# Synthesis of (*E*)- and (*Z*)-29-methylidyne-2,3-oxidosqualene derivatives as inhibitors of liver and yeast oxidosqualene cyclase

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The synthesis of (*E*)- and (*Z*)-29-methylidyne-2,3-oxidosqualene derivatives is described starting from the $C_{22}$ and $C_{17}$ squalene aldehyde monobromohydrins. The conversion was achieved by means of a Wittig reaction, followed by desilylation of the terminal acetylene. For trisubstituted 1,3-enynes, preliminary alkylation with a suitable allyl bromide was performed. A new procedure for the synthesis of squalene aldehyde $C_{27}$, $C_{22}$ and $C_{17}$ monobromohydrins is also described. Some of the new compounds behaved as inhibitors of pig liver and yeast oxidosqualene cyclase and were time-dependent inhibitors of the animal enzyme.

## Introduction

2,3-Oxidosqualene cyclases (OSCs) (EC 5.4.99.7) are widely distributed enzymes that catalyse the cyclization of (3*S*)-2,3-oxidosqualene (OS) **1** to lanosterol **3** in mammals and yeasts and to cycloartenol **4** (Scheme 1) or to a variety of tetracyclic and pentacyclic triterpenes in higher plants. OSCs are membrane-associated enzymes that have been purified and cloned from different species: *Candida albicans*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* among the fungi; higher plants, the rat, the pig and man.[1–12] The predicted molecular masses range from 80 to 90 kDa and the amino acid sequences determined show significant homology between rat, yeast and plant enzymes. Sequence comparison of OSCs with bacterial squalene hopene cyclase[13] show 17–27% homogeneity and reveals the existence of a highly conserved repetitive motif (the QW motif) rich in aromatic amino acids.[4]

The cyclization of OS **1** starts with the protonation of the epoxide by a suitable electrophilic residue of the enzyme, to give a C-2 carbonium ion intermediate and proceeds through the formation of a series of carbonium ion intermediates: the monocyclic, the bicyclic, the tricyclic with a five-membered cycle, the all six-membered tricyclic and finally the tetracyclic intermediate **2** or protosteryl ion. This latter undergoes back-

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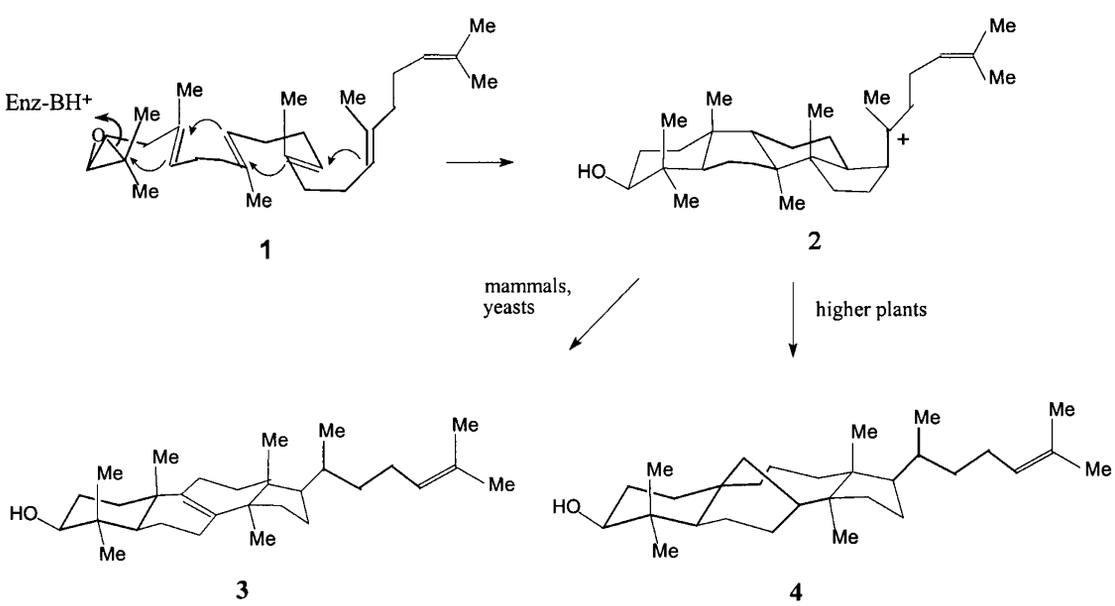


**Scheme 1** Mechanism of cyclization of 2,3-oxidosqualene **1** to lanosterol **3** and cycloartenol **4**.

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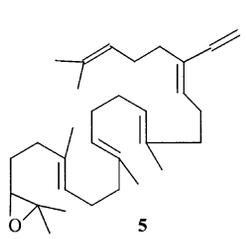
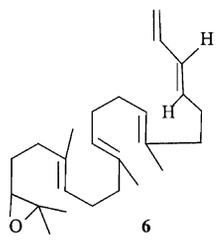
bone rearrangement to yield either lanosterol **3** in animals and yeasts or cycloartenol **4** in higher plants.[1,2,14,15]

For many years we and others have been studying OSC inhibitors. Initially, effective inhibitors were obtained by mimicking the carbocationic intermediates formed during cyclization of OS, designing squalene-derived structures in which the positively charged carbocation was replaced by a nitrogen.[1,16–19] Other research groups have developed different series of cyclised aza derivatives,[20] and sulfur-containing OS derivatives have been developed by the Oehlschlager group and by ourselves.[21–23] Another strategy to achieve new OSC inactivators is the introduction of a second epoxidic ring, replacing a carbon–carbon double bond in the natural substrate.[24]

Finally, a strategy that has been successfully adopted is to intercept the enzymatic active-site nucleophiles with a stable allylic cation, resulting in an irreversible covalent modification of OSC. Following this strategy, Prestwich,[25,26] Corey[27] and our[28,29] groups synthesized various series of 2,3-oxidosqualenoid dienes and vinyl epoxides, some of which were found to be selective and time-dependent inhibitors of yeast or animal OSCs, the most potent being dienes **5** and **6**.




**5**




**6**

Xiao and Prestwich succeeded in preparing an irreversible site-directed inhibitor of vertebrate OSC, (18*Z*)-29-methylidene-2,3-oxidosqualene (29-MOS) **5**. The proposed inhibition mechanism involves the initial cyclization to the 21-methylideneprotosterol cation, which instead of undergoing backbone rearrangement reacts with a nucleophilic site of the enzyme resulting in irreversible inactivation of the enzyme.[25] Tritiated 29-MOS was used in affinity labelling experiments to identify the 29-MOS binding site region of rat liver OSC, which is the DCTAEA motif, a well conserved region in all OSCs.[30]

Some years ago, in an attempt to achieve mechanism-based inhibitors able to trap the C-20 carbocation, the 22,23-dihydro-2,3-oxido-20-oxasqualene was synthesized.[31] Subsequently, using the same procedure, Corey[14,15,32] synthesized the 2,3-oxido-20-oxasqualene as a tool to provide information on the 17β-configuration of the protosteryl ion side chain.

It was considered that the syntheses of a new class of truncated and non-truncated methylidyne oxidosqualene derivatives might afford better insight into the function and reactivity of the nucleophiles of the active site of the enzyme that stabilize the C-2 and C-20 cationic intermediates.

## Results and discussion

### Synthesis of squalene aldehyde $C_{27}$, $C_{22}$ and $C_{17}$ monobromohydrins

The synthesis of many OS derivatives starts from the squalene aldehyde $C_{27}$, $C_{22}$ and $C_{17}$ monobromohydrins **19**, **20** and **21** (Schemes 2 and 3). Various methods of preparation have been developed, but they often suffer from low yield and tedious separation.[16,17,28,31]

$^1$H and $^{13}$C NMR and conformational and dynamical theoretical studies have been carried out on squalene, 2,3-oxidosqualene and derivatives[33] in various solvents, in order to explain the selective reactivity of the terminal double bond of squalene towards reagents such as *N*-bromosuccinimide (NBS). Squalene and derivatives in solution have been shown to undergo conformational equilibria caused by the mobile tails moving around the more rigid central portion, which gives the squalene chains the form of a dynamic precoil. Therefore, the centre of the chain is protected from electrophilic addition reactions.

Based on these studies, a general method for the protection of the internal double bonds of polyenic carbonyl compounds was developed.[28] Following this procedure, $C_{22}$ squalene aldehyde **11** was protected as the 1,3-dioxolane **14**, regioselectively reacted at the terminal double bond with NBS to form the bromohydrin **17** and finally deprotected with bis(acetonitrile)-palladium(II) dichloride to the $C_{22}$ squalene aldehyde bromohydrin **20**. This method may be separately applied to the synthesis of the three bromohydrins **19**, **20** and **21** (yield of **13**–**15** from squalene **7**: 30%). However, it requires a long separation procedure of the three aldehydes and each protection step is performed separately for each aldehyde.

