladium(II) dichloride in acetone in 72% yield.

We and others have attempted to find an explanation by reactivity studies in various solvents and with the aid of ^{1}H and ^{13}C NMR studies. Two main theories have been developed, based on the fact that the key factor for the selective reactivity of the terminal double bond appeared to be the presence of water in the reaction medium.

According to Van Tamelen,[29] conformational changes associated with increasing solvent polarity led to reduced accessibility of the internal double bonds of squalene. In aqueous organic solvents, coiling of the polyolefin may be effective in shielding the internal double bonds from oxidative attack. Nevertheless, a low selectivity toward the different double bonds was observed with neutral oxidizing species such as peracids, in contrast with the high selectivity of charged reactants, such as protonated *N*-bromosuccinimide or mercuric acetate. Conformational changes associated with increasing solvent polarity, leading to reduced accessibility of the "internal" double bonds, should be detectable by studies on ^{13}C NMR spin−lattice relaxation times.[30] The relaxation times of squalene are quite unaffected by dilution of deuterated THF with increasing quantities of water. NMR studies show that there are no significant changes in conformation on going to more aqueous media, as the increasing internal rigidity leading to shorter relaxation times is present in solution, either in the absence or in the presence of water. Other ^{13}C NMR studies on functionalized squalene derivatives have shown a steric interaction between the heteroatom and the "vicinal" double bond. Heterosubstituted squalenes thus seem to possess different precoiled

Scheme 3. Stereospecific Synthesis of (18*Z*)- (**8**) and (18*E*)-29-Methylidene-2,3-oxidohexanorsqualene (**21**)



conformations in solution than do underivatized squalene.[31]

Taking into account the above studies, in an attempt to explain the selectivity of the reaction, it seems more probable that the overall polarity of the reaction environment in the region of the terminal double bond, during the approach of a cationic reagent in an aqueous solvent, is higher than in the region of an internal double bond. As a consequence, the terminal double bond of squalene or C_{22} squalene dioxolane **13** are the favored points of electrophilic attack. In the direct bromination of C_{22} squalene aldehyde **12** with NBS under standard conditions, formation of a stable cyclic hemiacetal, due to the hydrobromination of the "vicinal" double bond, has been seen to be the driving force of the reaction.

Then we developed various stereospecific syntheses of 19-modified oxidosqualene analogues. Since the reactivity of the 19-linked functional groups is strongly influenced by their position in the active site in the enzyme, both stereospecifically pure 18*Z* and 18*E* isomers are needed. We thus planned the synthesis of (18*Z*)- (**8**) and (18*E*)-29-methylidene-2,3-oxidohexanorsqualene (**21**) in a fully stereospecific manner (Scheme 3).

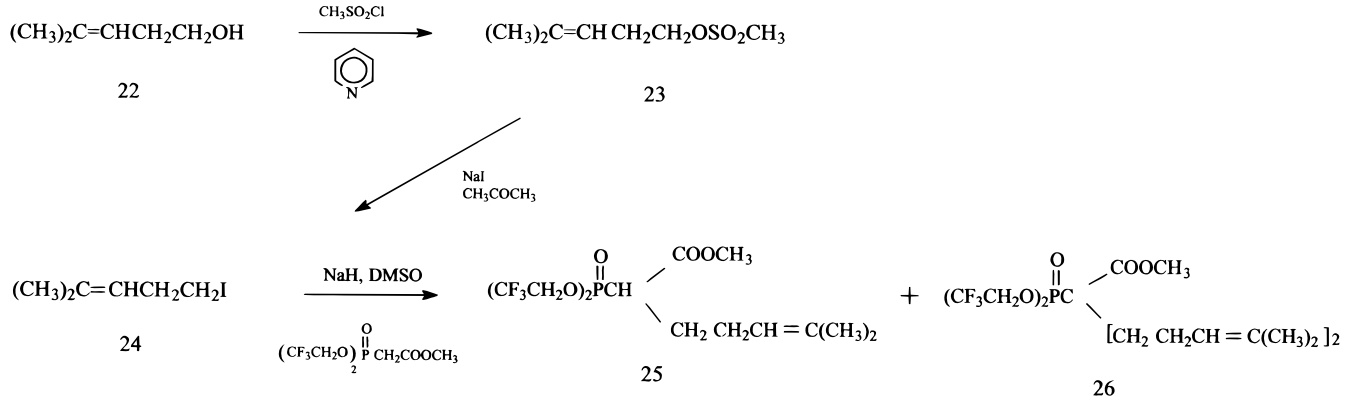
Most of methods reported in the literature were initially developed for regioselective syntheses and subsequently erythro or threo selectivity was afforded.[32] Methods reported in the literature were not found to be suitable for our syntheses, as the bromohydrin or aldehyde groups were often not stable in the reaction conditions used. We therefore developed a method for the reaction of aldehyde **15** with (trimethylsilyl)dicyclopentadienyltitanium **17**, which afforded exclusively (±)-(*R**,*S**)-*β*-hydroxytrimethylsilyl derivative **18**. Two different acidic or basic eliminations, followed or preceded by closure of the bromohydrin to epoxide, afforded (18*E*)- (**21**) or (18*Z*)-29-methylidene-2,3-oxidohexanorsqualene (**8**) in a fully stereospecific manner. The bromohydrin group in aldehyde **15** was found to be stable in the reaction conditions employed for synthesis of **18** and in the stereospecific acidic elimination conditions necessary to produce bromohydrin **20**, while for basic elimination it was previously closed to epoxide **19**.

The synthesis of (±)-(*R**,*S**) or *threo*-trimethylsilyl alcohol **18** consisted of six steps, which must be performed in the dark under a rigorously anhydrous atmosphere and solvents. The formation of the intermediates can easily be checked by their typical colors (see the Experimental Section). The first step consisted in reacting allyltrimethylsilane, *n*-butyllithium, and hexamethylphosphoramide to obtain allyltrimethylsilyllithium. Dicyclopentadienyltitanium(IV) dichloride was simultaneously reacted with isopropylmagnesium chloride to obtain dicyclopentadienyltitanium(III) monochloride.[33] The third stage consisted in the union of the two previously prepared reaction mixtures to obtain [(η^5-C_5H_5)$_2$Ti(η^3-1-(trimethylsilyl)allyl)] **17**, the key intermediate for the regioselectivity and stereoselectivity of the reaction with C_{22} aldehyde bromohydrin **15** (fourth stage). Addition of HCl allowed the stereoselective transformation of the dicyclopentadienyltitanium complex to (±)-(*R**,*S**)-trimethylsilyl alcohol **18** (fifth stage). The use of an acidic medium, HCl in this case, was seen to be essential. We found that, if water alone was used, the elimination of the dicyclopentadienyltitanium(III) monochloride was not stereospecific; the resulting trimethylsilyl alcohol is in a threo/erythro ratio of 75:25. In the sixth stage, under a flux of compressed air, dicyclopentadienyltitanium(III) monochloride is oxidized to dicyclopentadienyltitanium(IV) dichloride. The basic promoted and acidic promoted elimination reactions of (±)-(*R**,*S**)-*β*-hydroxysilane also occurred in a stereospecific manner, according to a syn or anti elimination mechanism.[34]

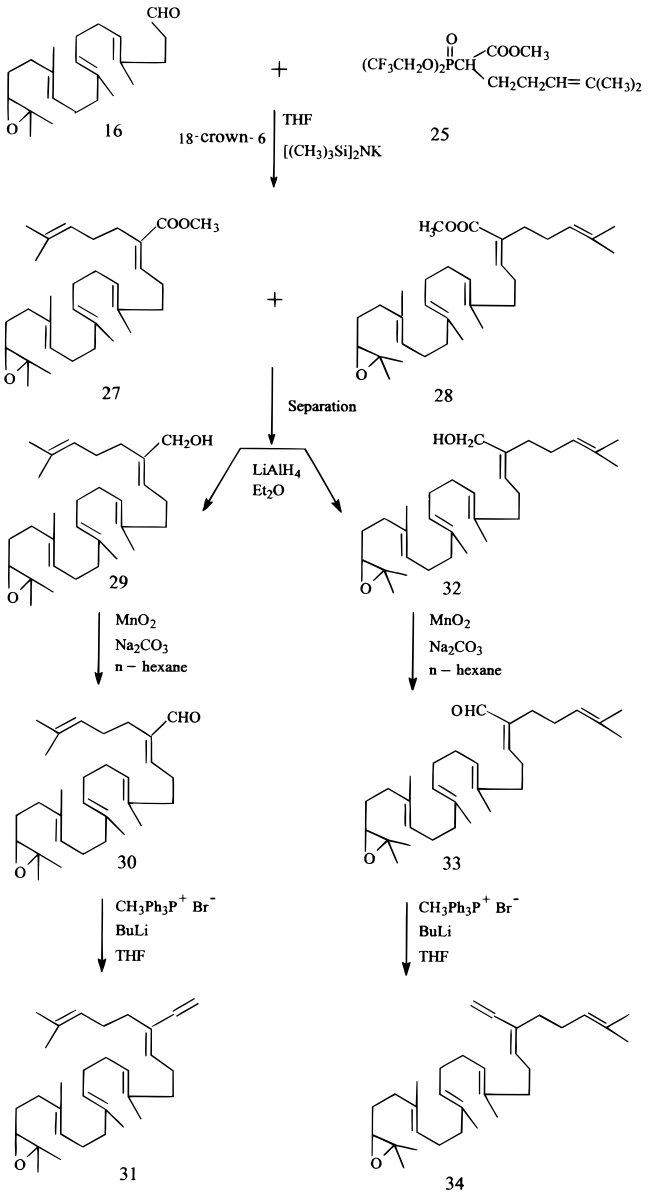
We thus obtained good yields of either (18*Z*)- (**8**) or (18*E*)-29-methylidene-2,3-oxidohexanorsqualene (**21**), from the same precursor (±)-(*R**,*S**)-trimethylsilyl alcohol **18**, since all three reactions, synthesis of alcohol and its syn and anti eliminations, proceeded in a fully stereospecific manner. Furthermore, the method, under the mild conditions we developed, allowed both *Z* and *E* terminal polyenes, containing labile groups, such as **8** or **21**, to be obtained stereospecifically.

We then obtained (18*Z*)- and (18*E*)-29-methylidene-2,3-oxidosqualene (29-MOS). The previous functionalization of the squalene moiety with a 2,3-epoxide, according to the new method, was seen to be essential to achieve good yields of (18*Z*)-29-MOS (**31**) and (18*E*)-29-MOS (**34**) separately (Schemes 4 and 5). Prestwich et al.[20,35,36] first developed a synthesis of the leader

Scheme 4. Synthesis of Bis(trifluoroethyl) Phosphonates **25** and **26**



Scheme 5. Stereospecific Synthesis of (18*Z*)- (**31**) and (18*E*)-29-Methylidene-2,3-oxidosqualene (**34**)



compound, (18*Z*)-29-MOS (**31**) and precursors, but (18*E*)-29-MOS has not been reported.

