Cyclohexenyl and Cyclohexylidene Inhibitors of 3-Dehydroquinate Synthase: Active Site Interactions Relevant to Enzyme Mechanism and Inhibitor Design

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Abstract: Cyclohexenyl and cyclohexylidene inhibitors possessing strategically placed olefinic residues, in general, bind to 3-dehydroquinate (DHQ) synthase more tightly than similarly substituted cyclohexyl inhibitors. All of the newly synthesized inhibitors were prepared from a common DHQ derivative. Cyclohexenyl phosphate **1** is the most potent inhibitor of DHQ synthase thus far identified with an inhibition constant ($K_i = 1.2 \times 10^{-10}$ M), indicating active site binding 1000-fold tighter relative to the corresponding cyclohexyl phosphate **5**. Cyclohexenyl tricarboxylate **2** binds 700-fold more tightly than similarly substituted cyclohexyl tricarboxylate **6** and is the first example of a nanomolar-level inhibitor ($K_i = 8.6 \times 10^{-9}$ M) possessing neither a phosphate monoester nor a phosphonic acid. Cyclohexenyl homophosphonate **4** ($K_i = 3.0 \times 10^{-8}$ M) and cyclohexylidene homophosphonate **10** ($K_i = 3.2 \times 10^{-9}$ M) bind 57- and 530-fold, respectively, more tightly than the corresponding cyclohexyl homophosphonate **8**. Cyclohexylidene homophosphonate **10** is the first example of a nanomolar-level, homophosphonic acid inhibitor of DHQ synthase. Cyclohexylidene phosphonate **9** ($K_i = 2.9 \times 10^{-10}$ M) is a 2.9-fold more potent inhibitor relative to cyclohexyl phosphonate **7** which was previously the most potent, slowly-reversible inhibitor of DHQ synthase. Cyclohexenyl phosphonate **3** ($K_i = 1.2 \times 10^{-9}$ M) is the only olefin-containing, carbocyclic inhibitor where improved binding over the corresponding cyclohexyl analogue was not observed. The impact of olefinic residues in inhibitors on active site binding may indicate that DHQ synthase plays an active catalytic role during E1cb elimination of inorganic phosphate from enzyme-bound substrate.

The role of 3-dehydroquinate synthase in catalyzing the conversion (Scheme 1) of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) into 3-dehydroquinate (DHQ) has been the subject of considerable discussion. The oxidation, elimination, reduction, pyranosyl ring opening, and intramolecular aldol condensation required for turnover of substrate into product were originally thought to be catalyzed by active site residues.[1] However, various experiments have more recently demonstrated that the structural components of reactive intermediates derived from DAHP provide a significant amount of the reactivity needed for conversion of DAHP into product DHQ.[2] A number of different lines of evidence now indicate that a basic residue at the enzyme's active site does not catalyze the elimination of inorganic phosphate from intermediate **B** (Scheme 1).[3] Instead, the phosphate monoester of intermediate **B** apparently mediates its own elimination. As for the intramolecular aldol condensation, independently synthesized intermediate **D** undergoes a spontaneous pyranosyl ring opening and primarily undergoes cyclization to 3-dehydroquinate with formation of only trace levels of 1-*epi*-DHQ.[4] This result indicates that DHQ synthase plays a catalytic role in establishing the proper stereochemistry in product DHQ. However, much of the stereoelectronic information required for proper cyclization already resides in intermediate **D**.

The elimination of inorganic phosphate from intermediate **A** remains a focal point for continued discussion of the respective roles played by enzyme active site residues versus the inherent reactivity of enzyme-bound intermediates in the turnover of DAHP into DHQ. Although the phosphate monoester of intermediate **A** is mediating its own elimination, the enzyme active site may still accelerate this elimination by selective stabilization of an E1cb intermediate (**B**, Scheme 1) or E1cb-like transition state. Along these lines, cyclohexenyl analogues **1**−**4** and cyclohexylidene analogues **9** and **10** were synthesized from a butane 2,3-bisacetal(BBA)-protected DHQ derivative. Inhibition constants were then measured for the cyclohexenyl and cyclohexylidene analogues and compared with those inhibition constants determined for cyclohexyl analogues **5**−**8**. With the exception of only cyclohexenyl phosphonate **3**, improved inhibition was observed for cyclohexenyl and cyclohexylidene analogues **1**, **2**, **4**, **9**, and **10** relative to the corresponding cyclohexyl analogues **5**−**8**. This suggests that the active site of DHQ synthase may not merely be a spectator during elimination of inorganic phosphate from intermediate **A** (Scheme 1). Improved inhibition observed with incorporation of a strategically placed olefin in the cyclohexenyl and cyclohexylidene analogues also points toward an important new structural element to be exploited in the design of potent inhibitors of DHQ synthase.

Results

Synthesis of Cyclohexenyl Inhibitors. All of the cyclohexenyl inhibitors (**1**−**4**) were derived from a single advanced

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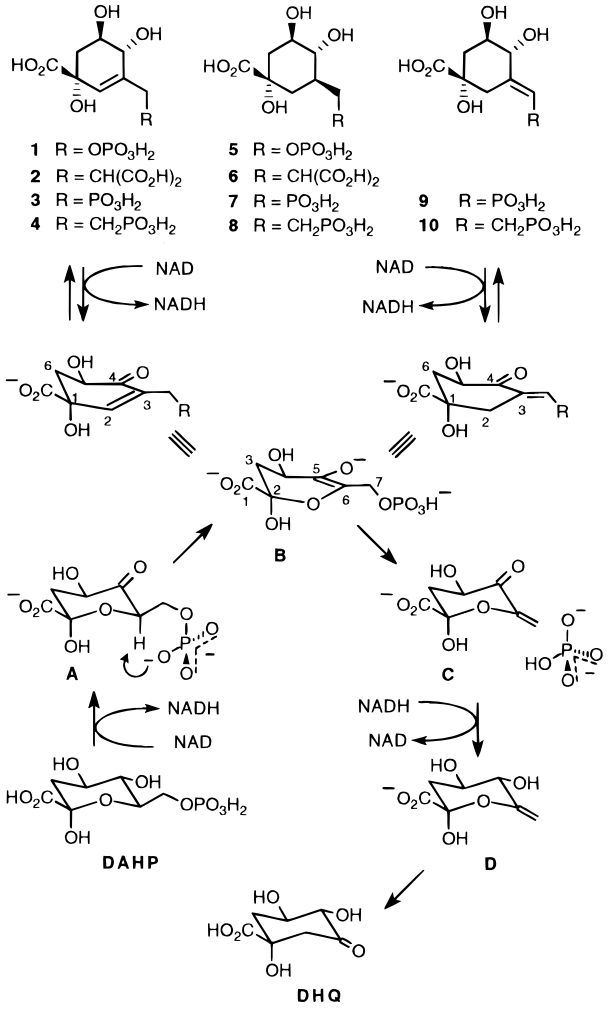
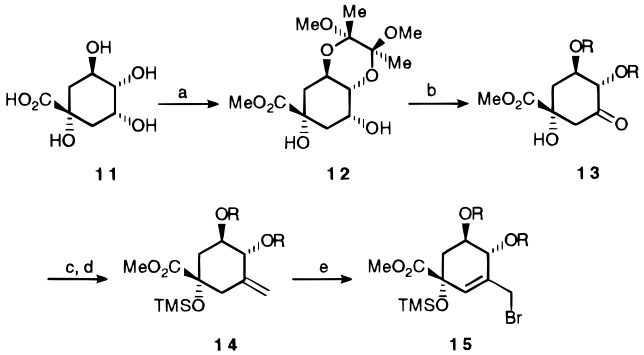
Scheme 1



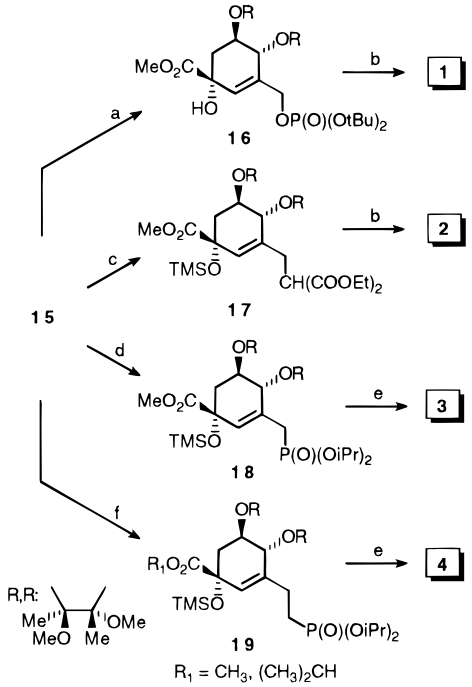
intermediate (**15**) which was synthesized (Scheme 2) from protected DHQ **13**. To circumvent the instability of DHQ during attachment of protecting groups, quinic acid **11** was esterified and then reacted with 2,2,3,3-tetramethoxybutane to selectively protect the vicinal diequatorial alcohols.[5] BBA-protected **12** was oxidized with catalytic $RuCl_3$ and KIO_4 to afford protected DHQ **13**.[6] Silylation of **13** with hexamethyldisilazane (HMDS) and TMSCl (TMS = trimethylsilyl) followed by olefination using Takai's reagent[7] produced exocyclic olefin **14**. Treatment of **14** with phenylselenyl bromide[8] under kinetic control, and subsequent oxidative elimination of the intermediate phenylselenide yielded allylic bromide **15**.

Scheme 2[a]



[a] Key: (a) (i) CH_3OH, Dowex 50 (H^+), reflux, (ii) 2,2,3,3-tetramethoxybutane, $(CH_3O)_3CH$, CH_3OH, camphorsulfonic acid, reflux, 87%; (b) KIO_4, K_2CO_3, $RuCl_3$, H_2O, $CHCl_3$, 77%; (c) TMSCl, HMDS, pyridine, 99%; (d) CH_2I_2, Zn, $TiCl_4$, THF, 92%; (e) (i) PhSeBr, Na_2CO_3, CH_2Cl_2, −78 °C, (ii) *m*CPBA, pyridine, CH_2Cl_2, −78 °C to rt, 83%.

