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Synthesis and Enzymatic Cyclization of (3*S*)-14-Fluoro-2,3-oxidosqualene

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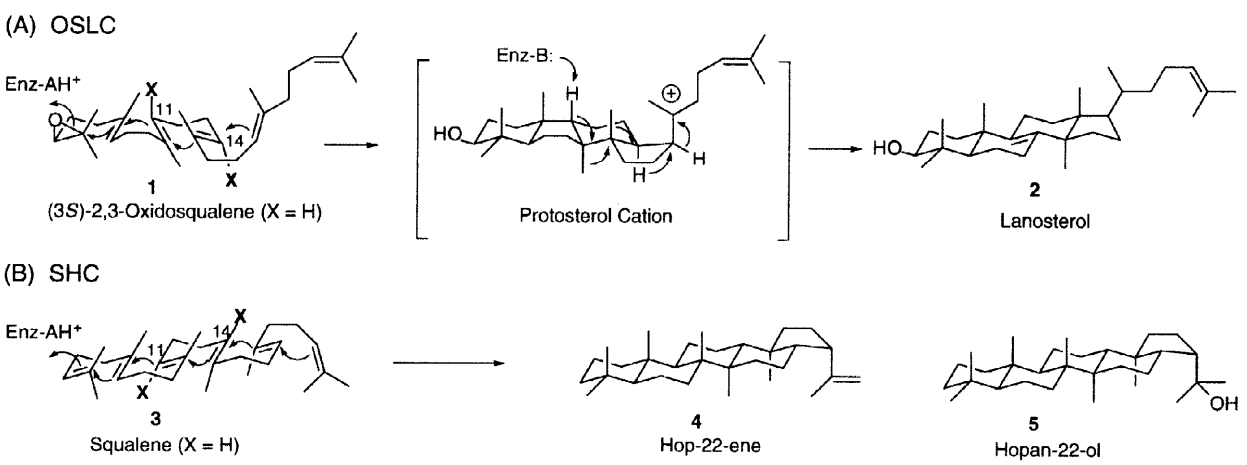
Abstract. A convergent asymmetric synthesis led to (3*S*)-14-fluoro-2,3-oxidosqualene (14-FOS, **16**), which was cyclized by bacterial squalene:hopane cyclase to a monocarbocyclic product with a bridged ether and a 2:3 mixture of bicyclic alcohols. 14-FOS was neither a substrate nor an inhibitor for vertebrate oxidosqualene:lanosterol cyclase.

Polyene cyclizations, both enzymatic and non-enzymatic, represent impressive examples of conformationally-orchestrated formation of new carbon-carbon bonds.[1] In enzymatic cyclizations, oxidosqualene:lanosterol cyclase (OSLC) (E.C. 5.4.99.7) and squalene:hopene cyclase (SHC) (E.C. 5.4.99.7) bind their respective substrates in chair-boat-chair or in all chair conformations. The enzyme catalyzes the initial protonation of the epoxide or alkene and then mediates the sequential ring-forming reactions through a progression of tightly-constrained carbocationic intermediates (Scheme 1). Eukaryotic OSLC and prokaryotic SHC show 17 to 27% identity, and each contains multiple repeats of a highly-conserved motif rich in aromatic amino acids (the QW motif).[2,3] Recently, the crystal structure of the SHC from a thermoacidophilic bacterium revealed an α-helix-rich dumbbell-shaped homodimer containing a large central cavity as the putative active site.[4] To-date, however, a structure containing bound substrate is unavailable. Accordingly, continued efforts to discover the subtleties of the enzymatic cyclization mechanism rely on studies using inhibitors and substrate analogs.



Scheme 1. Proposed mechanism for the cyclization of (3*S*)-2,3-oxidosqualene (**1**) to lanosterol (**2**) by OSLC (**A**) and squalene (**3**) to hop-22-ene (**4**) and hopan-22-ol (**5**) by SHC (**B**).

