Inhibitor Ionization as a Determinant of Binding to 3-Dehydroquinate Synthase

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Phosphinomethyl and carboxymethyl monoacids along with succinyl, malonyl ether, malonyl, and hydroxymalonyl diacids were substituted for phosphorylmethyl, phosphonoethyl, and phosphonomethyl groups in carbocyclic inhibitors of DHQ synthase. All but one of the carbocyclic inhibitors were synthesized via intermediacy of a 2,3-butane bisacetal-protected 3-dehydroquinic acid. Carbaphosphinate ($K_i = 20 \times 10^{-6}$ M) was a modest competitive inhibitor of DHQ synthase, while carbaacetate was a linear mixed-type inhibitor ($K_i = 3 \times 10^{-6}$ M, $K_i' = 20 \times 10^{-6}$ M). Carbasuccinate ($K_i = 5 \times 10^{-6}$ M), carbamalonate ether ($K_i = 7 \times 10^{-6}$ M), carbamalonate ($K_i = 0.7 \times 10^{-6}$ M), and carbahydroxymalonate ($K_i = 0.3 \times 10^{-6}$ M) were all competitive inhibitors. Carbaacetate was the only inhibitor that was not oxidized by DHQ synthase. On the basis of these data, carbocyclic inhibitors with malonyl and hydroxymalonyl groups are apparently bound by DHQ synthase as tightly as carbocyclic inhibitors possessing phosphorylmethyl and phosphonoethyl moieties.

Comparatively little attention[1] has been given to the impact of inhibitor ionization on binding to the active site of 3-dehydroquinate (DHQ) synthase (Scheme 1). To explore the inhibitory potency of dianionic analogues of substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP), carbaphosphinate **1** and carbaacetate **2** have been synthesized (Chart 1). Carbasuccinate **3**, carbamalonate ether **4**, carbamalonate **5**, and carbahydroxymalonate **6** were synthesized as trianionic carbocyclic substrate analogues (Chart 1). Carbacarboxylate **7** and spirocyclic carbaphosphodiester **12** are the only examples (Chart 1) in the literature[1b,2] of dianionic carbocyclic DHQ synthase inhibitors. Trianionic carbocyclic inhibitors of DHQ synthase include (Chart 1) carbocyclic phosphonates and phosphates having C-5 stereochemistry identical (**8**−**11**)[1a,3] and epimeric (**13**−**6**)[4] to the stereochemistry at C-6 of DAHP. Yet another example of a carbocyclic inhibitor having an accessible trianionic ionization state is C-1 epimeric carbaphosphonate **17**.[5] Newly synthesized carbocyclic tricarboxylates **3**−**6** are the first examples of trianionic DHQ synthase inhibitors possessing neither a phosphate monoester nor phosphonic acid.

A protected DHQ derivative proved to be an exceptionally useful intermediate in the syntheses of carbaphosphinate **1**, carbasuccinate **3**, carbamalonate ether **4**, carbamalonate **5**, and carbahydroxymalonate **6**. The utility of protected DHQ intermediacy was further demonstrated in the shortest, highest-yielding synthesis yet achieved of carbaphosphonate **11**, which is the most potent inhibitor known[3a] for DHQ synthase. Binding to DHQ synthase was evaluated by the magnitude of the measured inhibition constants, by the formation or absence of enzyme-bound NADH during inhibition, and by whether inhibition was competitive relative to substrate DAHP binding. Of the newly synthesized carbocyclic inhibitors, carbamalonate **5** and carbahydroxymalonate **6** were the most potent inhibitors with K_i values in the submicromolar range. DHQ synthase inhibition by carbamalonate **5** and carbahydroxymalonate **6** sug-

Scheme 1

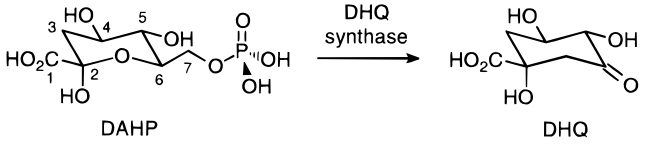
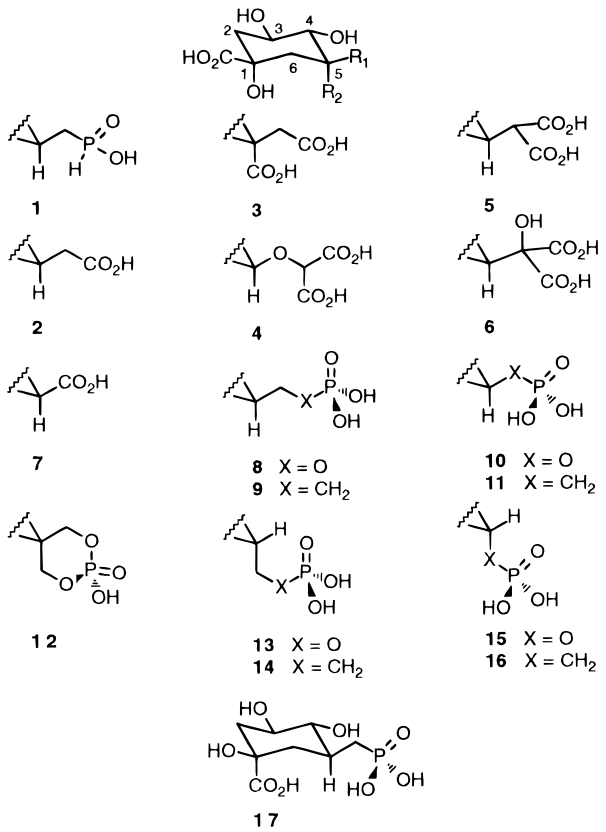