Scheme 3[a]



[a] Key: (a) $(tBuO)_2P(O)OH$, Ag_2O, CH_3CN, 63%; (b) (i) TFA/H_2O (20:1, v/v), CH_2Cl_2, (ii) 0.2 N aq NaOH, (iii) Dowex 50 (H^+), **1** 25%, **2** 100%; (c) $CH_2(CO_2Et)_2$, NaH, THF, 68%; (d) $(iPrO)_3P$, toluene, reflux; (e) (i) TFA/H_2O (20:1, v/v), CH_2Cl_2, (ii) TMSBr, Et_3N, CH_2Cl_2, (iii) 0.2 N aq NaOH, (iv) Dowex 50 (H^+), **3** 58% from **15**, **4** 48%; (f) $(iPrO)_2P(O)CH_2Li$, CuCN, THF, 0 °C, R_1 = Me 33%, R_1 = iPr 28%.

Allylic bromide **15** proved to be an extremely versatile intermediate (Scheme 3). Reaction of allylic bromide **15** with di-*tert*-butyl phosphate[9] (catalyzed by silver ion[10]), diethyl malonate anion, and triisopropyl phosphite gave, respectively, protected cyclohexenyl phosphate **16**, tricarboxylate **17**, and phosphonate **18**. Protected cyclohexenyl homophosphonate **19** resulted from coupling of **15** with the anion of esterified methylphosphonate. To achieve the necessary chemoselectivity in the presence of the reactive carboxylate ester, diisopropyl lithiomethylphosphonate was first reacted with CuCN followed by addition of allylic bromide **15**. Coupling occurred with concomitant partial transesterification yielding a mixture of carboxylate methyl and isopropyl esters.

The BBA protecting group was removed (Scheme 3) along with the *tert*-butyl phosphate esters in protected cyclohexenyl phosphate **16** and the TMS ether in protected cyclohexenyl tricarboxylate **17** upon treatment with aqueous trifluoroacetic acid in CH_2Cl_2. Cyclohexenyl phosphate **1** and cyclohexenyl tricarboxylate **2** were obtained after removal of the trifluoroacetic acid in vacuo and hydrolysis of the carboxylate methyl esters with dilute aqueous NaOH. Protected cyclohexenyl phosphonate **18** and cyclohexenyl homophosphonate **19** required an additional step after removal of the BBA protecting group involving transesterification of the phosphonate esters using TMSBr. A final base hydrolysis to remove the carboxylate ester

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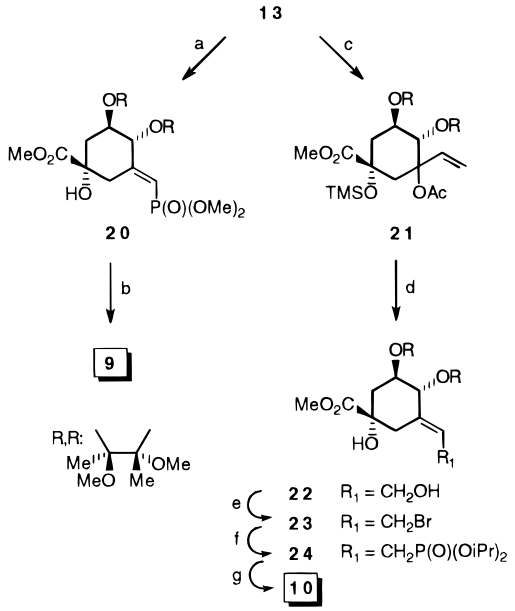
Scheme 4[a]



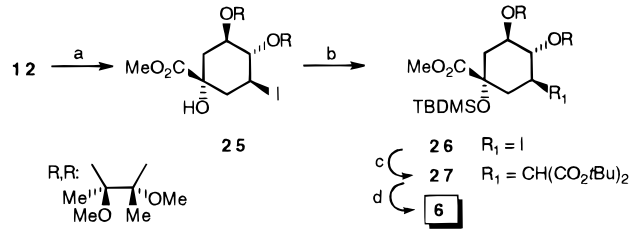
[a] Key: (a) nBuLi, $(CH_3O)_2P(O)CH_2P(O)(OCH_3)_2$, THF, −78 °C to rt, 56%; (b) 6 N aq HCl, reflux, 65%; (c) (i) TMSCl, HMDS, pyridine, (ii) CH_2═$CHMgBr$, $CeCl_3$, THF, −78 °C, (iii) Ac_2O, −78 °C to rt, 89%; (d) (i) MeOH, Dowex 50 (H^+), (ii) cat. $PdCl_2(CH_3CN)_2$, THF, (iii) MeONa, MeOH, 53%; (e) CBr_4, PPh_3, CH_3CN, 100%; (f) $(iPrO)_3P$, toluene, reflux, 47%; (g) (i) TFA/H_2O (20:1, v/v), CH_2Cl_2, (ii) TMSBr, Et_3N, CH_2Cl_2, (iii) 0.2 N aq NaOH, (iv) Dowex 50 (H^+), 50%.

yielded cyclohexenyl phosphonate **3** and cyclohexenyl homophosphonate **4**.

Synthesis of Cyclohexylidene Inhibitors. The synthesis of cyclohexylidene phosphonate **9** (Scheme 4) followed directly from the reaction of protected DHQ **13** with the lithium anion of tetramethyl methylenediphosphonate to afford (*E*)-cyclohexylidene phosphonate **20**.[6] Attempted Wadsworth−Emmons olefination of protected DHQ **13** using tetraisopropyl methylenediphosphonate failed to lead to cyclohexylidene phosphonate formation. Exhaustive hydrolysis of **20** was accomplished in a single step by refluxing with 6 N HCl. Under these conditions, cyclohexylidene phosphonate **9** was obtained cleanly without isomerization of the double bond. The assignment of the stereochemistry for protected cyclohexylidene phosphonate **20** and deprotected cyclohexylidene phosphonate **9** was based on ^{13}C NMR and the known relationship between $^3J(PC)$ and the dihedral angle.[11] Examination of the phosphorus couplings at C-4 (16−18 Hz) and C-2 (7−8 Hz) clearly supported the *E*-configuration of the double bond. Steric factors also support this assignment, given the predicted steric congestion involving the BBA group and the phosphonate diester in the *Z*-configuration as opposed to the *E*-configuration of intermediate **20**.

Synthesis of cyclohexylidene homophosphonate **10** (Scheme 4) required a somewhat different approach. Once again, protected DHQ **13** was employed as the key intermediate. Cerium-mediated 1,2-addition[12] of vinyl Grignard to the carbonyl group of TMS-protected **13** and trapping of the resulting alkoxide with acetic anhydride provided allylic acetate **21** in excellent yield. One diastereomer formed almost exclusively in this reaction, but the configuration at C-3 was not established since this stereocenter was destroyed in subsequent transformations. Allylic acetate **21** was treated with Dowex 50 (H^+) in MeOH to hydrolyze the TMS ether. Treatment of the resulting intermediate with catalytic $PdCl_2(CH_3CN)_2$ led to rearrangement and formation of a primary acetate.[13] Removal of the TMS ether was necessary to achieve rapid reaction rates and acceptable yields of allylic alcohol, although migration of the acetate from C-3 to C-1 was a competing reaction. Pd-catalyzed isomerization provided a single geometric isomer. Methanolysis of the primary acetate to give diol **22** was followed by conversion of the primary alcohol into bromide **23** by reaction of diol **22** with CBr_4·PPh_3 in acetonitrile. Subsequent Arbuzov condensation of **23** with triisopropyl phosphite in refluxing toluene and deprotection of the resulting homophosphonate **24** following the procedure used in the synthesis of cyclohexenyl phosphonate **3** afforded cyclohexylidene homophosphonate **10**.

Scheme 5[a]



[a] Key: (a) PPh_3, I_2, HMPA, C_6H_6, 41%; (b) TBDMSOTf, Et_3N, CH_2Cl_2, 69%; (c) $(Me_3Si)_3SiH$, H_2C═$C(CO_2tBu)_2$, AIBN, C_6H_6, reflux, 72%; (d) (i) TFA/H_2O (20:1, v/v), (ii) 1 N aq NaOH, reflux, (iii) Dowex 50 (H^+), 75%.

The configuration of the double bond in **10** could not be assigned from phosphorus−carbon coupling constants. However, comparison of chemical shifts in the ^{13}C NMR spectrum of desilylated olefin **14** with the spectra of diol **22**, bromide **23**, and homophosphonate **24** does provide confirmatory evidence for the indicated double bond configuration in **10**. The chemical shift of the C-2 carbon shifted from δ 43.0 in the reference unsubstituted olefin **14** to δ 36.4, 36.3, and 35.8 in compounds **22**, **23**, and **24** respectively, while the C-4 carbon remained constant at δ 72.3−72.4. The constant upfield shift of the C-2 resonance in the substituted olefins **22**−**24** is consistent with steric compression (γ-effect) and supports the *E*-configuration.[14] The Pd-catalyzed rearrangement of allylic acetates is also known to be *E*-selective.[13] A transition-state for attack of the acetate ion on the π-allyl palladium intermediate would likely involve the most stable π-allyl system in which steric constraints with the BBA group are avoided, and thus would lead to the *E*-configuration after rearrangement.