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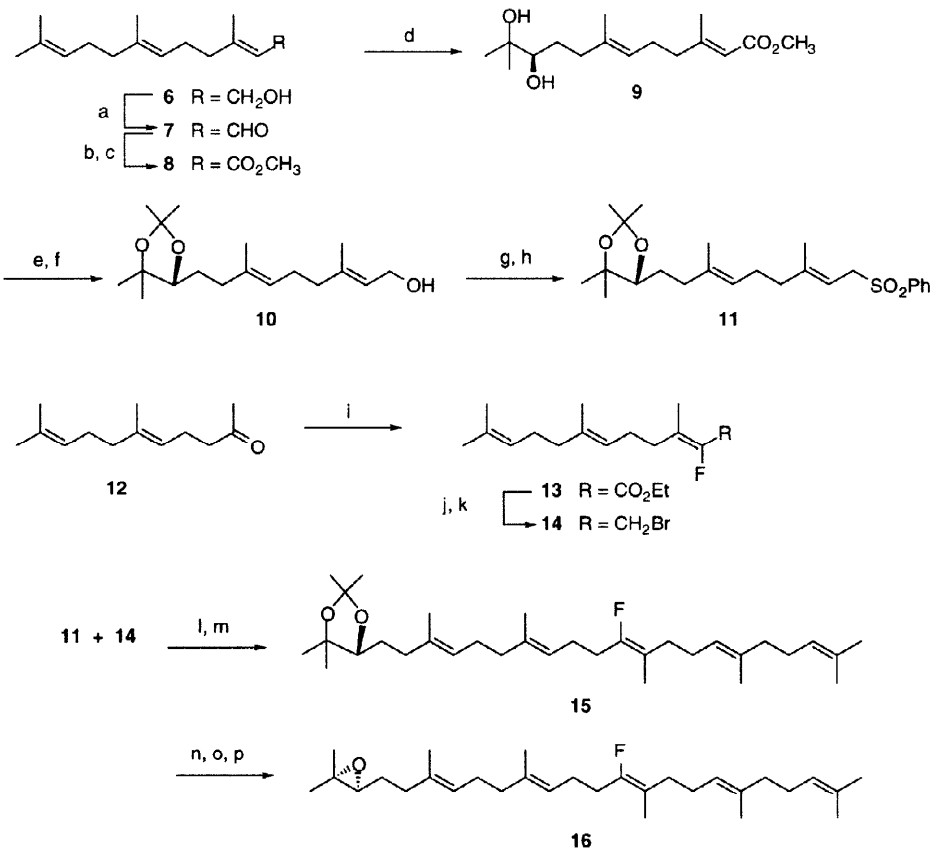
In order to test the effect of fluorine atom substitution on the enzymatic cyclization reactions, we previously reported the synthesis and enzymatic cyclization of (3*S*)-11-fluoro-2,3-oxidosqualene (11-FOS).[5] An unexpectedly dramatic stereoelectronic effect of a single fluorine-for-hydrogen substitution was observed. First, neither cyclization of 11-FOS nor inhibition of oxidosqualene cyclization by rat liver OSLC was observed. Second, with purified recombinant *Alicyclobacillus acidocaldarius* SHC, the only isolable cyclization product was the monocarbocyclic ether (**17**).[5] In this paper, we now describe the synthesis and enzymatic cyclization of (3*S*)-14-fluoro-2,3-oxidosqualene (14-FOS) (**16**) in which 14-H has been replaced by a fluorine atom. Based on the use of a fluorine atom by the late W.S. Johnson as a cation-stabilizing auxiliary that served to both enhance the cyclization reaction and control the regiochemistry of the product in a nonenzymatic process[6], we hypothesized that a 14-fluoro substituent would allow enzymatic cyclization to a tetracyclic protosterol-type intermediate, while subsequently interfering with the final hydride migration-deprotonation sequence leading to lanosterol.