Chart 1



[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1996.

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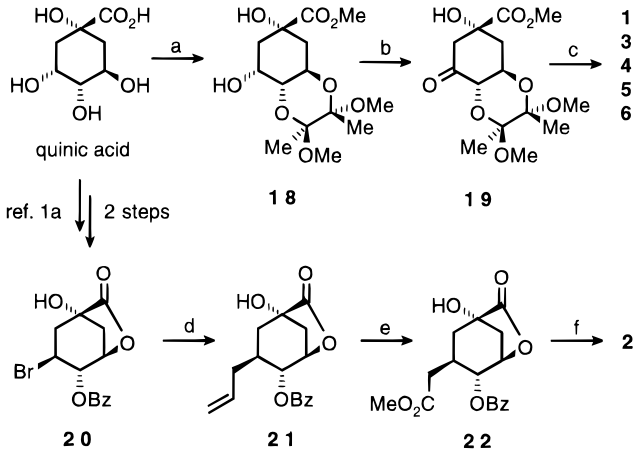
Scheme 2[a]



[a] Key: (a) (i) CH_3OH, Dowex 50 (H^+), reflux, (ii) 2,2,3,3-tetramethoxybutane, $CH(OCH_3)_3$, CH_3OH, CSA, reflux, 87%; (b) KIO_4, K_2CO_3, $RuCl_3$, H_2O, $CHCl_3$, 77%; (c) see Schemes 3 and 4; (d) allyltributyltin, AIBN, C_6H_6, reflux; (e) (i) $NaIO_4$, $RuCl_3$, CCl_4/CH_3CN/H_2O (2:2:3, v/v/v), (ii) CH_2N_2, Et_2O, CH_3OH, 65%; (f) (i) 0.2 N aqueous NaOH/THF (1:1, v/v), (ii) Dowex 50 (H^+), 100%.

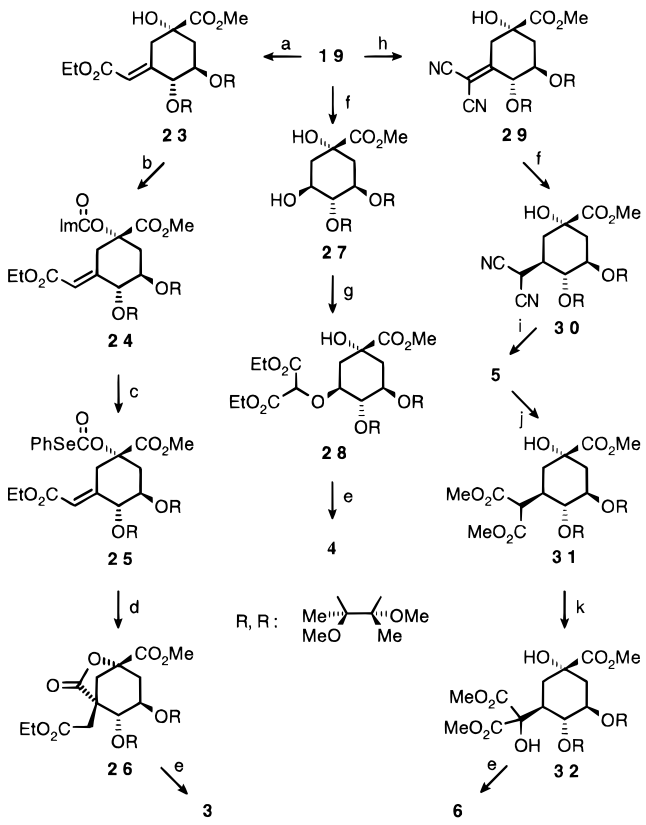
gests that C-5 malonyl and hydroxymalonyl moieties are effective mimics of C-5 phosphorylmethyl and phosphonoethyl groups in DHQ synthase inhibitors. This expansion in the types of structures that lead to in vitro DHQ synthase inhibition has ramifications important to ultimately achieving in vivo inhibition of this enzyme in pathogenic bacteria.

