Amidrazone and Amidoxime Inhibitors of Squalene Hopene Cyclase

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The cyclization of squalene catalyzed by the enzyme squalene-hopene cyclase (SHC) leads to the hopanoid family of pentacyclic triterpenes, which are widely found in bacteria as membrane constituents. SHC mediates a cascade of regio- and stereoselective cyclizations that has triggered considerable interest in understanding the enzyme's mechanism of action. This paper reports synthetic studies leading to the preparation of trienylamidrazone **8**, trienylamidoxime **9**, and tetraenylamidoxime **10** corresponding to a partially cyclized squalene chain. All three compounds displayed significant levels of inhibition when assayed against SHC, with **9** being more active than **8**, and amidoxime **10** being the most potent. Detailed profiles of their inhibition kinetics are also presented.

Introduction

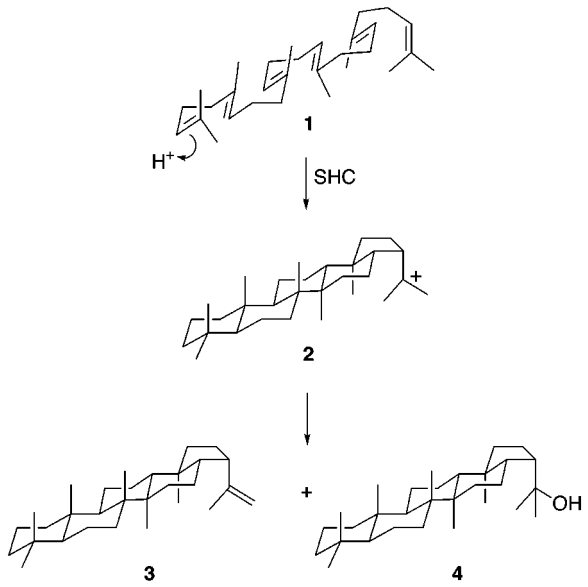
The cyclization of squalene **1** (Scheme 1) catalyzed by the enzyme squalene-hopene cyclase (SHC) leads to the hopanoid family of pentacyclic triterpenes.[1,2] These triterpenes are widely found in bacteria as membrane constituents, where they exert many of the same stabilizing effects as membrane sterols.[3] Like the enzyme oxidosqualene-lanosterol cyclase (OSLC),[1] which promotes the conversion of squalene oxide into lanosterol and other sterols, SHC mediates a cascade of regio- and stereoselective cyclizations that has triggered considerable interest in understanding the enzyme's mechanism of action.

In the SHC-catalyzed process, **1** is thought to bind to the enzyme in the all-chair conformation shown. Cyclization is initiated by protonation of the terminal alkene (Scheme 1) and proceeds through a series of alkene−cation additions leading to the pentacyclic cation **2**, the precursor of hop-22,29-ene **3** and hopan-22-ol **4**. A recent 2.9 Å resolution X-ray crystal structure[4] of SHC isolated from *Alicyclobacillus acidocaldarius* revealed a relatively nonpolar active site lined by aromatic amino acid residues that may play a role in stabilizing the various carbocationic intermediates propagated along the squalene backbone during cyclization.

The putative involvement of transient carbocations in the cyclization of oxidosqualene has triggered interest in the synthesis of polyisoprenylated cation mimics that inhibit OSLC.[5,6] Several aza-substituted mono-, bi-, and tricyclic structures representing partially cyclized sterol intermediates were evaluated as inhibitors of OSLC, the most potent of which displayed IC_{50} values in the single-digit micromolar range. However, the design and synthesis of specific SHC inhibitors has not been reported.

As part of our interest in the mechanism of enzyme-catalyzed glycoside hydrolysis, we have described several aza analogues of monosaccharide aldonolactams that are

Scheme 1



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(5) For nitrogen-containing monocyclic inhibitors of oxidosqualene cyclase, see: (a) Taton, M.; Benveniste, P.; Rahier, A.; Johnson, W. S.; Liu, H. T.; Sudhakar, A. R. *Biochemistry* **1992**, *31*, 7892−7898. (b) Dodd, D. S.; Oehlschlager, A. C. *J. Org. Chem.* **1992**, *57*, 2794−2803. (c) Rahier, A.; Taton, M.; Beneviste, P.; Place, P.; Anding, C. *Phytochemistry* **1985**, *24*, 1223−1232. (d) Rahier, A.; Narula, A. S.; Beneviste, P.; Schmitt, P. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 20−25.
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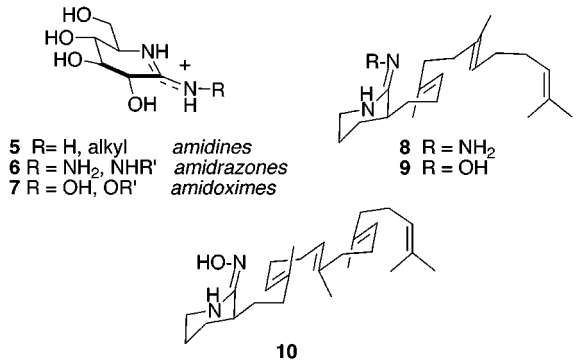
Scheme 2



