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Review Article

MECHANISM-BASED INHIBITORS AND OTHER ACTIVE-SITE TARGETED INHIBITORS OF OXIDOSQUALENE CYCLASE AND SQUALENE CYCLASE

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Enzymatic cyclizations of squalene and oxidosqualene lead to sterols and other triterpenoids in bacteria, fungi, plants, and animals. The cyclases for these reactions catalyze formation and stabilization of polycyclic carbocations and direct the enzyme-specific, templated formation of new carbon–carbon bonds in regio- and stereochemically defined contexts. The development of mechanism-based irreversible inhibitors, photoactivatable inhibitors, and numerous substrate analogs have helped to unravel the stepwise events occurring in the catalytic sites of these enzymes by covalent modification of specific amino acid residues.

Keywords: Suicide substrate; Cholesterol biogenesis; Polyolefin cyclization; Cation mimics; Photoaffinity label; Covalent modification

Oxidosqualene:lanosterol cyclase (OSLC) (E.C. 5.4.99.7) and bacterial squalene:hopene cyclase (SHC) (E.C. 5.4.99.7) catalyze a family of carbocyclizations in which a remarkable number of carbon–carbon bonds are formed regio- and stereospecifically during the biosynthesis of sterols and other polycyclic triterpenes.[1–3] These two enzymes bind their respective substrates in chair–boat–chair or in all chair conformations, activate the terminal epoxide or alkene by protonation, and then mediate sequential ring-forming

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reactions through a progression of partially cyclized carbocationic intermediates.[4–8] The formation of lanosterol is initiated by an acid-catalyzed opening of the oxirane ring of (3*S*)2,3-oxidosqualene (OS) (**1**); participation by neighboring π-bonds cascade to the tetracyclic C-20 protosteryl cation (**2**). This intermediate cation then undergoes a series of 1,2-methyl and hydride shifts, and subsequent proton abstraction by the enzyme yields the lanosterol (**3**) skeleton (Figure 1A). In contrast, SHC cyclization reaction proceeds from a different folding of squalene (**4**) and without carbon skeletal rearrangement to give the hopanyl C-22 carbocation (**5**). The two hopanoid products, hop-22(29)-ene (**6**) and hopan-22-ol (**7**), arise via loss of H-29 or via trapping by H_2O (Figure 1B). Bacterial SHCs also accept epoxides as substrates, and can catalyze the cyclization into pentacyclic triterpenes by initiating oxirane ring opening rather than by protonating the terminal double bond.[9–14]

OSLC and SHC are membrane-associated 70–85 kDa proteins showing 17–27% sequence identity to each other.[15–27] Rat liver OSLC was purified,[28] cloned, and functionally expressed[19] in our laboratory; it was a 83,321 Da protein with 733 amino acids, and showed a broad pH optimum within a range of 6.0 and 8.0. On the other hand, the SHC from a thermoacidophilic bacteria such as *Alicyclobacillus acidocaldarius* consists of 631 amino acids with a molecular mass of 71,524 Da, and shows its catalytic optimum at 60°C and pH 6.0.[23,29] The recently determined three-dimensional structure of the *A. acidocaldarius* SHC revealed an α-helix-rich dumbbell-shaped homodimer with membrane-binding characteristics.[30,31] The active site of the enzyme was proposed to be situated in a large central cavity lined with aromatic residues, as suggested by a bound molecule of *N*,*N*-dimethyldodecylamine-*N*-oxide, a detergent and competitive inhibitor of SHC ($K_I = 140$ nM). Interestingly, SHC and OSLC contain multiple repeats of a highly conserved motif rich in aromatic amino acids (the QW motif),

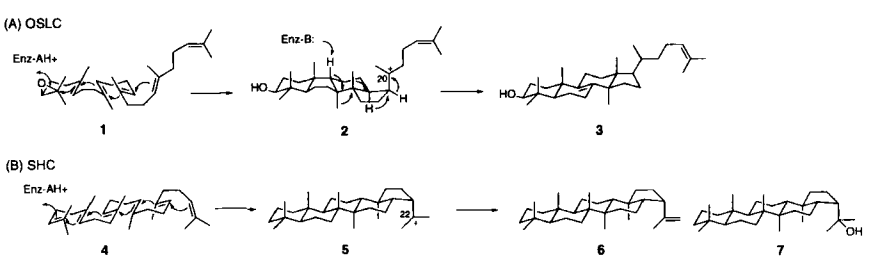


FIGURE 1 Proposed mechanism for the cyclization of (A) (3*S*)2,3-oxidosqualene (**1**) to lanosterol (**3**) by OSLC and (B) squalene (**4**) to hop-22-ene (**6**) and hopan-22-ol (**7**) by SHC.

which appears to stabilize the enzyme structure by connecting surface α-helices.[30,32,33]