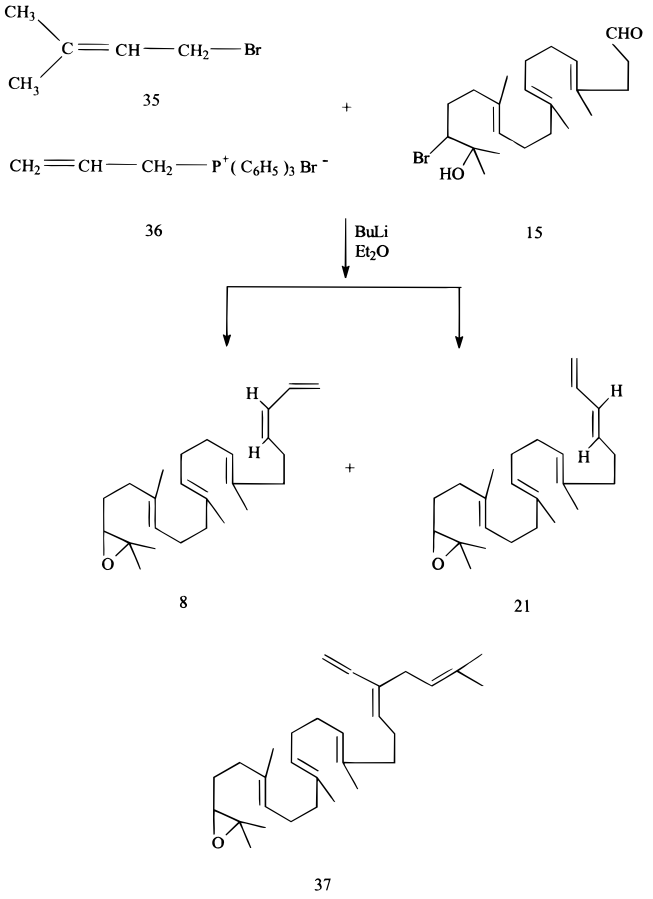
We prepared unsaturated esters **27** and **28** by the Horner−Emmons reaction of C_{22} epoxysqualene aldehyde **16** with bis(trifluoroethyl) phosphonate reagent **25**. We reacted 4-methyl-3-pentenyl methanesulfonate (**23**) with NaI in acetone, producing iodide **24**. Phosphonate **25** was prepared by coupling iodide **24** with methyl bis-(2,2,2-trifluoroethoxy)phosphoryl acetate,[37] in the presence of NaH. Direct coupling of mesylate **23** was not possible.

In our conditions, the Horner−Emmons olefination of bis(trifluoroethyl) phosphonoester **25** (see the experimental part) was stereoselective, but not stereospecific.[20,38,39] We obtained a mixture of methyl esters **27** and **28** in a *Z*:*E* ratio of about 70:30 directly in one step with a 61% yield, starting from epoxy aldehyde **16**. Flash chromatography easily separated the two geometrical isomers. Each ester, **27** or **28**, was reduced to alcohol **29** or **32** with $LiAlH_4$.[36] To overcome partial reduction of the ester α,β-double bond and of the epoxidic group, reduction was performed quickly at −30 °C until no more ester was detectable. Activated MnO_2 in the presence of Na_2CO_3 in *n*-hexane[36,40] at +5 °C allowed pure (18*Z*)- or (18*E*)-enals **30** or **33** to be obtained without isomerization. The Wittig reaction with the ylide of methyltriphenylphosphonium bromide afforded either (18*Z*)- (**31**) or (18*E*)-29-MOS (**34**) in a fully stereospecific manner.

For the synthesis of (18*E*)-29-methylidene-2,3-oxidonorsqualene [(18*E*)-nor-29-MOS], **37**, we focused our attention on a Wittig reaction between C_{22} squalene aldehyde monobromohydrin **15** and allyltriphenylphosphonium bromide (**36**), suitably alkylated (Scheme 6). According to the literature,[41] the ylide of allyltriphenylphosphonium bromide (**36**) is usually generated and reacted at room temperature. In our case, following these conditions, we obtained very low amounts of (18*E*)-nor-29-MOS (**37**). As the direct isolation of the alkylated phosphonium salt failed, allyltriphenylphosphonium bromide in diethyl ether was added with butyllithium at room temperature, and the resulting carbanion was alkylated under reflux with 4-bromo-2-methyl-2-butene (**35**) and reacted without isolation with C_{22} squalene aldehyde monobromohydrin **15**. This unusual Wittig reaction was also performed under reflux, at +40 °C, because of the low reactivity. In this "one-pot" reaction, (18*E*)-nor-29-MOS (**37**) formed stereospecifically with the contemporary closure of the bromohydrin to epoxide.

The same reaction was also tried, under the same conditions, to develop a "one-pot stereospecific synthesis" of (18*E*)-29-MOS (**34**). The alkylation with 5-bromo-2-methyl-2-pentene proceeded with great difficulty,

Scheme 6. Stereospecific Synthesis of (18*E*)-29-Methylidene-2,3-oxidonorsqualene (**37**)



affording, after the usual Wittig reaction, a mixture of (18*Z*)- (**8**) and (18*E*)- (**21**) and only very low amounts of (18*E*)-29-MOS (**34**).

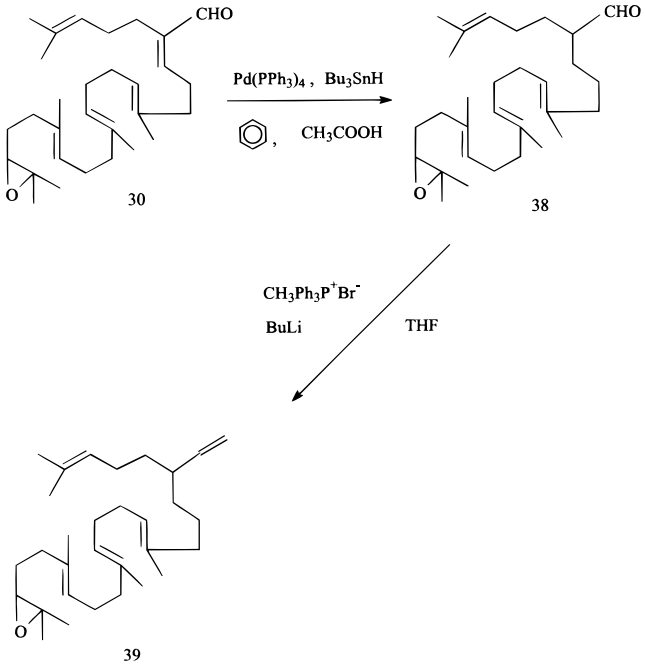
For the synthesis of 18,19-dihydro-29-methylidene-2,3-oxidosqualene (**39**), we needed to develop a method for the selective reduction of the 18,19-double bond, also compatible with the presence of the epoxidic and aldehydic groups present in the structure (Scheme 7). Direct reduction of the 18,19-double bond in ester **27** or in alcohol **29** was not satisfactory.

We therefore started from the α,β-unsaturated aldehyde **30**, having the 18,19-activated double bond. Reduction was performed with tributyltin hydride, in the presence of tetrakis(triphenylphosphine)palladium,[42] as catalyst. The reaction necessitated a proton donor, as coactivating agent, such as acetic acid. The epoxidic group was stable in the reaction conditions employed. Furthermore, no allylic or saturated alcohols or other reduced double bonds were detected by ^{1}H NMR and mass analysis. A Wittig reaction with the ylide of methyltriphenylphosphonium bromide with aldehyde **38** afforded compound **39**.

18-Heptanor-2,3-oxidosqualene **40** was obtained by reacting C_{22} aldehyde bromohydrin **15** with the ylide of methyltriphenylphosphonium bromide in THF (Scheme 8), in a one-pot reaction, with the contemporary closure of the bromohydrin to epoxide.

The good overall yields and the complete stereoselectivity of the methods made it easy to obtain 19-modified oxidosqualene analogues with various reactive groups

Scheme 7. Synthesis of 18,19-Dihydro-29-methylidene-2,3-oxidosqualene (**39**)



Scheme 8. Synthesis of 18-Heptanor-2,3-oxidosqualene (**40**)

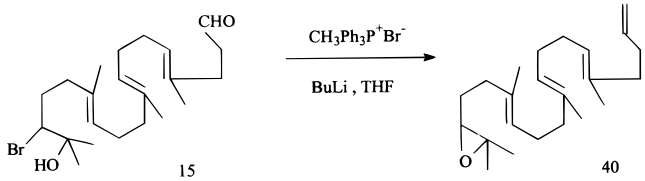


Table 1. IC_{50} and k_{inact}/K_I Values of Inhibition of Solubilized and Partially Purified OSC by 29-Methylidene-2,3-oxidosqualene Derivatives

	IC_{50} (μM)		k_{inact}/K_I[a] (min^{-1} μM^{-1})	
compd	*S. cerevisiae*	pig liver	*S. cerevisiae*	pig liver
8	15	20	0.35×10^{-3}	0.97×10^{-3}
21	1.5	3.5	28.9×10^{-3}	7.0×10^{-3}
31	1.0	0.4	3.08×10^{-3}	99.6×10^{-3}
34	5.0	4.0	nd	2.6×10^{-3}
37	18	32	nd	nd
39	>100	100	nd	nt
40	70	15	nd	nt

[a] nd = not determined; nt = not time-dependent.

either in the 2,3-oxidosqualene series or in the 2,3-oxidohexanorsqualene series.

Biological Results

Table 1 shows the IC_{50} of squalene methylidene derivatives **8**, **21**, **31**, **34**, **37**, **39**, and **40** obtained using partially purified pig liver and *Saccharomyces cerevisiae* OSC. From the IC_{50} values, it can be seen that the activity depends mainly on the geometrical isomerism of the diene functional group present in the oxidosqualene moiety and on the length of the chain.