Synthesis of Cyclohexyl Tricarboxylate. While cyclohexyl inhibitors **5**, **7**, and **8** are literature molecules,[3c,d,15] the cyclohexyl tricarboxylate **6** has not been previously reported. This compound required a different strategy for introduction of the C-3 side chain with the proper stereochemistry. Synthesis (Scheme 5) started from BBA-protected methyl quinate **12**. Treatment of **12** with iodine, PPh_3, and hexamethylphosphoramide (HMPA) afforded iodide **25**.[16] Reaction of **25** with *tert*-butyldimethylsilyl (TBDMS) triflate (OTf) gave TBDMS-protected iodide **26**, which was reacted under free radical conditions with di-*tert*-butyl methylene malonate.[17] Slow addition of a benzene solution of tris(trimethylsilyl)silane (TTMSS, 1.1 equiv) and 2,2′-azobisisobutyronitrile (AIBN) into a solution of di-*tert*-butyl methylene malonate (1.3 equiv) and

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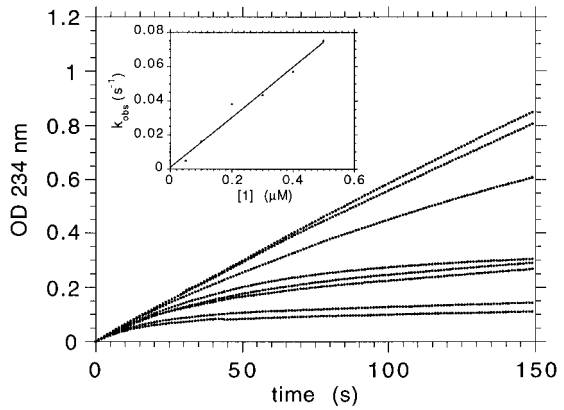

Figure 1. Progress curves for inhibition of DHQ synthase by cyclohexenyl phosphate **1**. Assay solutions contained DAHP (500 μM), NAD (10 μM), $CoCl_2$ (50 μM), MOPS (50 mM) at pH 7.5, varying concentrations of **1** (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 μM), and 3-dehydroquinase (1 unit). Reactions were initiated by the addition of DHQ synthase (0.024 units) to the assay solution thermally equilibrated at room temperature (rt). Inset: Observed first-order rate constants (k_{obsd}) obtained from nonlinear regression analysis of progress curves were plotted against inhibitor concentration.

iodide **26** (1 equiv) in refluxing benzene afforded a good yield of protected cyclohexyl tricarboxylate **27**. Use of TTMSS in the radical coupling was superior relative to use of tributyltin hydride in minimizing the amount of reductive cleavage of iodide from **26**. The choice of protecting group for the C-1 hydroxyl group was also critical. When iodide **25** was employed in the radical coupling reaction, a mixture of C-3 epimers was obtained. The same outcome was observed with TMS-protected **25**. Treatment of protected cyclohexyl tricarboxylate **27** with aqueous trifluoroacetic acid simultaneously hydrolyzed the BBA protecting group, the C-1 TBDMS ether, and the carboxylate *tert*-butyl esters. The remaining methyl ester was hydrolyzed under basic conditions to give cyclohexyl tricarboxylate **6**.

Enzymology. Formation of NADH was observed when cyclohexenyl, cyclohexyl, and cyclohexylidene inhibitors (**1**−**10**) were incubated with the enzyme. This suggests that all of these inhibitors are similarly positioned in the active site. Evaluation of the kinetic parameters employed spectrophotometric detection at 234 nm of the 3-dehydroshikimate produced by DHQ-dehydratase-catalyzed dehydration of DHQ. Time-dependent inhibition was observed with the cyclohexenyl inhibitors **1**−**4** and cyclohexylidene inhibitors **9** and **10**. Progress curves were generated and fitted to obtain the apparent first-order rate constant (k_{obsd}) for loss of activity.[18] A linear plot of k_{obsd} versus inhibitor concentration was observed in each case, indicating a single-step, slowly-reversible competitive mechanism.[18,19] The association (k_{on}) and dissociation rate constants (k_{off}) for the inhibitors could also be determined from the plot. However, the dissociation rate constant was determined more accurately[18] by incubating the enzyme with a near stoichiometric amount of inhibitor and by following the progress curve for recovery of enzyme activity upon 100-fold dilution into a high concentration of DAHP. This also excluded irreversible inhibition resulting from attack of an active site nucleophile on the Michael receptors (Scheme 1) generated at the active site upon binding and oxidation of the inhibitors. Typical experimental data is shown for cyclohexenyl phosphate **1** in Figure 1.

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Table 1. Kinetic Parameters for Cyclohexenyl, Cyclohexylidene, and Cyclohexyl Inhibitors of DHQ Synthase

	inhibition type	k_{on} (M^{-1} s^{-1}) k_{off} (s^{-1})	K_i (M)	relative inhibition[a]
1	slowly reversible	1.0×10^7 1.2×10^{-3}	1.2×10^{-10}	1000×
2	slowly reversible	8.4×10^5 7.2×10^{-3}	8.6×10^{-9}	700×
3	slowly reversible	6.0×10^5 7.0×10^{-4}	1.2×10^{-9}	0.69×
4	slowly reversible	1.4×10^5 4.2×10^{-3}	30×10^{-9}	57×
5	competitive		$1.2-1.6 \times 10^{-7}$ [b]	
6	competitive		6.0×10^{-6}	
7	slowly reversible	1.0×10^6 8.3×10^{-4}	8.3×10^{-10}	
8	competitive		1.7×10^{-6}	
9	slowly reversible	8.0×10^6 2.3×10^{-3}	2.9×10^{-10}	2.9×
10	slowly reversible	8.7×10^6 2.8×10^{-2}	3.2×10^{-9}	530×

[a] K_i cyclohexyl/K_i similarly substituted cyclohexenyl or cyclohexylidene analogue. [b] See refs 3d and 15.

Dissociation rate constants (k_{off}), association rate constants (k_{on}), and inhibition constants (K_i) for all of the cyclohexenyl and cyclohexylidene inhibitors are provided in Table 1. The same parameters for inhibition of DHQ synthase by cyclohexyl inhibitors **5**−**8** (Table 1) is also included for comparison. In the cyclohexenyl series **1**−**4**, cyclohexenyl phosphate **1**, cyclohexenyl tricarboxylate **2**, and cyclohexenyl homophosphonate **4** displayed 1000-, 700-, and 57-fold improvements, respectively, in binding affinity over similarly substituted cyclohexyl inhibitors. While slowly-reversible inhibition was observed only for phosphonate **7** in the cyclohexyl series **5**−**8**, all of the cyclohexenyl analogues **1**−**4** were slowly-reversible inhibitors. Cyclohexenyl phosphate **1** is, in fact, the most potent inhibitor of DHQ synthase thus far identified. Tricarboxylate **2** is by far the most tightly-bound inhibitor which lacks a phosphorus-containing functionality. Cyclohexenyl phosphonate **3** was the only instance where an inhibition constant associated with an unsaturated inhibitor was weaker than the inhibition constant measured for its saturated counterpart. For the cyclohexylidene analogues, cyclohexylidene phosphonate **9** is a 2.9-fold better inhibitor relative to cyclohexyl phosphonate **7**, which was the most potent, slowly-reversible inhibitor of DHQ synthase reported prior to this work. Cyclohexylidene homophosphonate **10**, a 530-fold better inhibitor relative to cyclohexyl homophosphonate **8**, is the most tightly bound homophosphonate inhibitor identified to date.

When incubated with the enzyme, all of the unsaturated inhibitors displayed an absorbance at 410 nm in addition to the absorbance increase at 340 nm. This longer wavelength absorbance is likely a charge-transfer absorption. The UV−vis spectrum for cyclohexenyl phosphate **1** is shown in Figure 2 and is representative of the other unsaturated inhibitors. Knowles and co-workers[15c] have also observed longer wavelength absorption (albeit much weaker) in the UV spectrum of a DHQ synthase−inhibitor complex and showed that these absorptions disappeared if the Co^{+2} cofactor was replaced with Mn^{+2}. When DHQ synthase was reconstituted with Mn^{+2} or Zn^{+2} and then incubated with cyclohexenyl phosphate **1**, spectra virtually identical to that of the Co^{+2} enzyme (Figure 2) were observed. Although the exact origin of the charge-transfer band remains a subject for further studies, these experiments indicated that the metal cofactor did not play a role in this phenomenon.

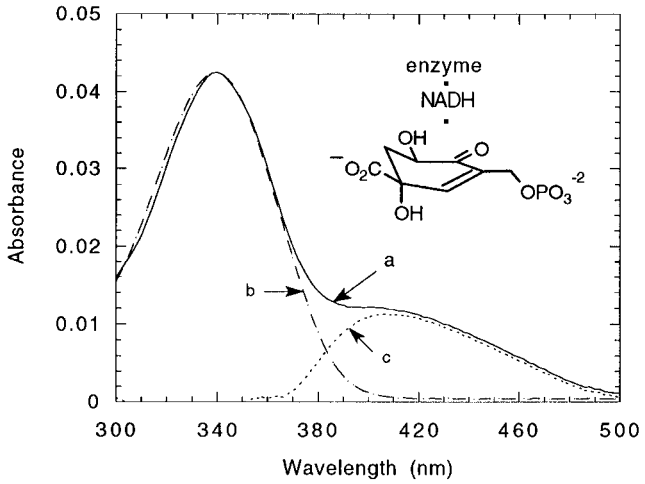
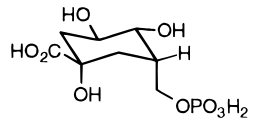


Figure 2. Curve a: UV−vis spectra of a solution of MOPS buffer (50 mM, pH 7.7) containing $CoCl_2$, DHQ synthase, and cyclohexenyl phosphate **1**. Curve b: UV−vis spectrum of NADH. Curve c: difference spectrum between DHQ synthase with bound cyclohexenyl phosphate **1** (curve a) and NADH (curve b).

Discussion

On the basis of the demonstrated *syn*-elimination of inorganic phosphate and the greater inhibitory potency of *Z*-vinyl homophosphonic acid versus *E*-vinyl homophosphonic acid, Knowles, Widlanski, and Bender[3b,c] proposed that the phosphate monoester of intermediate **A** mediated its own elimination. An alternate approach to ascertaining whether an active site residue was involved in elimination of inorganic phosphate focused on challenging DHQ synthase with C-5 *epi*-carbocyclic inhibitors such as 5-[(phosphonooxy)methyl]-5-deoxyquinate **28**.[20] DHQ



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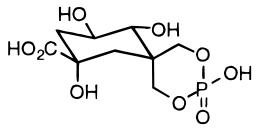
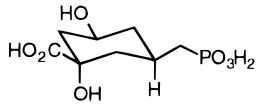
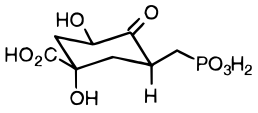


29



30



31

synthase would not be expected to tolerate the drastic repositioning of the phosphorylmethyl group in *epi*-carbocyclic analogue **28** relative to cyclohexyl phosphate **5** if an active site residue were employed during catalyzed elimination of inorganic phosphate. Synthesis and enzymological evaluation of 5-[(phosphonooxy)methyl]-5-deoxyquinate **28** revealed that this *epi*-carbocyclic analogue was not only an inhibitor of DHQ synthase but actually a superior inhibitor relative to cyclohexyl phosphate **5**.[20]