A new procedure was therefore developed, starting from a 1 : 1 : 1 mixture of the three aldehydes **10**, **11** and **12**, easily obtainable from squalene **7** (Schemes 2 and 3). The mixture was directly protected by treatment with ethane-1,2-diol in benzene, with toluene-*p*-sulfonic acid as catalyst, forming a mixture of the dioxolanes **13**, **14** and **15**. The dioxolanes were separated by reverse phase flash chromatography more easily and rapidly than the aldehydes and in higher total yield (yield of **13**–**15** from squalene **7**: 33%). Each dioxolane was then separately treated with NBS in aqueous THF, affording the corresponding dioxolane external bromohydrin **16**, **17** or **18**, which was deprotected to the $C_{27}$, $C_{22}$ or $C_{17}$ squalene aldehyde bromohydrin **19**, **20** or **21** with bis(acetonitrile)palladium(II) dichloride.

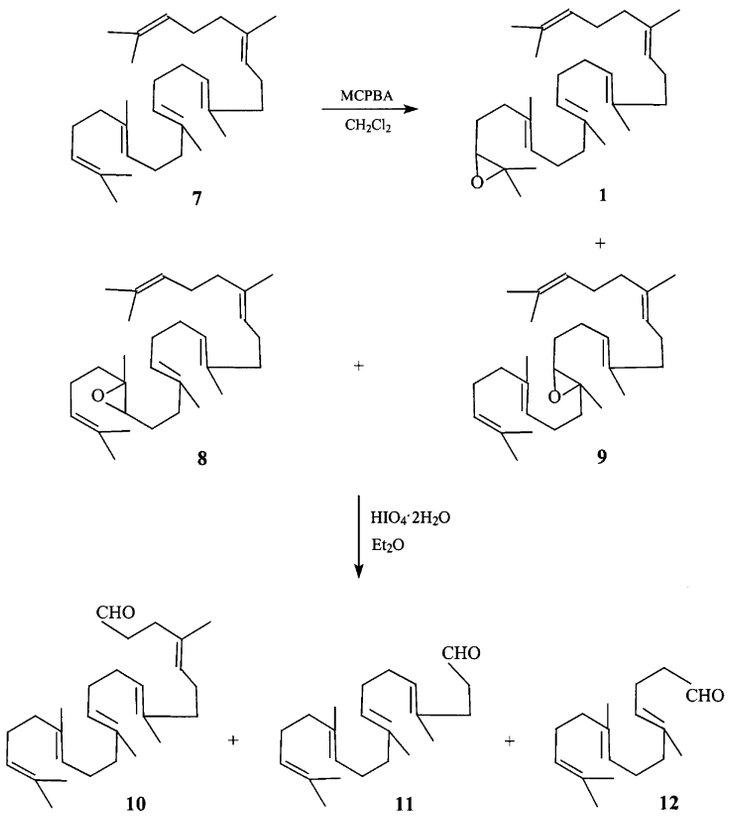
### Synthesis of 1,3-enynes

The synthesis was developed of truncated OS derivatives containing, at the terminal positions of the squalenoid skeleton, an unsubstituted 1,3-enyne unit or an alk-2-enyl substituted 1,3-enyne, starting from the $C_{22}$ or $C_{27}$ squalene aldehyde monobromohydrin **20** or **19**. Various preparative methods for derivatizing an aldehyde as a 1,3-enyne have been reported,[34] but few procedures are suitable for the present purposes.

The transformation of the $C_{22}$ and $C_{27}$ squalene aldehyde monobromohydrins **20** and **19** into the corresponding *E* and *Z* methylidyne derivatives was achieved in two steps according to the Corey[35] and the Masamune[34] procedures, which produce (*E*)-1,3-enynes with high *E* stereoselectivity. Other workers[36,37] have subsequently converted an aldehyde into an enyne following this method, in *E* stereoselectivity or *E* stereospecificity. In contrast, *Z* stereoselectivity was achieved when the enyne unit was linked to a dioxolane system.[38]

[3-(Trimethylsilyl)prop-2-ynyl]triphenylphosphonium bromide in anhydrous THF was treated with an excess of butyllithium and reacted at −80 °C with the $C_{22}$ or $C_{27}$ squalene aldehyde monobromohydrins **20** or **19** (Schemes 4 and 5) affording the *Z* and *E* trimethylsilyl derivatives **22a**, **22b** and **24a**, **24b** in 30% and 34% yield, respectively (*E* : *Z* = 2 : 1). During the Wittig reaction, the desired conversion of the bromohydrin to the epoxide was also achieved. The two isomers were separated by flash chromatography, eluting first the *Z* isomer and then the *E* isomer. The final conversion into the *E* or *Z* methylidyne derivatives of OS **23a**, **23b** and **25a**, **25b** was accomplished with tetrabutylammonium fluoride in anhydrous THF, in 77% and 78% yield respectively.[35–37]

The OS analogues having the methylidyne function at the C-29 position of the squalene skeleton (**29a** and **29b**) and the nor analogues (**27a** and **27b**) (Scheme 6) were synthesized, although a survey of the literature showed no previously reported method. [3-(Trimethylsilyl)prop-2-ynyl]triphenylphosphonium bromide in anhydrous THF was reacted with an excess of butyllithium (see the Experimental section) and subsequently with 1-bromo-3-methylbut-2-ene, followed by the




**Scheme 2** Synthesis of the $C_{27}$, $C_{22}$ and $C_{17}$ squalene aldehydes **10**, **11** and **12**.

$C_{22}$ squalene aldehyde monobromohydrin **20** (Scheme 6). By changing the reaction conditions during the one-pot reaction, the trimethylsilyl derivatives of (18*Z*)-**26a** and (18*E*)-29-methylidyne-20-nor-2,3-oxidosqualene (**26b**) (*E* : *Z* = 1 : 1) were obtained in 26% yield. During the Wittig reaction, conversion of the bromohydrin into the epoxide was also achieved. Alkylation with 5-bromo-2-methylpent-2-ene afforded the trimethylsilyl derivatives of (18*Z*)-**28a** and (18*E*)-29-methylidyne-2,3-oxidosqualene (**28b**) in very low yields (5%). The final conversion into the methylidyne derivatives of OS **27a**, **27b** and **29a**, **29b** was accomplished with tetrabutylammonium fluoride in anhydrous THF, in 81% and 72% yield respectively.[35–37] The *E* : *Z* ratio was determined to be 1 : 1 by $^1$H NMR analysis.[39]

By comparing the synthesis of the two series of compounds, it can be observed that when the 18,19-double bond is trisubstituted, the *E* and *Z* isomers are obtained in similar amounts, while enynes are obtained with *E* stereoselectivity when the terminal isoprene chain is absent. The synthetic procedure developed for the synthesis of the $C_{17}$, $C_{22}$ and $C_{27}$ squalene aldehyde monobromohydrins may be useful for the development of new OS derivatives functionalised at the C-15, C-19 and C-23 positions.

## Biological activity

Our and the previous findings of others showed that compounds such as the methylidene derivatives **5** and **6**, having a non-truncated or a truncated squalenoid structure and a correctly located reactive group adjacent to the C18–C19 double bond involved in the cyclization, are potent and selective inhibitors of OSCs.[25–30] The biological activity of the synthesized methylidyne derivatives of OS was studied on animal and yeast enzymes.