Results

Synthetic Strategy Options. Synthesis of carbocyclic DHQ synthase inhibitors **1** and **3**−**6** via protected DHQ intermediacy (Scheme 2) constitutes a significant departure from earlier syntheses of carbocyclic DHQ synthase inhibitors. Previous syntheses of such inhibitors have employed nucleophilic heteromethylation[5a,6] of oxiranes derived from quinic acid or routes strategically related[1a] to the one (Scheme 2) used to assemble carbaacetate **2** where bromo lactone **20** derived from quinic acid underwent Keck reaction[7] with allyltributyltin. Oxidation of the resulting C-5 allyl substituent of **21** with $NaIO_4$ and catalytic, in situ-generated RuO_4 was followed by esterification to expedite purification of the protected carbaacetate. Subsequent basic hydrolysis and neutralization afforded carbaacetate **2**.

Previous attempts to use DHQ as a starting material in the synthesis of carbocyclic DHQ synthase inhibitors failed due to DHQ's instability[8] under a variety of the conditions employed to selectively protect the molecule's hydroxyl groups. Access to protected DHQ has been made possible by use of the recently elaborated[9] 2,3-butane bisacetal (BBA) protecting group, which is highly selective for the protection of diequatorial diols. By selectively protecting the C-3 and C-4 hydroxyl groups of stable methyl quinate (Scheme 2), oxidation of the remaining secondary alcohol at C-5 provided a high-

Scheme 3[a]



[a] Key: (a) $Ph_3P{=}CHCO_2Et$, CH_3CN, reflux, 90%; (b) Im_2CO, $ClCH_2CH_2Cl$, reflux, 82%; (c) PhSeH, $ClCH_2CH_2Cl$, reflux, 87%; (d) Bu_3SnH, AIBN, C_6H_6, reflux, 27%; (e) (i) TFA/H_2O (20:1, v/v), (ii) 0.2 N aqueous NaOH/THF (1:1, v/v), (iii) Dowex 50 (H^+), 100%; (f) $NaBH(OAc)_3$, CH_3CN/HOAc (1:1, v/v), **27**: 86%, **30**: 92%; (g) $N_2{=}C(CO_2Et)_2$, $Rh_2(OAc)_4$, C_6H_6, reflux, 75%; (h) $CH_2(CN)_2$, NH_4OAc, HOAc, C_6H_6, 90%; (i) (i) TFA/H_2O (20:1, v/v), (ii) 6 N aqueous HCl, reflux, (iii) 0.2 N aq NaOH/THF (1:1, v/v), (iv) Dowex 50 (H^+), 95%; (j) (i) CH_2N_2, Et_2O, CH_3OH, (ii) 2,2,3,3-tetramethoxybutane, $CH(OCH_3)_3$, CH_3OH, CSA, reflux, 80% from **30**; (k) (i) $(CH_3)_3COK$, THF, 0 °C, (ii) 2-(benzenesulfonyl)-3-phenyloxaziridine, THF, −78 °C, 75%.

yielding "backdoor" route into the desired protected DHQ intermediate.

Starting material quinic acid was quantitatively converted (Scheme 2) into its methyl ester by refluxing in methanol with Dowex 50 (H^+). Reaction of methyl quinate with catalytic camphorsulfonic acid (CSA) and 2,2,3,3-tetramethoxybutane resulted in regioselective protection of the 3,4-*trans*-diol over the 4,5-*cis*-diol and afforded BBA-protected **18** in 87% yield. Oxidation of the C-5 hydroxyl group in **18** with tetrapropylammonium perruthenate[10] (TPAP) and 4-methylmorpholine *N*-oxide (NMO) furnished protected DHQ derivative **19** (Scheme 2). Large-scale (30 g) oxidations of **18** employed KIO_4 and catalytic $RuCl_3$ in $CHCl_3/H_2O$.