The first mechanism-based irreversible inhibitor of OSLC was 29-methylidene-2,3-oxidosqualene (29-MOS) (**8**) developed in our laboratories (Figure 2). It had an IC_{50} value of 0.5 μM for pig liver OSLC ($K_I = 4.4\,\mu M$) and showed extremely efficient time-dependent inactivation of this enzyme with $k_{inact} = 221\ min^{-1}$.[34,35] Vertebrate OSLCs were specifically labeled with the suicide substrate [^{3}H](3*S*)29-MOS, giving a single radioactive band on fluorograms of electrophoretically separated proteins.[28,34] In contrast, yeast and plant OSCs were not covalently modified, suggesting subtle species-specific differences in the active-site structures of the cyclases. The proposed mechanism of inhibition involves initial cyclization to the 21-methylidene-protosterol cation (**9**) as proposed for lanosterol formation. However, this allylic cation is trapped by an active-site nucleophile, resulting in covalent bond formation and concomitant irreversible inactivation (Figure 2A). An aspartate residue (D-456 in rat OSLC) contained in the highly conserved DCTAEA motif was identified as the site of covalent attachment of a cyclized inhibitor (structure **10**).[19,36,37] This implicated aspartate carboxylate in stabilization of the C-20 cationic center of the protosterol cation (**2**) during lanosterol-forming reaction. Indeed, site-directed mutagenesis experiments in OSLC and SHC have revealed that the homologous Asp of the DCTAEA motif was essential for catalytic function in each case.[38,39] For *A. acidocaldarius* SHC, both D-376 and D-377 of the homologous DDTAVV motif were shown to be crucial for enzyme activity.[40]

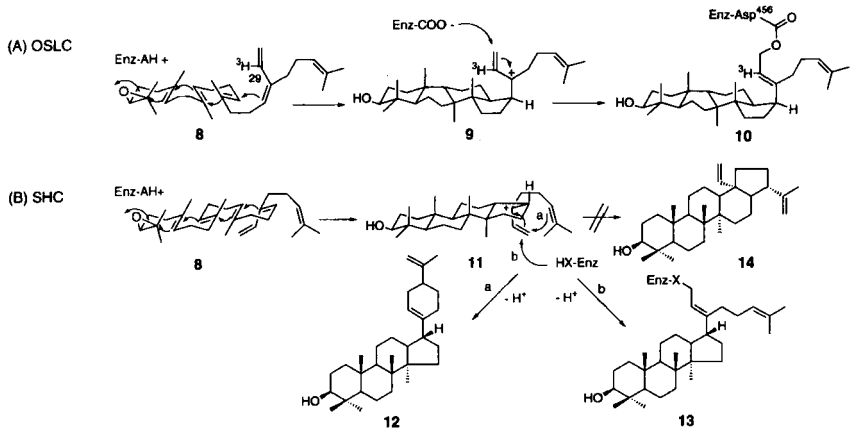
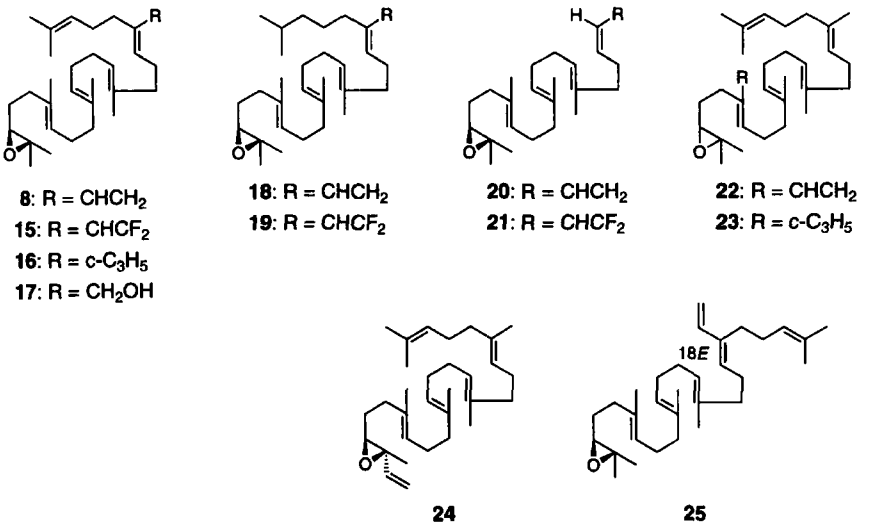


FIGURE 2 Proposed mechanism of (A) irreversible inhibition of OSLC and (B) cyclization and SHC inactivation by (3*S*)29-MOS (**8**).

Other 29-functionalized 2,3-oxidosqualene analogs such as 29-difluoromethylidene-2,3-oxidosqualene (**15**) ($IC_{50} = 3.0\,\mu M$, $K_I = 10.2\,\mu M$ for rat OSLC) were also irreversible inhibitors of OSLC (Figure 3, Table I). The rapid inactivation rates for these substrate analogs suggested (**15**, **18**, and **19**) that initiation, not termination, of cyclization was rate-limiting for these suicide substrates.[41] In contrast, truncation of the side chain such as 29-difluoromethylidene-hexanor-2,3-oxidosqualene (**21**) ($IC_{50} = 60\,\mu M$ for rat OSLC) suppressed inactivation and led to reversible inhibition.[41] Furthermore, both 29-cyclopropyl-2,3-oxidosqualene (**16**) and 26-cyclopropyl-2,3-oxidosqualene (**23**) failed to act as irreversible inhibitors ($IC_{50} > 400\,\mu M$);[42] 29-hydroxy-2,3-oxidosqualene (**17**) was quantitatively converted to the corresponding 21-hydroxy lanosterol analog.[43] 26-Methylidene-2,3-oxidosqualene (**22**)[34] and 1-methylidene-2,3-oxidosqualene (**24**)[44] were accepted as a substrate to give the corresponding vinyllanosterol products.



