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DESIGN AND SYNTHESIS OF SUBSTRATE ANALOGS FOR THE INHIBITION OF DEHYDROQUINATE SYNTHASE

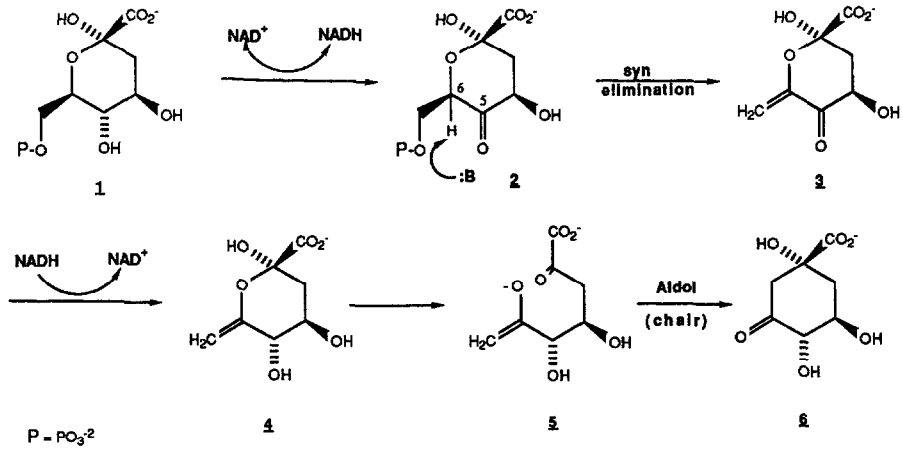
*Nicholas Nikolaides and Bruce Ganem**

Department of Chemistry, Baker Laboratory
Cornell University, Ithaca, NY 14853

Summary: *The synthesis of two enantiomerically pure substrate analogs, designed as potential inhibitors of dehydroquinate synthase on the basis of the proposed mechanistic pathway for this enzyme, is reported.*

Plants and microorganisms use the enzymes of the shikimate pathway to biosynthesize phenylalanine, tyrosine and tryptophan along with numerous other primary and secondary metabolites.[1] The second step of the pathway, conversion of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate 1 (DAHP) to dehydroquinate 6 (DHQ) with catalysis by dehydroquinate synthase, uses a well orchestrated sequence of redox changes[2] at C5 (Scheme 1) to facilitate β-elimination of phosphate with *syn*-stereochemistry.[3] Subsequent ring opening of 4 and intramolecular aldol reaction of 5 affords 6, although recent evidence suggests that these last two stages may not be catalyzed by DHQ synthase.[4] Because the main stem of the shikimate pathway represents an important target for the design of herbicides and antibiotics,[5] we now report synthetic studies aimed at the inhibition or inactivation of DHQ synthase based on the mechanism proposed for this enzyme.

SCHEME 1



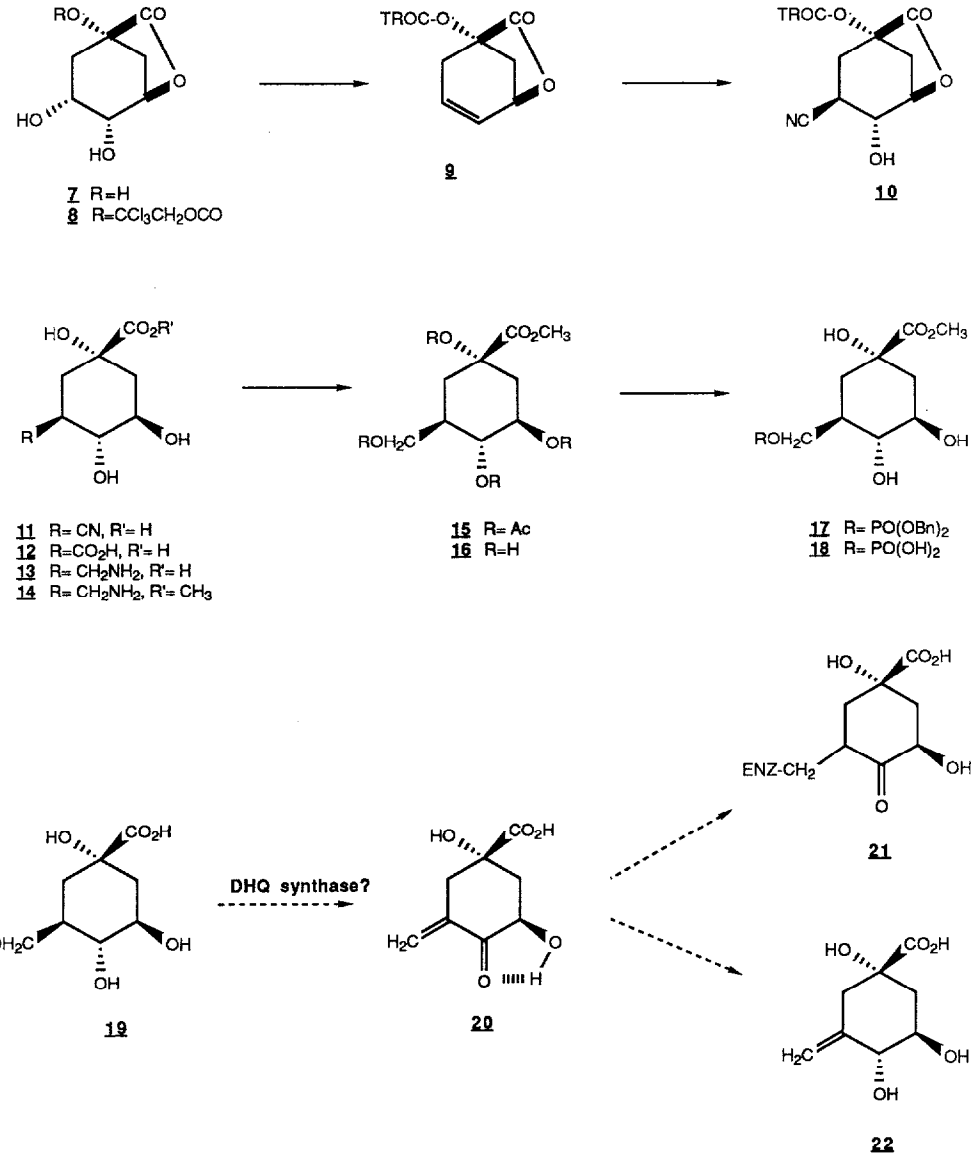
We reasoned that if 3 were a transient intermediate in dehydroquinate synthesis, then *carbocyclic* analog 20 (Scheme 2) with its more reactive H-bonded enone might trap the local base used for syn-elimination of P_i from 2. The enzyme would thus be inactivated as 21 as it processed 20 to 22. Therefore carbocyclic DAHP analog 19 became an attractive candidate for suicide inactivation of DHQ synthase.