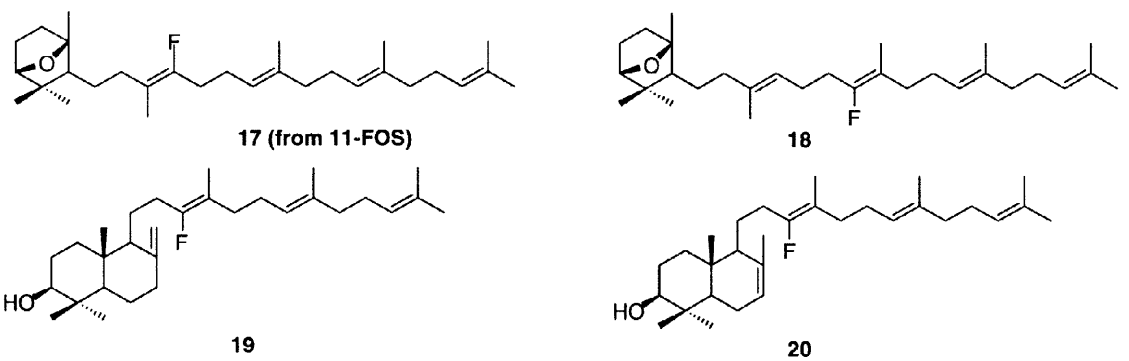


Scheme 2. (**a**) MnO_2, hexane, 85%; (**b**) KCN, AcOH, MeOH; (**c**) MnO_2, MeOH, 56%; (**d**) AD-mix-β, $(DHQD)_2$ DHAL, $K_2OsO_2(OH)_4$ aq. *t*-BuOH, $CH_3SO_2NH_2$, 12%; (**e**) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, 94%; (**f**) $LiAlH_4$, THF, 36%; (**g**) PBr_3, hexane, 84%; (**h**) $PhSO_2Na$, DMF, 65%; (**i**) $(EtO)_2P(O)CHFCO_2Et$, NaH, THF, 99%; (**j**) $LiAlH_4$, THF, 72%; (**k**) PBr_3, hexane, 77%; (**l**) *n*-BuLi, THF, -78 °C, 70%; (**m**) $PdCl_2$[dppp], $LiHBEt_3$, THF, 60%; (**n**) TsOH, MeOH, 80%; (**o**) DMAP, MsCl, TEA, CH_2Cl_2, 99%; (**p**) K_2CO_3, MeOH, 62%.

(3*S*)-14-FOS was synthesized from geranylacetone **12** and 2(*E*),6(*E*)-farnesol **6** (Scheme 2).[7] The synthesis employed the Sharpless asymmetric dihydroxylation[8] of methyl farnesoate **8** and the coupling of an

α-fluoro allylic bromide **14** with farnesyl phenylsulfone **11** as the key steps. (Efforts to couple an allylic bromide with the α-fluoro farnesyl phenylsulfone anion were unsuccessful due to the loss of fluoride.) The fluoroester **13** was obtained from geranylacetone **12** by the Horner-Wadsworth-Emmons reaction, giving an isomeric mixture from which the desired (2*Z*) isomer was readily separated[5]; the geometry of the fluoroolefin was established in the previous 11-FOS synthesis by NOESY and TOCSY 2-D NMR experiments. After the coupling reaction and dephenylsulfon-ation[5], **15** was deprotected to give a chiral diol, (3*R*)-2,3-diol, which was then converted to the methylsulfonate and the oxirane ring closed with inversion of configuration at C-3 to the desired (3*S*)-14-FOS (**16**).[9a]

As in the case of 11-FOS,[5] when cyclization of 14-FOS was attempted with purified rat liver OSLC, no cyclization product could be detected by TLC or GLC under conditions giving >50% conversion of the non-fluorinated substrate.[9b] The OSLC enzyme is particularly sensitive to structural changes by the fluorine atom substitution and thus fails to bind these analog compounds.[5] Indeed, in earlier experiments with a regioisomeric mixture of racemic 11- and 14-FOS, no inhibition of crude pig liver OSLC was observed at concentrations as high as 400 μM.[9d]



Scheme 3. Structures of substrate and SHC-derived cyclization products. Compounds **18** - **20** were obtained from 14-FOS (**16**) as substrate.

In contrast, recombinant *A. acidocaldarius* SHC converted (3*S*)-14-FOS into a mixture of monocarbocyclic product with a bridged ether **18** (33%) and a pair of bicyclic alcohols **19** and **20** (2:3 ratio 60%).[9b,c] Thus, the fluorine atom substitution interrupted the cyclization reaction, which was initiated by oxirane ring opening,[10] at the mono-or bicyclic carbocationic stage. A similar monocarbocyclic product **17** was obtained previously from (3*S*)-11-FOS in 27% isolated yield.[5] With other substrate analogs, bicyclic compounds were also obtained: two decalin derivatives with the ring B double bonds in identical positions were the major cyclization products of (18*E*)-(3*S*)-29-methylidene-2,3-oxidosqualene with *A. acidocaldarius* SHC.[10f] It appears that the electronic and/or steric perturbations by the fluorine atom may alter the incipient cation stability or the optimally folded conformation of the substrate in the active site of the enzyme. No evidence was found for tri-, tetra-, or pentacarbocyclic products in the reaction mixture.