8: R = $CHCH_2$
15: R = $CHCF_2$
16: R = c-C_3H_5
17: R = CH_2OH

18: R = $CHCH_2$
19: R = $CHCF_2$

20: R = $CHCH_2$
21: R = $CHCF_2$

22: R = $CHCH_2$
23: R = c-C_3H_5

24 **25**

FIGURE 3 Structures of side chain-modified and truncated analogs of 29-MOS (**8**).

TABLE I Fluorinated and truncated side chain analogs of 29-MOS (**8**)[41]

Compound	IC_{50} (μM)	K_I	*Apparent irreversibility*	k_{inact} (min^{-1})
29-MOS (**8**)	0.3	2.5	Yes	232
15	3.0	10.2	Yes	122
18	2.0	4.5	Yes	180
19	18	12	Yes	119
20	60	—	No	—
21	60	—	No	—

Interestingly, *A. acidocaldarius* SHC was also covalently modified by [^{3}H](3*S*)29-MOS.[13] Inhibition kinetics revealed that the inhibition was non-competitive and time-dependent ($IC_{50} = 1.2\,\mu M$, $K_I = 2.1\,\mu M$, $k_{inact} = 0.06\,min^{-1}$). Since the inactivation of SHC was over 1000-fold less rapid than for OSLC, we were able to isolate a major cyclization product; this product was characterized spectroscopically as a C_{31} dammarene–limonene hybrid with a 6.6.6.5 + 6 ring system (**12**).[13] No evidence was found for the methylidene-extended 3β-hydroxyhopene (**14**). Apparently, the presence of the methylidene residue interrupted the cyclization reaction by SHC at the tetracyclic 17-epi-dammarene C-20 cation (**11**) (with the 17α-side chain). Cation **11** could be partitioned between final ring closure to yield the unnatural product and being trapped by an active-site nucleophile to give a covalently modified enzyme such as **13** (Figure 2B). Comparison of the affinity labeling studies between bacterial SHC and eukaryotic OSC is now in progress.

Very recently, we have found that (18*E*)-(3*S*)29-MOS (**25**), the Δ^{18} regioisomer of the previous suicide substrate, (18*Z*)-(3*S*)29-MOS (**8**), also covalently modified the active site of rat OSLC.[68] (Note that the higher priority of the alkenyl substitutent renders an 18*Z* designation for the "*trans*"-type squalene.) Cattel and co-workers have reported recently that racemic (18*E*)-29-MOS is a time-dependent inhibitor with tenfold higher K_I value for crude pig and yeast OSLC.[45] Since it has been reported that the 2,3-oxidosqualene with the unnatural *Z*-stereochemistry at Δ^{18} was cyclized to the mixture of two 6.6.5-fused tricyclic products, both having *trans/syn/trans* A/B/C ring junctions,[46] (18*E*)-(3*S*)29-MOS may also be cyclized to a 6.6.5-fused tricyclic intermediate cation. This cation would then be trapped by active-site nucleophile, resulting in the covalent modification of the enzyme. It seems likely that the OSLC labeling site of the linked (18*E*)-isomer should be different from that of the extended (18*Z*)-isomer. Inhibition kinetics and active-site mapping with the tritium-labeled (18*E*)-(3*S*)29-MOS (**25**) will be reported in due course.

A number of sulfur-containing OS analogs in which a sulfur atom replaced carbons C-5, C-6, C-8, C-9, C-10, C-11, C-13, C-14, C-15, C-16, C-18, C-19, and C-20 were synthesized by Oehlschlager and co-workers; many of these thia analogs were potent inhibitors of OSLCs (Figure 4A).[47–50] In particular, OS analogs in which sulfur has replaced carbons C-18 and C-19 were reported to have extremely potent, subnanomolar IC_{50} values for both vertebrate and fungal OSLCs: S-18 (**29**) ($IC_{50} = 0.08\,nM$ for rat microsomal OSLC; 0.22 nM for *Candida albicans* microsomal OSLC) and S-19 (**30**) ($IC_{50} = 0.82\,nM$ for rat OSLC; 3.2 nM for *C. albicans* OSLC).[48,50] Sulfoxides