The Catalytic Role of DHQ Synthase. Recruitment of the phosphate monoester of intermediate **A** as an active site basic residue does not necessarily mean that DHQ synthase is a passive observer during elimination of inorganic phosphate. For example, the active site might accelerate the elimination of inorganic phosphate by restricting the conformational flexibility of the phosphorylmethyl group of intermediate **A**. To explore this possibility, spirocyclic phosphodiester **29** was synthesized.[21] When bound and oxidized by DHQ synthase, the resulting spirocyclic phosphodiester **29** would be a conformationally restrained mimic of the six-membered transition state wherein the methine proton in intermediate **A** is removed by the phosphate monoester. It appeared likely that the active site would accommodate binding of spirocyclic carbaphosphodiester **29** given the inhibition of DHQ synthase observed for cyclohexyl phosphate **5** and 5-[(phosphonooxy)methyl]-5-deoxyquinate **28**. Spirocyclic carbaphosphodiester was bound by DHQ synthase and oxidized as indicated by the formation of NADH during inhibition. However, the modest competitive inhibition ($K_i = 6.7 \times 10^{-5}$ M) observed for spirocyclic phosphodiester **29** was not consistent with active site restriction of the conformational freedom of the phosphorylmethyl group of intermediate **A** during elimination of inorganic phosphate.[21]

An alternate mechanism for DHQ synthase to accelerate the rate at which the phosphate monoester mediates its own elimination might entail stabilization of an E1cb intermediate (**B**, Scheme 1) or E1cb-like transition state. An sp^2-hybridized, C-6 carbon atom and a phosphorylmethyl group attached to this carbon are the defining structural features of an E1cb intermediate or transition state. Two different strategies were pursued to introduce the appropriate sp^2-hybridized center. In cyclohexenyl analogues **1**−**4**, the sp^2-hybridized carbon was part of an endocyclic olefin. Cyclohexylidene analogues **9** and **10** incorporated the sp^2-hybridized ring carbon into an exocyclic olefin. As for the charged appendage, a variety of different functional groups were examined in addition to the phosphorylmethyl group of cyclohexenyl phosphate **1**. Both homophosphonate (**4** and **10**) and phosphonate (**3** and **9**) analogues were synthesized in the cyclohexenyl and cyclohexylidene series. Cyclohexenyl tricarboxylate **2** was also synthesized by virtue of the recent successful inhibition of DHQ synthase with a malonyl diacid mimic of a phosphorylmethyl group.[6] Inhibition constants were then quantitated for cyclohexenyl analogues **1**−**4** and cyclohexylidene analogues **9** and **10**. Comparison with the inhibition constants measured for the corresponding cyclohexyl analogues **5**−**8** provided the basis for gauging the relative strength of active site interactions with an E1cb transition state or E1cb intermediate **B**. In absolute and relative terms, the improvement (57- to 1000-fold, Table 1) in active site binding upon introduction of an sp^2-hybridized center is striking.

Mimicry of an E1cb transition state or reactive intermediate is not the only possible explanation for the improvement in inhibitory potency upon introduction of a strategically placed olefin in carbocyclic inhibitors of DHQ synthase. A relatively constant 25- to 50-fold improvement has been observed for cyclohexyl relative to similarly substituted pyranosyl inhibitors of DHQ synthase.[3c] Knowles has suggested that this may reflect a thermodynamically more-favored oxidation arising from the substitution of a methylene group for an electronegative oxygen adjacent to the oxidized center.[3c] The unsaturation α,β to the oxidized center may similarly lead to stabilization and an increased rate of formation of enzyme-bound cyclohexenones (Scheme 1) derived from cyclohexenyl and cyclohexylidene inhibitors. However, the rate constant for release (k_{off}) from DHQ synthase of C-4 deoxy cyclohexyl phosphonate **30**, which completely lacks an oxidizable reaction center, is essentially the same as the k_{off} for cyclohexyl phosphonate **7**.[22] This observation is difficult to reconcile with the hypothesis of thermodynamic stabilization of the oxidized reaction center playing a major role in determining inhibitor potency. Furthermore, no significant differences in the percentage of DHQ synthase with bound NADH were observed for cyclohexenyl inhibitors **1**−**4**.

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Literature Precedent. Ample precedent exists for olefin-containing enzyme inhibitors where one of the olefin's carbon atoms corresponds to the position of an sp^3-hybridized, substrate carbon atom which undergoes rehybridization to an sp^2-hybridized center in a reactive intermediate or transition state. Examples include inhibition, respectively, of isocitrate lyase,[23] sucrase,[24] proline racemase,[25] and neuraminidase.[26] Inhibition of neuraminidase has recently been extended from olefin-containing 2,3-dehydrosialic acids to carbocyclic analogues synthesized from quinic acid which contain strategically placed olefinic residues.[26] These examples are suggestive that the improved inhibition of cyclohexenyl inhibitors **1**, **2**, and **4** relative to cyclohexyl inhibitors **5**, **6**, and **8**, as well as the improved inhibition of cyclohexylidene inhibitors **9** and **10** relative to cyclohexyl inhibitors **7** and **8**, can be reasonably ascribed to cyclohexenyl and cyclohexylidene mimicry of an E1cb reactive intermediate or transition state.

Another literature example relevant to the inhibition of DHQ synthase by cyclohexyl and cyclohexenyl inhibitors is the inhibition of *S*-adenosyl homocysteine hydrolase by aristeromycin[27] and neplanocin A.[28] Like DHQ synthase, *S*-adenosyl homocysteine hydrolase utilizes NAD as a catalyst.[1] Cyclopentyl aristeromycin, the carbocyclic analogue of adenosine, is a potent inhibitor of *S*-adenosyl homocysteinase.[27] The impact of replacing the furanosyl ring oxygen of adenosine with a methylene group is thus reminiscent of the impact on inhibition attendant with replacement of the pyranosyl ring oxygen of DAHP analogues. However, the inhibition constant for cyclopentenyl neplanocin A is only a fraction (0.6 times) of the magnitude of the inhibition constant for cyclopentyl aristeromycin.[28] Cyclohexenyl phosphonate **3** was the only example (Table 1) where incorporation of an olefin did not lead to improved inhibition of DHQ synthetase relative to the similarly substituted cyclohexyl analogue.

Designing DHQ Synthase Inhibitors. The importance of cyclohexenyl and cyclohexylidene analogues as inhibitors of DHQ synthase is best gauged by comparison with advances which have previously been achieved with design of DHQ synthase inhibitors. Relatively early in the elaboration of DHQ synthase enzymology, a nonisosteric phosphonate analogue of the phosphate monoester of DAHP was discovered to be a micromolar-level inhibitor of the enzyme.[29] Isosteric homophosphonic acid analogues of the phosphate monoester of DAHP have, in general, proven to be reasonably weak inhibitors of DHQ synthase.[29] Nanomolar-level inhibition was realized with the synthesis of cyclohexyl substrate analogues where pyranosyl ring oxygens were replaced with methylene groups.[3b−d] Epimerization of individual asymmetric centers in cyclohexyl[20,30] and 2,6-anhydro[31] substrate analogues has also proven to be a generally useful strategy for achieving DHQ synthase inhibition. One example of apparent irreversible inhibition of DHQ synthase by a ketocarbaphosphonate analogue of intermediate **B** has been reported.[32] Ketocarbaphosphonate **31** is a micromolar-level, competitive inhibitor when incubated with DHQ synthase for short periods of time and an irreversible inhibitor when incubated with the enzyme for longer periods of time.[32] Curiously, a finite amount of enzyme activity (~5% of the initial activity) remains even after prolonged incubation with DHQ synthase.

Relative to cyclohexyl phosphonate **7**, which epitomizes the structural features known to lead to potent DHQ synthase prior to this work, cyclohexenyl phosphate **1** is approximately 7-fold more potent an inhibitor of DHQ synthase. Cyclohexenyl **4** and cyclohexylidene **10** are the first instances where slowly-reversible inhibition of DHQ synthase has been achieved with homophosphonic acid analogues of a phosphate monoester. The general applicability of incorporating strategically placed double bonds in cyclohexyl inhibitors of DHQ synthase is best represented by the inhibition achieved with cyclohexenyl tricarboxylate **2**. This is the first example of nanomolar-level inhibition of DHQ synthase using an analogue lacking a phosphonic acid or phosphate monoester. All of the DHQ synthase inhibitors with epimerized asymmetric centers are significantly weaker inhibitors of DHQ synthase relative to the olefin-containing inhibitors of Table 1. In contrast to the complicated kinetics observed with ketocarbaphosphonate **31**,[32] the cyclohexenyl and cyclohexylidene inhibitors of Table 1 display straightforward, slowly-reversible inhibition kinetics. All of the cyclohexenyl and cyclohexylidene inhibitors are stable when incubated in buffered, aqueous solutions for prolonged periods of times. This is a marked contrast with the solution stability characteristics of ketocarbaphosphonate **31**.

When stable mimics of reactive intermediates and transition states are synthesized and the extent of enzyme inhibition which is achieved with these mimics is quantitated, insights can be gleaned into enzyme mechanism. However, potent enzyme inhibition achieved with a given reactive intermediate or transition state analogue is invariably subject to multiple interpretations. Ascribing DHQ synthase inhibition by cyclohexenyl and cyclohexylidene analogues to mimicry of an E1cb intermediate or transition state may ultimately be revisited as more structural information about the active site becomes available. What will remain invariant over the long term is the demonstration that a single strategically-placed olefin has transformed cyclohexyl phosphate **5**, tricarboxylate **6**, and homophosphonate **8** from micromolar-level, competitive inhibitors into nanomolar- and subnanomolar-level, slowly-reversible inhibitors. Judged in terms of general applicability and the absolute magnitudes of enzyme inhibition which are achieved, this conceptually simple structural modification has established a new generation of DHQ synthase inhibitor.

Experimental Section

General Chemistry. Organic solutions of products were dried over $MgSO_4$. See ref 21 for general experimental information. Spectrophotometric measurements were made on a Hewlett-Packard 8452A diode array spectrophotometer.