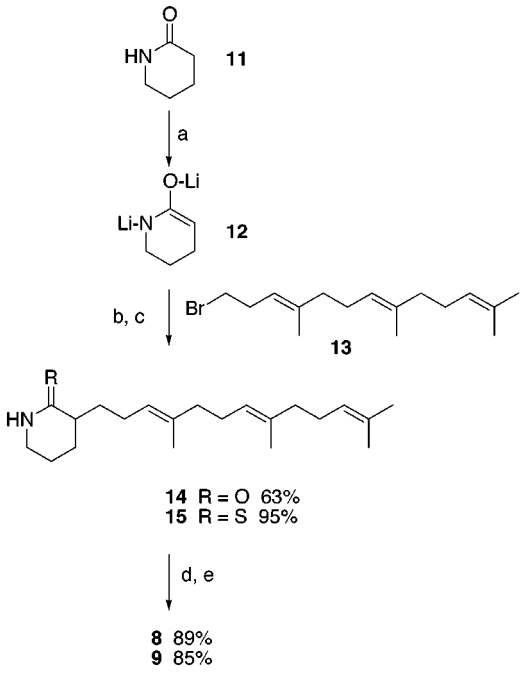
potent glycosidase inhibitors.[7] When protonated, such amidines, amidrazones, and amidoximes form resonance-stabilized cations (**5**−**7**, Scheme 2) that effectively mimic both the half-chair conformation and incipient positive charge of the resonance-stabilized glycosyl cation. With that same design strategy in mind, we reasoned that terpenoid-derived amidrazones or amidoximes representing partially cyclized intermediates might be effective inhibitors of squalene-cyclizing enzymes. Here, we describe synthetic studies leading to the preparation of trienylamidrazone **8**, trienylamidoxime **9**, and tetraenylamidoxime **10** corresponding to a partially cyclized squalene chain. All three compounds displayed significant levels of inhibition when assayed against SHC, with **9** being more active than **8** and amidoxime **10** being the most potent. Detailed profiles of their inhibition kinetics are also presented.

Discussion

Retrosynthetic analysis suggested that the desired target structures might be obtained by direct C-alkylation of 2-piperidone **11** (Scheme 3) using an appropriate homoallylic halide. After experimentation with a variety of dialkylamide bases generated in situ, it was found that deprotonation of **11** was best achieved directly using *n*-butyllithium (*n*-BuLi, 2.1 equiv, THF, 0 °C) to afford dianion **12**.[8] Addition of homofarnesyl bromide **13**, prepared according to a literature report,[5d] to **12** at 0 °C afforded the desired homofarnesylated lactam **14** in 63% yield after chromatography. Treatment of **14** with Lawesson's reagent (*p*-methoxyphenylthionophosphine sulfide dimer)[9] quantitatively produced thionolactam **15**. Following experimental protocols developed in our earlier work on saccharide mimics,[10] thionolactam **15** was converted either to amidrazone **8** (concd NH_2NH_2 in CH_3OH, 89% yield) or to amidoxime **9** (NH_2OH in CH_3OH, 95% yield).

In preliminary bioassays of their effect on homogeneous, recombinant *A. alcidocaldarius* SHC, amidrazone **8**, and amidoxime **9** displayed significant levels of inhibitory activity. For **8** and **9**, IC_{50} values of 220 and 56 nM, respectively, were recorded. Besides being the more potent inhibitor, amidoxime **9** was also more resistant

Scheme 3[a]



[a] (a) *n*-BuLi, THF, 0 °C; (b) **13**, 0 °C; (c) Lawesson's reagent, toluene, 50 °C; (d) NH_2NH_2, CH_3OH (for **8**); (e) NH_2OH, CH_3OH (for **9**).

to hydrolysis than amidrazone **8**, in accordance with earlier observations in our laboratory.[10] Therefore, the synthesis of amidoxime **10** embodying the complete, partially cyclized, squalene framework became our next objective.

A synthesis of **10** was developed (Scheme 4) that relied on an efficient and highly stereoselective assembly of tetraenyl bromide **24** from the known 4-benzyloxy-1-butyne **16**.[11] The route depicted in Scheme 4 is a higher-yielding modification of a previously published route to the corresponding tetraenyl iodide **25**[5b] in which assembly of the new trisubstituted alkene by carboalumination has been replaced with a highly stereoselective reductive coupling protocol using organocuprates.

Metalation and alkylation of **16** with formaldehyde afforded propargylic alcohol **17** in good yield. Aluminum hydride-mediated reductive iodination of this alkyne[12] gave *Z*-iodoalkene **18** as a single stereoisomer. Coupling of **18** with lithium dimethylcuprate then generated **19**. Treatment of **19** with PBr_3 afforded allylic bromide **20**, which was readily converted to phenyl sulfone **21**. The corresponding sulfone-stabilized allylic anion, generated from **21** using *n*-BuLi, reacted with farnesyl bromide to afford the coupled product **22** in 92% yield. Reductive desulfurization with concomitant debenzylation was achieved using lithium in ethylamine, giving tetraenol **23**. Reaction of **23** with Ph_3PBr_2 produced the corresponding homoallylic bromide **24**, which, when reacted with dianion **12**, formed lactam **26** in 85% yield. Unlike reactions with **13**, the alkylation of homoallylic bromide **24** was accompanied by almost no base-induced HBr elimination. The remarkable efficiency of this coupling attests to the pronounced effect of the newly installed,