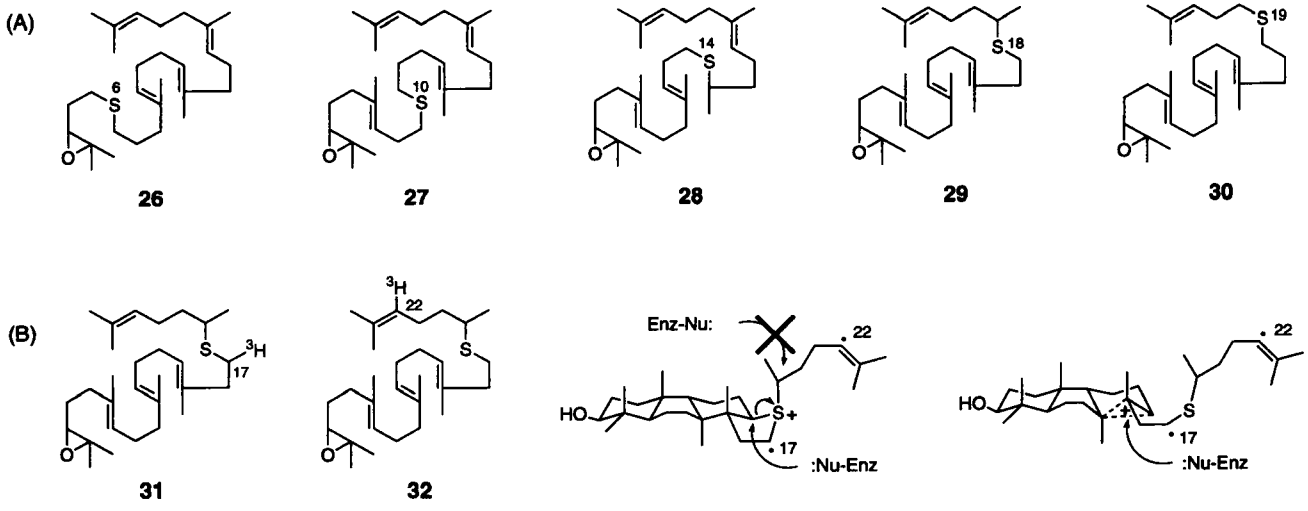


FIGURE 4 (A) Structures of sulfur-substituted-OS analogs (**26**–**30**) and (B) proposed mechanism of inhibition by S-18 (**29**).

TABLE II Sulfur-substituted-OS as inhibitors of rat liver OSLC and recombinant *A. acidocaldarius* SHC[51]

Compound	*Rat liver OSLC*			*A. acidocaldarius SHC*		
	IC_{50} (nM)	K_I (nM)	k_{inact} (min^{-1})	IC_{50} (nM)	K_I (nM)	k_{inact} (min^{-1})
S-6 (**26**)	500	520	0.085	150	127	0.0001
S-10 (**27**)	1000	2100	0.037	570	971	0.0001
S-14 (**28**)	11000	4200	0.042	86	109	0.058
S-18 (**29**)	50	37	0.0001	60	31	0.071
S-19 (**30**)	260	180	0.0001	78	83	0.054

were generally much less active than corresponding thioethers. In our enzyme assays with homogeneous OSLCs, these unusually low IC_{50} values have been revised in light of data obtained with purified rat OSLC: S-18 ($IC_{50} = 0.05\,\mu M$ for rat OSLC) and S-19 ($IC_{50} = 0.26\,\mu M$ for rat OSLC) (Table II).[50] Inhibition kinetics with purified vertebrate OSLCs demonstrated that the S-18 compound was a time-dependent, irreversible inhibitor of *pig* OSLC ($K_I = 1.5\,\mu M$, $k_{inact} = 0.06\,min^{-1}$, partition ratio = 16), while the inhibition by S-19 was reversible and not time-dependent.[50] Surprisingly, the inhibition of *rat* OSLC by the S-18 compound was reversible and more potent ($K_I = 0.037\,\mu M$).

When tested with recombinant *A. acidocaldarius* SHC, thia analogs of OS in which sulfur replaced carbons at C-6, C-10, C-14, C-18, or C-19 also showed potent inhibition of the enzyme at submicromolar level (Table II).[51] Among them, S-18 was the most potent inhibitor and showed time-dependent inhibition ($K_I = 31$ nM, $k_{inact} = 0.071\,min^{-1}$) of SHC. The potent inhibitory activities of these analogs may be attributed to the higher nucleophilicity of the sulfur atom compared to nitrogen and oxygen. Further, the more flexible and longer C–S bond in comparison with the C–C double bond may serve to bring the sulfur closer to the active-site nucleophilic residues important in cationic charge stabilization.

We have also demonstrated that both *pig* OSLC and *A. acidocaldarius* SHC could be covalently modified with [17-^{3}H]S-18 (**31**) and [22-^{3}H]S-18 (**32**), two tritium-labeled radioisotopomers of S-18.[51,52] The covalent modification appeared to require partial cyclization of S-18 at the active site of the enzyme with trapping of a cationic intermediate by an active-site nucleophile. Retention of the tritium label for both radioisotopomers excluded a possibility of an attack at C-20 with transfer of the side chain to the active site; alternatively, nucleophilic trapping could occur on a bicyclic or tricyclic intermediate (Figure 4B).[52] The structures of the S-18-derived material bound to the active site and the structures of the released S-18 cyclization products are currently under investigation.

The non-terpenoid benzophenone derivative Ro48-8071 (**33**) is an extremely potent, specific inhibitor of OSLC ($IC_{50} = 7$ nM for human liver OSLC), as recently disclosed by scientists at Hoffmann-La Roche.[53] The structural cousins BIBX79 (**34**)[54] and BIBB515 (**35**)[55] independently discovered at Karl Thomae/Boehringer Ingelheim also showed potent inhibition of OSLC ($IC_{50} = 6$ and 9 nM for human Hep G2 OSLC, respectively). These orally bioavailable compounds show considerable therapeutic promise as cholesterol lowering drugs (Figure 5A). The mechanism of inhibition for these compounds is not well-understood yet, although calculations suggested that the aromatic compounds could be similar in shape and size to the protosterol intermediate cation.[56] However, it has been reported that the protosterol cation analog is no longer strongly bound by the enzyme. The relatively modest inhibition of purified yeast OSLC by a 20-aza-protosterol analog ($IC_{50} = 22\ \mu M$) seemed to support a low affinity of the catalytic site for a late state high energy intermediate.[57] Finally, Ro48-8071 (**33**) also showed extremely potent, non-competitive inhibition of *A. acidocaldarius* SHC ($IC_{50} = 9.0$ nM, $K_I = 6.6$ nM); for homogeneous rat liver OSLC, values of $IC_{50} = 40$ nM, $K_I = 22$ nM were obtained.[58] We decided that additional information was required to localize these potent non-terpenoid OSLC inhibitors in the active site.