Acknowledgment. We thank Prof. K. Poralla (Universität Tübingen) for the plasmid encoding recombinant *A. acidocaldarius* SHC and Drs. J. Olsen and Y.F. Zheng (The University of Utah (UU)) for NMR on cyclization products and Professor A. Perez (University of Costa Rica) for determination of product ratios by GC. HRMS was performed either at Mass Spectroscopy Facilities at University of Illinois, Champaign-Urbana (synthetic intermediates) or at UU (cyclization products). We are grateful to the National Institutes of Health (Grant GM 44836 to G.D.P.) and UU for financial support.

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9. (**a**) (3*S*)-14-Fluoro-2,3-oxidosqualene (**16**).^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.12 (bm, 4H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.1 (brm, 19H), 1.75-1.5 (brm, 19H), 1.29 (s, 3H), 1.21 (s, 3H). ^{13}C-NMR (63 MHz, $CDCl_3$): δ 154.3 (d, *J* = 242 Hz), 135.9, 135.3, 134.1, 131.3, 124.8, 124.4, 124.0, 123.1, 111.3 (d, *J* = 17.7 Hz), 64.2, 58.3, 39.73, 39.65, 36.3, 29.7 (d, *J* = 7.3 Hz), 28.9 (d, *J* = 29.0 Hz), 27.5, 26.7, 26.6, 26.2, 25.7, 25.3, 24.9, 18.7, 17.7, 16.0 (x 3), 15.6 (d, *J* = 5.6 Hz). ^{19}F-NMR ($CDCl_3$, 235 MHz): δ -114.0 (t, *J* = 22.4 Hz). FTIR: 1249 cm^{-1}. HRMS (EI, 70 eV) $C_{30}H_{49}FO$: calcd 444.3762; found, 444.3756.

   (**b**) Enzymatic cyclizations. The enzyme tests with (3*S*)-14-FOS were carried out in essentially the same way as previously described for (3*S*)-11-FOS.[5] (3*S*)-14-FOS (8.0 mg) was incubated with purified rat liver OSLC in 100 ml of 100 mM Tris-HCl, pH 7.4, 0.1% Triton X-100, and incubated at 37 °C for 16 h. After extraction with EtOAc (300 mL × 2), no product was detected and (3*S*)-14-FOS (7.0 mg) was recovered unchanged. This was also confirmed by GC analysis. For SHC, the reaction mixture contained (3*S*)-14-FOS (8.0 mg) and recombinant *A. acidocaldarius* SHC (60 mg) in 200 ml of 50 mM Na-citrate, pH 6.0, 0.1% Triton X-100, was incubated at 60 °C for 16 h. The incubations were stopped by freezing and lyophilization, followed by extraction with EtOAc (300 mL × 2). The combined extracts were evaporated to dryness, and purified by SiO_2 TLC (10% EtOAc/Hexane) to give the monocarbocyclic product (**18**) (2.6 mg, R_f = 0.53), and the bicarbocyclic products (**19** and **20**) (4.8 mg, ratio = 2:3, R_f = 0.18).

   (**c**) Structure determination. The structures of **18-20** were confirmed comparison to similar mono- and bicarbocyclic cyclization products obtained in our laboratory.[5,9f] **18**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.20-5.06 (m, 3H), 3.69, (d, *J* = 5.5 Hz, 1H), 1.66 (s, 3H), 1.58 (s, 6H), 1.54 (s, 3H), 1.52 (s, 3H), 1.30 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H). HRMS (EI, 70 eV): $C_{30}H_{49}FO$: calcd 444.3756; found, 444.3761; **19**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.18-5.05 (m, 2H), 4.85 (s, 1H), 4.54 (s, 1H), 3.22, (m, 1H), 1.66 (s, 3H), 1.58 (s, 6H), 1.53 (s, 3H), 0.95 (s, 3H), 0.83 (s, 3H), 0.73 (s, 3H). **20**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.41 (bs, 1H), 5.18-5.05 (m, 2H), 4.85 (s, 1H), 4.54 (s, 1H), 3.22, (m, 1H), 1.66 (s, 3H), 1.58 (s, 6H), 1.56 (s, 3H), 1.53 (s, 3H), 0.97 (s, 3H), 0.75 (s, 3H), 0.66 (s, 3H). HRMS (EI, 70 eV) $C_{30}H_{49}FO$ for both **19** and **20**: calcd 444.3756; found, 444.3759; 500 MHz ^{1}H-NMR data are available for this manuscript by contacting the authors.

   (**d**) An inseparable mixture of racemic 11- and 14-FOS regioisomers was first chemically synthesized from 11-fluorosqualene: Xiao, X.-y. Ph.D. Dissertation, State University of New York at Stony Brook, Stony Brook, New York, 1991.
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