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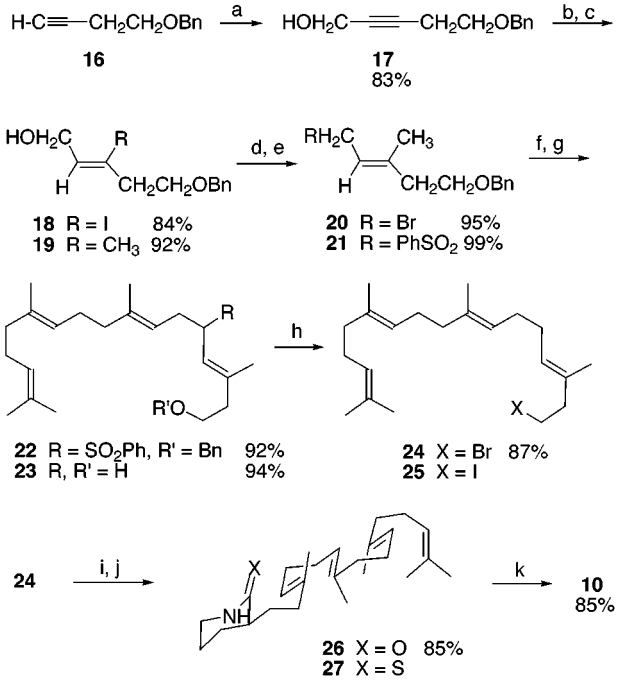
Scheme 4[a]



[a] (a) *n*-BuLi, −78 °C, then CH_2═O; (b) Red-Al, THF, rt, then *N*-iodosuccinimide, −78 °C; (c) Me_2CuLi, THF, 0 °C; (d) PBr_3, ether, 0 °C; (e) $NaSO_2Ph$, DMF, rt; (f) *n*-BuLi, −78 °C, then farnesyl bromide; (g) Li, $EtNH_2$, −78 °C; (h) Ph_3P, Br_2, pyr-CH_2Cl_2; (i) **12**, THF, 0 °C; (j) Lawesson's reagent, toluene, 50 °C; (k) NH_2OH, CH_3OH, 50 °C.

Table 1. Potency of Amidrazone and Amidoxime Inhibitors of Recombinant *A. Acidocaldarius* SHC

compd	IC_{50} (nM)	K_i (nM)	compd	IC_{50} (nM)	K_i (nM)
8	220	180	**28**	9.0[a]	6.6[a]
9	56	49	**29**	100[a]	ND[b]
10	30	90	**30**	60[a]	31[a]

[a] Data from refs 14 and 16. [b] ND: not determined.

cuprate-derived methyl group in screening the allylic hydrogens from adventitious base.

Biological Results

Amidrazone **8**, amidoxime **9**, and amidoxime **10** were designed as transition-state analogues that mimic the incipient monocyclic cation produced during the SHC-mediated cyclization of squalene. All three compounds displayed good to potent levels of inhibition of homogeneous, recombinant *A. acidocaldarius* SHC, with IC_{50} values ranging from 220 to 30 nM (Table 1). From analysis of their Lineweaver−Burk plots (Figures 1−3), it appeared that the three analogues acted as mixed, noncompetitive inhibitors of SHC, possibly binding in the nonpolar channel by which squalene enters the active site within the enzyme's central cavity.[4]

The observation that amidrazone **8** is significantly less potent than amidoximes **9** and **10** is of interest, since the SHC-mediated cyclization of squalene likely involves a progression of partially cyclized carbocationic intermediates. Amidrazones are typically 10^3 more basic than the corresponding amidoximes[10] and would be mostly protonated at pH 6.0, the pH optimum of SHC at which the assays reported here are conducted. By contrast, amidoximes **9** and **10** would exist predominantly in the unprotonated form at pH 6.0. The fact that amidoximes **9** and **10** are more potent than amidrazone **8** suggests that the unprotonated inhibitor binds to SHC.

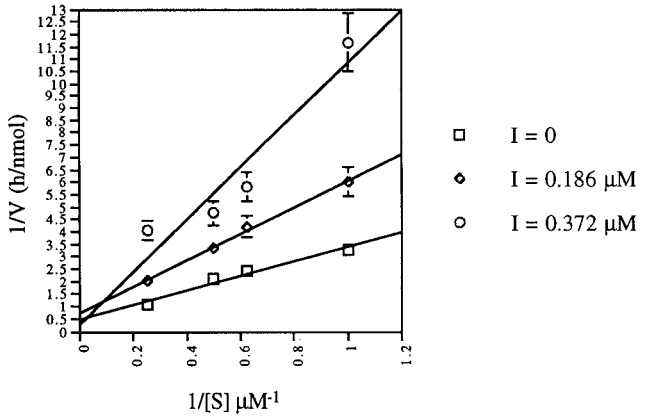


Figure 1. Lineweaver−Burk plot for amidrazone **8**.

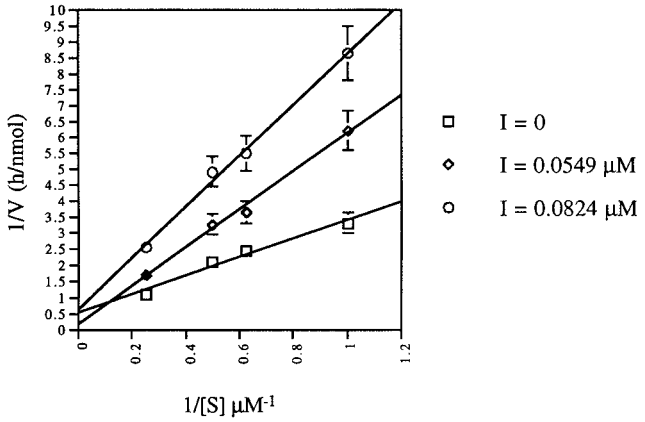


Figure 2. Lineweaver−Burk plot for amidoxime **9**.