To locate the regions of SHC and OSLC that interact with Ro48-8071, we took advantage of the benzophenone photophore as a photoaffinity label. In many biochemical systems, benzophenone derivatives have been employed as excellent photoaffinity probes with remarkable site specificity and highly efficient covalent modification of the target proteins.[59,60] Thus, tritium-labeled Ro48-8071 (**36**) (18.8 Ci/mmol) was chemically synthesized (Figure 5B); as expected, specific, efficient covalent modification of both OSLC and SHC enzymes was observed after 45 min irradiation at 360 nm.[58] The labeling of both OSLC and SHC by [^{3}H]Ro48-8071 was competitively displaced by co-incubation with a 1000-fold molar excess of S-18 or the non-terpenoid inhibitor BIBX79. Displacement of labeling of OSLC was also achieved with the suicide substrate (3*S*)29-MOS. Thus, the non-substrate Ro48-8071 and both terpenoid and non-terpenoid inhibitors of these enzymes appear to share a common binding site.

Recently, Corey and co-workers[61] reported seven irreversible, time-dependent inhibitors of yeast OSLC (**37**–**43**) including 20-oxa-2,3-oxidosqualene (**37**) and 10,15-didesmethyl-2,3-oxidosqualene (**42**) (Figure 6A); however, neither the details of the inhibition kinetics and efficiency nor the specificity of labeling were presented. In earlier work, 20-oxa-2,3-oxidosqualene was enzymatically transformed to protosterol derivatives having

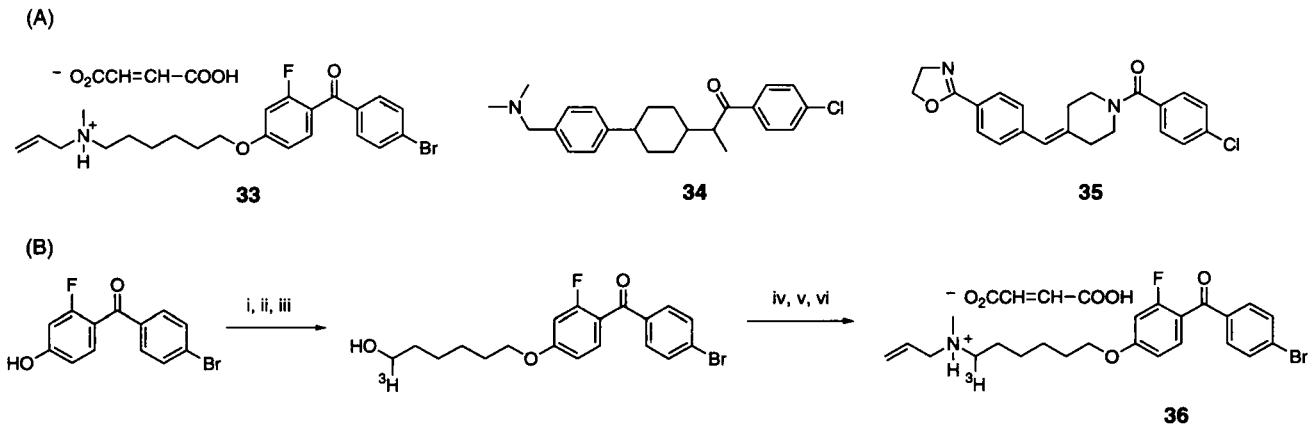


FIGURE 5 (A) Structures of Ro48-8071 (**33**), BIBX79 (**34**), and BIBB515 (**35**). (B) Synthesis of [^{3}H]Ro48-8071 (**36**). Reagents and conditions: (i) $HO(CH_2)_6Br$, K_2CO_3, acetone; (ii) TPAP, NMP, 4 Å molecular sieves, CH_2Cl_2; (iii) NaB^3H_4, EtOH; (iv) MsCl, Et_3N, DMAP, CH_2Cl_2; (v) *N*-allylmethylamine, DMA; (vi) fumaric acid, EtOH.