General Enzymology. Throughout this work, the K_m for DAHP has been taken as 4×10^{-6} M. The concentration of tricarboxylate inhibitors was quantified by NMR analysis as described in ref 6. The concentration of phosphorus-containing inhibitors was quantified by the methods of Avila and Ames.[33]

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DHQ Synthase activity was assayed as described previously.[3d,6] Enzyme-bound NADH formation during enzyme inhibition was detected as described in ref 6. Time-dependent inhibition was analyzed following literature procedures.[3d,18,19] Association rate constants (k_{on}) were determined after incubation of a range of inhibitor and substrate concentrations in the assay buffer at rt, followed by adding the enzyme (final concentration 0.04 μM) and monitoring the reaction over time. The progress curves were fitted to the equation: absorbance $= a + bt + ce^{-kt}$ (where a, b, and c are adjustable parameters, $k = k_{obsd}$ is the apparent first-order rate constant for loss of activity, and t is time), for portions of the progress curves where a control in the absence of inhibitor is linear. Initial velocities did not vary with inhibitor concentration. A plot of k_{obsd} versus [I] was linear and supported a one-step association mechanism of inhibition for which $k_{obsd} = k_{on}[I]/(1+ [S]/K_m) + k_{off}$. The association rate ($k_{on}$) was obtained from the slope of this plot. While the dissociation rate constant (k_{off}) can also be determined from the intercept of this plot, independent determination of k_{off} was more reliable.[18] Dissociation rate constants were determined by incubation of the enzyme and the inhibitor (in small excess) at rt. Aliquots were withdrawn at timed-intervals and diluted (100×) into assay buffer containing DAHP (0.5 mM) and dehydroquinase (1 unit). The progress curves were fitted to the equation: absorbance $= a + bt + ce^{-kt}$ (where $k = k_{off}$). The inhibition constant was then obtained from the equation $K_i = k_{off}/k_{on}$.

Exocyclic Alkene 14. To a solution of protected DHQ **13**[6] (8.18 g, 25.7 mmol) in pyridine (50 mL) were added hexamethyldisilazane (20 mL, 95 mmol) and TMSCl (15.0 mL, 118 mmol) at rt, under Ar. The resulting white suspension was stirred at rt (about 20 h). The reaction mixture was concentrated in vacuo, and the residue was partitioned between ether and water. The organic layer was washed successively with water (1×), aqueous $CuSO_4$ (1×), water (1×), and saturated aqueous $NaHCO_3$ (1×). Drying and concentration afforded the silyl ether as a slightly yellow oil (9.93 g, 99%) which slowly crystallized at rt. This could be used immediately in the next step. ^{1}H NMR ($CDCl_3$): δ 4.38 (dd, J = 10, 1 Hz, 1 H), 4.15 (ddd, J = 10, 10, 5 Hz, 1 H), 3.78 (s, 3 H), 3.26 (s, 3 H), 3.25 (s, 3 H), 2.84 (dd, J = 14, 1 Hz, 1 H), 2.64 (dd, J = 14, 3 Hz, 1 H), 2.20−2.35 (m, 2 H), 1.41 (s, 3 H), 1.31 (s, 3 H), 0.12 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 199.9, 172.8, 100.4, 99.5, 77.2, 67.1, 52.7, 49.9, 48.3, 47.9, 39.0, 17.7, 17.5, 1.6. Anal. Calcd for $C_{17}H_{30}O_8Si$: C, 52.28; H, 7.74. Found: C, 52.17; H, 7.67.

Neat CH_2I_2 (2.30 mL, 28.4 mmol) was added to a suspension of activated Zn dust (3.36 g, 51.0 mmol) in anhydrous THF (30 mL) at rt under Ar.[7] After 30 min, $TiCl_4$ (1.0 M in CH_2Cl_2, 6.25 mL, 6.25 mmol) was added slowly in portions at 0 °C to avoid boiling of the solvent. Removal of the ice bath was followed by stirring at rt for 30 min. The dark suspension was then cooled to 0 °C, and a solution of ketone (2.21 g, 5.67 mmol) in THF (20 mL) was added. The mixture was vigorously stirred at rt for 3 h, ether was added, and the mixture was carefully poured into ice-cold aqueous HCl (1 N). After the aqueous layer was extracted with ether (3×), the combined ether extracts were washed with saturated aqueous $NaHCO_3$ (1×). Drying and concentration afforded a yellow liquid. Purification by radial chromatography (4 mm thickness, hexane, EtOAc/hexane, 1:5, v/v) afforded the exocyclic alkene **14** as a colorless liquid (2.02 g, 92%). ^{1}H NMR ($CDCl_3$): δ 5.24 (s, 1 H), 4.87 (s, 1 H), 4.03 (d, J = 10 Hz, 1 H) 3.70−3.85 (m, 1 H), 3.74 (s, 3 H), 3.25 (s, 3 H), 3.24 (s, 3 H), 2.48 (s, 2 H), 2.00−2.05 (m, 2 H), 1.37 (s, 3 H), 1.31 (s, 3 H), 0.11 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 174.4, 139.4, 109.5, 99.8, 99.4, 77.2, 72.7, 68.2, 52.2, 47.8, 43.7, 39.1, 17.8 (2), 1.8. Anal. Calcd for $C_{18}H_{32}O_7Si$: C, 55.64; H, 8.30. Found: C, 55.74; H, 8.36.

Allylic Bromide 15. Powdered Na_2CO_3 (1.07 g, 10.1 mmol) was added to a solution of phenylselenyl bromide (1.72 g, 7.31 mmol) in CH_2Cl_2 (20 mL).[8] The mixture was cooled to −78 °C, and a solution of exocyclic alkene **14** (2.81 g, 7.23 mmol) in CH_2Cl_2 (20 mL) added via cannula under Ar. Stirring was continued at that temperature for 18 h and then pyridine (1.50 mL, 18.6 mmol) was added neat, followed by a solution of *m*CPBA (1.63 g, 9.43 mmol) in CH_2Cl_2 (20 mL). The cold bath was removed 15 min later, and the mixture was stirred at rt for 4 h. Ether and water were added, and the organic layer was washed with aqueous $Na_2S_2O_3$ (2×), aqueous $CuSO_4$ (2×), water (2×), and saturated aqueous $NaHCO_3$ (2×). Drying and concentrating gave an orange oil. Purification by radial chromatography (4 mm thickness, hexane, EtOAc/hexane, 1:5, v/v) afforded allylic bromide **15** as a slightly yellow oil (2.79 g, 83%). ^{1}H NMR ($CDCl_3$): δ 5.82 (s, 1 H), 4.38−4.43 (m, 2 H), 4.04 (ddd, J = 13, 9, 4 Hz, 1 H), 3.81 (d, J = 10 Hz, 1 H), 3.75 (s, 3 H), 3.41 (s, 3 H), 3.28 (s, 3 H), 2.19 (dd, J = 13, 13 Hz, 1 H), 2.02−2.08 (m, 1 H), 1.36 (s, 3 H), 1.32 (s, 3 H), 0.12 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 173.1, 137.9, 128.6, 100.4, 99.9, 75.8, 67.9, 65.4, 52.6, 48.7, 47.9, 37.4, 30.3, 17.8 (2), 1.8. Anal. Calcd for $C_{18}H_{31}BrO_7Si$: C, 46.25; H, 6.69. Found: C, 46.54; H, 6.77.

Protected Cyclohexenyl Phosphate 16. Freshly prepared di-*tert*-butyl phosphate[10] (0.400 g, 1.88 mmol) and silver(I) oxide (0.22 g, 0.95 mmol) were added to a solution of allylic bromide **15** (0.44 g, 0.94 mmol) in dry acetonitrile (5 mL) at rt under Ar.[9] The resulting dark suspension was vigorously stirred at rt for 23 h. Filtration through Celite and concentration of the resulting solution gave a solid powder which was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$. The organic layer was washed once with aqueous $NaHCO_3$, dried, and concentrated to an oil. Purification by radial chromatography (2 mm thickness, hexane, EtOAc/hexane, 1:5, 1:1, v/v, EtOAc) afforded protected cyclohexenyl phosphate **16** as a colorless oil (0.312 g, 63%). ^{1}H NMR ($CDCl_3$): δ 5.62 (s, 1 H), 4.64 (dd, J = 13, 6 Hz, 1 H), 4.56 (dd, J = 13, 6 Hz, 1 H), 4.30 (dd, J = 9, 2 Hz, 1 H), 4.08 (ddd, J = 13, 9, 4 Hz, 1 H), 3.77 (s, 3 H), 3.71 (s, 1 H), 3.30 (s, 3 H), 3.28 (s, 3 H), 2.19 (dd, J = 13, 13 Hz, 1 H), 1.95−2.05 (m, 1 H), 1.48 (s, 18 H), 1.33 (s, 3 H), 1.31 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 175.2, 137.5 (J_{POCC} = 9 Hz), 123.6, 100.2, 99.8, 82.2 (2, J_{POC} = 5 Hz), 72.9, 69.0, 65.4, 64.3 (J_{POC} = 6 Hz), 53.0, 48.0, 47.8, 36.7, 29.7 (J_{POCC} = 5 Hz), 17.7, 17.6. Anal. Calcd for $C_{23}H_{41}O_{11}P$: C, 52.66; H, 7.88. Found: C, 52.85; H, 7.95.

Cyclohexenyl Phosphate 1. Protected cyclohexenyl phosphate **16** (0.312 g, 0.590 mmol) in CH_2Cl_2 (5 mL) was stirred with CF_3CO_2H/H_2O (20:1, v/v, 1 mL) at rt. After 2 h, the solution was concentrated in vacuo, and the residue was treated with aqueous NaOH (0.2 N, 10 mL). Stirring was continued for 22 h, and the mixture was passed down a short column of Dowex 50 (H^+). The filtrate was neutralized to pH 7.7 with aqueous NaOH. The solution was applied to AG-1 X8 anion exchange resin (20 mL) which had been equilibrated with 200 mM $Et_3NH^+HCO_3^-$ (pH 7.5). The column was washed with water (40 mL) and eluted with a linear gradient (250 mL + 250 mL, 200−500 mM) of $Et_3NH^+HCO_3^-$ (pH 7.5). Fractions containing phosphorus were identified by the methods of Avila and Ames[33] and then concentrated to dryness. The resulting white residue was azeotroped six times with 2-propanol, dissolved in water, passed down a short column of Dowex 50 (H^+). The filtrate was neutralized to pH 7.5 with 0.1 N aqueous NaOH. Concentration in vacuo afforded cyclohexenyl phosphate **1** as a white foam. ^{1}H NMR (D_2O, pH 7.7): δ 5.74 (s, 1 H), 4.52 (dd, J = 13, 8 Hz, 1 H), 4.23 (dd, J = 13, 6 Hz, 1 H), 4.21 (d, J = 8 Hz, 1 H), 3.93 (ddd, J = 13, 8, 5 Hz, 1 H), 2.09 (dd, J = 13, 13 Hz, 1 H), 2.03 (dd, J = 13, 5 Hz, 1 H). ^{13}C NMR (D_2O, pH 7.7): δ 176.6, 135.8 (J_{POCC} = 6 Hz), 121.5, 69.4, 67.0, 64.9, 59.7, 34.8. HRMS (FAB) calcd for $C_8H_{11}O_9Na_2P$ (M + H^+): 329.0014. Found: 329.0024.