The most active compound, both in pig liver OSC (IC_{50} = 0.4 μM) and in yeast OSC (IC_{50} = 1 μM), was the *Z* isomer **31** of the 29-MOS, whereas the *E* isomer **34** was about 5−10 time less active. Differences in activity between *E* and *Z* isomers were also observed with the

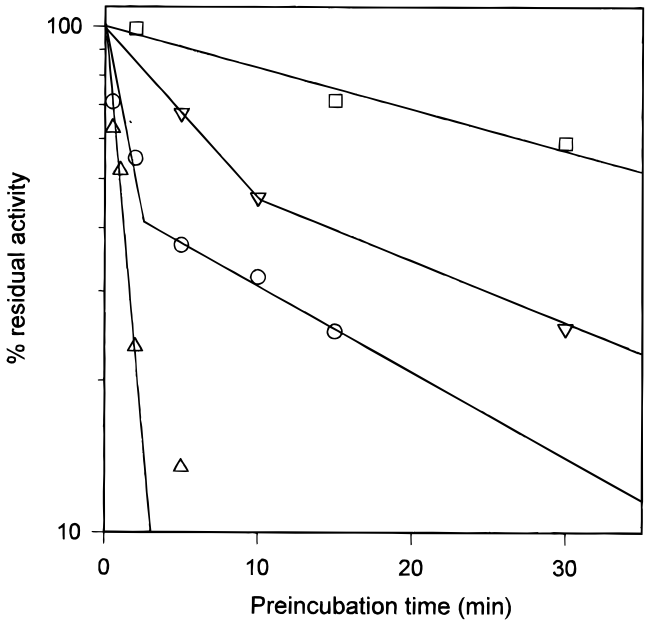


Figure 2. Time-dependent inhibition of pig liver OSC. Partially purified OSC was preincubated at 37 °C in the presence of (18*E*)- (**21**), 50 μM (○), (18*Z*)-29-methylidene-2,3-oxidohexanorsqualene (**8**), 50 μM (□), (18*Z*)- (**31**), 10 μM (△), and (18*E*)-29-methylidene-2,3-oxidosqualene (**34**) 50 μM (▽). Residual activity (percent of the control preincubated in the absence of inhibitors for the same time) was determined by withdrawing aliquots of 25 μL at time intervals and diluting to a final volume of 1 mL with substrate.

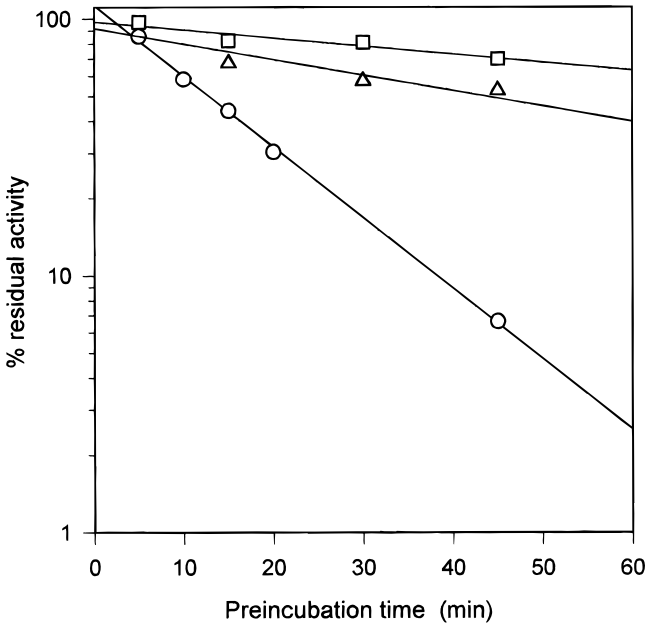


Figure 3. Time-dependent inhibition of yeast OSC by (18*E*)-(**21**), 2 μM (○), (18*Z*)-29-methylidene-2,3-oxidohexanorsqualene (**8**), 20 μM (□), and (18*Z*)-29-methylidene-2,3-oxidosqualene (**31**), 5 μM (△). Residual activity (percent of the control preincubated without the inhibitor at the same time) was determined by withdrawing aliquots of 50 mL at time intervals and diluting to a final volume of 0.5 mL with substrate.

truncated hexanor derivatives **8** and **21**. The more active isomer in this case was the *E* isomer **21** which showed in yeast an IC_{50} 1.5 μM comparable to compound **31** and 10 times lower than *Z*-hexanor derivative **8**. The IC_{50} observed with the pig liver enzyme was 3.5 μM for the *E* isomer **21** and 20 μM for the *Z* isomer **8**. The pig liver enzyme was less sensitive to the truncated derivatives, but the difference in activity between the *E* and *Z* isomers was almost the same as observed with the yeast enzyme.

It is interesting to note that Madden and Prestwich,[25] on testing the inhibition values of a series of squalene methylidene derivatives bearing the same *Z* isomerism in rat liver OSC, also found that the biological activity reduced drastically on passing from the 29-MOS *Z* isomer **31** to the *Z* hexanor derivative **8**. The activity of the nor derivative of the "unnatural" *E* isomer **37** of 29-MOS was also much lower (IC_{50} in OSC from pig liver = 32 μM) than that of the parent compound **34** (IC_{50} = 4 μM). Finally, if the reactive diene function was abolished by specific hydrogenation of the 18,19-double bond, such as in **39**, the activity was strongly reduced (IC_{50} from 0.4 to 90 μM). Instead, the 18-heptanor-2,3-oxidosqualene **40** retained some activity as inhibitor of OSC from pig liver (IC_{50} = 15 μM).

The time dependency of inactivation of pig liver OSC by squalene methylidene derivatives is shown in Figure 2. The most interesting result was the difference in the inactivation rates (k_{inact}/K_I) within each pair of geometrical isomers **8**, **21** and **31**, **34**. The (18*E*)-29-methylidene-2,3-oxidohexanorsqualene (**21**) is an effective time-dependent inhibitor, as is clear from plot of residual activity vs preincubation times. Residual activity was reduced by 50% in less than 5 min at 50 μM concentration on pig liver OSC. Inactivation follows a biphasic kinetic with a fast inactivation phase within the first 10 min of preincubation. The second-order inactivation rate constant was calculated from the half-life ($t_{1/2}$) in the fast phase of inactivation. The second-order inactivation rate is 7×10^{-3} μM^{-1} min^{-1} for the *E* isomer **21**, whereas the *Z* isomer **8** rate constant is 10 times lower (0.97×10^{-3} μM^{-1} min^{-1}). Moreover, with the *Z* isomer, very high concentrations of inhibitor (200−300 μM) are necessary to obtain a sensible time-dependent inhibition.

The differences between the *E* and *Z* isomers **8** and **21** are even more evident in yeast OSC (Figure 3). In this case, the second-order inactivation rate constant of the *E* isomer **21** is 28.9×10^{-3} μM^{-1} min^{-1}, 100 times higher than the constant of isomer *Z* **8** (0.35×10^{-3} μM min^{-1}) and 10 times higher than the constant of (*Z*)-29-MOS **31** (3.08×10^{-3} μM min^{-1}). Last we used a chromatographic method to check the irreversibility of time-dependent inhibition of pig and yeast OSC, using small DEAE-agarose columns. Enzyme that had been preincubated with the inhibitor for different increased times was adsorbed onto the column with a low-strength buffer and, after washing with the same buffer, eluted by increasing the ionic strength of the buffer. In neither case was activity restored after preincubation with the *E* isomer, whereas in yeast it was almost completely restored with the *Z* isomer.

Taking in account the other pair of isomers, (*Z*)- (**31**) and (*E*)-29-MOS (**34**), we again see a big difference in the IC_{50} activity of the two geometrical isomers, but with an inversion in the regiospecific activity. In fact, the *E* isomer shows an inactivation rate constant in pig liver OSC which is about 50 time lower than the "natural" *Z* isomer (k_{inact}/K_I = 99.6 × 10^{-3} vs 2.6 × 10^{-3} μM^{-1} min^{-1}). Moreover, in our experimental conditions (using

solubilized partially purified pig liver OSC), the (*Z*)-29-MOS showed a much lower inactivation rate than that found by Prestwich et al. using sonicated pig liver microsomes[20,25] (99.6×10^{-3} vs 50 μM^{-1} min^{-1}). In purified yeast OSC enzyme, neither the (*Z*)- nor the (*E*)-29-MOS isomers were found to be irreversible inactivators up to 5 μM inhibitor concentration (i.e., this value is 10 times that found for the IC_{50}).

In the present work, we showed that the *E* isomer of the 29-methylidenehexanoroxidosqualene **21** behaved as an irreversible inhibitor of both pig liver and *S. cerevisiae* OSCs, and it was the only irreversible inhibitor of the series on the yeast enzyme, showing a high potency. A nucleophilic residue, able to interact with or near protosteryl cation **5**, responsible for the inactivation by *E* derivative **21**, could exist in both pig liver and *S. cerevisiae* OSCs. On the other hand, Corey, by affinity labeling experiments,[24] found that H234 was the conserved amino acid residue of yeast OSC, which interacted irreversibly with many squalene-type inhibitors (included the isomeric mixture of **8** and **21**).

We also found that the "natural" *Z* isomer of 29-MOS acted as an irreversible inhibitor of pig liver cyclase, whereas in yeast it is a poor time-dependent, but not irreversible inhibitor. Moreover, the IC_{50} activity of the *Z* isomer was much higher than that of the *E* isomer in both enzymes. This might mean that (1) the interaction of methylidene derivatives with the nucleophilic site of the enzyme is sterically very specific; (2) the aspartate D456, identified in vertebrate OSC[21] as the nucleophile that interacts with the 21-methylidene protosterol ion **9**, may have a different function in yeast and animal cyclases. However, the definitive function of D-456 in yeast or animal OSC must await X-ray crystallographic analysis of crystalline OSC.

In conclusion, the stereospecific synthesis of the two pairs of *E* and *Z* isomers **8**, **21** and **31**, **34** enabled us to obtain compounds with very high and specific activities as irreversible inhibitors of animal and yeast OSC. Moreover, these compounds are good tools for a better insight into the complex mechanism of cyclization of squalene 2,3-epoxide. In particular, we ascertained some differences in the mechanism shown by yeast and animal OSCs that may facilitate the design of more specific hypocholesterolemic or antifungal drugs.

Experimental Section

The ^{1}H NMR spectra were recorded either on a JEOL EX 400 or a Bruker AC 200 instrument in $CDCl_3$ solution at room temperature, with $SiMe_4$ as internal standard. Mass spectra were obtained on a VG Analytical 7070 EQ-HF or a Finnigan MAT TSQ 700 spectrometer, by electron impact or chemical ionization. IR spectra were recorded on a Perkin-Elmer 781 spectrophotomer. Microanalyses were performed on an elemental analyzer 1106 (Carlo Erba Strumentazione), except in the case of P, which was analyzed according to the method of Schôniger.