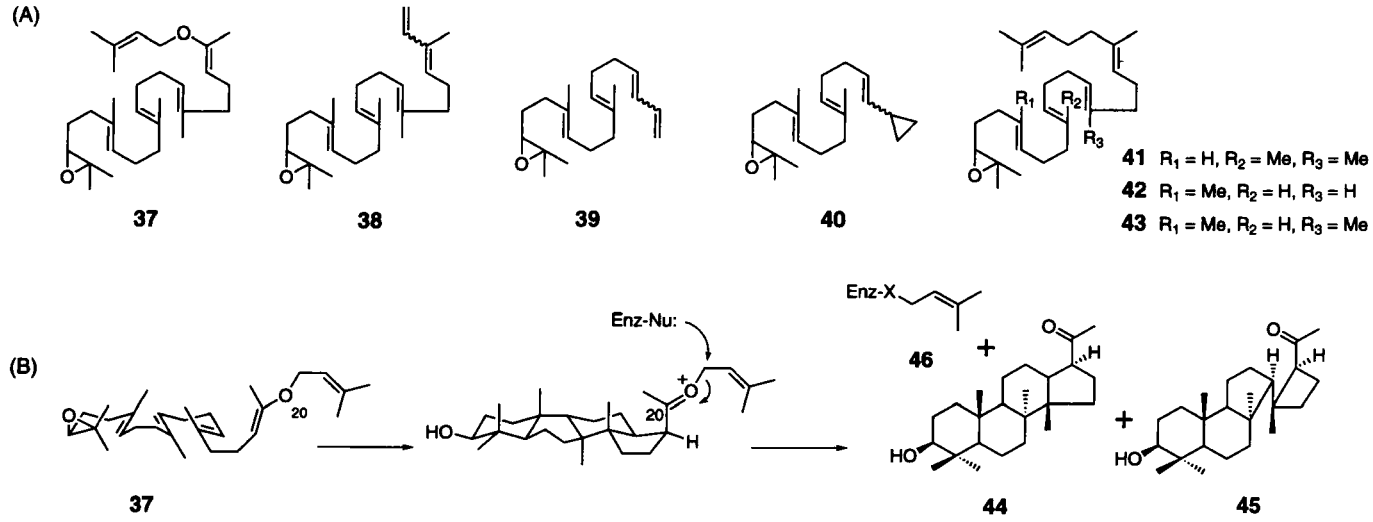


FIGURE 6 (A) Irreversible, time-dependent yeast OSLC inhibitors (**37**–**43**) developed by Corey and co-workers. (B) Proposed mechanism of cyclization and OSLC inactivation by 20-oxa-2,3-oxidosqualene (**37**).

17β-oriented side chains (**44**).[7] In addition, a unique tetracyclic compound with a 6.6.5-fused A/B/C ring system and a pendant four-membered "D" ring (**45**) was identified as a minor cyclization product of 20-oxa-2,3-oxidosqualene (Figure 6B).[62] The 10,15-didesmethyl-2,3-oxidosqualene was also enzymatically converted to a similar compound with the 6.6.5 + 4 ring system.[63]

These seven analogs were employed in tritium-labeled form for active-site mapping studies. Trypsin digestion of the labeled OSLC followed by identification of the peptide fragments by mass spectroscopy or Edman degradation revealed that (i) the first four OS analogs covalently modified residues W-232 and H-234 within the 231–236 segment (yeast numbering), while (ii) the last three analogs lacking methyl groups covalently modified the 486–512 segment.[61] Using systematic site-directed mutagenesis of a total of 76 amino acid residues that are conserved in five different species, six mutants (D456N, H146A, H234A/K/R, M532A) were inactive. The authors concluded that residues D-456, H-146, H-234, and M-532 were essential for catalytic activity,[22,38,61] and they proposed a working model of some aspects of the activation and binding of 2,3-oxidosqualene by OSLC. In this hypothetical model, D-456 and protonated H-146 initiate cyclization, and the domains containing 231–236 and 486–512 make contact with the reacting substrate.[61] This hypothesis fails to account for the result that D-456 was alkylated by a partially cyclized suicide substrate,[36] 29-MOS. Moreover, this hypothesis does not incorporate the importance of cation stabilization at the end of the polycyclization as the "pull" of a push–pull cyclization process.

Finally, in a continued investigation of the ability of fluorine to facilitate, inhibit, or redirect polycyclizations, we have prepared several vinylic fluoro analogs of OS. The late W.S. Johnson had shown that a fluorine atom could act as a cation-stabilizing auxiliary that served to both enhance the cyclization reaction and control the regiochemistry of the product.[64–67] In order to test the effect of fluorine atom substitution on the cyclase reaction, (3*S*)11-fluoro-2,3-oxidosqualene (11-FOS) (**47**)[14] and (3*S*)14-fluoro-2,3-oxidosqualene (14-FOS) were synthesized (Robustell and Prestwich, unpublished results). Surprisingly, when cyclization of 11-FOS with purified rat liver OSLC was attempted, no cyclization product could be detected.[14] The OSLC enzyme is particularly sensitive to structural changes on the pro-β-face and thus fails to bind (3*S*)11-FOS. In contrast, recombinant *A. acidocaldarius* SHC converted (3*S*)11-FOS into a carbocyclic compound with a bridged ether (**48**) in 27% yield (Figure 7). For SHC, 11-FOS was accepted in the catalytic site, but the presence of the fluorine atom interrupted the cyclization reaction at the monocyclic cationic intermediate stage. Partial

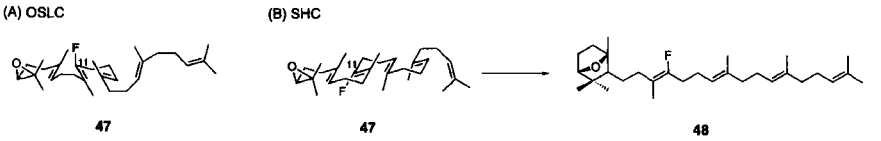


FIGURE 7 Enzymatic cyclization of (3*S*)11-fluoro-2,3-oxidosqualene (**47**) by (A) OSLC and (B) SHC.

cyclization may be attributed to the strong electron-withdrawing effect of the 11-F atom or to the distortion of the substrate folding. The synthesis of additional fluorinated OS analogs and the processing of these as substrates by OSLC and SHC will be reported in due course.

In summary, mechanism-based inhibitors and other active-site targeted inhibitors for eukaryotic OSLC and bacterial SHC have afforded important insights into the cyclization mechanism. The next generation of results will place the mechanistic understanding into a structural context, and the roles for specific active-site residues will be elucidated through peptide mapping and crystallographic studies. This is an exciting time: after forty years of study, the mysteries of how these fascinating enzymes handle multiple carbocations in water may soon be revealed.