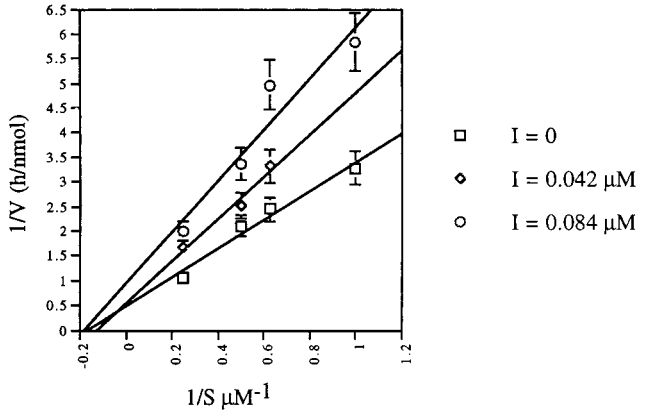


Figure 3. Lineweaver−Burk plot for amidoxime **10**.

For comparative purposes, Table 1 also includes data on the inhibition of SHC by three other inhibitors of squalene-cyclizing enzymes. Benzophenone derivative **28** (Scheme 5), known as Ro48-8071, is a new, orally active, extremely potent, nonterpenoid inhibitor of OSLC recently reported by Hoffmann-La Roche.[13] The inhibition of SHC by **28** has been shown to be mixed, noncompetitive.[14] Propiophenone **29** is a structurally related non-

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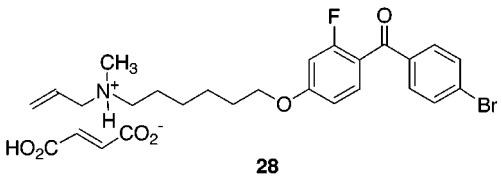
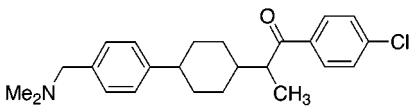
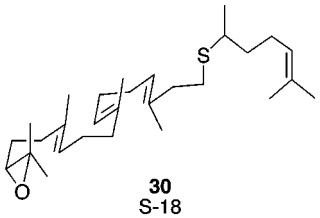
Scheme 5



28
Ro48-8071



29
BIBX79



30
S-18

terpenoid inhibitor known as BIBX79.[15] The terpenoid inhibitor S-18 **30** is the most potent of a series of thia-substituted analogues of 2,3-oxidosqualene.[16]

In summary, we have demonstrated that amidrazone and amidoxime mimics of a monocyclic intermediate in the enzyme-catalyzed cyclization of squalene are highly effective inhibitors of SHC. Moreover, the compounds reported herein represent the first designed transition-state analogues in the study of SHC inhibition. The inhibition data for amidoximes **9** ($IC_{50} = 30$ nM, $K_i = 90$ nM) and **10** ($IC_{50} = 56$ nM, $K_i = 49$ nM) indicate biological activity comparable to 18-thia-2,3-oxidosqualene ($IC_{50} = 60$ nM, $K_i = 31$ nM) and only slightly weaker than Ro48-8071 ($IC_{50} = 9$ nM, $K_i = 6.6$ nM), the best inhibitors of SHC known to date.

Experimental Section[17]

Trienyllactam (14). A solution of *n*-butyllithium (1.5 M, 1.27 mL, 1.9 mmol) in hexane was added dropwise to a solution of *δ*-valerolactam **11** (90 mg, 0.9 mmol, 0.2 M) in dry THF (3 mL) at 0 °C. After the mixture was stirred at 0 °C for 2 h, homofarnesyl bromide **13** (0.29 mL, 1.0 mmol) was added neat to the dianion solution. The resulting mixture was stirred at 0 °C for 3 h and then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was extracted with ether, and the extracts were dried over $MgSO_4$, concentrated, and purified by chromatography (49:1 CH_2Cl_2/CH_3OH) to give the lactam **14** (182 mg, 63%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62 (br s, 1 H), 5.20−5.00 (m, 3 H), 3.37−3.17 (m, 2 H), 2.30−2.17 (m, 1 H), 2.17−1.75 (m, 13 H), 1.75−1.37, 1.65, 1.57 and 1.56 (overlapping m and 3 s, 15 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.3, 135.5, 134.8, 131.1, 124.3, 124.1, 123.7, 42.3, 40.4, 39.6, 31.5, 26.7, 26.6, 26.0, 25.6, 25.2, 21.3, 17.6, 16.0, 15.9; IR (film) 3300, 3200, 2920, 1670 cm^{-1}; FABMS *m*/*z* 318 (M + 1, 100).

Trienylthionolactam (15). Lawesson's reagent (65 mg, 0.16 mmol) was added to a solution of trienyllactam **14** in dry toluene (3 mL), and the resulting mixture was heated at 50 °C for 30 min. Water (5 mL) was added, and the mixture was extracted with ether. The combined extracts were washed with brine (5 mL), dried over $MgSO_4$, and concentrated. The residue was taken up in CH_2CH_2 and stirred for 1 h to decompose the excess Lawesson's reagent, which had the same R_f as **15**. After concentration, the residue was purified by chromatography (4:1 petroleum ether/ethyl acetate) to give the trienylthiolactam **15** (52 mg, 98%) as a semisolid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (br s, 1 H), 5.20−5.00 (m, 3 H), 4.40−3.17 (m, 2 H), 2.70−2.57 (m, 1 H), 2.25−2.19 (m, 1 H), 2.19−1.68 (m, 13 H), 1.68−1.50, 1.65, 1.59 and 1.56 (overlapping m and 3 s, 14 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.8, 136.2, 135.2, 131.5, 124.7, 124.4, 123.7, 46.3, 45.0, 40.0, 35.4, 27.0, 26.9, 26.0, 25.7, 24.7, 19.4, 18.0, 16.4, 16.3; IR (film) 3190, 2920, 1570 cm^{-1}; FABMS *m*/*z* 334 (M + 1, 100).