Protected Cyclohexenyl Tricarboxylate 17. Diethyl malonate (0.20 mL, 1.3 mmol) was carefully added to a suspension of NaH (60% w/w dispersion in mineral oil, 0.046 g, 1.1 mmol) in anhydrous THF (5 mL) at rt. After 5 min, allylic bromide **15** (0.490 g, 1.05 mmol) in THF (5 mL) was added to the clear solution at rt. After 16 h, saturated aqueous NH_4Cl was added, followed by water and ether. The organic layer was dried and concentrated to a yellow oil. Purification by radial chromatography (2 mm thickness, hexane, hexane/EtOAc, 5:1, v/v) afforded protected cyclohexenyl tricarboxylate **17** as a colorless oil (0.393 g, 68%). ^{1}H NMR ($CDCl_3$): δ 5.35 (s, 1 H), 4.00−4.10 (m, 5 H), 3.86 (ddd, J = 13, 9, 4 Hz, 1 H), 3.58 (s, 3 H), 3.52 (dd, J = 9, 7 Hz, 1 H), 3.20 (s, 3 H), 3.14 (s, 3 H), 2.78 (dd, J = 15, 7 Hz, 1 H), 2.56 (dd, J = 15, 9 Hz, 1 H), 2.00 (dd, J = 13, 13 Hz, 1 H), 1.88 (dd, J = 13, 4 Hz, 1 H), 1.22 (s, 3 H), 1.19 (s, 3 H), 1.10−1.20 (m, 6 H), 0.00 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 173.5, 168.6, 137.7, 125.7, 100.3, 99.5, 75.7, 69.8, 65.3, 61.2, 61.1, 52.1, 50.2, 48.0, 47.6, 30.0, 17.6 (2), 13.8, 1.6. Anal. Calcd for $C_{25}H_{42}O_{11}Si$: C, 54.92; H, 7.74. Found: C, 55.21; H, 7.78.

Cyclohexenyl Tricarboxylate 2. Protected cyclohexenyl tricarboxylate **17** (0.352 g, 0.64 mmol) was stirred in CF_3CO_2H/H_2O (20:1, v/v, 5 mL) for 3 h. After concentration the brown residue was dissolved in aqueous NaOH (0.2 N, 20 mL) and THF (3 mL). After 20 h, the solution was passed down Dowex 50 (H^+), and the pH was adjusted

to 7.7 with aqueous NaOH. Concentration afforded cyclohexenyl tricarboxylate **2** as a white foam (0.228 g, 100%). ^{1}H NMR (D_2O, pH 7.7): δ 5.43 (s, 1 H), 4.01 (d, J = 8 Hz, 1 H), 3.87 (ddd, J = 13, 8, 4 Hz, 1 H), 3.34 (dd, J = 7, 7 Hz, 1 H), 2.50−2.65 (m, 2 H), 2.09 (dd, J = 13, 13 Hz, 1 H), 1.98 (dd, J = 13, 4 Hz, 1 H). ^{13}C NMR (D_2O, pH 7.7): δ 183.9, 181.9, 144.1, 127.7, 77.0, 75.9, 72.6, 59.6, 41.6, 35.5. HRMS (FAB) calcd for $C_{11}H_{11}O_9Na_3$ (M + H^+): 357.0174. Found: 357.0184.

Cyclohexenyl Phosphonate 3. A solution of allylic bromide **15** (0.561 g, 1.20 mmol) and $(iPrO)_3P$ (2.0 mL, 8.1 mmol) in toluene (10 mL) was refluxed under Ar for 25 h. After cooling, the reaction mixture was concentrated in vacuo to afford **18** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 5.67 (d, J = 6 Hz, 1 H), 4.55−4.80 (m, 2 H), 4.20−4.25 (m, 1 H), 3.97 (ddd, J = 13, 9, 4 Hz, 1 H), 3.71 (s, 3 H), 3.35 (s, 3 H), 3.27 (s, 3 H), 3.02 (dd, J = 21, 15 Hz, 1 H), 2.45 (dd, J = 23, 15 Hz, 1 H), 2.23 (dd, J = 13, 13 Hz, 1 H), 1.95−2.05 (m, 1 H), 1.20−1.40 (m, 18 H), 0.13 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 173.5, 132.8 (J_{PCC} = 10 Hz), 127.8 (J_{PCCC} = 11 Hz), 100.5, 99.7, 76.1, 70.3 (J_{POC} = 7 Hz), 70.1 (J_{POC} = 7 Hz), 69.6, 65.6, 52.2, 48.3, 47.8, 37.6, 27.4 (J_{PC} = 140 Hz), 23.9 (J_{POCC} = 3 Hz), 17.8, 1.8. Anal. Calcd for $C_{24}H_{45}O_{10}PSi$: C, 52.16; H, 8.21. Found: C, 52.10; H, 8.24.

The oil was dissolved in CH_2Cl_2 (20 mL) and stirred with CF_3CO_2H/H_2O (20:1, v/v, 5 mL) at 0 °C. After 2 h, the solution was concentrated in vacuo, and the solid residue was redissolved in CH_2Cl_2 (20 mL). Anhydrous Et_3N (1.0 mL, 7.2 mmol) and TMSBr (2.0 mL, 15.2 mmol) were added at 0 °C. After 16 h at rt, the brown reaction mixture was concentrated in vacuo. Aqueous NaOH (0.2 N, 20 mL) and THF (10 mL) were then added, and the resulting heterogeneous mixture was stirred vigorously at rt. After 24 h, the mixture was passed down a short column of Dowex 50 (H^+), the eluant was washed with CH_2Cl_2 (2×) and Et_2O (1×), and the pH was adjusted to 7.1 with aqueous NaOH. The solution was applied to AG-1 X8 anion exchange resin (20 mL) which had been equilibrated with 200 mM $Et_3NH^+HCO_3^-$ (pH 7.5). The column was washed with water (40 mL) and eluted with a linear gradient (250 mL + 250 mL, 200−500 mM) of $Et_3NH^+HCO_3^-$ (pH 7.5). Fractions containing phosphorus were identified by the methods of Avila and Ames[33] and then concentrated to dryness. The resulting white residue was azeotroped six times with 2-propanol, dissolved in water, and passed down a short column of Dowex 50 (H^+). The eluant was neutralized to pH 7.5 with 0.1 N aqueous NaOH. Concentration in vacuo afforded cyclohexenyl phosphonate **3** as a white foam (0.218 g, 57%). ^{1}H NMR (D_2O, pH 7.5): δ 5.46 (s, 1 H), 4.14 (dd, J = 8, 2 Hz, 1 H), 3.88 (ddd, J = 11, 8, 4 Hz, 1 H), 2.59 (dd, J = 21, 14 Hz, 1 H), 2.40 (dd, J = 19, 14 Hz, 1 H), 1.95−2.15 (m, 2 H). ^{13}C NMR (D_2O, pH 7.5): δ 183.8, 141.0 (J_{PCC} = 9 Hz), 128.5 (J_{PCCC} = 10 Hz), 77.0, 76.5, 72.2, 42.2, 36.8 (J_{PC} = 122 Hz). HRMS (FAB) calcd for $C_8H_{11}O_8Na_2P$ (M + H^+): 313.0065. Found: 313.0054.

Protected Cyclohexenyl Homophosphonate 19. A solution of nBuLi in hexane (1.6 M, 5.1 mL, 8.1 mmol) was added dropwise to diisopropyl methanephosphonate (1.47 g, 8.13 mmol) in THF (20 mL) at −78 °C. The mixture was stirred for 20 min, and CuCN (0.364 g, 4.07 mmol) was rapidly added. After 5 min, the dry ice/acetone bath was replaced with an ice bath and stirring was continued at 0 °C. After 30 min, a solution of allylic bromide **15** (1.58 g, 3.39 mmol) in THF (20 mL) was added via cannula. The initially heterogeneous mixture slowly became clear, and after 11 h at 0 °C, saturated aqueous NH_4Cl was added. The mixture was extracted with EtOAc (2×), and the combined organic layers were dried and concentrated to a yellow oil. Purification by radial chromatography (4 mm thickness, hexane/EtOAc, 1:1, v/v, EtOAc) afforded two major components. The slower eluting methyl ester was obtained as a yellow oil (0.63 g, 33%). ^{1}H NMR ($CDCl_3$): δ 5.43 (s, 1 H), 4.60−4.75 (m, 2 H), 4.14 (d, J = 9 Hz, 1 H), 4.00 (ddd, J = 13, 9, 4 Hz, 1 H), 3.73 (s, 3 H), 3.28 (s, 3 H), 3.27 (s, 3 H), 2.40−2.50 (m, 2 H), 2.15 (dd, J = 13, 13 Hz, 1 H), 2.03 (dd, J = 13, 4 Hz, 1 H), 1.75−1.90 (m, 2 H), 1.30−1.35 (m, 18 H), 0.12 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 173.8, 140.9 (J_{PCCC} = 19 Hz), 123.5, 100.3, 99.6, 75.8, 70.2, 69.9 (J_{POC} = 6 Hz), 65.5, 52.3, 48.0, 47.8, 37.6, 25.3 (J_{PC} = 141 Hz), 23.9 (J_{POCC} = 3 Hz), 23.8, 17.7, 1.8. Anal. Calcd for $C_{25}H_{47}O_{10}PSi$: C, 52.98; H, 8.36. Found: C, 53.06; H, 8.41. The faster eluting fraction provided the isopropyl ester as a yellow oil (0.56 g, 29%). ^{1}H NMR ($CDCl_3$): δ 5.42 (s, 1 H), 4.60−4.75 (m, 3 H), 4.14 (d, J = 9 Hz, 1 H), 4.00 (ddd, J = 13, 9, 4 Hz, 1 H), 3.29 (s, 3 H), 3.27 (s, 3 H), 2.30−2.50 (m, 2 H), 2.14 (dd, J = 13, 13 Hz, 1 H), 1.95−2.10 (m, 1 H), 1.80−1.95 (m, 2 H), 1.30−1.35 (m, 18 H), 1.26 (d, J = 6 Hz, 3 H), 1.25 (d, J = 6 Hz, 3 H), 0.13 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 172.8, 140.6 (J_{PCCC} = 19 Hz), 123.7, 100.2, 99.5, 75.8, 70.2, 69.8 (J_{POC} = 7 Hz), 68.9, 65.5, 48.0, 47.7, 37.6, 25.4 (J_{PC} = 141 Hz), 23.9 (J_{POCC} = 4 Hz), 21.5, 17.7, 1.9.