References

[1] Abe, I., Rohmer, M. and Prestwich, G.D. (1993) *Chem. Rev.*, **93**, 2189.
[2] Abe, I. and Prestwich, G.D. (1998) in *Comprehensive Natural Products Chemistry*, Barton, D.H.R. and Nakanishi, K. (Eds.), Vol. 2, Elsevier Science, Ltd, Oxford, UK (in press).
[3] Abe, I., Tomesch, J.C., Wattanasin, S. and Prestwich, G.D. (1994) *Nat. Prod. Rep.*, **11**, 279.
[4] Eschenmoser, A., Ruzicka, L., Jeger, O. and Arigoni, D. (1955) *Helv. Chim. Acta*, **38**, 1890.
[5] Stork, G. and Burgstahler, A.W. (1955) *J. Am. Chem. Soc.*, **77**, 5068.
[6] van Tamelen, E.E. (1982) *J. Am. Chem. Soc.*, **104**, 6480.
[7] Corey, E.J. and Virgil, S.C. (1991) *J. Am. Chem. Soc.*, **113**, 4025.
[8] Corey, E.J., Virgil, S.C. and Sarshar, S. (1991) *J. Am. Chem. Soc.*, **113**, 8171.
[9] Rohmer, M., Anding, C. and Ourisson, G. (1980) *Eur. J. Biochem.*, **112**, 541.
[10] Rohmer, M., Bouvier, P. and Ourisson, G. (1980) *Eur. J. Biochem.*, **112**, 557.
[11] Bouvier, P., Berger, Y., Rohmer, M. and Ourisson, G. (1980) *Eur. J. Biochem.*, **112**, 549.
[12] Abe, I. and Rohmer, M. (1994) *J. Chem. Soc. Perkin Trans. 1*, 783.
[13] Abe, I., Dang, T., Zheng, Y.F., Madden, B.A., Feil, C., Poralla, K. and Prestwich, G.D. (1997) *J. Am. Chem. Soc.*, **119**, 11333.
[14] Robustell, B., Abe, I. and Prestwich, G.D. (1998) *Tetrahedron Lett.*, **39**, 957.
[15] Kelly, R., Miller, S.M., Lai, M.H. and Kirsch, D.R. (1990) *Gene*, **87**, 177.
[16] Buntel, C.J. and Griffin, J.H. (1992) *J. Am. Chem. Soc.*, **114**, 9711.
[17] Corey, E.J., Matsuda, S.P.T. and Bartel, B. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 2211.
[18] Shi, Z., Buntel, C.J. and Griffin, J.H. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 7370.
[19] Abe, I. and Prestwich, G.D. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 9274.
[20] Kusano, M., Shibuya, M., Sankawa, U. and Ebizuka, Y. (1995) *Biol. Pharm. Bull.*, **18**, 195.
[21] Baker, C.H., Matsuda, S.P.T., Liu, D.R. and Corey, E.J. (1995) *Biochem. Biophys. Res. Commun.*, **213**, 154.