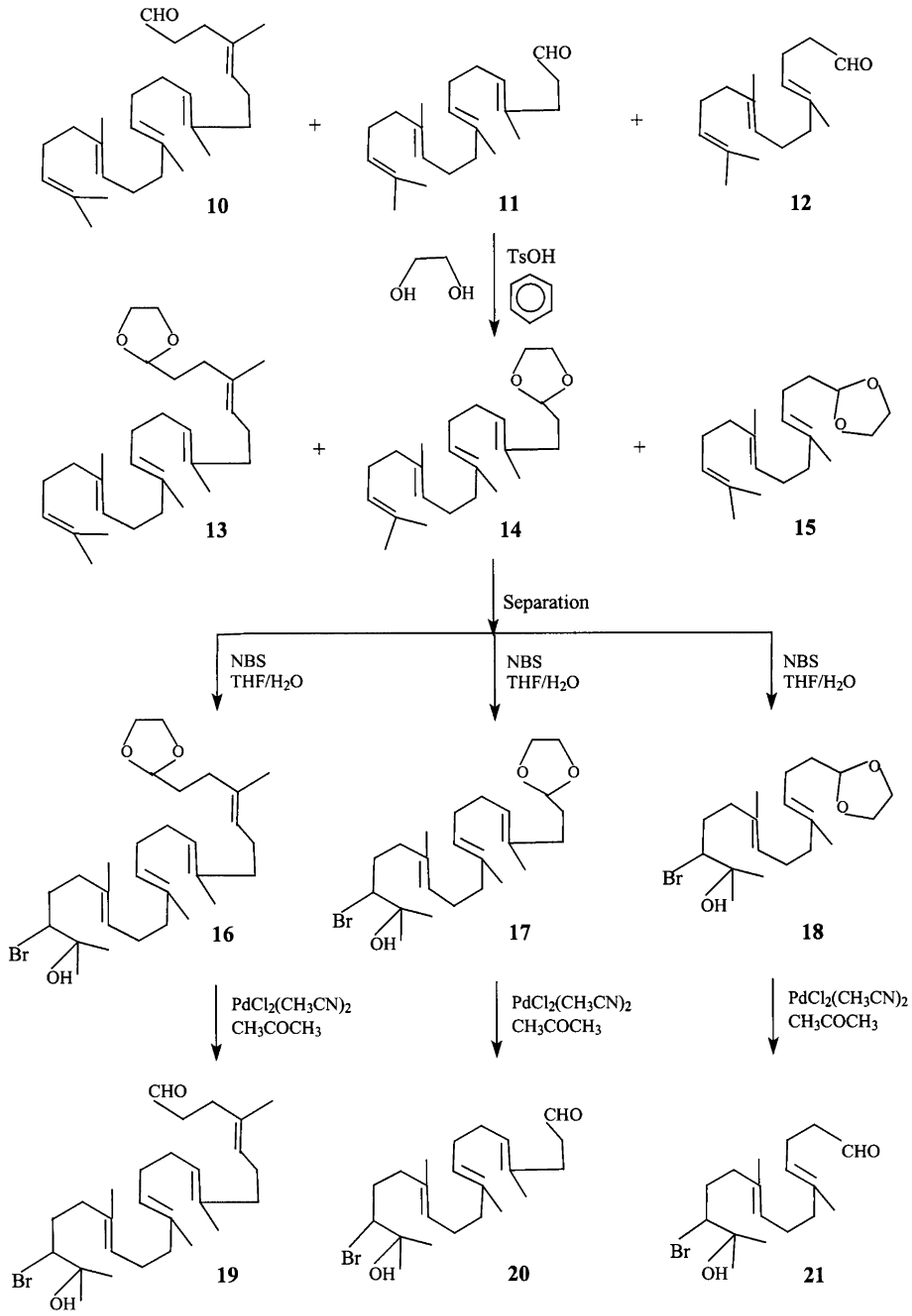
Table 1 reports the $IC_{50}$ inhibition values obtained during testing for OSC activity with solubilized and partially purified pig liver OSC and a microsomal suspension of *S. cerevisiae*, using compound **6** as a control compound.[28] Most of the compounds, as expected, are inhibitors of OSCs. The more active compounds are the non-truncated acetylenes **27a** + **27b** and **29a** + **29b**, which showed an $IC_{50}$ of 20–30 μM on pig liver OSC and of 30–50 μM on the yeast enzyme. Compounds **27a** and **29a** have the same isomeric structure as OS and they should be cyclized by the enzyme to analogues of the 21-methylidyneprotosterol cation, **30** and **31**, which should react with a nucleophilic site of the enzyme, resulting in irreversible inactivation (Scheme 7). The resulting inhibitor is probably converted into unstable allenic derivatives **32** or **33**, which then undergo further degradation. Possibly, the lower biological activity of the 29-methylidyne derivatives compared to 29-methylidene derivatives depends on preferential cyclization to the 29-methylidyne lanosterol derivatives **34** and **35**, instead of covalent linkage to the enzyme.

**Table 1** Inhibition values ($IC_{50}$/μM) of pig liver and *Saccharomyces cerevisiae* oxidosqualene cyclase (OSC) by methylidyne derivatives of oxidosqualene

| | $IC_{50}$/μM[a] | |
|---|---|---|
| Compound | OSC Pig liver | OSC *S. cerevisiae* |
| **6** (control) | 3.5 | 1.5 |
| **23a** | 60 | 50 |
| **23b** | 50 | 30 |
| **25a** | >100 | >100 |
| **25b** | >100 | >100 |
| **26a** + **26b** | 60 | ND[b] |
| **27a** + **27b** | 20 | 50 |
| **29a** + **29b** | 30 | 30 |

[a] $IC_{50}$, inhibitor concentration reducing enzymatic conversion by 50%. [b] ND = not determined.

Concerning (18*Z*)-**23a** and (18*E*)-29-methylidynehexanor-2,3-oxidosqualene (**23b**), the two isomers showed modest and similar activity on the two cyclases. On the other hand, the corresponding (18*E*)-29-methylidenehexanor-2,3-oxidosqualene (**6**), previously synthesized by us,[28] was a potent and time-




**Scheme 3** Synthesis of the $C_{27}$, $C_{22}$ and $C_{17}$ squalene aldehyde monobromohydrins **19**, **20** and **21**.

dependent inhibitor of yeast OSC, while the 18*Z*-isomer was much less active. Compounds **25a** and **25b** did not inhibit the animal and yeast cyclases even at 100 μM concentration, thus confirming that the inhibitors require an epoxide and a reactive function near the crucial positions involved in the cyclization.

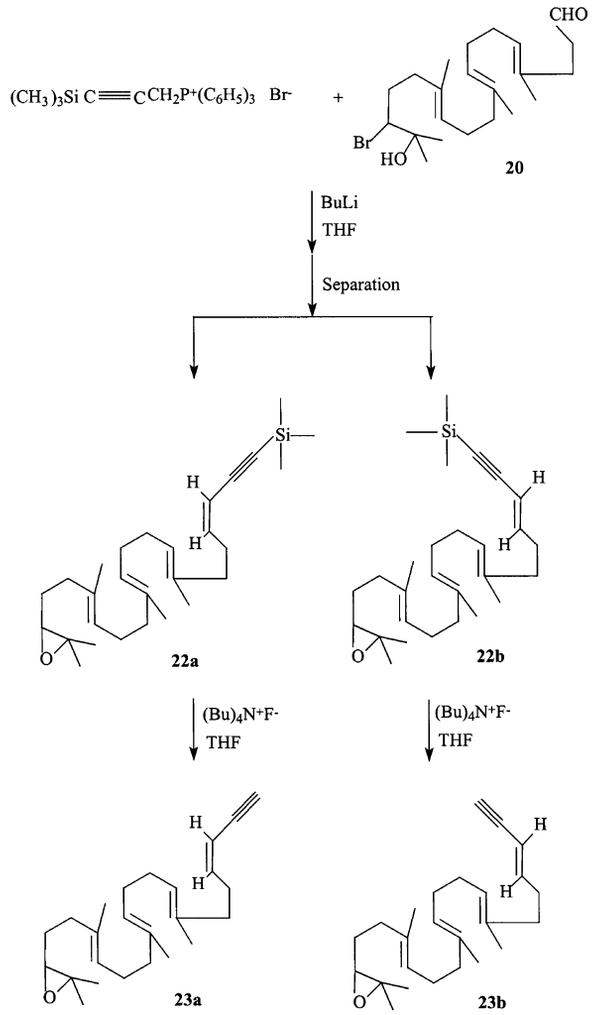
Time-dependent inhibition experiments were performed by pre-incubating the pig liver enzyme in the presence of the inhibitors at 200 μM concentration (see the Experimental section). Aliquots were withdrawn at time intervals, diluted at least 40 times and added to substrate, to check the residual enzymatic activity with respect to controls that were pre-incubated in the absence of inhibitors for the same length of time. The time-dependent decrease of activity did not depend on insufficient dilution, as non-pre-incubated controls never showed more than a 10% decrease of activity when tested in the presence of inhibitor at the same final dilution used in the tests.

Pig liver OSC was inhibited in a time-dependent manner by the methylidyne derivatives tested (**23b** and **27a** + **27b**), the best compounds being again the nor-methylidyne derivative **27a** + **27b**, while the (18*E*)-hexanormethylidyne derivative **23b** was less active. The *t*/2 values obtained at 200 μM concentration were 20 min for **27a** + **27b** and 55 min for **23b**.

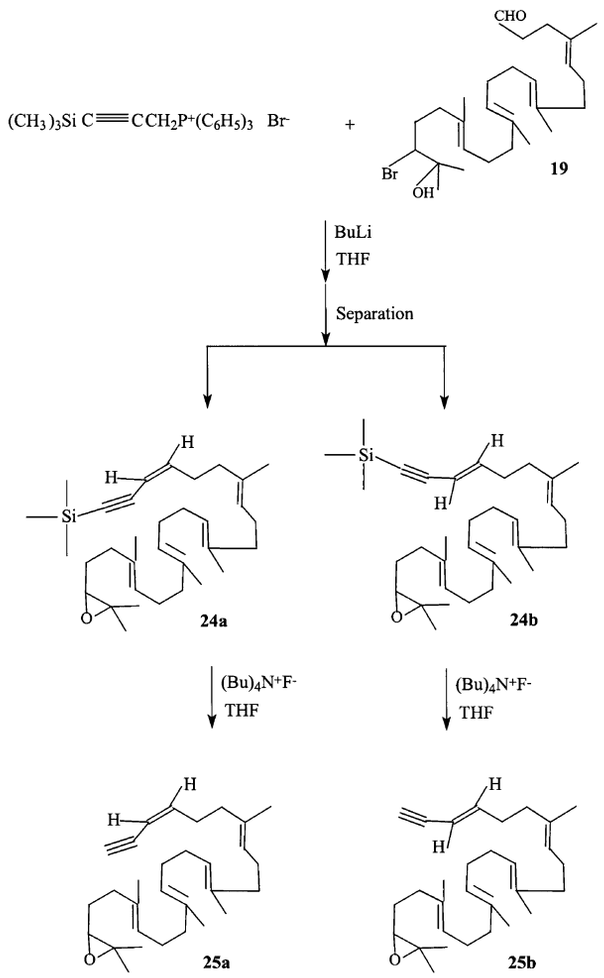
The time-dependence of inhibition of *S. cerevisiae* OSC was evaluated for compounds **23b** and **27a** + **27b** at concentrations similar to their $IC_{50}$ values, but the low specific activity found in yeast microsomes did not allow the dilution necessary to test the residual activity after pre-incubation. A search for a more active and suitable source of yeast enzyme is underway. Recently it was shown that OSC activity is almost exclusively associated with yeast lipid particles, while the occurrence of OSC in other organelles, including the endoplasmic reticulum, is negligible.[6] Work is in progress to prepare lipid particle fractions and to the test time-dependence of the more interesting inhibitors on yeast enzyme systems.