Cyclohexenyl Homophosphonate 4. Fully protected cyclohexenyl homophosphonate **19** (0.56 g, 0.98 mmol) in CH_2Cl_2 (10 mL) was stirred with CF_3CO_2H/H_2O (20:1, v/v, 10 mL) at rt. After 45 min, the solution was concentrated in vacuo and azeotroped with toluene (3×). The crude material was dissolved in CH_2Cl_2 (10 mL), and then Et_3N (1.0 mL, 7.2 mmol) and TMSBr (2.0 mL, 15.2 mmol) were added at rt. After 18 h, the brown reaction mixture was concentrated in vacuo. Aqueous NaOH (0.5 N, 20 mL) was added, and the resulting solution was stirred vigorously at rt. After 25 h, the solution was passed down a short column of Dowex 50 (H^+). After the pH was adjusted 7.3 with aqueous NaOH, the solution was purified by anion exchange chromatography as described for cyclohexenyl phosphonate **3**. Cyclohexenyl homophosphonate **4** was obtained as a colorless film (0.153 g, 48%). ^{1}H NMR (D_2O, pH 7.6): δ 5.48 (s, 1 H), 4.05 (d, J = 8 Hz, 1 H), 3.89 (ddd, J = 13, 8, 5 Hz, 1 H), 2.30−2.40 (m, 2 H), 1.90−2.10 (m, 2 H), 1.50−1.80 (m, 2 H). ^{13}C NMR (D_2O, pH 7.6): δ 184.1, 146.2 (J_{PCCC} = 16 Hz), 126.4, 76.8, 75.6, 72.5, 41.7, 29.4, 29.4 (J_{PC} = 131 Hz). HRMS (FAB) calcd for $C_9H_{13}O_8Na_2P$ (M + H^+): 327.0222. Found: 327.0217.

Cyclohexylidene Phosphonate 9. Protected cyclohexylidene phosphonate **20**[6] (0.261g, 0.615 mmol) was refluxed in 6 N HCl for 2 h. After the solvent was removed in vacuo, the residue was dissolved in water and neutralized. Purification by anion exchange chromatography was conducted as described for cyclohexenyl phosphonate **3** to afford cyclohexylidene phosphonate **9** as a white foam (0.125 g, 65%). ^{1}H NMR (D_2O, pH 7.5): δ 5.93 (d, J = 13 Hz), 3.97 (d, J = 9 Hz, 1 H), 3.61 (ddd, J = 13, 9, 4 Hz, 1 H), 3.40 (d, J = 14 Hz, 1 H), 1.90−2.10 (m, 3 H). ^{13}C NMR (D_2O, pH 7.5): δ 184.7, 150.3 (J_{PCC} = 6 Hz), 123.4 (J_{PC} = 171 Hz), 79.8 (J_{PCCC} = 16 Hz), 78.8, 74.8, 43.4, 40.8 (J_{PCCC} = 7 Hz). HRMS (FAB) calcd for $C_8H_{11}O_8Na_2P$ (M + H^+): 313.0065. Found: 313.0062.

Allylic Acetate 21. Cerium chloride heptahydrate (11.5 g, 30.8 mmol) was dried in vacuo at 145 °C for 2 h. Ar was introduced while the flask was still hot. After the reaction mixture was cooled to 0 °C, THF (25 mL) was added and the white suspension was stirred overnight at rt. The suspension was cooled to −78 °C, and vinylmagnesium bromide in THF (1.0 M, 26 mL, 26 mmol) was added via syringe. Stirring was continued for 2 h, and then a solution of silylated (see Exocyclic Alkene **14**) DHQ **13** (4.00 g, 10.2 mmol) in THF (25 mL) was added via cannula. After an additional 4 h at −78 °C, acetic anhydride (6.0 mL, 64 mmol) was added neat via syringe. The resulting mixture was stirred at −78 °C for 1.5 h and then at rt for 2 h. The thick reaction mixture was poured into saturated aqueous $NaHCO_3$, and Et_2O was added. The aqueous suspension was extracted with Et_2O (1×), and the combined organic layers were washed with saturated aqueous Na_2CO_3 (2×) and brine (1×). Drying over $MgSO_4$ and concentrating afforded a yellow liquid. Purification by radial chromatography (4 mm thickness, hexane, hexane/EtOAc, 5:1, 1:1, v/v) afforded allylic acetate **21** (4.19 g, 89%) as a slightly yellow gum. ^{1}H NMR ($CDCl_3$): δ 6.10 (dd, J = 18, 11 Hz, 1 H), 5.19 (d, J = 11 Hz, 1 H), 5.13 (d, J = 18 Hz, 1 H), 4.34 (ddd, J = 12, 10, 5 Hz, 1 H), 3.73 (s, 3 H), 3.40 (d, J = 10 Hz, 1 H), 3.30 (s, 3 H), 3.17 (s, 3 H), 2.00−2.30 (m, 3 H), 2.08 (s, 3 H), 1.78 (d, J = 15 Hz, 1 H), 1.33 (s, 3 H), 1.29 (s, 3 H), 0.14 (s, 9 H). ^{13}C NMR ($CDCl_3$): δ 174.3, 170.1, 139.3, 113.9, 100.1, 99.1, 80.9, 77.3, 77.0, 63.4, 52.3, 47.9, 47.8, 38.4, 22.5, 17.8, 17.7, 1.6. Anal. Calcd for $C_{21}H_{36}O_9Si$: C, 54.76; H, 7.88. Found: C, 54.85; H, 7.89.

Diol 22. Allylic acetate **21** (1.40 g, 3.03 mmol) was dissolved in MeOH (10 mL), and Dowex 50 (H^+) (0.2 g) was added. After 5 h at rt, the suspension was filtered, and the solvent was evaporated to afford a foamy gum. THF (30 mL) was added, followed by $PdCl_2(CH_3CN)_2$ (0.038 g, 0.15 mmol). The resulting orange solution was stirred overnight at rt. After concentration of the solution under reduced pressure, the residue was taken up in EtOAc, and the solution washed with brine (2×). Drying over $MgSO_4$ and concentrating afforded an orange oil. MeOH (30 mL) and NaOMe (0.158 g, 2.92 mmol) were added. After 2 h at rt, Dowex 50 (H^+) was added, and the suspension

was filtered. Concentration afforded a light brown foam. Purification by radial chromatography (2 mm thickness, hexane/EtOAc, 1:1, v/v, EtOAc) afforded diol **22** (0.561 g, 53%) as a white solid: mp = 110−111 °C. ^{1}H NMR ($CDCl_3$): δ 6.11 (dd, J = 8, 7 Hz, 1 H), 4.21 (dd, J = 11, 8 Hz, 1 H), 4.00−4.10 (m, 2 H), 3.80−3.90 (m, 1 H), 3.80 (s, 3 H), 3.25 (s, 3 H), 3.22 (s, 3 H), 2.84 (dd, J = 14, 2 Hz, 1 H), 2.65 (br, 2 H), 2.33 (d, J = 14 Hz, 1 H), 1.96 (ddd, J = 13, 5, 2 Hz, 1 H), 1.35 (s, 3 H), 1.30 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 175.2, 134.4, 122.8, 99.9, 99.4, 74.1, 72.4, 68.7, 57.3, 53.0, 47.9, 47.8, 37.8, 36.4, 17.7 (2). HRMS (FAB) calcd for $C_{16}H_{26}O_8$ (M + Na^+): 369.1525. Found: 369.1528.

Bromide 23. Diol **22** (1.15 g, 3.32 mmol) was dissolved in anhydrous CH_3CN (50 mL) and PPh_3 (0.959 g, 3.65 mmol) followed by CBr_4 (1.270 g, 3.65 mmol) added at rt under Ar. After 3 h, the solution was concentrated under reduced pressure, and the resulting oil was purified by radial chromatography (4 mm thickness, hexane, hexane/EtOAc, 5:1, 1:1, v/v) to afford allylic bromide **23** (1.36 g, 100%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 6.15 (dd, J = 8, 7 Hz, 1 H), 4.05−4.15 (m, 2 H), 3.96 (dd, J = 10, 7 Hz, 1 H), 3.86 (ddd, J = 13, 10, 5 Hz, 1 H), 3.82 (s, 3 H), 3.25 (s, 3 H), 3.22 (s, 3 H), 3.06 (br, 1 H), 2.78 (dd, J = 14, 2 Hz, 1 H), 2.36 (d, J = 14 Hz, 1 H), 2.08 (dd, J = 13, 13 Hz, 1 H), 1.93 (ddd, J = 13, 5, 2 Hz, 1 H), 1.36 (s, 3 H), 1.30 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 175.4, 136.5, 119.8, 99.9, 99.4, 74.2, 72.3, 68.2, 53.2, 48.0, 47.9, 37.8, 36.3, 27.0, 17.7 (2). HRMS (FAB) calcd for $C_{16}H_{25}O_7Br$ (M + Na^+): 431.0681. Found: 431.0682.

Protected Cyclohexylidene Homophosphonate 24. A solution of bromide **23** (1.36, 3.32 mmol) and ($iPrO_3$)P (3.74 g, 18.0 mmol) in anhydrous toluene (20 mL) was refluxed for 24 h under Ar. The reaction mixture was then concentrated in vacuo with heating to afford a partially crystalline yellow residue. Purification by radial chromatography (4 mm thickness, hexane/EtOAc, 1:1, v/v, EtOAc) afforded protected homophosphonate **24** (0.776 g, 47%) as a white solid: mp = 129−132 °C. ^{1}H NMR ($CDCl_3$): δ 6.01 (br, 1 H), 5.74 (ddd, J = 8, 7, 7 Hz, 1 H), 4.55−4.80 (m, 2 H), 4.00 (dd, J = 10, 7 Hz, 1 H), 3.90 (ddd, J = 12, 10, 5 Hz, 1 H), 3.76 (s, 3 H), 3.23 (s, 3 H), 3.16 (s, 3 H), 2.98 (dd, J = 14, 2 Hz, 1 H), 2.65 (ddd, J = 22, 14, 8 Hz, 1 H), 2.47 (ddd, J = 23, 14, 7 Hz, 1 H), 2.36 (dd, J = 14, 7 Hz, 1 H), 2.12 (ddd, J = 13, 5, 2 Hz, 1 H), 1.93 (dd, J = 13, 12 Hz, 1 H), 1.25−1.35 (m, 18 H). ^{13}C NMR ($CDCl_3$): δ 175.1, 136.3 (J_{PCCC} = 13 Hz), 111.2 (J_{PCC} = 12 Hz), 99.6, 99.2, 74.9 (J_{PCCCCC} = 2 Hz), 72.5 (J_{PCCCCC} = 4 Hz), 72.3 (J_{PCCCC} = 7 Hz), 70.4 (J_{POC} = 8 Hz), 68.1 (J_{POC} = 6 Hz), 52.3, 47.7, 47.5, 37.4, 35.8 (J_{PCCCC} = 10 Hz), 25.6 (J_{PC} = 139 Hz), 23.9 (J_{POCC} = 5 Hz), 23.8 (J_{POCC} = 3 Hz), 23.5 (J_{POCC} = 5 Hz), 23.2 (J_{POCC} = 3 Hz), 17.6, 17.5. Anal. Calcd for $C_{22}H_{39}O_{10}P$: C, 53.43; H, 7.95. Found: C, 53.29; H, 7.90.