The reactions were monitored by TLC on F_{254} silica gel precoated sheets (Merck); after development, the sheets were exposed to iodine vapor. Flash column chromatography was performed on 230−400 mesh silica gel (Merck). Petroleum ether refers to the fraction boiling in the range 40−60 °C. Diethyl ether and THF were dried over sodium benzophenone ketyl. All solvents and bases were distilled prior to flash chromatography.

We have named the new compounds as 29-methylidene derivatives, as in the first report[20] for compound **31**. According to the IUPAC rule, the name 29-methylene should be recommended.

C_{22} squalene aldehyde: (4*E*,8*E*,12*E*)-4,9,13,17-tetramethyl-4,8,12,16-octadecatetraenal (12) was synthesized and purified as reported in the literature.[14]

2-[(3*E*,7*E*,11*E*)-3,8,12,16-Tetramethyl-3,7,11,15-heptadecatetraenyl]-1,3-dioxolane (13). A solution of C_{22} squalene aldehyde **12** (5 g, 15.8 mmol), ethylene glycol (3 equiv, 2.94 g, 47.4 mmol) and *p*-toluenesulfonic acid monohydrate, as catalyst (0.1 equiv, 300 mg, 1.58 mmol) in benzene (300 mL) was refluxed for 4 h in a refrigerator equipped with a Markusson connector. It was then cooled, and a small amount of solid $NaHCO_3$ was added to salify the catalyst. The reaction mixture was diluted with benzene (200 mL), washed with saturated $NaHCO_3$ (2 × 100 mL) and brine (1 × 100 mL), dried over anhydrous sodium sulfate, and evaporated to dryness. The resulting oil was purified by flash chromatography (petroleum ether/diethyl ether, 99:1, then 98:2, finally 97:3) to give dioxolane **13** (4.90 g, 86% yield from **12**) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.58−1.76 (m, 17 H, allylic CH_3 and CH_2-dioxolane), 1.98−2.13 (m, 14 H, allylic CH_2), 3.82−3.97 (m, 4 H, OCH_2CH_2O), 4.85 (t, J = 4.8 Hz, 1 H, dioxolane CH), 5.02−5.18 (m, 4 H, vinylic CH); IR (liquid film) 2930, 2860, 1450, 1380, 1140 cm^{-1}; EIMS *m*/*z* 360 (72), 317 (30), 303 (25), 298 (30), 291 (92), 278 (30), 249 (38), 229 (100), 210 (84); HRMS *m*/*z* 360.3032 (calcd for $C_{24}H_{40}O_2$ 360.3028). Anal. ($C_{24}H_{40}O_2$) C, H, O.

(6*E*,10*E*,14*E*)-3-Bromo-17-(2-dioxolanyl)-2,6,10,15-tetramethyl-6,10,14-heptadecatrien-2-ol (14). Dioxolane **13** (1.0 g, 2.77 mmol) was dissolved in THF (80 mL) in a two-necked flask and stirred under nitrogen at 0 °C. Water was added until the solution became lightly opalescent. *N*-Bromosuccinimide (1.1 equiv, 542 mg, 3.05 mmol) was added at small portions with vigorous stirring within 15 min, at intervals adding a few drops of water to maintain the reaction mixture lightly opalescent. It was allowed to stand for 15 min at 0 °C, again adding a few drops of water at intervals when it began to clear. The reaction mixture was quenched with cold 10% $NaHCO_3$ (80 mL), extracted with diethyl ether (3 × 80 mL), washed with 10% $NaHCO_3$ (1 × 80 mL) and brine (1 × 80 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 97:3, to remove unreacted dioxolane (352 mg, 35%), then petroleum ether/diethyl ether, 80:20, to give pure dioxolane bromohydrin **14** (518 mg, 41% yield from **13**) as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.30 [2 s, 6 H, (CH_3)$_2$COH], 1.50−1.70 (m, 13 H, allylic CH_3, CH_2CHBr and CH_2-dioxolane), 1.98−2.13 (m, 12 H, allylic CH_2), 3.80−3.95 (m, 5 H, OCH_2CH_2O and C*H*Br), 4.85 (t, J = 4.8 Hz, 1 H, dioxolane CH), 5.00−5.20 (m, 3 H, vinylic CH); IR (CCl_4) 3570, 2980, 2930, 2855, 1550, 1450, 1385, 1140 cm^{-1}; EIMS *m*/*z* 458 (30), 456 (32), 443 (8), 441 (8), 396 (28), 394 (30), 359 (46), 316 (23), 314 (25), 297 (45), 289 (53), 287 (53), 277 (39), 257 (55), 249 (100); HRMS *m*/*z* 456.2238 (calcd for $C_{24}H_{41}BrO_3$ 456.2239). Anal. ($C_{24}H_{41}BrO_3$) C, H, Br, O.

C_{22} Squalene Aldehyde External Bromohydrin: (4*E*,8*E*,-12*E*)-16-Bromo-17-hydroxy-4,9,13,17-tetramethyl-4,8,12-octadecatrienal (15). Dioxolane **14** (400 mg, 0.87 mmol) was dissolved in acetone (200 mL) under dry nitrogen, with stirring. Bis(acetonitrile)palladium(II) dichloride [$PdCl_2$(CH_3-$CN)_2$] (0.2 equiv, 44 mg, 0.17 mmol) was added and was allowed to stand for 8 h under nitrogen, with stirring. Controls on silica gel TLC revealed that, in most eluants, dioxolane **14** and aldehyde **15** had about the same R_f, while dichloromethane/ethyl acetate, 95:5 differentiated the two compounds. The reaction mixture was quenched with cold 10% $NaHCO_3$ (100 mL) and extracted with diethyl ether (2 × 100 mL). The combined extracts were washed with 10% $NaHCO_3$ (1 × 50 mL) and brine (1 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/

diethyl ether, 95:5, to remove impurities, then 92:8, and finally 90:10 to give 259 mg (72% yield from **14**) of aldehyde bromohydrin **15** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.28 [2 s, 6 H, $(CH_3)_2COH$], 1.48−1.62 (m, 11 H, allylic CH_3 and CH_2CHBr), 1.85−2.20 (m, 12 H, allylic CH_2), 2.35−2.40 (m, 2 H, CH_2CHO), 3.84 (m, 1 H, $CHBr$), 4.98−5.23 (m, 3 H, vinylic CH), 9.78 (m, 1 H, CHO) (lit.[15]); IR (liquid film) 3500−3400, 2960, 2920, 2860, 1725 (CO), 1450, 1390, 1110 cm^{-1}; EIMS *m*/*z* 414 (0.5), 412 (0.5), 332 (3), 316 (1), 247 (1), 153 (6), 135 (15), 111 (16), 93 (38), 81 (90), 43 (100); HRMS *m*/*z* 412.1980 (calcd for $C_{22}H_{37}BrO_2$ 412.1977). Anal. ($C_{22}H_{37}BrO_2$) C, H, Br, O.

C_{22} Squalene Aldehyde External Epoxide: (4*E*,8*E*,12*E*)-16,17-Epoxy-4,9,13,17-tetramethyl-4,8,12-octadecatrienal (16). K_2CO_3 (3 equiv, 495 mg, 3.6 mmol) was dissolved in methanol (20 mL), C_{22} squalene aldehyde external bromohydrin **15** (500 mg, 1.2 mmol) was added, and the mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with diethyl ether (3 × 50 mL), and the combined extracts were washed with saturated brine (2 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 95:5, to give 290 mg (73% yield from **15**) of aldehyde **16** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.24 and 1.28 (2 s, 6 H, epoxidic CH_3), 1.59−1.70 (m, 11 H, allylic CH_3 and epoxide-CH_2) 1.96−2.08 (m, 12 H, allylic CH_2), 2.35−2.40 (m, 2 H, CH_2CHO), 2.70 (t, 1 H, epoxidic CH), 5.05−5.15 (m, 3 H, vinylic CH), 9.73 (m, 1 H, CHO) (lit.[22]); IR (liquid film) 2950, 2920, 2840, 1730 (CO), 1460, 1360, 1280, 1170 cm^{-1}; CIMS (isobutane) *m*/*z* 333 (52), 315 (100), 297 (20); HRMS *m*/*z* 332.2721 (calcd for $C_{22}H_{36}O_2$ 332.2715). Anal. ($C_{22}H_{36}O_2$) C, H, O.

(6*E*,10*E*,14*E*,18*R,19*S**)-3-Bromo-2,6,10,15-tetramethyl-19-(trimethylsilyl)-6,10,14,20-henicosatetraene-2,18-diol (18). Reaction 1.** Allyltrimethylsilane (1.20 equiv, 174 mg, 1.52 mmol) was dissolved in anhydrous THF (5 mL) in a two-necked flask equipped with a perforable cap and stirred under a flux of dry argon. The reaction mixture was cooled at −60 °C, and only a faint light was allowed in the laboratory. *n*-Butyllithium (1.6 M solution in hexane, 1.30 equiv, 1 mL, 1.65 mmol) was added; during this time the solution remained colorless. Hexamethylphosphoramide (0.4 mL) was added after 10 min, and the color turned to orange. The mixture was then stirred for 1 h at −60 °C.

Reaction 2. In another two-necked flask equipped with a perforable cap, dicyclopentadienyltitanium(II) dichloride [(η^5-$C_5H_5)_2TiCl_2$] (1.30 equiv, 411 mg, 1.65 mmol) was rapidly added, dissolved in anhydrous THF (5 mL), and stirred at room temperature under a flux of dry argon. Isobutylmagnesium chloride (2.0 M solution in diethyl ether, 1.30 equiv, 0.83 mL, 1.65 mmol) was added to the orange suspension. During the addition, effervescence occurred and the color turned to dark olive green. The flask was stirred in an oil bath at +40 °C for 30 min.

Reaction 3. Both the flasks containing reaction 1 and reaction 2 were cooled at −90 °C, and reaction 1 was transferred into reaction 2 using a cold glass syringe. The color turned immediately to dark purple, indicating the formation of the intermediate allyltrimethylsilyl dicyclopentadienyl titanium **17**; if the color turned brown, sufficient amount of this intermediate had not formed. The reaction mixture was stirred for 30 min at −90 °C.

Reaction 4. C_{22} squalene aldehyde external bromohydrin **15** (1 equiv, 525 mg, 1.27 mmol) dissolved in anhydrous THF (1 mL) was slowly added with vigorous stirring at −90 °C and slowly allowed to reach room temperature, within 2 h, in the acetone bath, and maintained for 30 min at room temperature. During this time the color turned to dark brown.

Reaction 5. 4 N HCl (5 mL) was slowly added at 0 °C, while the reaction mixture turned to orange. The mixture was left for 30 min at this temperature, forming stereospecifically *threo*-trimethylsilyl alcohol **18**.