Trienylamidrazone Acetic Acid Salt (8). Anhydrous hydrazine (0.1 mL, 3.2 mmol) was added to a solution of **15** (21 mg, 0.06 mmol) in anhydrous CH_3OH (1 mL) at 0 °C. The resulting mixture was kept at 4 °C for 12 h. The solvent was removed in vacuo, and the residue was purified by chromatography with $CH_3CN/H_2O/HOAc$ (94:5:1 to 91:8:1). After concentration of the fractions, the residue was dissolved in CH_3OH (5 mL) and treated with decolorizing carbon for 30 min at room temperature. After filtration and concentration, trienylamidrazone acetic acid salt **8** (22 mg, 89%) was obtained as a pale yellow oil: ^{1}H NMR (300 MHz, D_2O) δ 5.12−4.92 (m, 3 H), 3.48−3.20 (m, 2 H), 2.70−2.56 (m, 1 H), 2.10−1.60, 1.85, 1.56, 1.54, 1.49 and 1.49 (overlapping m and 5 s, 32 H); ^{13}C NMR (75 MHz, D_2O) δ 177.9, 164.2, 133.3, 131.7, 127.8, 121.7, 121.6, 120.4, 38.1, 36.9, 30.8, 29.6, 24.0, 22.6, 22.2, 20.3, 19.5, 15.2, 14.5, 12.9; IR (film) 3200, 2920, 1685, 1620, 1450 cm^{-1}; FABMS *m*/*z* 332 (M^+, 100).

Trienylamidoxime (9). A solution of anhydrous hydroxylamine (1 M, 0.63 mL, 0.6 mmol) in CH_3OH was added to a solution of **15** (21 mg, 0.06 mmol) in CH_3OH (1 mL), and the resulting mixture was heated at 50 °C for 15 h. The solvent was removed *in vacuo*, and the residue was chromatographed with a gradient 49:1 to 19:1 CH_2Cl_2/CH_3OH as the eluent to give trienylamidoxime **9** (20 mg, 95%) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31 (br s, 1 H), 5.17−5.00 (m, 3 H), 3.18 (br t, 2 H, J = 5.6 Hz), 2.58−2.44 (m, 1 H), 2.12−1.72 (m, 13 H), 1.72−1.36, 1.67, 1.58 (overlapping m and 2 s, 15 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 135.4, 134.9, 131.2, 124.4, 124.2, 123.9, 41.8, 39.7, 35.3, 32.2, 26.8, 26.7, 26.3, 25.7, 25.4, 21.4, 17.7, 16.1, 16.0; IR (film) 3400, 3200, 2920, 2850, 1650, 1450 cm^{-1}; FABMS *m*/*z* 333 (M + 1, 100).

5-Benzyloxy-2-pentyn-1-ol (17). A solution of *n*-butyllithium (1.55 M, 9.0 mL, 14.0 mmol) in hexane was added dropwise to a solution of 4-benzyloxybutyne (2.13 g, 13.3 mmol) in dry THF (40 mL). The yellow solution was stirred at −78 °C for 2 h and then treated with dry paraformaldehyde (600 mg, 20 mmol) at once. The resulting mixture was allowed to warm slowly to room temperature and then stirred for 12 h. Saturated aqueous NH_4Cl (20 mL) was added, and the layers were separated. The aqueous layer was extracted with ether. The combined organic layers were dried over $MgSO_4$, concentrated, and chromatographed with a gradient 9:1 to 4:1 to 7:3 petroleum ether/ethyl acetate as the eluent to give **17** (2.1 g, 83%) as a pale liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.21 (m, 5 H), 4.54 (s, 2 H), 4.20 (dt, 2 H, J = 5.9, 2.1 Hz), 3.57 (t, 2 H, J = 7.0 Hz), 2.51 (tt, 2 H, J = 7.0, 2.1 Hz), 2.33 (t, 1 H, J = 5.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.2, 128.7, 128.0, 83.2, 79.9, 73.2, 68.5, 51.4, 20.4; IR (film) 3400, 3070, 2925, 2870, 2230, 1475, 1450, 1350 cm^{-1}; FABMS *m*/*z* 195 (M + $2H_2$, 20), 135 ($C_6H_5CH_2OC_2H_4^+$, 67).

5-Benzyloxy-(*Z*)-3-iodo-2-penten-1-ol (18). A solution of Red-Al (3.33 *M*, 4.7 mL, 15.6 mmol) in toluene was added dropwise to a solution of **17** (1.78 g, 9.4 mmol) in dry THF (25 mL). After being stirred at room temperature for 12 h, the reaction mixture was then cooled to −78 °C and treated with a solution of *N*-iodosuccinimide (3.79 g, 16.9 mmol) in dry THF (15 mL). The resulting mixture was stirred at −78 °C for 30 min and then allowed to warm to 0 °C. Saturated aqueous Rochelle's salt (50 mL) was added, followed by saturated

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(17) General procedures: King, S. B.; Ganem, B. *J. Am. Chem. Soc.* **1994**, *116*, 562−570.

aqueous sodium thiosulfate (50 mL), and the aqueous layer was extracted with ether. The combined organic extracts were dried over $MgSO_4$, concentrated, and chromatographed using 4:1 petroleum ether/ethyl acetate to give **18** (2.57 g, 86%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.21 (m, 5 H), 5.93 (t, 1 H, J = 5.6 Hz), 4.52 (s, 2 H), 4.16 (br t, 2 H, J = 4.8 Hz), 3.61 (t, 2 H, J = 6.4 Hz), 2.78 (t, 2 H, J = 6.4 Hz), 2.02 (br t, 1 H, J = 4.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.3, 136.2, 128.7, 128.0, 105.3, 73.4, 68.9, 67.5, 45.5; IR (film) 3400, 2820, 1680, 1450, 1350 cm^{-1}; FABMS m/z 211 (M − $C_6H_5CH_2O$, 3), 135 ($C_6H_5CH_2OC_2H_4^+$, 42).