Tricarboxylate Syntheses. An essential feature of all tricarboxylate syntheses was the use of the C-1 alcohol to direct critical reactions. For example, the C-1 alcohol was left unprotected during reaction of DHQ intermediate **19** with (carbethoxymethylene)triphenylphosphorane (Scheme 3). Protection of the C-1 alcohol of DHQ intermediate **19** as a TMS ether gave a slower condensation reaction that was accompanied by dehydration involving elimination of the protected C-1 alcohol. Phos-

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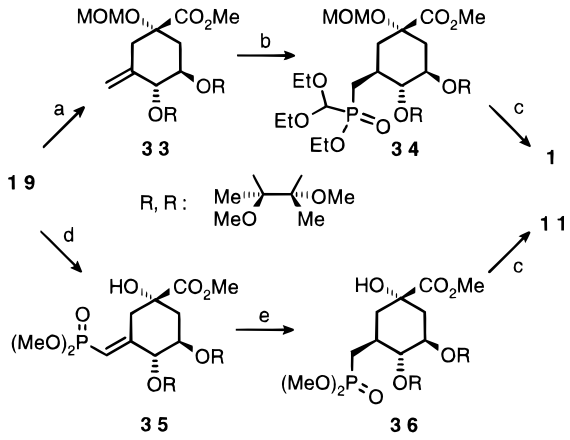
phorane complexation with the C-1 alcohol was likely responsible for directing and accelerating the rate of the condensation reaction.

The C-1 alcohol was also essential to establishing carbasuccinate's C-5 stereochemistry (Scheme 3) during radical cyclization of the C-1 phenylselenocarbonate **25**. Because of its steric environment, functionalization of the C-1 tertiary alcohol in dicarboxylate **23** required a two-step sequence. Initial reaction with 1,1′-carbonyldiimidazole was followed by interception of the imidazoyl carbamate **24** with phenylselenol to afford phenylselenocarbonate **25**. Radical cyclization provided lactone **26** in 27% yield.[11] Deoxygenation products epimeric at C-1 were also isolated which accounts for the modest yield of **26** and likely reflects C-1 carboxylate stabilization of a C-1 tertiary radical resulting from loss of CO_2.[12] The BBA protecting group was easily removed in 95% aqueous trifluoroacetic acid at room temperature. Subsequent hydrolysis of the esters and lactone under basic conditions followed by protonation using Dowex 50 (H^+) provided carbasuccinate **3**.

The C-5 stereochemistry of carbamalonate ether **4**, carbamalonate **5**, and carbahydroxymalonate **6** all depended on C-1 alcohol direction of $NaBH(OAc)_3$ reductions. For example (Scheme 3), reaction of DHQ intermediate **19** with $NaBH(OAc)_3$ exclusively gave *epi*-quinate **27**. The extremely high stereoselectivity of this reaction can best be explained by delivery of a hydride to the face of the carbonyl dictated by complexation of the C-1 alcohol with $NaBH(OAc)_3$.[1a,13] Coupling of *epi*-quinate **27** with diethyl diazomalonate to give protected malonate ether **28** was easily accomplished using $Rh_2(OAc)_4$ catalysis. Subsequent ester and BBA removal using the aforementioned reaction conditions yielded carbamalonate ether **4**.

Reduction of the activated olefin of α,β-unsaturated carbamalonitrile **29** to give carbamalonitrile **29** (Scheme 3) is also consistent with C-1 alcohol complexation of $NaBH(OAc)_3$.[13] α,β-Unsaturated carbamalonitrile **29** was obtained by reaction of DHQ intermediate **19** with malononitrile catalyzed by NH_4OAc and HOAc. Attempts to react DHQ intermediate **19** with diethyl malonate under basic conditions failed to yield a condensation product. Removal of the BBA protection group of **30** was followed by hydrolysis of the nitrile groups in refluxing 6 N hydrochloric acid, which also resulted in lactonization of the C-4 hydroxyl and malonyl carboxylate. This lactone was hydrolyzed under basic conditions prior to Dowex 50 (H^+) treatment to give the carbamalonate **5** triacid. Carbamalonate **5** was also converted into carbahydroxymalonate **6**. Esterification of **5** with CH_2N_2 and reprotection of the C-3,4 *trans*-diol provided triester **31**, which underwent oxaziridine hydroxylation[14] yielding protected hydroxymalonate **32**. TFA-catalyzed removal of the BBA protecting group, base-catalyzed hydrolysis of the methyl esters, and treatment with Dowex 50 (H^+) led to carbahydroxymalonate **6**.

Scheme 4[a]



[a] Key: (a) (i) $CH_2(OCH_3)_2$, P_2O_5, $CHCl_3$, (ii) CH_2I_2, Zn, $TiCl_4$, THF, 73%; (b) ethyl (diethoxymethyl)phosphinate, $(PhCOO)_2$, dioxane, reflux; (c) 6 N aqueous HCl, reflux, **1**: 19% from **33**, **11**: 100% from **36**; (d) *n*-BuLi, $(CH_3O)_2P(O)CH_2P(O)(OCH_3)_2$, THF, −78 °C to rt, 56%; (e) H_2, 10% Pd/C, EtOAc, 82%.