## Experimental

$^1$H NMR spectra were recorded on a Jeol EX 400 instrument for samples in $CDCl_3$ solution at room temperature, with




**Scheme 4** Synthesis of the 18*Z* and 18*E* isomers of 29-methylidyne-20,21,22,23,24,30-hexanor-2,3-oxidosqualene **23a** and **23b**.




**Scheme 5** Synthesis of the 22*Z* and 22*E* isomers of 24-methylidyne-30-nor-2,3-oxidosqualene **25a** and **25b**.

$Me_4Si$ (TMS) as internal standard. Coupling constants (*J*) are given in Hz. IR spectra were recorded on a PE 781 spectrophotometer. Mass spectra were obtained on a Finnigan MAT TSQ 700 spectrometer. Microanalyses were determined on an elemental analyser 1106 and were within ±0.3% of the theoretical values. The reactions were monitored by TLC on $F_{254}$ silica gel precoated sheets; after development, the sheets were exposed to iodine vapour. Flash-column chromatography was performed on 230–400 mesh silica gel. THF and diethyl ether were dried over sodium benzophenone ketyl. All solvents were distilled prior to flash chromatography.

### Squalene monoepoxides: (6*E*,10*E*,14*E*,18*E*)-22,23-epoxy-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18-pentaene 1, (6*E*,10*E*,14*E*)-*trans*-18,19-epoxy-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,22-pentaene 8 and (6*E*,10*E*,18*E*)-*trans*-14,15-epoxy-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,18,22-pentaene 9 (Scheme 2)

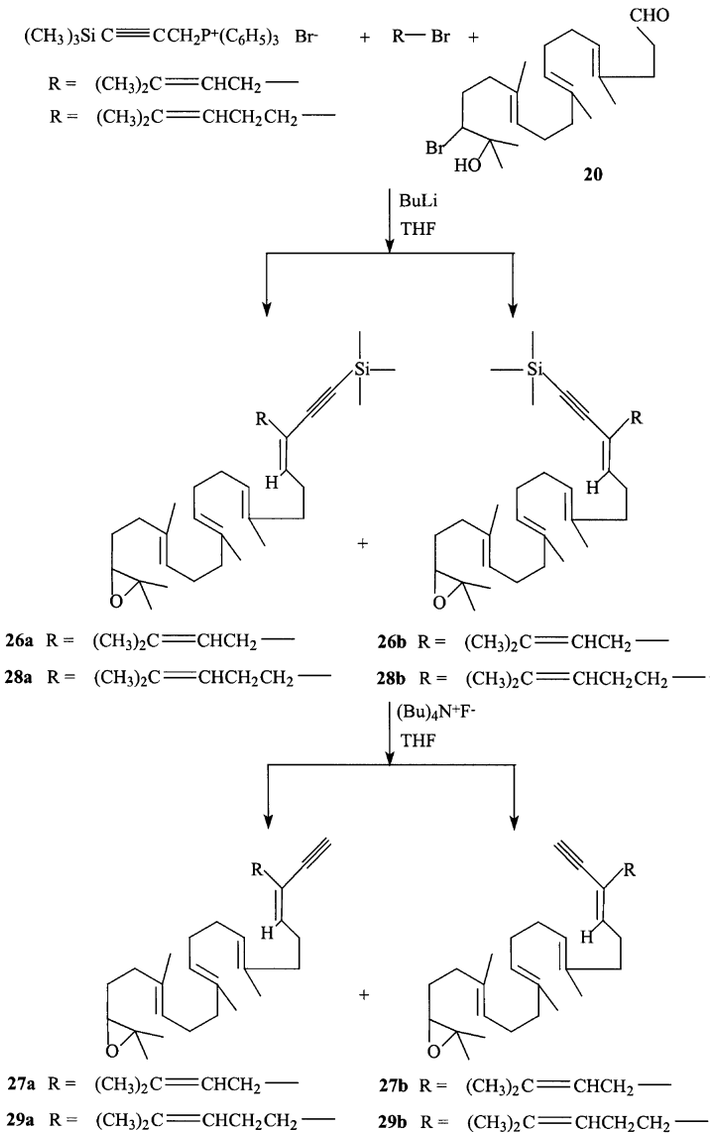
A solution of squalene **7** (10 g, 24.3 mmol) dissolved in $CH_2Cl_2$ (250 $cm^3$) at 0 °C was stirred, while *m*-chloroperbenzoic acid (MCPBA) (85% purity, 1.5 equiv., 6.30 g, 36.5 mmol) was added over a period of 30 min; it was then allowed to react for further 30 min with continued stirring. The reaction mixture was washed with 20% aqueous $NaHCO_3$ (3 × 100 $cm^3$) and brine (2 × 100 $cm^3$), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The resulting oil was purified by flash chromatography (petroleum ether–diethyl ether 90 : 10) to give a 1 : 1 : 1 mixture of the three monoepoxides (4.65 g, 45%) as a colourless oil. A sample of the mixture was separated and characterised as previously reported,[31] to give a 1 : 1 mixture of the two internal monoepoxides **8** and **9** and the external monoepoxide **1**.

### $C_{27}$, $C_{22}$ and $C_{17}$ Squalene aldehydes: (4*E*,8*E*,12*E*,16*E*)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaenal 10, (4*E*,8*E*,12*E*)-4,9,13,17-tetramethyloctadeca-4,8,12,16-tetraenal 11 and (4*E*,8*E*)-5,9,13-trimethyltetradeca-4,8,12-trienal 12 (Scheme 2)

$HIO_4 \cdot 2H_2O$ (1.5 equiv., 1.60 g, 7.04 mmol) was added to diethyl ether (250 $cm^3$) with vigorous stirring and, when dissolution was almost complete, the 1 : 1 : 1 mixture of squalene epoxides **1**, **8** and **9** (2.0 g, 4.69 mmol) in diethyl ether (5 $cm^3$) was added. Stirring was continued for 15 min after which the reaction mixture was washed with saturated brine (3 × 100 $cm^3$), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The resulting oil was purified by flash chromatography (petroleum ether–diethyl ether 80 : 20) to give a 1 : 1 : 1 mixture of the $C_{27}$, $C_{22}$ and $C_{17}$ aldehydes **10**, **11** and **12**, (1.27 g, 86%) as a colourless oil. A sample of the mixture was separated and characterised as previously reported,[16,31] to give sequentially the $C_{17}$ aldehyde **12**, the $C_{22}$ aldehyde **11** and finally the $C_{27}$ aldehyde **10**.

### Squalene aldehyde dioxolanes: 2-[(3*E*,7*E*,11*E*,15*E*)-3,7,12,16,20-pentamethylhenicosa-3,7,11,15,19-pentaenyl]-1,3-dioxolane 13, 2-[(3*E*,7*E*,11*E*)-3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl]-1,3-dioxolane 14 and 2-[(3*E*,7*E*)-4,8,12-trimethyltrideca-3,7,11-trienyl]-1,3-dioxolane 15 (Scheme 3)

A solution of the 1 : 1 : 1 mixture of the $C_{27}$, $C_{22}$ and $C_{17}$ squalene aldehydes **10**, **11** and **12** (5 g, 15.8 mmol), ethylene




**Scheme 6** Synthesis of the 18*Z* and 18*E* isomers of 29-methylidyne-20-nor-2,3-oxidosqualene **27a** and **27b** and 29-methylidyne-2,3-oxidosqualene **29a** and **29b**.