5-Benzyloxy-(*E*)-3-methyl-2-penten-1-ol (19). A solution of methyllithium (1.4 M, 54.4 mL, 76.1 mmol) in ether was added to a suspension of freshly prepared CuI (7.54 g, 39.6 mmol) in dry THF (50 mL) at 0 °C dropwise. A solution of vinyl iodide **18** (2.42 g, 7.6 mmol) in dry THF (10 mL) was added by cannula to the solution of lithium dimethylcuprate generated above. After being stirred at 0 °C for 12 h, the reaction mixture was poured into ice-cold saturated aqueous NH_4Cl (50 mL) covered with ether (25 mL). The organic layer was washed with 3% aqueous NH_4OH until clear, and the combined aqueous layers were extracted with ether. The extracts were dried over $MgSO_4$, concentrated, and then chromatographed with 7:3 petroleum ether/ethyl acetate to give **19** (1.44 g, 92%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.21 (m, 5 H), 5.46 (tq, 1 H, J = 7.0 Hz), 4.50 (s, 2 H), 4.14 (d, 2 H, J = 7.0 Hz), 3.56 (t, 2 H, J = 6.7 Hz), 2.34 (t, 2 H, J = 6.7 Hz), 1.68 (s, 3 H), 1.40 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.3, 136.5, 128.3, 127.6, 127.5, 125.1, 72.8, 68.5, 59.2, 39.4, 16.4; IR (film) 3400, 2810, 1670, 1450, 1350 cm^{-1}; FABMS m/z 189 (M + 1 − H_2O, 100).

5-Benzyloxy-(*E*)-1-bromo-3-methyl-2-pentene (20). A solution of phosphorus tribromide (0.25 mL, 2.7 mmol) in dry ether (2 mL) was added dropwise to a solution of alcohol **19** (1.24 g, 6.0 mmol) in dry ether (20 mL) at 0 °C. After the solution was stirred at 0 °C for 10 min, the ice bath was removed and stirring continued for another 10 min. The reaction mixture was quenched with brine (30 mL). The aqueous layer was extracted with ether, and the combined ether layers were washed with saturated aqueous sodium bicarbonate then brine until neutral. The organic layer was dried over $MgSO_4$ and concentrated to give bromide **20** (1.46 g, 91%) as a pale yellow liquid used directly for next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.21 (m, 5 H), 5.59 (tt, 1 H, J = 8.1, 1.1 Hz), 4.50 (s, 2 H), 4.01 (d, 2 H, J = 8.1 Hz), 3.56 (t, 2 H, J = 6.7 Hz), 1.74 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.6, 138.3, 128.3, 127.6, 127.5, 122.0, 72.8, 68.3, 39.5, 29.2, 16.2; IR (film) 2920, 2820, 1670, 1450 cm^{-1}; FDMS m/z 189 (M^+ − Br, 100).

5-Benzyloxy-(*E*)-3-methyl-1-phenylsulfinyl-2-pentene (21). Sodium phenylsulfinate (1.34 g, 8.2 mmol) was added once to a solution of bromide **20** (1.46 g, 5.4 mmol) in dry DMF (20 mL). After being stirred at room temperature for 12 h, the reaction mixture was poured into water (25 mL) and extracted with ether. The combined extracts were washed with brine (25 mL) once, dried over $MgSO_4$, concentrated, and chromatographed with a gradient 9:1 to 4:1 petroleum ether/ethyl acetate to give **21** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (br d, 2 H, J = 7.5 Hz), 7.58 (br t, 1 H, J = 7.0 Hz), 7.45 (br t, 1 H, J = 7.5 Hz), 7.38−7.20 (m, 5 H), 5.24 (t, 1 H, J = 8.1 Hz), 3.80 (d, 1 H, J = 8.1 Hz), 3.46 (t, 2 H, J = 6.7 Hz), 2.29 (t, 2 H, J = 6.5 Hz), 1.32 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.8, 138.8, 138.5, 133.8, 129.2, 128.8, 128.7, 127.9, 112.3, 73.2, 68.5, 56.3, 39.9, 16.7; IR (film) 3070, 3020, 2910, 2860, 1450, 1300 cm^{-1}; FABMS m/z 331 (M + 1, 100).