Reaction 6. The reaction mixture was transferred into an Erlenmeyer flask; water (50 mL) and diethyl ether (50 mL) were added, followed by oxidation with a flux of compressed air for 15 min. The mixture was extracted with diethyl ether (2 × 50 mL) after addition of brine (50 mL). The combined extracts were washed with brine (1 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The crude orange oil was purified by flash chromatography with petroleum ether/diethyl ether, 95:5, then 97:3, finally 90:10 to give 348 mg (52% yield from **15**) of (±)-(*R**,*S**) isomer **18** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.03 [s, 9 H, $Si(CH_3)_3$], 1.33 and 1.35 [2 s, 6 H, $(CH_3)_2COH$], 1.56−1.71 (m, 13 H, allylic CH_3, CH_2CHBr and CH_2CHOH), 1.99−2.30 (m, 13 H, allylic CH_2 and $CHSi$), 3.82 (m, 1 H, $CHOH$), 3.98 (m, 1 H, $CHBr$), 4.87−5.06 (m, 2 H, CH_2=CH), 5.02−5.23 (m, 3 H, vinylic CH), 5.82 (m, 1 H, CH_2=CH); IR (CCl_4) 3620−3560, 2955, 2930, 2855, 1535, 1480, 1350, 1250 cm^{-1}; CIMS (isobutane) *m*/*z* 529 (5), 527 (6), 523 (10), 521 (12), 511 (85), 509 (100); HRMS *m*/*z* 526.2839 (calcd for $C_{28}H_{51}BrO_2Si$ 526.2842). Anal. ($C_{28}H_{51}BrO_2Si$) C, H, Br, O, Si.

(3*R,4*S**,7*E*,11*E*,15*E*)-19,20-Epoxy-7,12,16,20-tetramethyl-3-(trimethylsilyl)-1,7,11,15-henicosatetraen-4-ol (19).** K_2CO_3 (3 equiv, 117 mg, 0.85 mmol) was dissolved in methanol (10 mL), compound **18** (150 mg, 0.284 mmol) was added, and the mixture was stirred for 2 h at room temperature. It was extracted with diethyl ether (3 × 50 mL), and the combined extracts were washed with saturated brine (2 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 95:5, to give 104 mg (82% yield from **18**) of compound **19** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.04 [s, 9 H, $Si(CH_3)_3$], 1.259 and 1.300 (2 s, 6 H, epoxidic CH_3), 1.62−1.71 (m, 13 H, allylic CH_3, CH_2-epoxide and CH_2-$CHOH$), 1.99−2.25 (m, 13 H, allylic CH_2 and $CHSi$), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 3.80 (m, 1 H, $CHOH$), 4.87−5.05 (m, 2 H, CH_2=CH), 5.02−5.23 (m, 3 H, vinylic CH), 5.82 (m, 1 H, CH_2=CH); IR (CCl_4) 3620−3570, 2955, 2925, 2855, 1450, 1380 cm^{-1}; CIMS *m*/*z* 447 (2), 429 (20), 391 (18), 371 (12), 357 (15), 339 (35), 315 (22), 191 (40), 149 (50), 135 (88), 91 (100); HRMS *m*/*z* 446.3585 (calcd for $C_{28}H_{50}O_2Si$ 446.3580). Anal. ($C_{28}H_{50}O_2Si$) C, H, O, Si.

(18*Z*)-29-Methylidene-2,3-oxidohexanorsqualene: (3*Z*,7*E*,11*E*,15*E*)-19,20-epoxy-7,12,16,20-tetramethyl-1,3,7,11,15-henicosapentaene (8). KH (35% suspension in oil) was washed with pentane and rapidly dried. Pure KH (3 equiv, 22 mg, 0.54 mmol) was added in a two-necked flask, anhydrous THF (5 mL) was added, and the reaction mixture was stirred under dry nitrogen. Silyl alcohol **19** (80 mg, 0.18 mmol) was added at +10 °C and allowed to react for 15 min. The mixture was then poured into cold 10% NH_4Cl/diethyl ether (1:1, 50 mL) and extracted with diethyl ether (3 × 30 mL). The combined extracts were washed with saturated brine (1 × 30 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 99:1, to remove impurities, then 98:2 to give 54 mg (84% yield from **19**) of (18*Z*)-29-methylidene-2,3-oxidohexanorsqualene **8** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.266 and 1.308 (2 s, 6 H, epoxidic CH_3), 1.58−1.70 (m, 11 H, allylic CH_3 and CH_2-epoxide), 2.00−2.20 (m, 12 H, allylic CH_2), 2.30 (q, J = 6.3 Hz, 2 H, CH_2CH=CH), 2.71 (t, J = 6.2 Hz, 1 H, epoxidic CH), 5.00−5.24 (m, 5 H, trisubstituted double bond vinylic CH and CH_2=CH), 5.45 (m, 1 H, CH_2CH=CH), 6.00 (m, 1 H, CH_2-CH=CH), 6.65 (m, 1 H, CH_2=CH); IR (CCl_4) 2960, 2925, 2850, 2440, 1450, 1380 cm^{-1}; EIMS *m*/*z* 356 (3), 341 (3), 315 (3), 229 (5), 203 (8), 147 (30), 135 (50), 93 (60), 81 (100); HRMS *m*/*z* 356.3075 (calcd for $C_{25}H_{40}O$ 356.3079). Anal. ($C_{25}H_{40}O$) C, H, O.

(6*E*,10*E*,14*E*,18*E*)-3-Bromo-2,6,10,15-tetramethyl-6,10,14,18,20-henicosapentaen-2-ol (20). Silyl alcohol **18** (150 mg, 0.284 mmol) was dissolved in THF (10 mL) and stirred under dry nitrogen. Two drops of concentrated H_2SO_4 were added, and it was allowed to react at room temperature for 24 h with stirring. The reaction mixture was poured in a separator funnel containing 10% cold $NaHCO_3$/diethyl ether, 1:1 (20 mL), and extracted with diethyl ether (3 × 30 mL). The combined extracts were washed with saturated brine (2

× 30 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 97:3, then 94:6, to give 81 mg (65% yield from **18**) of derivative **20** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.29 and 1.30 [2 s, 6 H, $(CH_3)_2$-COH], 1.45−1.69 (m, 11 H, allylic CH_3 and CH_2CHBr), 1.99−2.20 (m, 14 H, allylic CH_2), 3.90 (m, 1 H, CHBr), 4.93−5.10 (m, 2 H, CH_2CH), 5.03−5.20 (m, 3 H, trisubstituted double bond vinylic CH), 5.70 (m, 1 H, CH_2CH═CH), 6.06 (m, 1 H, CH_2CH═C*H*), 6.30 (m, 1 H, CH_2═C*H*); IR (CCl_4) 3620−3560, 2960, 2925, 2850, 1450, 1380 cm^{-1}; HRMS *m*/*z* 436.2341 (calcd for $C_{25}H_{41}BrO$ 436.2341). Anal. ($C_{25}H_{41}BrO$) C, H, Br, O.

(18*E*)-29-Methylidene-2,3-oxidohexanorsqualene: (3*E*,7*E*,11*E*,15*E*)-19,20-Epoxy-7,12,16,20-tetramethyl-1,3,7,-11,15-henicosapentaene (21). K_2CO_3 (3 equiv, 70 mg, 0.51 mmol) was dissolved in methanol (10 mL), compound **20** (75 mg, 0.17 mmol) was added, and the mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with diethyl ether (3 × 50 mL), and the combined extracts were washed with saturated brine (2 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 99:1, to remove impurities, then 98:2 to give 56 mg (93% yield from **20**) of (18*E*)-29-methylidene-2,3-oxidohexanorsqualene (**21**) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.265 and 1.307 (2 s, 6 H, epoxidic CH_3), 1.53−1.70 (m, 11 H, allylic CH_3 and CH_2-epoxide), 2.00−2.24 (m, 14 H, allylic CH_2), 2.71 (t, J = 6.2 Hz, 1 H, epoxidic CH), 4.93−5.10 (m, 2 H, CH_2CH), 5.05−5.20 (m, 3 H, trisubstituted double bond vinylic CH), 5.70 (m, 1 H, CH_2CH═CH), 6.06 (m, 1 H, CH_2CH═C*H*), 6.31 (m, 1 H, CH_2═C*H*); IR (CCl_4) 2960, 2925, 2850, 2440, 1450, 1380 cm^{-1}; EIMS *m*/*z* 356 (2), 341 (2), 315 (2), 229 (5), 203 (7), 147 (32), 81 (100); HRMS *m*/*z* 356.3082 (calcd for $C_{25}H_{40}O$ 356.3079). Anal. ($C_{25}H_{40}O$) C, H, O.

4-Methyl-3-pentenyl Methanesulfonate (23). 4-Methyl-3-penten-1-ol (**22**) (4.00 g, 40 mmol) was dissolved in anhydrous pyridine (15 mL) and stirred at 0 °C under dry nitrogen. Methanesulfonyl chloride (1.2 equiv, 5.5 g, 48 mmol) was added at 0 °C over 15 min, the reaction mixture became cloudy and frothy, and it was allowed to react for 2 h at room temperature. *n*-Hexane (100 mL) was added, the suspension was filtered, and the solid was washed with *n*-hexane and evaporated to dryness. The resulting oil was dissolved in petroleum ether (100 mL), washed with cold diluted HCl (1 × 50 mL), and extracted with petroleum ether (2 × 50 mL). The combined extracts were washed with 10% $NaHCO_3$ (3 × 50 mL) and saturated brine (2 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. A flash chromatography column was preeluted with petroleum ether/isopropylamine, 99:1, until basic eluate, followed by petroleum ether/isopropylamine, 99.8:0.2 (500 mL). The crude product was eluted with petroleum ether/isopropylamine, 99.8:0.2, to remove impurities, then petroleum ether/diethyl ether/isopropylamine, 98.8:1:0.2, to give 6.2 g (87% yield from **22**) of compound **23** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.65 and 1.72 (2 s, 6 H, allylic CH_3), 2.45 (m, 2 H, CH_2CH═), 3.00 (s, 3 H, SO_2CH_3), 4.18 (t, J = 7.0 Hz, 2 H, CH_2O), 5.10 (broad t, 1 H, vinylic CH); IR (liquid film) 2970, 2920, 2860, 1450, 1355, 1175 cm^{-1}; CIMS (isobutane) *m*/*z* 179 (100), 165 (10), 153 (22), 139 (14), 121 (15); HRMS *m*/*z* 178.0666 (calcd for $C_7H_{14}O_3S$ 178.0664). Anal. ($C_7H_{14}O_3S$) C, H, O, S.