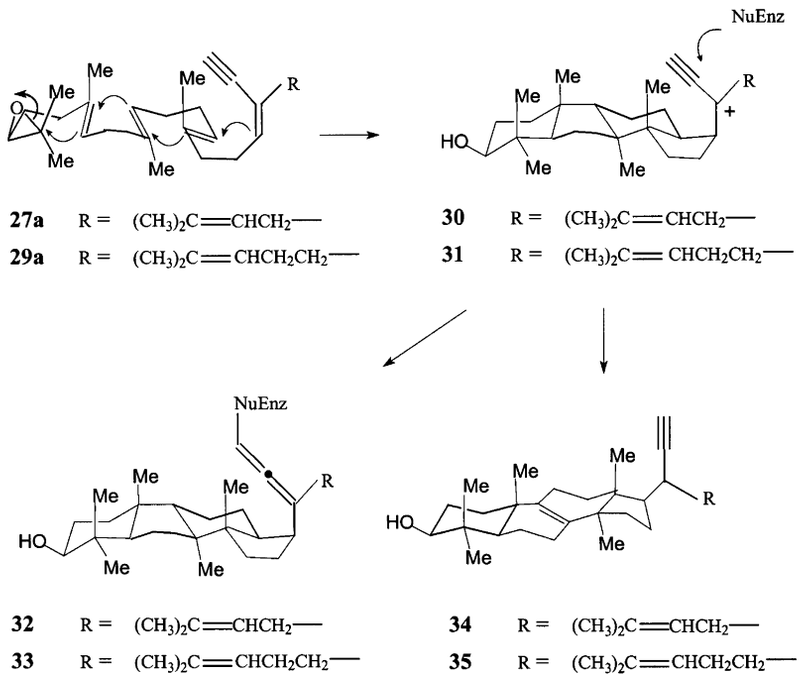
glycol (3 equiv., 2.94 g, 47.4 mmol) and toluene-*p*-sulfonic acid monohydrate as catalyst (0.1 equiv., 300 mg, 1.58 mmol) in benzene (300 $cm^3$) was refluxed for 4 h in a refrigerator equipped with a Markusson connector. It was then cooled and a small amount of solid $NaHCO_3$ was added. The reaction mixture was diluted with benzene (200 $cm^3$), washed with saturated $NaHCO_3$ (2 × 100 $cm^3$) and brine (1 × 100 $cm^3$), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The resulting oil was purified by flash chromatography (petroleum ether–diethyl ether 97 : 3), to give a 1 : 1 : 1 mixture of dioxolanes **13**, **14** and **15** (4.90 g, 86%) as a colourless oil. A sample of the mixture was separated by flash chromatography (petroleum ether–diethyl ether 99 : 1, then 98 : 2 and finally 97 : 3) to give sequentially the $C_{27}$ squalene aldehyde dioxolane **13**, the $C_{22}$ squalene aldehyde dioxolane **14** and finally the $C_{17}$ squalene aldehyde dioxolane **15**. Analytical data for $C_{22}$ squalene aldehyde dioxolane **14** were identical to those previously reported.[28]

$C_{27}$ squalene aldehyde dioxolane **13**: $\nu_{max}$(film)/$cm^{-1}$ 2940, 2865, 1450, 1380 and 1140; $\delta_H$(200 MHz, $CDCl_3$) 1.60–1.75 (20 H, m, allylic $CH_3$ and C$H_2$-dioxolane), 1.97–2.15 (18 H, m, allylic $CH_2$), 3.82–3.98 (4 H, m, OC$H_2$C$H_2$O), 4.86 (1 H, t, *J* 4.8, dioxolane CH) and 5.00–5.18 (5 H, m, vinylic CH); *m*/*z*(EI) 428 (2%), 371 (0.7), 359 (1.6), 291 (2), 229 (4.2), 203 (6), 161 (18), 135 (22), 93 (98) and 69 (100); (Found: C, 81.28; H, 11.30; O, 7.44. $C_{29}H_{48}O_2$ requires: C, 81.25; H, 11.29; O, 7.46%).

$C_{17}$ squalene aldehyde dioxolane **15**: $\nu_{max}$(film)/$cm^{-1}$ 2940, 2870, 1450, 1385 and 1140; $\delta_H$(200 MHz, $CDCl_3$) 1.57–1.76 (14 H, m, allylic $CH_3$ and C$H_2$-dioxolane), 1.94–2.14 (10 H, m, allylic $CH_2$), 3.80–4.00 (4 H, m, OC$H_2$C$H_2$O), 4.85 (1 H, t, *J* 4.8, dioxolane CH) and 5.05–5.18 (3 H, m, vinylic CH); *m*/*z*(EI) 292 (8%), 249 (5), 223 (7), 204 (10), 161 (12), 142 (40), 121 (18) and 99 (100); *m*/*z*(CI, isobutane) 293 (100%); (Found: C, 78.01; H, 11.05; O, 10.93. $C_{19}H_{32}O_2$ requires: C, 78.03; H, 11.03; O, 10.94%).

### Squalene aldehyde dioxolane bromohydrins: (6*E*,10*E*,14*E*,18*E*)-3-bromo-21-(1,3-dioxolan-2-yl)-2,6,10,15,19-pentamethylhenicosa-6,10,14,18-tetraen-2-ol 16, (6*E*,10*E*,14*E*)-3-bromo-17-(1,3-dioxolan-2-yl)-2,6,10,15-tetramethylheptadeca-6,10,14-trien-2-ol 17 and (6*E*,10*E*)-3-bromo-13-(1,3-dioxolan-2-yl)-2,6,10-trimethyltrideca-6,10-dien-2-ol 18 (Scheme 3)

The 1 : 1 : 1 mixture of dioxolanes **13**, **14** and **15** (1.0 g, 2.77 mmol) was dissolved in THF (80 $cm^3$) in a two-necked flask and stirred under nitrogen at 0 °C. Water was added until the solution became lightly opalescent. NBS (1.1 equiv.,




**Scheme 7** Hypothetical inhibition mechanism of oxidosqualene cyclase by (18*Z*)-29-methylidyne-2,3-oxidosqualene **29a** and its 20-nor derivative **27a**.

542 mg, 3.05 mmol) was added in small portions with vigorous stirring over 15 min, with, at intervals, addition of a few drops of water to keep the reaction mixture lightly opalescent. The mixture was allowed to stand for 15 min at 0 °C, again with addition of a few drops of water at intervals when it began to clear. The reaction mixture was quenched with cold 10% $NaHCO_3$ (80 $cm^3$), extracted with diethyl ether (3 × 80 $cm^3$), washed with 10% $NaHCO_3$ (1 × 80 $cm^3$) and brine (1 × 80 $cm^3$), dried with anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The resulting oil was purified by $RP_{18}$ reverse phase flash chromatography (acetonitrile–water 80 : 20, then 90 : 10, finally pure acetonitrile) to give sequentially the $C_{17}$ squalene aldehyde dioxolane bromohydrin **18** (185 mg), the $C_{22}$ squalene aldehyde dioxolane bromohydrin **17** (215 mg) and finally the $C_{27}$ squalene aldehyde dioxolane bromohydrin **16** (242 mg), in 51% total yield, as light yellow oils.

Analytical data for $C_{22}$ aldehyde dioxolane bromohydrin **17** were identical to those previously reported.[28]

$C_{27}$ squalene aldehyde dioxolane bromohydrin **16**: $\nu_{max}(CCl_4)$/$cm^{-1}$ 3570, 2980, 2930, 2855, 1550, 1450, 1385 and 1140; $\delta_H$(200 MHz, $CDCl_3$) 1.28 and 1.32 [6 H, 2 s, $(CH_3)_2COH$], 1.50–1.72 (16 H, m, allylic $CH_3$, $CH_2CHBr$ and $CH_2$-dioxolane), 1.96–2.13 (16 H, m, allylic $CH_2$), 3.78–3.95 (5 H, m, $OCH_2CH_2O$ and CHBr), 4.85 (1 H, t, *J* 4.8, dioxolane CH) and 5.02–5.20 (4 H, m, vinylic CH); *m*/*z*(EI) 526 (0.7%), 524 (0.7), 462 (0.6), 444 (1), 427 (1.2), 365 (1.1), 291 (3.5), 255 (1.5), 161 (22), 135 (35) and 93 (100); *m*/*z*(CI, isobutane) 527 (72%) and 525 (100); (Found: C, 66.30; H, 9.38; Br, 15.20; O, 9.11. $C_{29}H_{49}BrO_3$ requires: C, 66.27; H, 9.40; Br, 15.20; O, 9.13%).

$C_{17}$ squalene aldehyde dioxolane bromohydrin **18**: $\nu_{max}(CCl_4)$/$cm^{-1}$ 3570, 2970, 2930, 2855, 1550, 1450, 1390 and 1140; $\delta_H$(200 MHz, $CDCl_3$) 1.28 and 1.32 [6 H, 2 s, $(CH_3)_2COH$], 1.52–1.75 (10 H, m, allylic $CH_3$, $CH_2CHBr$ and $CH_2$-dioxolane), 1.97–2.15 (8 H, m, allylic $CH_2$), 3.84–3.96 (5 H, m, $OCH_2CH_2O$ and CHBr), 4.85 (1 H, t, *J* 4.8, dioxolane CH) and 5.02–5.18 (2 H, m, vinylic CH); *m*/*z*(EI) 390 (2%), 388 (2), 375 (1), 373 (1), 309 (6), 291 (8), 229 (4), 223 (6), 181 (6), 142 (42) and 99 (100); *m*/*z*(CI, isobutane) 391 (95%) and 389 (100); (Found: C, 58.64; H, 8.56; Br, 20.50; O, 12.32. $C_{19}H_{33}BrO_3$ requires: C, 58.61; H, 8.54; Br, 20.52; O, 12.33%).