Benzyloxytetraene (22). A solution of *n*-butyllithium (1.6*M*, 1.67 mL, 2.7 mmol) in hexane was added dropwise to a solution of **21** (800 mg, 2.4 mmol) in dry THF (15 mL) at −78 °C. The resulting yellow solution was stirred at −78 °C for 1 h, and then a solution of farnesyl bromide (0.73 mL, 2.7 mmol) in dry THF (5 mL) was added dropwise to the anion solution at −78 °C. After 4 h at −78 °C, the reaction mixture was quenched with CH_3OH (1 mL), water (25 mL), and saturated aqueous NH_4Cl (25 mL) and then warmed to 0 °C. The aqueous layer was extracted with ether, and the combined extracts were dried over $MgSO_4$, concentrated, and chromatographed with 9:1 petroleum ether/ethyl acetate to give **22** (1.87 g, 92%) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2 H, J = 7.8 Hz), 7.55 (t, 1 H, J = 7.0 Hz), 7.41 (t, 2 H, J = 7.8 Hz), 7.38−7.22 (m, 5 H), 5.16−4.89 (m, 4 H), 4.46 (s, 2 H), 3.72 (td, 1 H, J = 10.5, 3.2 Hz), 3.40 (t, 2 H, J = 7.0 Hz), 2.87 (ddd, 1 H, J = 14.0, 7.0, 3.2 Hz), 2.33 (ddd, 1 H, J = 14.0, 7.0, 3.2 Hz), 2.26 (t, 2 H, J = 7.0 Hz), 2.12−1.85 (m, 8 H), 1.66 (s, 3 H), 1.58 & 1.55 (overlapping 2 s, 9 H), 1.21 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.2, 138.7, 138.2, 137.9, 135.1, 133.3, 131.2, 129.1, 128.6, 128.3, 127.5, 124.2, 123.8, 118.7, 118.4, 72.8, 68.4, 64.7, 39.6, 26.7, 26.5, 26.4, 25.6, 17.6, 16.8, 16.3, 15.9; IR (film) 3080, 3030, 2920, 2860, 1670, 1580 cm^{-1}; FDMS m/z 534 (M^+, 100).

Tetraenol (23). Ethylamine (30 mL) was condensed at −78 °C to a flask containing **22** (1.02 g, 1.9 mmol) in dry THF (5 mL). Lithium wire (135 mg, 19.0 mmol) was rinsed with hexane, cut into small pieces, and added under argon. The reaction mixture turned deep blue. After 30 min at −78 °C, solid NH_4Cl was added to the reaction mixture until the blue color disappeared. The mixture was then diluted with ether (20 mL) and water (30 mL), and the aqueous layer was extracted with ether. The combined extracts were dried over $MgSO_4$, concentrated, and chromatographed with 9:1 petroleum ether/ethyl acetate to give alcohol **23** (540 mg, 93%) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.30−5.17 and 5.17−5.00 (2 m, 4 H), 3.62 (br t, 2 H, J = 6.2 Hz), 2.23 (t, 2 H, J = 6.4 Hz), 2.15−1.90 (m, 12 H), 1.66 (s, 3 H), 1.62 (s, 3 H), 1.58 (s, 9 H), 1.52 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.8, 135.2, 131.6. 131.5, 128.2, 124.7, 124.5, 124.2, 60.2, 43.0, 40.0, 28.5, 28.3, 27.0, 26.9, 25.9, 17.9, 16.3, 16.2, 16.0; IR (film) 3350, 2920, 1660, 1440, 1380 cm^{-1}; FDMS m/z 304 (M^+, 100).

Bromotetraene (24). To a solution of Ph_3P (642 mg, 2.4 mmol) in dry CH_2Cl_2 (10 mL) under argon at 0 °C was added bromine (0.13 mL, 2.4 mmol) dropwise until an orange color persisted, and then a few crystals of Ph_3P were added to discharge the excess bromine. Pyridine (0.25 mL, 3.1 mmol) was added followed by the addition of alcohol **23** (620 mg, 2.0 mmol) in dry CH_2Cl_2 (5 mL). After the mixture was stirred at room temperature for 4 h, the solvent was removed in vacuo, and the solid residue was diluted with hexane (20 mL) and filtered through a pad of Celite. The solid was rinsed well with hexane. The combined extracts were dried over sodium sulfate, concentrated, and chromatographed with hexane as the eluent to give bromide **24** (653 mg, 87%) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28−5.17 and 5.17−5.00 (2 m, 4 H), 3.41 (t, 2 H, J = 7.5 Hz), 2.52 (t, 2 H, J = 7.5 Hz), 2.13−1.90 (m, 12 H), 1.67 (s, 3 H), 1.61 and 1.59 (overlapping 2 s, 12 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7, 135.2, 132.2, 131.5, 128.0, 124.7, 124.5, 124.2, 43.2, 40.0, 32.0, 28.5, 28.2, 27.0, 26.9, 26.0, 17.9, 16.3, 16.3, 15.9; IR (film) 2920, 1670, 1450 cm^{-1}; FDMS m/z 366 (M^+ − 1, 100), 368 (M + 1, 96).

Tetraenyllactam (26). A solution of *n*-butyllithium (1.6 *M*, 1.23 mL, 2.0 mmol) in hexane was added dropwise to a solution of δ-valerolactam **12** (93 mg, 0.94 mmol, 0.2 M) in dry THF (3 mL) at 0 °C. After the solution was stirred at 0 °C for 2 h, bromide **24** (0.36 mL, 1.0 mmol) was added neat to the dianion solution generated above. The resulting mixture was stirred at 0 °C for 2 h and then quenched with saturated aqueous NH_4Cl (10 mL). The mixture was extracted with ether, and the extracts were dried ($MgSO_4$), concentrated, and purified by chromatography (99:1 CH_2Cl_2/CH_3OH) to give **26** (305 mg, 85%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (s, 1 H), 5.18−5.00 (m, 4 H), 3.32−3.16 (m, 2 H), 2.29−1.74 (m, 18 H), 1.74−1.38, 1.64, 1.57 and 1.56 (overlapping m and 3 s, 18 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 135.3, 135.1, 134.9, 131.4, 125.0, 124.7, 124.5, 124.4, 42.5, 40.7, 40.0, 37.2, 30.1, 28.5, 28.5, 27.0, 26.9, 26.3, 25.9, 21.6, 17.9, 16.3, 16.2, 16.2; IR (film) 3300, 3200, 2920, 2860, 1670 cm^{-1}; FABMS m/z 386 (M + 1, 100).