5-Iodo-2-methyl-2-pentene (24). Mesylate **23** (6 g, 33.7 mmol) was dissolved in acetone (20 mL), and NaI (5 equiv, 25 g, 168.5 mmol), dissolved in acetone (100 mL), was added. The reaction mixture was left for 3 h at +40 °C under dry nitrogen with stirring. During this time it became cloudy and bright yellow. It was then cooled, poured into cold water/pentane, 1:1 (200 mL), and extracted with pentane (3 × 80 mL). The combined extracts were washed with water (2 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo at room temperature just until ^{1}H NMR controls revealed no more solvent, giving 6.87 g (97% yield from **23**) of iodide **24** as a pale yellow oil. The crude product was pure (^{1}H NMR and TLC analysis), and because of its volatility, it was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.62 and 1.70 (2 s, 6 H, allylic CH_3), 2.57 (m, 2 H, CH_2CH_2I), 3.11 (t, J = 7.4 Hz, 2 H, CH_2I), 5.09 (broad t, 1 H, vinylic CH); IR (liquid film) 2965, 2930, 1670, 1450, 1380, 1240, 1210 cm^{-1}. A satisfactory EI or CI mass spectrum has not been obtained.

Methyl α-[Bis(2,2,2-trifluoroethoxy)phosphoryl]-α-(4-methyl-3-pentenyl)acetate (25) and Methyl α-[Bis(2,2,2-trifluoroethoxy)phosphoryl]-α,α-bis(4-methyl-3-pentenyl)-acetate (26). NaH (60% suspension in oil, washed with pentane) (1.5 equiv, 515 mg, 21.4 mmol) was added to anhydrous DMSO (15 mL) and left under dry argon with stirring. Methyl bis(2,2,2-trifluoroethoxy)phosphoryl acetate (1 equiv, 4.55 g, 14.3 mmol) was added in small portions within 15 min, with cooling in a water bath at room temperature to avoid excessive foaming. The bath was removed, and after an additional 15 min, iodide **24** (1 equiv, 3.0 g, 14.3 mmol) was added in small portions. The pale yellow solution was left for 2 days at room temperature under argon, with stirring; during this time it progressively became light orange. It was then poured into a cold water/diethyl ether, 1:1 (100 mL), biphasic system and extracted with diethyl ether (3 × 80 mL). The combined organic layers were washed with saturated brine (2 × 50 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 95:5, then 90:10 to give 965 mg (14% yield) of dialkylated derivative **26**, and then 85:15 to give 2.63 g (46% yield) of the desired monoalkylated compound **25** as colorless oils.

25: ^{1}H NMR ($CDCl_3$) (δ) 1.58 and 1.69 [2 s, 6 H, $C(CH_3)_2$], 1.80−2.16 (m, 4 H, $CHCH_2CH_2CH$), 3.03−3.23 (m, 1 H, C*H*PO), 3.77 (s, 3 H, $COOCH_3$), 4.32−4.51 [m, 4 H, $(CF_3CH_2O)_2$], 5.03 (broad t, 1 H, C*H*═C); IR (liquid film) 2970, 2930, 2860, 2360, 1745, 1440, 1420, 1300, 1260, 1175, 1100, 1075 cm^{-1}; EIMS *m*/*z* 400 (14), 369 (12), 331 (5), 319 (48), 318 (27), 287 (24), 286 (17), 260 (11), 258 (14), 219 (15), 123 (12), 82 (100); HRMS *m*/*z* 400.0871 (calcd for $C_{13}H_{19}F_6O_5P$ 400.0874). Anal. ($C_{13}H_{19}F_6O_5P$) C, H, F, O, P.

26: ^{1}H NMR ($CDCl_3$) (δ) 1.59 and 1.68 [2 s, 12 H, bisC-$(CH_3)_2$], 1.79−2.15 [m, 8 H, bis(CH_2CH_2)], 3.78 (s, 3 H, $COOCH_3$), 4.34−4.52 [m, 4 H, $(CF_3CH_2O)_2$], 5.06 [(broad t, 2 H, bis(C*H*═C)]; IR (liquid film) 2970, 2930, 2860, 1745, 1725, 1455, 1290, 1255, 1175, 1105, 1070 cm^{-1}; EIMS *m*/*z* 482 (1), 400 (3), 369 (1), 331 (100), 299 (32), 245 (4); HRMS *m*/*z* 482.1655 (calcd for $C_{19}H_{29}F_6O_5P$ 482.1657). Anal. ($C_{19}H_{29}F_6O_5P$) C, H, F, O, P.

Methyl (2*Z*,6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-2,6,10,14-icosatetraenoate (27) and Methyl (2*E*,6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-2,6,10,14-icosatetraenoate (28). To a solution of 18-crown-6 (5 equiv, 1.98 g, 7.5 mmol) in anhydrous THF (40 mL) under argon, with stirring at room temperature was added phosphonium acetate **25** (1 equiv, 600 mg, 1.5 mmol) in THF (2 mL). The mixture was cooled to −80 °C, and potassium bis(trimethylsilyl)amide (1.3 equiv, 0.5 M solution in toluene, 1.95 mmol, 3.9 mL) was slowly added; during the addition, the mixture progressively turned yellow. After 30 min, C_{22} squalene aldehyde external epoxide **16** (1 equiv, 500 mg, 1.5 mmol) in THF (2 mL) was slowly added with vigorous stirring. The reaction mixture was left for 2 h at −80 °C, then brought to −40 °C, and left for an additional 15 min at this temperature. It was poured into 80 mL of an iced brine/diethyl ether, 1:1, two-phase system, and extracted with diethyl ether (3 × 40 mL). The combined extracts were washed with saturated brine (2 × 30 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with a petroleum ether/diethyl ether gradient, 98:2, 97:3, 96:4, 95:5, 94:6, to give initially *Z* isomer **27** (290 mg), then *E* isomer **28** (112 mg) completely separated in 57% total yield from **16** (*Z*:*E* = 72:28).

27: ^{1}H NMR ($CDCl_3$) δ 1.257 and 1.299 (2 s, 6 H, epoxidic CH_3), 1.58−1.68 (m, 17 H, allylic CH_3 and epoxide-CH_2), 1.98−2.15 (m, 14 H, allylic CH_2), 2.26 [t, 2 H, $CH_2C(COOCH_3)$], 2.52 [m, 2 H, CH_2CH═$C(COOCH_3)$], 2.70 (t, J = 6.2 Hz, 1 H,

epoxidic CH), 3.73 (s, 3 H, COOCH_3), 5.00−5.16 (m, 4 H, vinylic CH), 5.86 [t, J = 7.3 Hz, 1 H, CH=C(COOCH$_3$)]; IR (liquid film) 2960, 2925, 2855, 1715 (CO), 1440, 1380 cm^{-1}; CIMS (isobutane) m/z 471 (100), 453 (65), 439 (14), 315 (70), 291 (18), 273 (34); HRMS 470.3762 (calcd for $C_{31}H_{50}O_3$ 470.3760). Anal. ($C_{31}H_{50}O_3$) C, H, O.

28: ^{1}H NMR (CDCl$_3$) δ 1.257 and 1.299 (2 s, 6 H, epoxidic CH$_3$), 1.58−1.69 (m, 17 H, allylic CH$_3$ and epoxide-CH_2), 1.98−2.14 (m, 14 H, allylic CH$_2$), 2.29 [m, 4 H, CH_2CH=CCH_2-(COOCH$_3$)], 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 3.72 (s, 3 H, COOCH_3), 5.00−5.20 (m, 4 H, vinylic CH), 6.74 [t, J = 7.4 Hz, 1 H, CH=C(COOCH$_3$)]; IR (liquid film) 2960, 2925, 2850, 1720 (CO), 1440, 1375 cm^{-1}; CIMS (isobutane) m/z 471 (100), 453 (71), 439 (13), 333 (15), 319 (87), 291 (34), 273 (59); HRMS 470.3754 (calcd for $C_{31}H_{50}O_3$ 470.3760). Anal. ($C_{31}H_{50}O_3$) C, H, O.

(2*Z*,6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-2,6,10,14-icosatetraen-1-ol (29). Li-AlH$_4$, washed with pentane before use (5 equiv, 61 mg, 1.60 mmol), was added to dry diethyl ether (10 mL) with stirring and cooled to −30 °C under argon. *Z*-Ester **27** (1 equiv, 150 mg, 0.32 mmol) in dry diethyl ether (1 mL) was added with vigorous stirring. After 30 min, the reaction was stopped, as no more ester was present. Saturated aqueous NH$_4$Cl (100 μL) was added with a syringe to the mixture at −30 °C, under argon, whereupon effervescence appeared. A very small amount of anhydrous sodium sulfate was added with stirring, and the suspension was filtered on a syntherized porous septum and evaporated to dryness. A flash chromatography column was preeluted with petroleum ether/diethyl ether/isopropylamine, 98:1:1, until basic eluate and then with petroleum ether/diethyl ether/isopropylamine, 98.9:1:0.1 (300 mL). The crude product was eluted with petroleum ether/diethyl ether/isopropylamine, 98.9:1:0.1, to remove impurities, then petroleum ether/diethyl ether/isopropylamine, 94.9:5:0.1, finally 89.9:10:0.1, to obtain 133 mg (94% yield from **27**) of compound **29** as a colorless oil: ^{1}H NMR (CDCl$_3$) δ 1.258 and 1.300 (2 s, 6 H, epoxidic CH$_3$), 1.58−1.70 (m, 17 H, allylic CH$_3$ and epoxide-CH_2), 2.00−2.27 (m, 18 H, allylic CH$_2$), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 4.11 (broad s, 2 H, CH_2OH), 5.03−5.23 (m, 4 H, vinylic CH), 5.31 [t, J = 7.2 Hz, 1 H, CH=C(CH$_2$-OH)]; IR (liquid film) 3450, 2960, 2925, 2855, 1450, 1380 cm^{-1}; CIMS (isobutane) m/z 443 (24), 425 (100), 407 (23), 291 (77), 273 (69), 221 (44), 207 (47); HRMS 442.3813 (calcd for $C_{30}H_{50}O_2$ 442.3811). Anal. ($C_{30}H_{50}O_2$) C, H, O.