Cyclohexylidene Homophosphonate 10. Protected cyclohexylidene homophosphonate **24** (0.758 g, 1.53 mmol) in CH_2Cl_2 (10 mL) was stirred with CF_3CO_2H/H_2O (20:1, v/v, 10 mL) at rt. After 1.5 h, the solution was concentrated in vacuo, and the residue was azeotroped with toluene (3×). The crude product was dissolved in CH_2Cl_2 (20 mL), and then Et_3N (1.0 mL, 7.2 mmol) and TMSBr (2.0 mL, 15 mmol) were added at rt. After 24 h, the brown reaction mixture was concentrated in vacuo. Aqueous NaOH (0.5 N, 20 mL) was added, and the resulting solution was stirred vigorously at rt. After 24 h, the solution was passed down a short column of Dowex 50 (H^+). After the pH was adjusted to 7.2 with aqueous NaOH, the solution was concentrated to afford cyclohexylidene homophosphonate **10** as a white solid (0.216 g, 50%). ^{1}H NMR (D_2O, pH 7.2): δ 5.74 (ddd, J = 7, 6, 6 Hz, 1 H), 3.98 (dd, J = 7, 7 Hz, 1 H), 3.63 (ddd, J = 15, 7, 5 Hz, 1 H), 2.75 (d, J = 14 Hz, 1 H), 2.25−2.45 (m, 3 H), 1.90−2.10 (m, 2 H). ^{13}C NMR (D_2O, pH 7.2): δ 184.3, 137.6 (J_{PCCC} = 13 Hz), 119.8 (J_{PCC} = 11 Hz), 79.3, 78.5, 74.4, 43.2, 38.3, 30.9 (J_{PC} = 127 Hz); HRMS (FAB) calcd for $C_9H_{15}O_8P$ (M + H^+): 283.0583. Found: 283.0558.

Iodide 25. Iodine (1.82 g, 7.17 mmol) was added to a mixture of diol **12**[5] (1.10 g, 3.43 mmol), PPh_3 (1.82 g, 6.94 mmol), and HMPA (2.4 mL, 14 mmol) in benzene (30 mL).[16] The dark mixture was stirred for 12 h at rt. Aqueous $NaHCO_3$ and ether were added, and the organic layer was washed successively with aqueous $Na_2S_2O_3$ (1×), water (1×), and brine (1×). Drying and concentrating afforded a yellow residue. Most Ph_3PO was removed by crystallization from ether and hexane. Purification by radial chromatography (4 mm thickness, hexane/EtOAc, 5:1, 1:1, v/v) afforded iodide **25** as a white foam (0.634 g, 41%). ^{1}H NMR ($CDCl_3$): δ 4.25 (ddd, J = 12, 12, 5 Hz, 1 H), 3.99 (ddd, J = 12, 9, 5 Hz, 1 H), 3.81 (s, 3 H), 3.62 (dd, J = 12, 9 Hz, 1 H), 3.38 (s, 3 H), 3.25 (s, 3 H), 3.00−3.25 (br, 1 H), 2.49 (dd, J = 12, 12 Hz, 1 H), 2.39 (ddd, J = 12, 5, 3 Hz, 1 H), 2.03 (dd, J = 12, 12 Hz, 1 H), 1.92 (ddd, J = 12, 5, 3 Hz), 1.36 (s, 3 H), 1.30 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 174.7, 100.7, 99.9, 76.2, 75.3, 66.0, 53.4, 48.3, 48.0, 46.7, 38.0, 22.7, 17.6, 17.4. Anal. Calcd for $C_{14}H_{23}IO_7$: C, 39.08; H, 5.39. Found: C, 39.14; H, 5.33.

TBDMS Ether 26. Iodide **25** (4.26 g, 9.54 mmol) was dissolved in CH_2Cl_2 (20 mL), and anhydrous Et_3N (1.6 mL, 11 mmol) was added. The resulting solution was cooled to 0 °C, and TBDMSOTf (2.2 mL, 9.6 mmol) was added dropwise under Ar. After the reaction mixture was stirred at rt for 43 h, water and Et_2O were added. The resulting organic layer was washed with water (1×), aqueous $CuSO_4$ (1×), and brine (1×). Drying, concentration, and purification by radial chromatography (4 mm thickness, hexane/EtOAc, 5:1, v/v) afforded TBDMS ether **26** as a yellow oil (3.67 g, 69%). ^{1}H NMR ($CDCl_3$): δ 4.18 (ddd, J = 12, 11, 4 Hz, 1 H), 3.95 (ddd, J = 12, 9, 5 Hz, 1 H), 3.72 (s, 3 H), 3.56 (dd, J = 11, 9 Hz, 1 H), 3.36 (s, 3 H), 3.23 (s, 3 H), 2.60 (ddd, J = 13, 4, 3 Hz, 1 H), 2.40 (dd, J = 13, 12 Hz, 1 H), 2.10 (ddd, J = 13, 5, 3 Hz, 1 H), 1.98 (dd, J = 13, 12 Hz), 1.36 (s, 3 H), 1.30 (s, 3 H), 0.90 (s, 9 H), 0.09 (s, 3 H), 0.07 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 172.6, 100.7, 99.7, 78.3, 76.3, 66.0, 52.3, 48.3, 48.0, 47.9, 39.0, 25.9, 23.2, 18.5, 17.7, 17.4, −3.4, −3.7. Anal. Calcd for $C_{20}H_{37}IO_7Si$: C, 44.11; H, 6.85. Found: C, 44.29; H, 6.86.

Protected Cyclohexyl Tricarboxylate 27. TBDMS ether **26** (0.501 g, 0.920 mmol) and di-*tert*-butyl methylenemalonate[17] (0.299 g, 1.20 mmol) were dissolved in distilled benzene (15 mL) and Ar was bubbled through the solution for 15 min. The solution was heated to reflux under Ar, and a solution of tris(trimethylsilyl)silane (TTMSS, 0.250 g, 1.01 mmol) and AIBN (0.033 g, 0.20 mmol) in benzene (5 mL) was added over 2.25 h via syringe pump (0.45 mmol of TTMSS/h) through the top of the reflux condenser. Once the addition was complete, the reaction mixture was refluxed for an additional 1.5 h. Heating was stopped, and the solvent removed under reduced pressure. Purification by radial chromatography (2 mm thickness, hexane, hexane/EtOAc, 5:1, v/v) afforded protected cyclohexyl tricarboxylate **27** as a white foam (0.43 g, 72%). ^{1}H NMR ($CDCl_3$): δ 3.94 (ddd, J = 12, 9, 4 Hz, 1 H) 3.68 (s, 3 H), 3.65−3.75 (m, 1 H), 3.34 (dd, J = 7, 6 Hz, 1 H), 3.26 (s, 3 H), 3.23 (s, 3 H), 2.29 (ddd, J = 14, 6, 5 Hz, 1 H), 1.80−2.05 (m, 5 H), 1.50−1.70 (m, 1 H), 1.46 (s, 9 H), 1.44 (s, 9 H), 1.31 (s, 3 H), 1.28 (s, 3 H), 0.90 (s, 9 H), 0.09 (s, 3 H), 0.08 (s, 3 H). ^{13}C NMR ($CDCl_3$): δ 173.9, 169.1, 168.6, 99.4, 99.1, 81.1, 76.3, 75.2, 66.7, 52.1, 51.8, 47.7, 40.5, 38.5, 33.4, 30.3, 27.7, 25.9, 18.4, 17.7 (2), −3.4, −3.7. Anal. Calcd for $C_{32}H_{58}IO_{11}Si$: C, 59.78; H, 9.09. Found: C, 59.51; H, 8.98.

Cyclohexyl Tricarboxylate 6. Protected cyclohexyl tricarboxylate **27** (0.40 g, 0.63 mmol) was stirred in CF_3CO_2H/H_2O (20:1, v/v, 10 mL) for 3 h. After the solution was concentrated, the residue was dissolved in water (10 mL), and the resulting solution was washed with CH_2Cl_2 (1×) and Et_2O (1×). Aqueous NaOH (1 N, 10 mL) was then added to the aqueous layer, and the mixture was refluxed for 9 h. The cool reaction mixture was washed with Et_2O (1×), and the resulting aqueous phase was brought to pH 7.3 by the addition of Dowex 50 (H^+) in small portions. Concentration afforded cyclohexyl tricarboxylate **6** as a white foam (0.169 g, 75%). ^{1}H NMR (D_2O, pH 7.3): δ 3.70 (ddd, J = 12, 9, 5 Hz, 1 H), 3.14 (dd, J = 9, 9 Hz, 1 H), 3.05−3.20 (m, 1 H), 2.20−2.35 (m, 1 H), 1.96 (ddd, J = 13, 5, 3 Hz, 1 H), 1.82 (dd, J = 13, 12 Hz, 1 H), 1.50−1.85 (m, 3 H), 1.56 (dd, J = 13, 12 Hz, 1 H). ^{13}C NMR (D_2O, pH 7.3): δ 188.4, 186.0, 185.7, 84.4, 81.6, 77.3, 62.0, 46.5, 44.2, 42.7, 38.4. HRMS (FAB) calcd for $C_{11}H_{13}O_9Na_3$ (M + H^+): 359.0331. Found: 359.0333.

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