Tetrathiolactam (27). Lawesson's reagent (32 mg, 0.08 mmol) was added to a solution of **26** in dry toluene (2 mL), and the resulting mixture was heated at 50 °C for 30 min. Water (5 mL) was added, and the mixture was extracted with ether. The combined extracts were dried over $MgSO_4$ and concentrated. The residue was taken in CH_2Cl_2 and stirred

for 1 h to decompose the excess Lawesson's reagent. After concentration, the residue was purified by chromatography with 4:1 petroleum ether/ethyl acetate to afford **27** (52 mg, 98%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.92 (br s, 1 H), 5.22−5.02 (m, 4 H), 3.39−3.20 (m, 2 H), 2.70−2.55 (m, 1 H), 2.47−2.32 (m, 1 H), 2.20−1.68 (m, 17 H), 1.68−1.52, 1.66, 1.63 and 1.58 (overlapping m and 3 s, 17 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.3, 135.4, 135.1, 134.6, 131.5, 125.5, 124.7, 124.5, 124.5, 46.0, 45.1, 40.0, 37.2, 33.7, 28.5, 28.5, 27.0, 26.9, 26.0, 24.5, 19.4, 17.9, 16.3, 16.3, 16.1; IR (film) 3190, 2920, 2860, 1670, 1570, 1500 cm^{-1}; FDMS *m*/*z* 401 (M^+, 100).

Tetraenylamidoxime (10). A solution of anhydrous hydroxylamine (1 M, 0.62 mL, 0.6 mmol) in CH_3OH was added to a solution of tetraenylthiolactam **27** (25 mg, 0.06 mmol) in anhydrous CH_3OH (1 mL), and the resulting mixture was heated at 50 °C for 15 h. The solvent was removed in vacuo, and the residue was chromatographed using a gradient 99:1 to 49:1 to 19:1 CH_2Cl_2/CH_3OH to give tetraenylamidoxime **10** (21.5 mg, 86%) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.30 (br s, 1 H), 3.18 (br t, 2 H, J = 5.7 Hz), 2.28 (heptet, 1 H, J = 4.2 Hz), 2.13−1.77 (m, 17 H), 1.77−1.40, 1.67, 1.58 and 1.58 (overlapping m and 3 s, 18 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.0, 135.1, 134.9, 134.7, 131.2, 124.6, 124.4, 124.3, 41.8, 39.7, 37.0, 35.2, 30.4, 28.3, 28.2, 26.8, 26.7, 26.2, 25.7, 21.3, 17.7, 16.0, 16.0, 15.9; IR (film) 3380, 3240, 2920, 2850, 1650, 1450 cm^{-1}; FABMS *m*/*z* 401 (M + 1, 100).

Enzyme Purification and Assay. The recombinant *A. acidocaldarius* SHC was expressed in *E. coli* and purified according to the published method.[18] The enzyme converted squalene into a 17:1 mixture of hop-22(29)-ene and hopan-22-ol and showed an apparent K_m = 1.8 μM and k_{cat} = 2.4 min^{-1} in the presence of 0.1% Triton X-100 in the assay mixture.

The reaction mixture contained 100 mM sodium citrate, pH 6.0, 0.1% (v/v) Triton X-100, 5 μM [^{14}C]squalene (7.0 mCi/mmol), and 0.5 μg of purified recombinant SHC in a final volume of 1 mL. Incubations were carried out at 60 °C for 30 min, stopped by extraction of 1 mL of CH_2Cl_2. The extract was concentrated using a Speed-Vac and subjected to silica gel TLC (Whatmann LK6D). The TLC plates were developed twice: 5 cm in $CHCl_3$ and then 15 cm in hexane. The R_f values of squalene, hopene, and hopan-2-ol were 0.45, 0.77, and 0.15, respectively. The reaction mixtures were then analyzed using a radio-TLC scanner (Bio-Scan, System 500), which has an instrumental reproducibility of ±5−10% for repeated scans of the same sample lanes. Quantification of activity was obtained using only the squalene to hopene (major product) conversion; the small, variable amount of hopanol was generally in the baseline noise and was not included in the calculations. The percentage of activity was plotted against inhibitor concentration to determine the IC_{50} value. All assays were carried out in duplicate.[19]

Inhibition Kinetics. The experiments were carried out in duplicate using three concentrations of each inhibitor (**8**: 0, 186, 372 nM; **9**: 0, 55, 82 nM; **10**: 0, 42, 84 nM). For each inhibitor concentration, [^{14}C]squalene was added to give four substrate concentrations: 1, 1.6, 2.0, and 4.0 μM. The mixtures were incubated at 60 °C for 30 min and analyzed using a radio-TLC scanner as described. Reaction velocities (V) obtained at different substrate concentrations for each inhibitor concentration were plotted as double reciprocal plots of 1/V versus 1/[S] as shown in Figures 1−3. The slopes from each Lineweaver−Burk analysis were replotted against [I] to obtain the enzyme−inhibitor dissociation constant K_i.[18]

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Supporting Information Available: ^{13}C NMR spectra for **8**−**10**, **14**, **15**, **17**−**24, 26**, and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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