Phosphinate and Phosphonate Syntheses. The versatility of protected DHQ **19** as an intermediate in the synthesis of carbocyclic DHQ synthase inhibitors was further demonstrated (Scheme 4) with the synthesis of carbaphosphinate **1** and carbaphosphonate **11**. Protection of the C-1 alcohol of DHQ intermediate **19** as a methoxymethyl (MOM) ether was followed by reaction with $TiCl_4/Zn/CH_2I_2$ using the Takai procedure[15] to afford exocyclic olefin **33**. Radical addition of ethyl (diethoxymethyl)phosphinate[16] to exocyclic olefin **33** yielded masked phosphinate **34**. Acid hydrolysis simultaneously removed the BBA and MOM protecting groups, hydrolyzed the methyl ester, and unmasked the phosphinic acid. Product carbaphosphinate **1** was purified by anion exchange chromatography.

Assignment of the C-5 stereochemistry in carbaphosphinate **1** followed from analysis of the ^1H NMR coupling constants associated with the C-4 methine at δ 3.15. Coupling constants ($J = 9$ Hz) diagnostic of *trans*-diaxial relationships were observed between both the C-5 and C-4 methine protons and the C-3 and C-4 methine protons. The coupling constant ($J = 9$ Hz) between the C-5 and C-4 protons in **1** markedly differs from the smaller coupling constant ($J = 3$ Hz) observed in the C-5 epimeric series[4] between the C-5 and C-4 methine protons. Additional corroboration of the C-5 stereochemistry in carbaphosphinate **1** synthesized via Scheme 4 follows from independent synthesis of **1** from a C-5 bromomethyl intermediate with known C-5 stereochemistry. Arbuzov condensation of the in situ generated product[17] of ethyl (diethoxymethyl)phosphinate reaction with hexamethyldisilazane and previously synthesized[1a] methyl [1*S*-(1α,3β,4α,5β)]-5-(bromomethyl)-1,3,4-tris(benzoyloxy)cyclohexane-1-carboxylate in refluxing mesitylene followed by exhaustive hydrolysis afforded a product that was spectroscopically identical to carbaphosphinate **1**.

Carbaphosphonate **11** synthesis began with the condensation of DHQ intermediate **19** with tetramethyl

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Table 1. Inhibition of DHQ Synthase by Carbocyclic Inhibitors 1−6[a]

inhibitor	type of inhibiiton	E-NADH formation	K_i (M)	K_i/K_m
1	competitive	+	20×10^{-6}	5
2	linear mixed-type	−	3×10^{-6} [a]	0.8
3	competitive	+	5×10^{-6}	1.3
4	competitive	+	7×10^{-6}	1.8
5	competitive	+	0.7×10^{-6}	0.2
6	competitive	+	0.3×10^{-6}	0.08

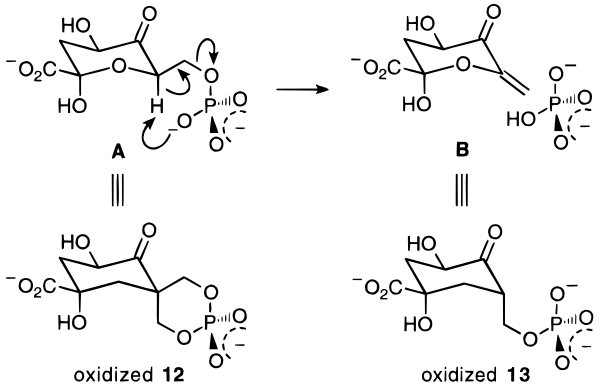
[a] $K_i' = 20 \times 10^{-6}$ M slopes and *y*-axis intercepts derived from double reciprocal plots were plotted as a function of inhibitor concentration. The base-line intercepts of the slope and *y*-axis intercepts, respectively, provided inhibition constants K_i and K_i'.[22]

methylenediphosphonate (Scheme 4). After hydrogenation of phosphonylmethylidene **35** using 10% Pd on C to give phosphonomethyl **36**, refluxing 6 N HCl was used to remove the BBA protecting group, carbmethoxy ester, and both phosphonate methyl esters in a single step. The absence of clearly resolved, diagnostic ^{1}H NMR resonances precluded establishing the C-5 stereochemistry of phosphonomethyl **36** obtained from the hydrogenation of phosphonomethylidene **35**. However, the final product obtained after deprotection of **36** was identical, based on ^{1}H and ^{13}C NMR, to independently synthesized carbaphosphonate **11**. Synthesis (Scheme 4) of carbaphosphonate **11** from quinic acid via protected DHQ **19** compares very favorably with two previously reported syntheses of **11** from quinic acid. The first reported synthesis[1a] of carbaphosphonate **11** required 14 steps and afforded a 12% overall yield while a later synthesis[5a] required 12 steps and gave a 5% overall yield. The synthesis of Scheme 4 leads to carbaphosphonate **11** after only 5 steps with an overall yield of 30%.