(2*Z*,6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-2,6,10,14-icosatetraenal (30). *Z*-Alcohol **29** (120 mg, 0.27 mmol) dissolved in *n*-hexane (40 mL) was left at +5 °C under nitrogen, with stirring. Na$_2$CO$_3$ (50 equiv, 1.43 g, 13.5 mmol) and activated MnO$_2$ (20 equiv, 85% purity, 552 mg, 5.4 mmol) were added. After 2 h of reaction at +5 °C, additional Na$_2$CO$_3$ (25 equiv, 715 mg, 6.75 mmol) and activated MnO$_2$ (10 equiv, 85% purity, 276 mg, 2.7 mmol) were added, and the mixture was allowed to react for a further 2 h. The reaction mixture was filtered on a porous septum and evaporated to dryness. A flash chromatography column was preeluted with petroleum ether/isopropylamine, 99:1, until basic eluate, followed by petroleum ether/isopropylamine, 99.9:0.1 (300 mL). The crude product was quickly eluted with petroleum ether/diethyl ether/isopropylamine, 98.9:1:0.1, to remove impurities, then petroleum ether/diethyl ether/isopropylamine, 96.9:3:0.1, to give 105 mg (88% yield from **29**) of pure *Z*-enal **30** as a colorless oil. As the crude product is essentially pure (^{1}H NMR and TLC analysis), it may be used directly in the next step: ^{1}H NMR (CDCl$_3$) δ 1.258 and 1.300 (2 s, 6 H, epoxidic CH$_3$), 1.56−1.69 (m, 17 H, allylic CH$_3$ and epoxide-CH_2), 1.98−2.25 (m, 18 H, allylic CH$_2$), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 5.00−5.18 (m, 4 H, vinylic CH), 6.46 [t, J = 8.1 Hz, 1H, CH=C(CHO)], 10.09 (s, 1 H, CHO); IR (liquid film) 2960, 2925, 2855, 1678 (CO), 1455, 1380 cm^{-1}; CIMS (isobutane) m/z 441 (100), 423 (92); EIMS m/z 440 (3), 422 (8), 407 (2), 371 (4), 353 (3), 289 (5), 271 (4), 217 (8), 203 (18), 153 (23), 135 (55), 109 (52), 81 (80), 69 (100); HRMS 440.3655 (calcd for $C_{30}H_{48}O_2$ 440.3654). Anal. ($C_{30}H_{48}O_2$) C, H, O.

(18*Z*)-29-MOS: (6*Z*,10*E*,14*E*,18*E*)-22,23-Epoxy-2,10,15,-19,23-pentamethyl-6-vinyl-2,6,10,14,18-tetracosapentaene (31). Methyltriphenylphosphonium bromide (10 equiv, 810 mg, 2.27 mmol) in anhydrous THF (10 mL) was left at −20 °C, under nitrogen, with stirring. *n*-Butyllithium (1.6 M solution in hexane, 10 equiv, 1.4 mL, 2.27 mmol) was added; the color progressively turned to yellow and then to orange. It was allowed to stand for 30 min at −20 °C and then placed in another acetone bath at −80 °C. *Z*-Enal **30** (100 mg, 0.227 mmol) was dissolved in anhydrous THF (50 mL) and cooled to −80 °C. The orange solution of the ylide at −80 °C was slowly transferred with a glass syringe into the solution of *Z*-enal **30** at −80 °C under dry nitrogen. After 30 min of stirring at −80 °C, it was left at room temperature for 30 min. The mixture was poured into saturated iced NH$_4$Cl/diethyl ether, 1:1 (100 mL), upon which the orange-yellow color disappeared. The mixture was then extracted with diethyl ether (3 × 40 mL). The combined extracts were washed with saturated brine (2 × 30 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether/diethyl ether, 99:1, to remove impurities, then 98:2, finally 97:3, affording 87 mg (88% yield from *Z*-enal **30**) of (18*Z*)-29-MOS (**31**) as a colorless oil. ^{1}H NMR analysis showed pure *Z*-isomerism in the conjugated system. On TLC it had identical R_f to 2,3-oxidosqualene: ^{1}H NMR (CDCl$_3$) δ 1.256 and 1.301 (2 s, 6 H, epoxidic CH$_3$), 1.56−1.69 (m, 17 H, allylic CH$_3$ and CH_2-epoxide), 1.98−2.28 (m, 18 H, allylic CH$_2$), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), for (H^1)(H^2)C=C(H^3)(R): H^1 cis to H,[3] 5.08 [broad d, $J_{1,3}$ = 11.1 Hz, 1 H, (H^1)(H^2)C=C(H^3)(R)], 5.05−5.19 (m, 4 H, non conjugated vinylic CH), 5.24 [broad d, $J_{2,3}$ = 17.4 Hz, 1 H, (H^1)(H^2)C=C(H^3)(R)], 5.37 [broad t, J = 7.3 Hz, 1 H, CH=C(R)-CH=CH$_2$], 6.68 [dd, $J_{3,1}$ = 11.1 Hz and $J_{3,2}$ = 17.4 Hz, 1 H, (H^1)(H^2)C=C(H^3)(R)]; IR (liquid film) 2925, 2855, 1665, 1595, 1450, 1380 cm^{-1}; EIMS m/z 438 (1.4), 410 (0.6), 395 (2.3), 377 (2), 351 (1), 337 (1), 309 (2), 271 (9), 243 (7), 215 (15), 203 (20), 173 (20), 161 (38), 149 (63), 135 (47), 93 (100); HRMS m/z 438.3865 (calcd for $C_{31}H_{50}O$ 438.3862). Anal. ($C_{31}H_{50}O$) C, H, O.

(2*E*,6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-2,6,10,14-icosatetraen-1-ol (32). Compound **32** was obtained from *E*-acetate **28** following the same method and purification as compound **29**, with 90% yield: ^{1}H NMR (CDCl$_3$) δ 1.258 and 1.299 (2 s, 6 H, epoxidic CH$_3$), 1.43−1.68 (m, 17 H, allylic CH$_3$ and epoxide-CH_2), 1.99−2.27 (m, 18 H, allylic CH$_2$), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 3.54 (m, 1 H, OH), 4.03 (broad s, 2 H, CH_2OH), 5.00−5.20 (m, 4 H, vinylic CH), 5.41 [t, J = 6.7 Hz, 1 H, CH=C(CH$_2$OH)]; IR (liquid film) 3450, 2960, 2925, 2855, 1450, 1380 cm^{-1}; CIMS (isobutane) m/z 443 (15), 427 (35), 425 (100), 407 (30); HRMS 442.3806 (calcd for $C_{30}H_{50}O_2$ 442.3811). Anal. ($C_{30}H_{50}O_2$) C, H, O.

(2*E*,6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-2,6,10,14-icosatetraenal (33). Compound **33** was obtained from *E*-alcohol **32** following the same method and purification as compound **30**, with 87% yield: ^{1}H NMR (CDCl$_3$) δ 1.256 and 1.300 (2 s, 6 H, epoxidic CH$_3$), 1.56−1.68 (m, 17 H, allylic CH$_3$ and epoxide-CH_2), 2.00−2.52 (m, 18 H, allylic CH$_2$), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 5.05−5.23 (m, 4 H, vinylic CH), 6.44 [t, J = 7.2 Hz, 1H, CH=C-(CHO)], 9.35 (s, 1 H, CHO); IR (liquid film) 2960, 2925, 2855, 1690 (CO), 1455, 1375 cm^{-1}; CIMS (isobutane) m/z 441 (100), 423 (70), 407 (6), 383 (10); HRMS 440.3658 (calcd for $C_{30}H_{48}O_2$ 440.3654). Anal. ($C_{30}H_{48}O_2$) C, H, O.

(18*E*)-29-MOS: (6*E*,10*E*,14*E*,18*E*)-22,23-Epoxy-2,10,15,-19,23-pentamethyl-6-vinyl-2,6,10,14,18-tetracosapentaene (34). (18*E*)-29-MOS (**34**) was obtained from *E*-aldehyde **33** following the same method and purification as compound **31**, with 85% yield. ^{1}H NMR analysis showed pure *E*-isomerism in the conjugated system: ^{1}H NMR (CDCl$_3$) δ 1.258 and 1.301 (2 s, 6 H, epoxidic CH$_3$), 1.59−1.70 (m, 17 H, allylic

CH_3 and C*H*$_2$-epoxide), 1.99−2.28 (m, 18 H, allylic CH_2), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), for (H^1)(H^2)C=C(H^3)(R): H^1 cis to H,[3] 4.92 [broad d, $J_{1,3}$ = 10.7 Hz, 1 H, (*H*^{1})(H^2)C=C(H^3)-(R)], 5.07 [partially covered part of a doublet, 1 H, (H^1)-(*H*^{2})C=C(H^3)(R)], 5.05−5.20 (m, 4 H, non conjugated vinylic CH), 5.45 [t, J = 7.0 Hz, 1 H, C*H*=C(R)-CH=C*H*$_2$], 6.25 [dd, $J_{3,1}$ = 10.7 Hz and $J_{3,2}$ = 17.5 Hz, 1 H, (H^1)(H^2)C=C(*H*^{3})(R)]; IR (liquid film) 2925, 2855, 1450, 1380 cm^{-1}; CIMS (isobutane) *m*/*z* 439 (100), 421 (55), 411 (10), 397 (10); EIMS *m*/*z* 438 (0.7), 410 (0.3), 395 (0.4), 368 (0.4), 353 (0.2), 297 (0.2), 271 (0.6), 229 (0.4), 203 (2), 161 (4), 149 (15), 107 (25), 93 (75), 69 (100); HRMS *m*/*z* 438.3861 (calcd for $C_{31}H_{50}O$ 438.3862). Anal. ($C_{31}H_{50}O$) C, H, O.

(5*E*,9*E*,13*E*,17*E*)-21,22-Epoxy-2,9,14,18,22-pentamethyl-5-vinyl-2,5,9,13,17-tricosapentaene (37). Allyltriphenylphosphonium bromide **36** (1.5 equiv, 276 mg, 0.72 mmol) was dissolved in anhydrous diethyl ether (20 mL), and *n*-butyllithium (1.6 M solution in hexane, 3.5 equiv, 1.05 mL, 1.7 mmol) was added at room temperature, under nitrogen, and left to react for 1 h, forming a red solution. 1-Bromo-3-methyl-2-butene (**35**) (2 equiv, 143 mg, 0.96 mmol) in diethyl ether (1 mL) was added, and the mixture was refluxed for 3 h in an oil bath at 40−50 °C. After the mixture was cooled to room temperature, C_{22} aldehyde bromohydrin **15** (1 equiv, 200 mg, 0.48 mmol) in diethyl ether (1 mL) was added, and the mixture was left for 30 min at room temperature and refluxed for 1 h. It was then poured into iced NH_4Cl/petroleum ether, 1:1 (100 mL), and extracted with petroleum ether (3 × 80 mL). The combined extracts were washed with saturated brine (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether to remove impurities, then petroleum ether/diethyl ether, 99:1, finally 98:2 to give 60 mg (30% yield from **15**) of compound **37**, as a colorless oil and then 58 mg of a mixture of (*Z*)- and (*E*)-hexanormethylidenes **8** and **21** (40:60, 34% yield from **15**). ^{1}H NMR and mass data of the mixture of compounds **8** and **21** was identical with that of (*Z*)- and (*E*)-hexanormethylidenes **8** and **21** prepared separately according to the previously described stereospecific method, and then mixed (40:60).