### $C_{22}$ squalene aldehyde bromohydrin: (4*E*,8*E*,12*E*)-16-bromo-17-hydroxy-4,9,13,17-tetramethyloctadeca-4,8,12-trienal 20 (Scheme 3)

Dioxolane bromohydrin **17** (400 mg, 0.87 mmol) was dissolved in acetone (200 $cm^3$) under dry nitrogen, with stirring. Bis-(acetonitrile)palladium(II) dichloride $[PdCl_2(CH_3CN)_2]$ (0.2 equiv., 44 mg, 0.17 mmol) was added and the mixture allowed to stand for 8 h under nitrogen, with stirring. Controls on silica gel TLC revealed that in most eluants, the dioxolane **17** and the aldehyde **20** 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 $cm^3$) and extracted with diethyl ether (2 × 100 $cm^3$). The combined extracts were washed with 10% $NaHCO_3$ (1 × 50 $cm^3$) and brine (1 × 50 $cm^3$), dried with anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The resulting oil was purified by flash chromatography (petroleum ether–diethyl ether, 95 : 5 to remove impurities, then 92 : 8, finally 90 : 10) to give 259 mg (72%) of $C_{22}$ squalene aldehyde bromohydrin **20** as a colourless oil.[28] $\nu_{max}$(film)/$cm^{-1}$ 3500–3400, 2965, 2920, 2860, 1725, 1450, 1385 and 1110; $\delta_H$(200 MHz, $CDCl_3$) 1.28 and 1.32 [6 H, 2 s, $(CH_3)_2COH$], 1.50–1.70 (11 H, m, allylic $CH_3$ and $CH_2CHBr$), 1.88–2.16 (12 H, m, allylic $CH_2$), 2.35–2.40 (2 H, m, $CH_2CHO$), 3.84 (1 H, m, CHBr), 5.00–5.23 (3 H, m, vinylic CH) and 9.78 (1 H, m, CHO); *m*/*z*(EI) 414 (0.5%), 412 (0.5), 332 (3), 316 (1), 247 (1), 153 (6), 135 (15), 111 (16), 93 (38), 81 (90) and 43 (100); (Found: C, 63.88; H, 9.02; Br, 19.31; O, 7.75. Calc. for $C_{22}H_{37}BrO_2$: C, 63.91; H, 9.02; Br, 19.33; O, 7.74%).

### $C_{27}$ squalene aldehyde bromohydrin: (4*E*,8*E*,12*E*,16*E*)-20-bromo-21-hydroxy-4,8,13,17,21-pentamethyldocosa-4,8,12,16-tetraenal 19 (Scheme 3)

Compound **19** was obtained and purified using the method described for **20**, starting from the dioxolane **16**, in 70% yield. $\nu_{max}$(film)/$cm^{-1}$ 3500–3400, 2960, 2920, 2860, 1725, 1450, 1390 and 1110; $\delta_H$(200 MHz, $CDCl_3$) 1.28 and 1.32 [6 H, 2 s, $(CH_3)_2COH$], 1.48–1.70 (14 H, m, allylic $CH_3$ and $CH_2CHBr$), 1.87–2.16 (16 H, m, allylic $CH_2$), 2.34–2.40 (2 H, m, $CH_2CHO$), 3.84 (1 H, m, CHBr), 5.00–5.24 (4 H, m, vinylic CH) and 9.78 (1 H, m, CHO); *m*/*z*(EI) 482 (0.3%), 480 (0.3), 383 (2.5), 301

(1.8), 273 (2), 229 (2.2), 217 (8), 203 (6), 135 (36), 93 (50) and 81 (100); *m*/*z*(CI, isobutane) 483 (36%), 481 (38), 465 (95) and 463 (100); (Found: C, 67.31; H, 9.45; Br, 16.61; O, 6.66. $C_{27}H_{45}BrO_2$ requires: C, 67.34; H, 9.42; Br, 16.59; O, 6.65%).

### $C_{17}$ squalene aldehyde bromohydrin: (4*E*,8*E*)-12-bromo-13-hydroxy-5,9,13-trimethyltetradeca-4,8-dienal 21 (Scheme 3)

Compound **21** was obtained and purified using the method described for **20**, starting from the dioxolane **18**, in 73% yield. $\nu_{max}$(film)/cm$^{-1}$ 3500–3400, 2970, 2920, 2850, 1725, 1445, 1385 and 1115; $\delta_H$(200 MHz, $CDCl_3$) 1.28 and 1.32 [6 H, 2 s, $(CH_3)_2COH$], 1.50–1.72 (8 H, m, allylic $CH_3$ and $CH_2CHBr$), 1.88–2.20 (8 H, m, allylic $CH_2$), 2.35–2.41 (2 H, m, $CH_2CHO$), 3.85 (1 H, m, C*H*Br), 5.02–5.20 (2 H, m, vinylic CH) and 9.77 (1 H, m, CHO); *m*/*z*(EI) 346 (3%), 344 (3), 328 (3), 326 (3), 302 (1), 300 (1), 264 (5), 243 (4), 229 (2), 203 (1), 135 (32), 107 (15), 93 (35) and 81 (100); (Found: C, 59.10; H, 8.47; Br, 23.09; O, 9.25. $C_{17}H_{29}BrO_2$ requires: C, 59.13; H, 8.47; Br, 23.14; O, 9.27%).

### (3*Z*,7*E*,11*E*,15*E*)-19,20-Epoxy-7,12,16,20-tetramethyl-1-(trimethylsilyl)henicosa-3,7,11,15-tetraen-1-yne 22a and (3*E*,7*E*,11*E*,15*E*)-19,20-epoxy-7,12,16,20-tetramethyl-1-(trimethylsilyl)henicosa-3,7,11,15-tetraen-1-yne 22b (Scheme 4)

In a three-necked flask containing anhydrous THF (25 cm$^3$), [3-(trimethylsilyl)prop-2-ynyl]triphenylphosphonium bromide (1.2 equiv., 465 mg, 1.02 mmol) was suspended at −80 °C, under a flux of dry nitrogen, with stirring. Butyllithium (1.6 M solution in hexane, 3 equiv., 1.6 cm$^3$, 2.55 mmol) was added, during which the reaction mixture turned red. It was left for 15 min at −40 °C and then cooled to −80 °C. $C_{22}$ squalene aldehyde monobromohydrin **20** (1 equiv., 351 mg, 0.85 mmol) in anhydrous THF (2 cm$^3$) was added after 5 min and allowed to react for 1 h at −80 °C, during which time the colour turned orange. It was then gradually allowed to reach room temperature, poured into cold 10% $NH_4Cl$–diethyl ether (1 : 1, 50 cm$^3$) and extracted with diethyl ether (3 × 30 cm$^3$). The combined extracts were washed with saturated brine (2 × 30 cm$^3$), dried with anhydrous sodium sulfate, filtered and evaporated *in vacuo* at +35 °C. The resulting oil was purified by flash chromatography (petroleum ether to remove impurities, then petroleum ether–diethyl ether, 99 : 1, 98 : 2 and finally 95 : 5) to give 36 mg of the *Z* isomer of silyl acetylene **22a** and 73 mg of the *E* isomer **22b**, as colourless oils, in 30% total yield (*E* : *Z* = 2 : 1).

**22a** (*Z*): $\nu_{max}$(KBr pellet)/cm$^{-1}$ 2970, 2920, 2850, 1450, 1380 and 1245; $\delta_H$(200 MHz, $CDCl_3$) 0.19 [9 H, s, $(CH_3)_3Si$], 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.70 (11 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.90–2.20 (14 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 5.05–5.20 (3 H, m, vinylic CH), 5.47 (1 H, br d, *Z* CH=C*H*–C≡C) and 5.92 (1 H, dt, *J* 10.7 and *J* 6.8, *Z* C*H*=CH–C≡C); *m*/*z*(CI, isobutane) 427 (100%); (Found: C, 78.82; H, 10.85; O, 3.76; Si, 6.55. $C_{28}H_{46}OSi$ requires: C, 78.80; H, 10.86; O, 3.75; Si, 6.58%).

**22b** (*E*): $\nu_{max}$(KBr pellet)/cm$^{-1}$ 2970, 2920, 2850, 1450, 1380 and 1245; $\delta_H$(200 MHz, $CDCl_3$) 0.19 [9 H, s, $(CH_3)_3Si$], 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.70 (11 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.90–2.20 (14 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 5.05–5.20 (3 H, m, vinylic CH), 5.51 (1 H, br d, *E* CH=C*H*–C≡C) and 6.20 (1 H, dt, *J* 15.9 and *J* 6.8, *E* C*H*=CH–C≡C); *m*/*z*(CI, isobutane) 427 (100%); (Found: C, 78.81; H, 10.88; O, 3.74; Si, 6.55. $C_{28}H_{46}OSi$ requires: C, 78.80; H, 10.86; O, 3.75; Si, 6.58%).