Enzymology. DHQ dehydratase[18] was used as the coupling enzyme for assay of DHQ synthase activity. Dehydratase-catalyzed conversion of DHQ into 3-dehydroshikimate (DHS) and the increase in optical density at 234 nm resulting from formation of DHS provided a means for continuous quantitation of DHQ formation. The measured value of the Michaelis constant (K_m) for DAHP binding to DHQ synthase has been reported to depend on the assay method.[19] For this work, the K_m for DAHP has been taken as 4×10^{-6} M. Inhibition constants (K_i) were determined (Table 1) for carbocyclic inhibitors **1**−**6**. Interactions with DHQ synthase were further evaluated by whether observed inhibition was competitive, noncompetitive, or linear mixed-type relative to substrate DAHP binding. Formation of NADH was measured by increases in absorbance at 340 nm. This measure of whether the carbocyclic inhibitor's C-4 alcohol was in close enough proximity to the bound NAD for C-4 alcohol oxidation to occur provided an indication of the carbocyclic inhibitor's alignment in the active site.

Carbocyclic diacids carbaphosphinate **1** and carbaacetate **2** were modest inhibitors of DHQ synthase. Carbaacetate **2** was a linear mixed-type inhibitor (Table 1). While carbaphosphinate **1** was a significantly weaker inhibitor (Table 1) of DHQ synthase, its binding to the active site was entirely competitive relative to substrate DAHP. NADH formation was observed during carbaphosphinate **1** inhibition but not during carbaacetate **2** inhibition. Of the carbocyclic inhibitors **1**−**6** synthesized in this account, carbaacetate's inhibition of DHQ synthase was the only instance where NADH formation was not observed (Table 1). All of the tricarboxylates **3**−**6** were competitive inhibitors (Table 1) with carbamalonate **5** and carbahydroxymalonate **6** displaying the most potent inhibition of DHQ synthase.

Scheme 5



Discussion

Trianionic Ionization States. Considerable experimental evidence now supports the proposal that the phosphate monoester of intermediate **A** (Scheme 5) mediates its own elimination.[1a,4,20] Even though spirocyclic carbaphosphodiester **12** is oxidized by DHQ synthase, the putative transition state analogue (Scheme 5) generated at the active site is a modest inhibitor with $K_i = 67 \times 10^{-6}$ M.[1b] This relatively weak active site interaction is consistent with a rather passive role for DHQ synthase during phosphate monoester elimination whereby the enzyme merely ensures that either DAHP or intermediate **A** is in its trianionic ionization state. Other indications of the possible importance of a trianionic ionization state include the 560-fold weaker inhibition of DHQ synthase by spirocyclic carbaphosphodiester[1b] **12** relative to carbaDAHP[2,3b] **8** ($K_i = 0.12 \times 10^{-6}$ M) and the 25 000-fold weaker inhibition measured for carbaphosphinate **1** relative to carbaphosphonate[1a,3a] **11** ($K_i = 0.8 \times 10^{-9}$ M). While carbaDAHP **8** and carbaphosphonate **11** can exist in the active site as trianions, spirocyclic carbaphosphodiester **12** and carbaphosphinate **1** are limited to dianionic ionization states.

Even though a dibasic phosphate monoester (and hence an overall trianionic ionization state) at the active site of DHQ synthase would seem to ensure the optimum reaction environment for conversion of intermediate **A** to intermediate **B**, elimination of a monobasic phosphate monoester is also a mechanistic possibility. This leads to an alternate explanation for the weaker inhibition of DHQ synthase by carbaphosphinate **1** relative to carbaphosphonate **11**. A monobasic phosphonate hydroxyl group in enzyme-bound, dianionic carbaphosphonate **11** can be viewed as having been replaced with a hydrogen in the dominant tautomeric form of the phosphinate of enzyme-bound, dianionic carbaphosphinate **1**. Loss of active site interactions with a hydroxyl group might then explain the severely attenuated inhibitor potency of carbaphosphinate **1**. Active site interactions with hydroxyl groups in carbaphosphonate **11** can be approxi-

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mated by the 41-fold and 100-fold reduction in inhibitor potency observed, respectively, for C-3 and C-4 monodeoxycarbaphosphonates.[21] This reduction in inhibitor potency due to loss of active site interactions with a hydroxyl group is substantially less than the 25 000-fold loss of inhibition potency observed for carbaphosphinate **1** relative to carbaphosphonate **11**. The lack of access to a trianionic ionization state thus appears to be a better explanation for weaker inhibition observed for carbaphosphinate **1**.