[22] Corey, E.J., Matsuda, S.P.T., Baker, C.H., Ting, A.Y. and Cheng, H. (1996) *Biochem. Biophys. Res. Commun.*, **219**, 327.
[23] Ochs, D., Kaletta, C., Entian, K.-D., Beck-Sickinger, A. and Poralla, K. (1992) *J. Bacteriol.*, **174**, 298.
[24] Reipen, I.G., Poralla, K., Sahm, H. and Sprenger, G.A. (1995) *Microbiology*, **141**, 155.
[25] Perzl, M., Müller, P., Poralla, K. and Kannenberg, E.L. (1997) *Microbiology*, **143**, 1235.
[26] Sung, C.K., Shibuya, M., Sankawa, U. and Ebizuka, Y. (1995) *Biol. Pharm. Bull.*, **18**, 1459.
[27] Young, M., Chen, H.M., Lalioti, M.D. and Antonarakis, S.E. (1996) *Human Genetics*, **97**, 620.
[28] Abe, I., Bai, M., Xiao, X.-y. and Prestwich, G.D. (1992) *Biochem. Biophys. Res. Commun.*, **187**, 32.
[29] Ochs, D., Tappe, C.H., Gärtner, P., Kellner, R. and Poralla, K. (1990) *Eur. J. Biochem.*, **194**, 75.
[30] Wendt, K.U., Poralla, K. and Schulz, G.E. (1997) *Science*, **277**, 1811.
[31] Wendt, K.-U., Feil, C., Lenhart, A., Poralla, K. and Schulz, G.E. (1997) *Protein Sci.*, **6**, 722.
[32] Poralla, K., Hewelt, A., Prestwich, G.D., Abe, I., Reipen, I. and Sprenger, G. (1994) *Trends Biochem. Sci.*, **19**, 157.
[33] Poralla, K. (1994) *Bioorg. Med. Chem. Lett.*, **4**, 285.
[34] Xiao, X.-y. and Prestwich, G.D. (1991) *J. Am. Chem. Soc.*, **113**, 9673.
[35] Madden, B.A. and Prestwich, G.D. (1994) *J. Org. Chem.*, **59**, 5488.
[36] Abe, I. and Prestwich, G.D. (1994) *J. Biol. Chem.*, **269**, 802.
[37] Abe, I. and Prestwich, G.D. (1995) *Lipids*, **30**, 231.
[38] Corey, E.J., Cheng, H., Baker, C.H., Matsuda, S.P.T., Li, D. and Song, X. (1997) *J. Am. Chem. Soc.*, **119**, 1277.
[39] Griffin, J.H., Buntel, C.J. and Siregar, J.J. (1998) *Proc. Natl. Acad. Sci. USA* (in press).
[40] Feil, C., Süssmuth, R., Jung, G. and Poralla, K. (1996) *Eur. J. Biochem.*, **242**, 51.
[41] Madden, B.A. and Prestwich, G.D. (1997) *Bioorg. Med. Chem. Lett.*, **7**, 309.
[42] Xiao, X.-y. (1991) Ph. D. Thesis, State University of New York, Stony Brook.
[43] Xiao, X.-y. and Prestwich, G.D. (1991) *Tetrahedron Lett.*, **32**, 6843.
[44] Xiao, X.-y., Sen, S.E. and Prestwich, G.D. (1990) *Tetrahedron Lett.*, **31**, 2097.
[45] Ceruti, M., Rocco, F., Viola, F., Balliano, G., Milla, P., Arpicco, S. and Cattel, L. (1998) *J. Med. Chem.*, **41**, 540.
[46] Krief, A., Schauder, J.-R., Guittet, E., Herve du Penhoat, C. and Lallemand, J.-Y. (1987) *J. Am. Chem. Soc.*, **109**, 7910.
[47] Zheng, Y.F., Oehlschlager, A.C. and Hartman, P.G. (1994) *J. Org. Chem.*, **59**, 5803.
[48] Zheng, Y.F., Oehlschlager, A.C., Georgopapadakou, N.H., Hartman, P.G. and Scheliga, P. (1995) *J. Am. Chem. Soc.*, **117**, 670.
[49] Zheng, Y.F., Dodd, D.S., Oehlschlager, A.C. and Hartman, P.G. (1995) *Tetrahedron*, **51**, 5255.
[50] Stach, D., Zheng, Y.F., Perez, A.L., Oehlschlager, A.C., Abe, I., Prestwich, G.D. and P.G. Hartman (1997) *J. Med. Chem.*, **40**, 201.
[51] Zheng, Y.F., Abe, I. and Prestwich, G.D. (1998) *Biochemistry*, **37**, 5981–5987.
[52] Abe, I., Liu, W., Oehlschlager, A.C. and Prestwich, G.D. (1996) *J. Am. Chem. Soc.*, **118**, 9180.
[53] Morand, O.H., Aebi, J.D., Dehmlow, H., Ji, Y.-H., Gains, N., Lengsfeld, H. and Himber, J. (1997) *J. Lipid Res.*, **38**, 373.
[54] Mark, M., Müller, P., Mainer, R. and Eisele, B. (1996) *J. Lipid Res.*, **37**, 148.
[55] Eisele, B., Budzinski, R., Müller, P.R.M. and Mark, M. (1997) *J. Lipid Res.*, **38**, 564.
[56] Jolidon, S., Polak-Wyss, A., Hartman, P.G. and Guerry, P. (1993) in *Recent Advances in the Chemistry of Anti-infective Agents*, Bentley, P.H. and Ponsfer, R. (Eds.), The Royal Society of Chemistry, London.
[57] Corey, E.J., Daley, D.C. and Cheng, H. (1996) *Tetrahedron Lett.*, **37**, 3287.
[58] Abe, I., Zheng, Y.F. and Prestwich, G.D. (1998) *Biochemistry*, **37**, 5779–5784.
[59] Dormán, G. and Prestwich, G.D. (1994) *Biochemistry*, **33**, 5661.
[60] Prestwich, G.D., Dormán, G., Elliott, J.T., Marecak, D.M. and Chaudhary, A. (1997) *Photochem. Photobiol.*, **65**, 222.

[61] Corey, E.J., Cheng, H., Baker, C.H., Matsuda, S.P.T., Li, D. and Song, X. (1997) *J. Am. Chem. Soc.*, **119**, 1289.
[62] Corey, E.J., Virgil, S.C., Cheng, H., Baker, C.H., Matsuda, S.P.T., Singh, V. and Sarshar, S. (1995) *J. Am. Chem. Soc.*, **117**, 11819.
[63] Corey, E.J., Virgil, S.C., Liu, D.R. and Sarshar, S. (1992) *J. Am. Chem. Soc.*, **114**, 1524.
[64] Johnson, W.S., Buchanan, R.A., Bartlett, W.R., Tham, F.S. and Kullnig, R.K. (1993) *J. Am. Chem. Soc.*, **115**, 504.
[65] Johnson, W.S., Chenera, B., Tham, F.S. and Kullnig, R.K. (1993) *J. Am. Chem. Soc.*, **115**, 493.
[66] Johnson, W.S., Fletcher, V.R., Chenera, B., Bartlett, W.R., Tham, F.S. and Kullnig, R.K. (1993) *J. Am. Chem. Soc.*, **115**, 497.
[67] Johnson, W.S., Plummer, M.S., Reddy, S.P. and Bartlett, W.R. (1993) *J. Am. Chem. Soc.*, **115**, 515.
[68] Zheng, Y.F., Abe, I. and Prestwich, G.D. (1998) *J. Org. Chem.* (in press).