### (18*Z*)-29-Methylidyne-20,21,22,23,24,30-hexanor-2,3-oxidosqualene: (3*Z*,7*E*,11*E*,15*E*)-19,20-epoxy-7,12,16,20-tetramethylhenicosa-3,7,11,15-tetraen-1-yne 23a (Scheme 4)

(*Z*)-Silyl acetylene **22a** (1 equiv., 50 mg, 0.117 mmol) was dissolved in anhydrous THF (3 cm$^3$) at 0 °C under a flux of dry argon. Tetrabutylammonium fluoride (1.0 M solution in THF, 1.15 equiv., 135 µl, 0.135 mmol) was added and the mixture was stirred for 15 min at 0 °C and concentrated *in vacuo*. It was then poured into cold 10% $NH_4Cl$-diethyl ether (1 : 1, 50 cm$^3$) and extracted with diethyl ether (3 × 30 cm$^3$). The combined extracts were washed with saturated brine (2 × 30 cm$^3$), dried with anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The resulting oil was purified by flash chromatography (petroleum ether–diethyl ether, 99.8 : 0.2 to remove impurities, then 95 : 5) to give 32 mg (77%) of the (18*Z*)-methylidyne derivative **23a**, as a colourless oil. $\nu_{max}$($CCl_4$)/cm$^{-1}$ 3310, 2965, 2930, 2860, 1450, 1380 and 1250; $\delta_H$(200 MHz, $CDCl_3$) 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.58–1.72 (11 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.92–2.22 (14 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 3.08 (1 H, d, *J* 2.3, C≡C*H*, *Z* isomer), 5.06–5.21 (3 H, m, vinylic CH), 5.44 (1 H, br d, *Z* CH=C*H*–C≡CH) and 5.97 (1 H, dt, *J* 11.2 and *J* 6.9, *Z* C*H*=CH–C≡CH); *m*/*z*(EI) 354 (0.2%), 325 (1.2), 297 (2), 279 (3), 203 (6), 149 (44), 135 (50), 81 (100); *m*/*z*(CI, isobutane) 355 (100%); (Found: C, 84.72; H, 10.80; O, 4.48. $C_{25}H_{38}O$ requires: C, 84.69; H, 10.80; O, 4.51%).

### (18*E*)-29-Methylidyne-20,21,22,23,24,30-hexanor-2,3-oxidosqualene: (3*E*,7*E*,11*E*,15*E*)-19,20-epoxy-7,12,16,20-tetramethylhenicosa-3,7,11,15-tetraen-1-yne 23b (Scheme 4)

*E*-isomer **23b** was obtained starting from the silyl derivative **22b**, using the method described for compound **23a**, in 75% yield. $\nu_{max}$($CCl_4$)/cm$^{-1}$ 3310, 2970, 2930, 2860, 1450, 1380 and 1250; $\delta_H$(200 MHz, $CDCl_3$) 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.58–1.72 (11 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.92–2.22 (14 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 2.78 (1 H, d, *J* 2.2, C≡C*H*, *E* isomer), 5.06–5.21 (3 H, m, vinylic CH), 5.48 (1 H, br d, *E* CH=C*H*–C≡CH) and 6.23 (1 H, dt, *J* 16.1 and *J* 6.9, *E* C*H*=CH–C≡CH); *m*/*z*(EI) 354 (0.2%), 325 (1.2), 297 (2), 279 (3.2), 203 (6), 149 (40), 135 (55), 81 (100); *m*/*z*(CI, isobutane) 355 (100%); (Found: C, 84.66; H, 10.82; O, 4.49. $C_{25}H_{38}O$ requires: C, 84.69; H, 10.80; O, 4.51%).

### (3*Z*,7*E*,11*E*,15*E*,19*E*)-23,24-Epoxy-7,11,16,20-tetramethyl-1-(trimethylsilyl)pentacosa-3,7,11,15,19-pentaen-1-yne 24a and (3*E*,7*E*,11*E*,15*E*,19*E*)-23,24-epoxy-7,11,16,20-tetramethyl-1-(trimethylsilyl)pentacosa-3,7,11,15,19-pentaen-1-yne 24b (Scheme 5)

Compounds **24a** and **24b** were obtained in 34% total yield (*E* : *Z* = 2 : 1) and separated using the method described for the silyl derivatives **22a** and **22b**, using the $C_{27}$ squalene aldehyde bromohydrin **19** instead of the $C_{22}$ squalene aldehyde bromohydrin **20**.

**24a** (*Z*): $\nu_{max}$(KBr pellet)/cm$^{-1}$ 2970, 2920, 2850, 1450, 1380 and 1245; $\delta_H$(200 MHz, $CDCl_3$) 0.19 [9 H, s, $(CH_3)_3Si$], 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.70 (14 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.94–2.22 (18 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 5.00–5.16 (4 H, m, vinylic CH), 5.47 (1 H, br d, *Z* CH=C*H*–C≡C) and 5.92 (1 H, dt, *J* 10.7 and *J* 6.8, *Z* C*H*–CH–C≡C); *m*/*z*(EI) 494 (1%), 479 (0.5), 451 (0.7), 421 (0.8), 341 (3), 273 (6), 267 (2), 245 (2), 225 (3), 204 (12), 199 (18), 189 (33), 135 (35), 93 (47), 81 (80) and 73 (100); (Found: C, 80.07; H, 11.02; O, 3.25; Si, 5.68. $C_{33}H_{54}OSi$ requires: C, 80.09; H, 11.00; O, 3.23; Si, 5.68%).

**24b** (*E*): $\nu_{max}$(KBr pellet)/cm$^{-1}$ 2965, 2920, 2850, 1450, 1385 and 1245; $\delta_H$(200 MHz, $CDCl_3$) 0.19 [9 H, s, $(CH_3)_3Si$], 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.70 (14 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.94–2.22 (18 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 5.00–5.16 (4 H, m, vinylic CH), 5.51 (1 H, br d, *E* CH=C*H*–C≡C) and 6.21 (1 H, dt, *J* 15.9 and *J* 6.8, *E* C*H*=CH–C≡C); *m*/*z*(EI) 494 (1.2%), 479 (0.6), 451 (0.6), 421 (0.8), 341 (4), 273 (5), 267 (2), 225 (2), 204 (10), 199 (18), 189 (35), 93 (47), 81 (90) and 73 (100); (Found: C, 80.11; H, 11.02;

O, 3.22; Si, 5.69. $C_{33}H_{54}OSi$ requires: C, 80.09; H, 11.00; O, 3.22; Si, 5.68%).

**(22*Z*)-24-Methylidyne-30-nor-2,3-oxidosqualene: (3*Z*,7*E*,11*E*,15*E*,19*E*)-23,24-epoxy 7,11,16,20-tetramethylpentacosa-3,7,11,15,19-pentaen-1-yne 25a (Scheme 5)**

Compound **25a** was obtained in 78% yield, using the method described for **23a**, starting from the silyl derivative **24a**. $\nu_{max}$(KBr pellet)/cm$^{-1}$ 3310, 2970, 2930, 2855, 1445, 1380 and 1250; $\delta_H$(200 MHz, $CDCl_3$) 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.58–1.73 (14 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.92–2.20 (18 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 3.09 (1 H, d, *J* 2.3, C≡C*H*, *Z* isomer), 5.06–5.20 (4 H, m, vinylic CH), 5.45 (1 H, br d, *Z* CH=C*H*–C≡CH) and 5.98 (1 H, dt, *J* 11.2 and *J* 6.9, *Z* C*H*=CH–C≡CH); *m*/*z*(CI, isobutane) 423 (100%); (Found: C, 85.27; H, 10.95; O, 3.81. $C_{30}H_{46}O$ requires: C, 85.25; H, 10.97; O, 3.79%).

**(22*E*)-24-Methylidyne-30-nor-2,3-oxidosqualene: (3*E*,7*E*,11*E*,15*E*,19*E*)-23,24-epoxy-7,11,16,20-tetramethylpentacosa-3,7,11,15,19-pentaen-1-yne 25b (Scheme 5)**

Compound **25b** was obtained in 75% yield, using the method described for **23a**, starting from the silyl derivative **24b**. $\nu_{max}$(KBr pellet)/cm$^{-1}$ 3310, 2970, 2930, 2850, 1445, 1385 and 1250; $\delta_H$(200 MHz, $CDCl_3$) 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.58–1.73 (14 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.92–2.20 (18 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 2.79 (1 H, d, *J* 2.2, C≡C*H*, *E* isomer), 5.06–5.20 (4 H, m, vinylic CH), 5.48 (1 H, br d, *E* CH=C*H*–C≡CH) and 6.23 (1 H, dt, *J* 16.1 and *J* 6.9, *E* C*H*=CH–C≡CH); *m*/*z*(CI, isobutane) 423 (100%); (Found: C, 85.28; H, 10.99; O, 3.79. $C_{30}H_{46}O$ requires: C, 85.25; H, 10.97; O, 3.79%).