Di- and Tricarboxylate Inhibitors. The carbocyclic inhibitors **2**−**6** synthesized in this account can be viewed as evolutionary extensions of the previously synthesized carbacarboxylate **7**, which was reported[2] to be a competitive inhibitor with $K_i = 100 \times 10^{-6}$ M. By inserting a methylene carbon between the carbocyclic ring and the carboxylate, inhibition of DHQ synthase improves (Table 1) by a factor of approximately 30-fold based on the competitive inhibition constant K_i for carbaacetate **2** relative to carbacarboxylate **7**. The noncompetitive portion (K_i') of the linear mixed-type inhibition observed for carbaacetate **2** suggests that this inhibitor's interactions with DHQ synthase are not restricted to the enzyme's active site.[22] Providing access to a trianionic ionization state led to competitive inhibition for carbasuccinate **3**, carbamalonate ether **4**, carbamalonate **5**, and carbahydroxymalonate **6**. Employment of a malonate-type charged appendage results in improved active site interactions as indicated (Table 1) by the approximately 10-fold improvement in K_i values observed for tricarboxylates **5** and **6** relative to tricarboxylates **3** and **4**. Titration of carbamalonate **5** indicated a single, large inflection point indicative of multiple proton dissociation at an apparent $pK_a = 4.40$. For comparison, two inflection points indicating apparent acid dissociation constants at $pK_{a1} = 2.55$ and $pK_{a2} = 6.25$ were observed during titration of DAHP.

Carbahydroxymalonate **6**, the most potent of the newly synthesized tricarboxylate inhibitors, is approximately as potent a competitive inhibitor of DHQ synthase as carbaDAHP[2,3b] **8** ($K_i = 0.12 \times 10^{-6}$ M). Both carbamalonate **5** and carbahydroxymalonate **6** are better competitive inhibitors than carbahomophosphonate[1a,4] **9** ($K_i = 1.7 \times 10^{-6}$ M), which is the isosteric phosphonic acid analogue of carbaDAHP **8**. Other carbocyclic phosphate esters such as carbaphosphate[1a,4] **10** ($K_i = 1.7 \times 10^{-6}$ M) and 3-(phosphonooxy)quinate[4] **15** ($K_i = 53 \times 10^{-6}$ M) are weaker competitive inhibitors of DHQ synthase. The only phosphate monoester synthesized to date that is a substantially more potent competitive inhibitor than carbahydroxymalonate **6** is C-5 epimeric 5-[(phosphonooxy)methyl]-5-deoxyquinate[4] **13** ($K_i = 7.0 \times 10^{-9}$ M). Organophosphonic acid analogues such as carbaphosphonate[1a,3a] **11** ($K_i = 0.8 \times 10^{-9}$ M), C-1 epimeric carbaphosphonate[5] **17** ($K_i = 1.8 \times 10^{-9}$ M), and C-5 epimeric 5-(phoshonomethyl)-5-deoxyquinate[4] **16** ($K_i = 13 \times 10^{-9}$ M) are more potent inhibitors of DHQ synthase.

Designing inhibitors to structurally mimic substrate DAHP does not appear to lead to the most potent DHQ synthase inhibition. By contrast, nonisosteric carbaphosphonate **11**, C-1 epimeric carbaphosphonate **17**, C-5 epimeric phosphate **13**, and C-5 epimeric phosphonate **16** are all nanomolar-level, competitive inhibitors. The nonisosteric phosphonic acid moieties of carbaphosphonate **11** and C-1 epimeric carbaphosphonate **17** may well be interacting with active site residues that bind to DHQ synthase transition states and/or reactive intermediates selectively over substrate DAHP (or phosphate monoester of carbaDAHP **8** and isosteric phosphonoethyl group of **9**). 5-[(Phosphonooxy)methyl]-5-deoxyquinate **13** and 5-(phosphonomethyl)-5-deoxyquinate **16** after oxidation by DHQ synthase might be best viewed (Scheme 5) as intermediate **B** covalently attached to inorganic phosphate. These C-5 epimers may be taking advantage of active site residues that bind to intermediate **B** concurrently with separate active site residues that bind to inorganic phosphate prior to its release from the enzyme. The C-5 epimers 5-(phosphonoethyl)-5-deoxyquinate (**14**) ($K_i = 3.0 \times 10^{-5}$ M) and 3-(phosphonooxy)quinate (**15**) ($K_i = 5.0 \times 10^{-5}$ M) are much weaker inhibitors.[4]

The hydroxymalonic acid and malonic acid moieties of, respectively, carbahydroxymalonate **6** and carbamalonate **5** are excellent analogues of a phosphate monoester. This follows from the competitive nature of these tricarboxylates' inhibition relative to substrate DAHP, the magnitude of their inhibition constants relative to those of carbaDAHP **8** and carbahomophosphonate **9**, and formation of NADH during DHQ synthase inhibition. The hydroxymalonic acid and malonic acid moieties are, not surprisingly, poor analogues of a nonisosteric phosphonic acid moiety of carbaphosphonate **11**. How well a C-5 epimeric hydroxymalonic acid and a C-5 epimeric malonic acid might approximate the inhibition of DHQ synthase observed for C-5 epimeric phosphate 5-[(phosphonooxy)-methyl]-5-deoxyquinate (**13**) and 5-(phosphonomethyl)-5-deoxyquinate (**16**) remains for future investigation.