(-)-Quinide 7[6] was converted via its acetonide to trichloroethoxycarbonyl (TROC) derivative 8 (70%).[7] Reduction of the derived thionocarbonate using trimethyl phosphite furnished alkene 9 (85%). Oxidation of 9 (CF_3CO_3H, CH_2Cl_2, rt) gave exclusively the α-epoxide which proved extremely resistant to nucleophilic attack. However trans-diaxial opening with Et_2AlCN (CH_2Cl_2, rt) led to the 1,2-cyanohydrin 10 in 40-45% yield. Saponification of 10 (NaOH-CH_3OH) gave cyanoacid 11, while acidic hydrolysis (1:1 conc. HCl:H_2O, 100°C) afforded 12 (both yields >99%). Raney nickel hydrogenation of nitrile 11 (CH_3OH-NH_3) gave amine 13 which was esterified (CH_3OH-HCl) to 14 in 85% overall yield. Peracetylation, then N-nitrosation and thermal rearrangement according to White[8] afforded tetraacetate 15 in 70% yield. After exhaustive hydrolysis (KOH-CH_3OH), tetraol 16 could be selectively phosphorylated to 17 by the elegant method of Fraser-Reid [$(iPr)_2NP(OBn)_2$-tetrazole-MCPBA, CH_2Cl_2; 65%].[9] Hydrogenolysis of 17 to 18 (97%) and saponification in aqueous NaOH furnished the desired carbocyclic DAHP analog 19 in 85% yield after Dowex 50X8 chromatography, $[\alpha]_D = +8.3^\circ$ (c=0.6, H_2O).

DHQ synthase was assayed at pH 7.4 using substrate (DAHP 1; $K_M = 4\ \mu M$) in the presence of Co^{++} and NAD^+ by coupling with dehydroquinase.[10] At saturating levels of 19, the rate of P_i release was reduced to 0.3% of that observed with 1 alone under saturation conditions. The K_I for 19 was determined to be 0.16 μM, indicating that 19 binds some 25 times more tightly than natural substrate. However no inactivation of the enzyme was observed; instead DHQ synthase processed 19 to 22, which was identified by its ^{1}H-NMR spectrum.[11]

Diacid 12[12] was also of interest as a substrate for DHQ synthase. Enzymatic oxidation of 12 and deprotonation of the resulting ketone (as in 2) would result in a stable enol, thus creating an "internal state block."[13] With the coenzyme locked in its reduced form, neither the forward nor the reverse enzymatic reaction would be possible. In fact 12 was a modest competitive inhibitor of DHQ synthase ($K_I \simeq 100\ \mu M$), however UV absorption measurements at 340 nm using high enzyme concentrations failed to detect bound NADH.

SCHEME 2



7 R=H
8 R=CCl_3CH_2OCO

9

10

11 R= CN, R'= H
12 R=CO_2H, R'= H
13 R= CH_2NH_2, R'= H
14 R= CH_2NH_2, R'= CH_3

15 R= Ac
16 R=H

17 R= $PO(OBn)_2$
18 R= $PO(OH)_2$

19

20

21

22

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11. These enzymatic assays and kinetic measurements were conducted by T.S. Widlanski, S.L. Bender and J.R. Knowles, who have independently synthesized and studied 19 as a DHQ synthase inhibitor. These workers also established the identity of 22 by comparison with an authentic sample they prepared (Biochemistry, submitted).
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