**(5*Z*,9*E*,13*E*,17*E*)-21,22-Epoxy-2,9,14,18,22-pentamethyl-5-(trimethylsilylethynyl)tricosa-2,5,9,13,17-pentaene 26a and (5*E*,9*E*,13*E*,17*E*)-21,22-epoxy-2,9,14,18,22-pentamethyl-5-(trimethylsilylethynyl)tricosa-2,5,9,13,17-pentaene 26b (Scheme 6)**

In a three-necked flask containing anhydrous THF (15 cm$^3$), [3-(trimethylsilyl)prop-2-ynyl]triphenylphosphonium bromide (1.2 equiv., 274 mg, 0.60 mmol) was suspended at −80 °C, under a flux of dry nitrogen, with stirring. Butyllithium (1.6 M solution in hexane, 4 equiv., 1.3 cm$^3$, 2.0 mmol) was added, during which the reaction mixture turned red. It was left for 15 min at −40 °C and then 1-bromo-3-methylbut-2-ene (1.5 equiv., 112 mg, 0.75 mmol) was added, during which the solution turned orange. The solution was then cooled to −80 °C and additional butyllithium (1.6 M solution in hexane, 2 equiv., 0.6 cm$^3$, 1.0 mmol) was added, during which the solution turned dark red. After 15 min of stirring at −80 °C, the $C_{22}$ squalene aldehyde monobromohydrin **20** (1 equiv., 207 mg, 0.50 mmol) in anhydrous THF (1 cm$^3$) was added and allowed to react for 1 h at −80 °C, during which time the colour turned pink. It was then gradually allowed to reach room temperature, poured into cold 10% $NH_4Cl$–diethyl ether (1 : 1, 50 cm$^3$) and extracted with diethyl ether (3 × 30 cm$^3$). The combined extracts were washed with saturated brine (2 × 30 cm$^3$), dried with anhydrous sodium sulfate, filtered and evaporated *in vacuo* at +35 °C. The resulting oil was purified by flash chromatography (petroleum ether to remove impurities, then petroleum ether–diethyl ether, 99.5 : 0.5) to give 64 mg (26%) of the two silyl acetylenes **26a** and **26b** (*E* : *Z* = 1 : 1), as a colourless oil. $\nu_{max}$($CCl_4$)/cm$^{-1}$ 2965, 2930, 2850, 1450, 1380 and 1250; $\delta_H$(200 MHz, $CDCl_3$) 0.18 [9 H, s, $(CH_3)_3Si$], 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.58–1.75 (17 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.90–2.22 (16 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 5.05–5.22 (4 H, m, vinylic CH) and 5.34–5.41 (1 H, m, C*H*=C–C≡C); *m*/*z*(CI, isobutane) 495 (100%); (Found: C, 80.10; H, 10.98; O, 3.23; Si, 5.66. $C_{33}H_{54}OSi$ requires: C, 80.09; H, 11.00; O, 3.23; Si, 5.68%).

**(18*Z*)- and (18*E*)-29-Methylidyne-20-nor-2,3-oxidosqualene: (5*Z*,9*E*,13*E*,17*E*)-21,22-epoxy-5-ethynyl-2,9,14,18,22-pentamethyltricosa-2,5,9,13,17-pentaene 27a and (5*E*,9*E*,13*E*,17*E*)-21,22-epoxy-5-ethynyl-2,9,14,18,22-pentamethyltricosa-2,5,9,13,17-pentaene 27b (Scheme 6)**

Compounds **27a** and **27b** were obtained in 81% yield (*E* : *Z* = 1 : 1), starting from the silyl derivatives **26a** and **26b**, using the method described for compound **23a**. $\nu_{max}$($CCl_4$)/cm$^{-1}$ 3310, 2965, 2930, 2850, 1450 and 1380; $\delta_H$(200 MHz, $CDCl_3$) 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.73 (17 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.91–2.20 (16 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 2.72 (1 H × 1/2, br s, C≡C*H*, *E* isomer), 2.97 (1 H × 1/2, br s, C≡C*H*, *Z* isomer), 5.20 (4 H, m, vinylic CH) and 5.35–5.42 (1 H, m, C*H*=C–C≡C); *m*/*z*(EI) 422 (0.3%), 353 (0.4), 279 (1), 267 (2), 245 (4), 217 (5), 203 (6), 135 (42) and 81 (100); *m*/*z*(CI, isobutane) 423 (100%); (Found: C, 85.28; H, 10.99; O, 3.77. $C_{30}H_{46}O$ requires: C, 85.25; H, 10.97; O, 3.79%).

**(6*Z*,10*E*,14*E*,18*E*)-22,23-Epoxy-2,10,15,19,23-pentamethyl-6-(trimethylsilylethynyl)tetracosa-2,6,10,14,18-pentaene 28a and (6*E*,10*E*,14*E*,18*E*)-22,23-epoxy-2,10,15,19,23-pentamethyl-6-(trimethylsilylethynyl)tetracosa-2,6,10,14,18-pentaene 28b (Scheme 6)**

Compounds **28a** and **28b** were obtained in *E* : *Z* = 1 : 1 ratio, using the method described for the silyl derivatives **26a** and **26b**, using 5-bromo-2-methylpent-2-ene instead of 1-bromo-3-methylbut-2-ene. In this case, they were obtained in very low yield (5%), together with the non-alkylated silyl derivatives **22a** and **22b**, in 21% yield. The alkylated silyl derivatives **28a** and **28b** were obtained first, separated from the non-alkylated silyl derivatives **22a** and **22b** by flash chromatography (petroleum ether to remove impurities, then petroleum ether–diethyl ether 99.5 : 0.5).

**28a** and **28b**: $\nu_{max}$(KBr pellet)/cm$^{-1}$ 2965, 2930, 2850, 1450, 1380 and 1245; $\delta_H$(200 MHz, $CDCl_3$) 0.18 [9 H, s, $(CH_3)_3Si$], 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.72 (17 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.93–2.21 (18 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 5.06–5.22 (4 H, m, vinylic CH) and 5.33–5.41 (1 H, m, C*H*=C–C≡C); *m*/*z*(CI, isobutane) 509 (100%); (Found: C, 80.25; H, 11.11; O, 3.12; Si, 5.50. $C_{34}H_{56}OSi$ requires: C, 80.25; H, 11.09; O, 3.14; Si, 5.52%).

**(18*Z*)- and (18*E*)-29-Methylidyne-2,3-oxidosqualene: (6*Z*,10*E*,14*E*,18*E*)-22,23-epoxy-6-ethynyl-2,10,15,19,23-pentamethyltetracosa-2,6,10,14,18-pentaene 29a and (6*E*,10*E*,14*E*,18*E*)-22,23-epoxy-6-ethynyl-2,10,15,19,23-pentamethyltetracosa-2,6,10,14,18-pentaene 29b (Scheme 6)**

Compounds **29a** and **29b** were obtained in 72% yield (*E* : *Z* = 1 : 1), starting from the silyl derivatives **28a** and **28b**, using the method described for compound **23a**. $\nu_{max}$(film)/cm$^{-1}$ 3310, 2970, 2930, 2850, 1450 and 1380; $\delta_H$(200 MHz, $CDCl_3$) 1.26 and 1.31 (6 H, 2 s, epoxidic $CH_3$), 1.56–1.75 (17 H, m, allylic $CH_3$ and epoxidic $CH_2$), 1.92–2.22 (18 H, m, allylic $CH_2$), 2.71 (1 H, t, *J* 6.3, epoxidic CH), 2.72 (1 H × 1/2, br s, C≡C*H*, *E* isomer), 2.98 (1 H × 1/2, br s, C≡C*H*, *Z* isomer), 5.05–5.20 (4 H, m, vinylic CH) and 5.35–5.42 (1 H, m, C*H*=C–C≡C); *m*/*z*(CI, isobutane) 437 (100%); (Found: C, 85.22; H, 11.08; O, 3.64. $C_{31}H_{48}O$ requires: C, 85.26; H, 11.08; O, 3.66%).

## Enzyme assays

**Solubilisation and purification of OSCs.** Partially purified OSCs from pig liver microsomes and yeast microsomes were obtained as previously described.[16]

**Assay of OSC activity and kinetic determination.** The 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-$^3$H]-3-(*R,S*)-2,3-oxidosqualene (50000 cpm), as previously described.[19] $IC_{50}$ values (the concentration of inhibitor that reduces the enzymatic conversion of OS to lanosterol by 50%) were determined at 25 μM substrate concentration in the presence of different concentrations of inhibitors, using (18*E*)-29-methylidene-hexanor-2,3-oxidosqualene **6** as a control compound.[28]

**Time-dependent inactivation of the OSC.** Time-dependent inactivation was determined at 37 °C by adding the inhibitors to the enzyme solution in the absence of substrate. Aliquots were withdrawn at time intervals from 5 min to 40 min and diluted 40-fold for pig enzyme or 10-fold for yeast enzyme, by transfer to test tubes containing cold and labelled substrate OS (25 μM) and Tween-80 (0.5 mg ml$^{-1}$) in Na–K phosphate buffer. Residual activity was determined by incubating pig or yeast enzyme under the same conditions. The *t*/2 values were calculated by plotting the log of the residual activity against time.[19]

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