In Vivo Inhibition. The virulence of pathogenic microbes is greatly attenuated upon introduction of genomic mutations that eliminate the catalytic activity of enzymes in the common pathway of aromatic amino acid biosynthesis.[23] Might chemical inhibition of a common pathway enzyme likewise attenuate the virulence of pathogenic microbes? DHQ synthase is one possible target given that some of the most potent inhibitors of common pathway enzymes are available for this enzyme. Carbaphosphonate **11**, C-1 epimeric carbaphosphonate **17**, 5-[(phosphonooxy)methyl]-5-deoxyquinate (**13**), and 5-(phosphonomethyl)-5-deoxyquinate (**16**) are appealing candidates for "arobiotics" given their nanomolar, slowly-reversible inhibition of DHQ synthase. However, the ionization states of these inhibitors at physiologically relevant pH are not compatible with effective penetration into the bacterial cytosol, which is a prerequisite for in vivo DHQ synthase inhibition.

Prodrug strategies will need to be explored whereby inhibitor **11**, the most potent in vitro inhibitor of DHQ synthase, is covalently modified to derivatives that can penetrate into intact bacteria. Upon reaching the bacterial cytosol, the covalently attached groups would be enzymatically hydrolyzed, thereby releasing the DHQ synthase inhibitor. Exploring such prodrug strategies

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has been complicated by the long syntheses and low overall yields of carbaphosphonate **11**. Simultaneous pursuit of several different in vivo inhibition strategies or examination of strategies that might require several steps for covalent modification of carbaphosphonate **11** has not been possible. With synthesis via protected DHQ intermediacy leading to a 30% yield in just five steps from quinic acid, gram quantities of carbaphosphonate **11** can now be conveniently obtained.

The availability of tricarboxylate inhibitors of DHQ synthase such as carbamalonate **5** and carbahydroxymalonate **6** is also important given the wider spectrum of prodrug strategies that are available for carboxylic acids versus phosphate esters and phosphonic acids. Carbamalonate **5** and carbahydroxymalonate **6** are already more potent inhibitors than α-C-(1,5-anhydro-7-amino-2,7-dideoxy-D-*manno*-heptopyranosyl)carboxylate ($K_i = 4.0 \times 10^{-6}$ M), a competitive inhibitor[24] of CMP-KDO synthetase that possesses chemotherapeutic activity against Gram-negative bacteria. A prodrug strategy was used to drastically increase the concentrations of this essentially nonpermeant ulosonic acid inhibitor that penetrated into bacterial cytosols.[24] Related prodrug strategies can now be employed for DHQ synthase that exploit the expanded structural diversity of inhibitory molecules (tricarboxylates **5** and **6**) and improved synthetic accessiblity of the most potent in vitro inhibitor (carbaphosphonate **11**). In this fashion, the chemotherapeutic utility of in vivo DHQ synthase inhibition can ultimately be evaluated.

Experimental Section

General Chemistry. Organic solutions of products were dried over $MgSO_4$. See ref 1b for general experimental information. All air- and moisture-sensitive reactions were conducted under Ar. 1,4-Dioxane and $ClCH_2CH_2Cl$ were distilled from calcium hydride under Ar. CH_3CN was dried over activated 3A molecular sieves. Melting points were uncorrected and were determined using a Mel-Temp II melting point apparatus. Spectrophotometric measurements were made on a Hewlett-Packard 8452A diode array spectrophotometer.

General Enzymology. To quantify the concentration of a given dicarboxylate or tricarboxylate inhibitor, the carboxylic acid solution was concentrated to dryness, exchanged twice with D_2O, and redissolved in a known volume of D_2O. From this stock solution, an aliquot was mixed with a known volume of sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP) solution (5.0 mM in D_2O). The concentration of the carboxylic acid was quantitated by comparison of the integration of selected proton resonances of the carboxylic acid with the integrated resonance of the internal TSP standard.

DHQ synthase activity was assayed in a 1.0 mL solution of 3-(*N*-morpholino)propanesulfonate (MOPS) buffer (50 mM, pH 7.7) containing $CoCl_2$ (50 μM), NAD^+ (10 μM), dehydroquinase (1 unit), and varying amounts of DAHP and of the enzyme inhibitor. After equilibration at rt, DHQ synthase (0.024 units) was added, and the increase in absorbance at 234 nm was monitored over time. