# Synthesis and Evaluation of Aziridine Analogues of Presqualene Diphosphate as Squalene Synthase Inhibitors

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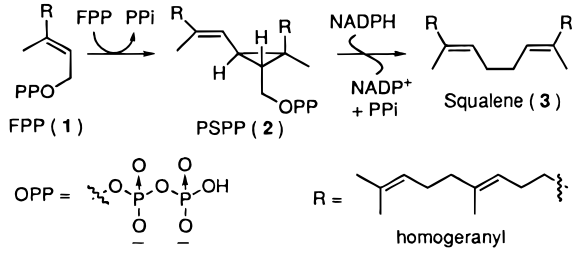
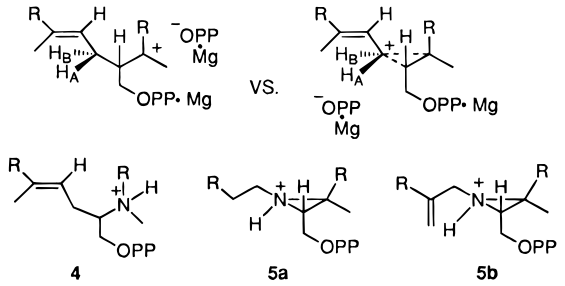
The membrane-associated enzyme squalene synthase (SS, EC 2.5.1.21) catalyzes the first committed step on the biosynthetic pathway to cholesterol and steroid hormones.[1,2] The recognition of its location downstream from metabolic flow toward other crucial isoprenoid products has stimulated a search for specific inhibitors of this enzyme.[2,3] The reductive coupling of two molecules of (*E*,*E*)-farnesyl diphosphate (**1**, FPP) catalyzed by SS takes place in two steps through the formation of a cyclopropane intermediate, presqualene diphosphate (**2**, PSPP), and its reductive rearrangement to squalene (**3**) (Scheme 1).[4] Recently, PSPP has been reported to serve as an intercellular signal for down-regulation of superoxide formation in neutrophils.[5]

**Scheme 1**



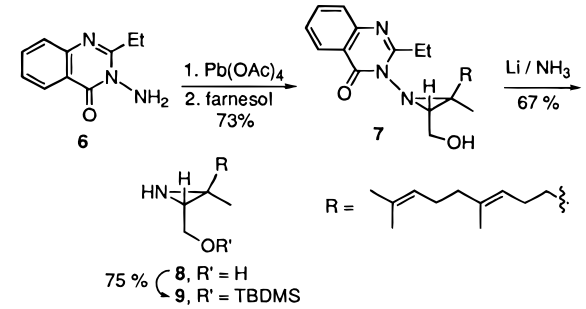

Homogeneous SS from yeast is a single polypeptide that catalyzes both steps, producing PSPP and squalene.[6] The gene for yeast SS has been cloned,[7] and a soluble truncated recombinant form has been expressed, purified, and characterized.[8] Insights into the catalytic mechanism of SS have been gained from investigations of ammonium[9−11] and sulfonium ion[12] inhibitors designed to resemble the substrate, various carbocationic intermediates, and the product of the reaction. Other types of potent SS inhibitors[2,3] include the zaragozic acids,[13] isoprenoid bisphosphonates and (phosphinylmethyl)phosphonates,[14] and α-(phosphino)sulfonates.[15]

**Scheme 2**




It seems reasonable to suppose that the cyclopropane ring of PSPP is formed by electrophilic attack of C-1 of one FPP on the 2,3 double bond of the second.[1,16,17] In one scenario, the alkylation would give rise to a tertiary carbocationic intermediate that would undergo 1,3 elimination of $H_B$ (Scheme 2). Alternatively, the intermediate might be better represented as a protonated cyclopropane having a more dispersed charge distribution. Previously, we reported the synthesis of aza bifarnesyl diphosphate **4** designed to mimic an open carbocation intermediate.[10] The racemic analogue inhibited recombinant SS from yeast with an $IC_{50}$ of 20−25 $\mu$M in the presence of inorganic pyrophosphate. In this paper, we describe the synthesis of novel aziridine diphosphates **12a**,**b**, the aziridinium forms of which (**5a**,**b**) resemble a protonated cyclopropane precursor to PSPP, and preliminary evaluation of their potency as SS inhibitors.

**Scheme 3**




*N*-Alkylaziridino alcohol precursors to **12a**,**b** were prepared in racemic form by regioselective aziridination of farnesol and *N*-alkylation as shown in Schemes 3 and 4. Hydroxyl-directed aziridination of (*E*,*E*)-farnesol was accomplished by Atkinson's method[18] with 3-amino-4(3*H*)-quinazolone ($Pb(OAc)_4$, $CH_2Cl_2$, −23 °C, 73%). The hetero-

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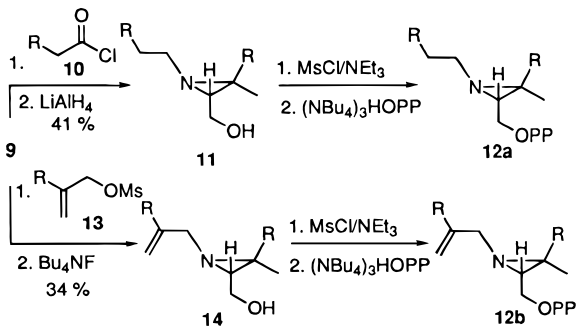
**Scheme 4**




cyclic substituent on nitrogen was detached by reductive cleavage (6 equiv of Li, $NH_3$−THF, −33 °C, 67%), conditions found to be general for a series of *N*-quinazolonylaziridines.[19]

Installation of the *N*-(*E*)-6,10-dimethyl-5,9-undecadienyl substituent in **12a** and its 2-methylene analogue in **12b** resembling the triene isoprenoid chains of PSPP was effected in three steps by O-silylation (*t*-$BuMe_2SiCl$, imidazole, DMF, 75%),[20] *N*-acylation,[21] and hydride reduction (10 equiv of $LiAlH_4$, ether, reflux, 48h, 68%) or by *O*-silylation, *N*-alkylation[21] (ether, rt), and desilylation ($Bu_4NF{\cdot}3H_2O$, THF, rt; 77%), respectively. $S_N2$ displacements of the corresponding aziridino mesylates with pyrophosphate anion [2 equiv of ($n$-$Bu_4N)_3HP_2O_7$, $CH_3CN$, rt, 48 h][22] afforded aziridino diphosphates **12a** (66%) and **12b** (64%) as $n$-$Bu_4N^+$ salts that were characterized by $^1H$ and $^{31}P$ NMR spectroscopy.[23]

Although the aziridino diphosphates underwent slow solvolytic ring opening in aqueous solution at pH 7 ($D_2O$, rt, $t_{1/2}$ ca. 24 h), the compounds were sufficiently stable for evaluation of their inhibitory properties toward squalene synthase. Kinetic assays of recombinant yeast SS activity with [$^3$H]FPP as substrate at pH 7.2 (see Table 1, footnote a for complete conditions)[8] were carried out at various concentrations of **4**,[10] **12a**, and **12b** as $Bu_4N^+$ salts. The $IC_{50}$ values for each compound were measured in the absence and presence of inorganic pyrophosphate (1.5 mM $PP_i$) on the expectation that the ammonium/diphosphate ion pairs might interact more strongly with the catalytic site on the enzyme.[9] In fact, synergistic effects of the $PP_i$ additive on the inhibitory potencies of the aziridino diphosphates **12a** (ca. 4-fold) and **12b** (ca. 2-fold) were observed, although the $IC_{50}$ of the aza bifarnesyl inhibitor was unaffected by $PP_i$ addition within experimental error. A $K_i$ value of 0.21 $\mu$M (competitive against FPP, $K_m^{FPP} = 20$ $\mu$M) was determined for the most potent aziridino diphosphate inhibitor **12a** in the presence of PPi.

**Table 1. Inhibition of Recombinant Yeast Squalene Synthase by Aza Bifarnesyl Diphosphate (4) and Aziridino Diphosphates 12a,b in the Absence and Presence of Inorganic Diphosphate at pH 7.2**[a]

| | $IC_{50}$[b] ($\mu$M) | | |
|---|---|---|---|
| inhibitor | $-PP_i$ | $+PP_i$[c] | $K_i$[d] ($\mu$M) |
| **4** | 9.7 ± 2.7 | 11.4 ± 2.2 | |
| **12a** | 7.2 ± 0.8 | 1.9 ± 0.2 | 0.21[c] |
| **12b** | 6.9 ± 1.6 | 4.0 ± 0.4 | |

[a] Assay conditions: 50 mM MOPS, pH 7.2, 20 mM $MgCl_2$, 2% (v/v) Tween 80, 1 mM DTT, 1 mg/mL of bovine serum albumin, 1.0 mM NADPH, 0 or 1.5 mM $PP_i$, 100 $\mu$M [$^3$H]FPP (7.5 $\mu$Ci/$\mu$mol), 0−300 $\mu$M inhibitor, and 0.1 $\mu$g protein in a total volume of 200 $\mu$L. Samples were incubated at 30 °C for 5 min before enzyme was added and stopped after an additional 10 min by adding 40% aqueous KOH in methanol. Product [$^3$H]squalene was isolated by extraction with hexanes, purified by filtration over alumina, and analyzed by liquid scintillation spectrometry.[7] [b] $IC_{50}$ values were interpolated from plots of specific activity at [I] = 0.001, 0.50, 1.00, 3.00, 10.0, 30.0, 100, and 300 $\mu$M using Grafit (Erithicus Software, Staines, UK). Deviations shown are standard errors. [c] Incubations in the presence of 1.5 mM PPi. [d] 5−100 $\mu$M [$^3$H]FPP (7.5 $\mu$Ci/$\mu$mol).

The selectivity of the interactions of these three aza analogue inhibitors with SS is validated by the much lower affinity exhibited by a similar aziridino diphosphate having a straight-chain $N$-$C_{11}H_{23}$ substituent (55% inhibition of SS at 219 $\mu$M) instead of the unsaturated isoprenoid groups in **12a**,**b**. It is noteworthy that the two aziridino diphosphates are substantially stronger inhibitors than their acyclic analogue **4**, despite the lack of the proximal double bond, the additional absence of a methyl group in **12a**, and the lower basicity of the heterocyclic nitrogen. The enhanced potency of the aziridine inhibitors presumably manifests better spacial congruency with the SS active site than that of the acyclic aza analogue and may indicate a nonclassical, protonated cyclopropane intermediate in the formation of **2**. It is not known whether the aziridine inhibitors bind to SS in the aziridinium ion forms (**5a** and **5b**)[24] or the aziridine forms (**12a** and **12b**). If the latter is true, the aziridine diphosphates may be better regarded as PSPP analogues rather than mimics for a charged transition state. In any case, aziridines structurally analogous to bridged nonclassical ions proposed as intermediates in cyclizations catalyzed by terpene synthases are interesting candidates for novel, mechanism-based inhibitors of these enzymes.[25]

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**Supporting Information Available:** The detailed experimental procedures and characterization data for compounds **7**−**9**, **11**, **14**, and **12a**,**b**, and unnumbered intermediates; $^1H$ NMR spectra for **7**−**9**, **11**, **14**, and **12a**,**b**, and unnumbered intermediates; and $^{31}P$ NMR spectra for **12a**,**b** (24 pages).

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