37: ^{1}H NMR ($CDCl_3$) δ 1.259 and 1.302 (2 s, 6 H, epoxidic CH_3), 1.58−1.69 (m, 17 H, allylic CH_3 and C*H*$_2$-epoxide), 1.98−2.38 (m, 16 H, allylic CH_2), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), for (H^1)(H^2)C=C(H^3)(R): H^1 cis to H,[3] 4.95 [broad d, 1 H, (*H*^{1})(H^2)C=C(H^3)(R)], 5.00−5.22 [m, 5 H, non conjugated vinylic CH and (H^1)(*H*^{2})C=C(H^3)(R)], 5.48 [t, 1 H, C*H*=C(R)-CH=C*H*$_2$], 6.30 [dd, 1 H, (H^1)(H^2)C=C(*H*^{3})(R)], IR (CCl_4) 2960, 2925, 2850, 2440, 1450, 1380 cm^{-1}; EIMS *m*/*z* 424 (20), 379 (8), 309 (10), 283 (10), 271 (20), 229 (15), 203 (40), 135 (100); HRMS 424.3701 (calcd for $C_{30}H_{48}O$ 424.3705). Anal. ($C_{30}H_{48}O$) C, H, O.

(6*E*,10*E*,14*E*)-18,19-Epoxy-6,11,15,19-tetramethyl-2-(4-methyl-3-pentenyl)-6,10,14-icosatrienal (38). Acetic acid (1 equiv, 7.9 mg, 0.132 mmol) was dissolved in benzene (1 mL) at room temperature, under argon, with stirring. *Z*-Enal **30** (1 equiv, 58 mg, 0.132 mmol) was added, followed by $Pd(Ph_3)_4$ (0.01 equiv, 1.5 mg, 0.00132 mmol). Bu_3SnH (1.2 equiv, 46 mg, 43 μL, 0.158 mmol) was added dropwise over a period of 1 min and allowed to react for 1 h at room temperature, forming a light brown solution. It was poured into iced water−dichloromethane (20 mL × 3), washed with saturated brine (20 mL × 1), dried with anhydrous sodium sulfate, and evaporated in vacuo. A flash chromatography column was preeluted with petroleum ether/isopropylamine, 99:1, until basic eluate, followed by petroleum ether (100 mL). The crude product was eluted with petroleum ether/diethyl ether, 99:1, then 98:2 to give 37 mg (63% yield from **30**) of aldehyde **38** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.263 and 1.305 (2 s, 6 H, epoxidic CH_3), 1.35−1.50 (m, 6 H, alkylic CH_2), 1.55−1.68 (m, 17 H, allylic CH_3 and epoxide-C*H*$_2$), 1.98−2.25 (m, 15 H, allylic CH_2 and C*H*CHO), 2.71 (t, J = 6.2 Hz, 1 H, epoxidic CH), 5.03−5.18 (m, 4 H, vinylic CH), 9.56 (d, J = 3.0 Hz, 1 H, CHO); IR (liquid film) 2960, 2925, 1720 (CO), 1455, 1375 cm^{-1}; HRMS 442.3809 (calcd for $C_{30}H_{50}O_2$ 442.3811). Anal. ($C_{30}H_{50}O_2$) C, H, O.

18,19-Dihydro-29-methylidene-2,3-oxidosqualene: (10*E*,-14*E*,18*E*)-22,23-Epoxy-2,10,15,19,23-pentamethyl-6-vinyl-2,10,14,18-tetracosatetraene (39). Methyltriphenylphosphonium bromide (10 equiv, 243 mg, 0.68 mmol) in anhydrous THF (5 mL) was allowed to stand at −20 °C, under nitrogen, with stirring. *n*-Butyllithium (1.6 M solution in hexane, 10 equiv, 425 μL, 0.68 mmol) was added; the color progressively turned yellow and then orange. It was allowed to stand for 30 min at −20 °C and then placed in another acetone bath at −80 °C. Compound **38** (30 mg, 0.068 mmol), dissolved in anhydrous THF (5 mL), was slowly added. After 30 min of stirring at −80 °C, it was stood at room temperature for 30 min. It was poured into saturated iced NH_4Cl/diethyl ether, 1:1 (50 mL), whereupon the orange-yellow color disappeared; it was then extracted with diethyl ether (3 × 30 mL). The combined extracts were washed with saturated brine (2 × 20 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether, then petroleum ether/diethyl ether, 99:1, affording 17 mg (57% yield from **38**) of compound **39** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.26−1.45 (m, 12 H, alkylic CH_2 and epoxidic CH_3), 1.55−1.70 (m, 17 H, allylic CH_3 and C*H*$_2$-epoxide), 1.87−2.20 (m, 15 H, allylic CH_2 and C*H*CH=C*H*$_2$), 2.71 (t, J = 6.2 Hz, 1 H, epoxidic CH), 4.90−4.99 (two broad d, overlapped at 4.99, J =10.5 and 18.5 Hz, 2 H, C*H*$_2$=CH), 5.03−5.18 (m, 4 H, vinylic CH), 5.45−5.60 (m, 1 H, CH$_2$=C*H*); IR (CCl_4) 2960, 2930, 2850, 1740, 1460, 1380 cm^{-1}; CIMS *m*/*z* 441 (68), 423 (100); EIMS *m*/*z* 440 (2), 422 (3), 397 (2), 372 (2), 355 (4), 313 (10), 245 (15), 217 (15), 203 (25), 135 (90), 81 (100); HRMS *m*/*z* 440.4013 (calcd for $C_{31}H_{52}O$ 440.4018). Anal. ($C_{31}H_{52}O$) C, H, O.

18-Heptanor-2,3-oxidosqualene: (5*E*,9*E*,13*E*)-17,18-Epoxy-5,10,14,18-tetramethyl-1,5,9,13-nonadecatetraene (40). Methyltriphenylphosphonium bromide (2 equiv, 86 mg, 0.24 mmol) in anhydrous THF (5 mL) was allowed to stand at −20 °C, under nitrogen, with stirring. *n*-Butyllithium (1.6 M solution in hexane, 3.2 equiv, 240 μL, 0.38 mmol) was added; the color progressively turned yellow and then orange. It was allowed to stand for 30 min at −20 °C and then placed in another acetone bath at −80 °C. Compound **15** (50 mg, 0.12 mmol), dissolved in anhydrous THF (5 mL), was slowly added. After 30 min of stirring at −80 °C, it was left at room temperature for 30 min. The mixture was poured into saturated iced NH_4Cl/diethyl ether, 1:1 (50 mL), whereupon the orange-yellow color disappeared. The mixture was then extracted with diethyl ether (3 × 30 mL). The combined extracts were washed with saturated brine (2 × 20 mL), dried with anhydrous sodium sulfate, and evaporated in vacuo. The resulting oil was purified by flash chromatography with petroleum ether, then petroleum ether/diethyl ether, 99:1, affording 34 mg (85% yield from **15**) of compound **40** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.258 and 1.299 (2 s, 6 H, epoxidic CH_3), 1.55−1.70 (m, 11 H, allylic CH_3 and C*H*$_2$-epoxide), 1.98−2.18 (m, 14 H, allylic CH_2), 2.70 (t, J = 6.2 Hz, 1 H, epoxidic CH), 4.90−5.06 (two broad d, overlapped at 4.96, 2 H, C*H*$_2$=CH), 5.02−5.20 (m, 3 H, vinylic CH), 5.70−5.90 (m, 1 H, CH$_2$=C*H*); IR (liquid film) 3080, 2960, 2930, 2860, 1650, 1450, 1380, 1250 cm^{-1}; CIMS *m*/*z* 331 (80), 313 (100); EIMS *m*/*z* 330 (3), 312 (4), 289 (2), 269 (3), 257 (2), 203 (20), 175 (25), 153 (30), 135 (74), 81 (100); HRMS *m*/*z* 330.2918 (calcd for $C_{23}H_{38}O$ 330.2923). Anal. ($C_{23}H_{38}O$) C, H, O.

Biological Assays. (A) Microsomes. Yeast and pig liver microsomes were prepared according to the methods previously described.[43]

(B) Solubilization and Purification of OSC. The pig enzyme was purified according to the method described.[44] For the solubilization of yeast enzyme, washed microsomes were diluted with 0.5 M Na/K phosphate buffer (pH 6.20) to a protein concentration of 12 mg/mL. The solution was gently stirred for 30 min at 4 °C and then centrifuged at 150000*g* for 60 min, giving a supernatant fraction, containing the periph-

eral proteins, and a pellet containing the integral proteins. The yeast OSC was then solubilized, resuspending the pellet in 0.1 M Na/K phosphate buffer (pH 7.05) containing 10 mg/mL of Polidocanol, to a protein concentration of 12 mg/mL. The solution was gently stirred for 60 min at 4 °C and then centrifuged at 150000*g* for 60 min, giving a supernatant containing the enzymatic activity.

(C) Assay of OSC Activity and Kinetic Determination. Enzyme activity of OSC was determined by incubating the partially purified pig enzyme for 30 min at 45 °C, and the solubilized yeast enzyme for 30 min at 35 °C, with [^{3}H]-(*R*,*S*)-2,3-oxidosqualene (50000 cpm), as previously described.[43] IC_{50} values (the concentration of inhibitor that reduced the enzymatic conversion of 2,3-oxidosqualene to lanosterol by 50%) were determined at 25 μM substrate concentration, in the presence of different concentrations of inhibitors.

(D) Time-Dependent Inactivation of the OSC. Time-dependent inactivation was determined at 35 °C by adding the inhibitors to the enzyme solution in the absence of the substrate. Aliquots were withdrawn at time intervals from 30 s to 45 min and diluted 40-fold for pig enzyme or 10-fold for yeast enzyme by transfer to test tubes containing cold and labeled substrate 2,3-oxidosqualene (25 μM) and Tween-80 (0.5 mg/mL) in Na/K phosphate buffer. Residual activity was determined by incubating pig or yeast enzyme under the same conditions as above. Second-order inactivation constants were determined from *t*/2 values obtained in the time-dependent inactivation experiments.

Acknowledgment. This work was supported by grants from the Ministero della Ricerca Scientifica e Tecnologica and from CNR, Progetto Finalizzato Chimica Fine. Thanks are due to Prof. G. Prestwich and Prof. I. Abe for an authentic sample of 29-MOS. Thanks are also due to Mr. Daniele Zonari.

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JM970534J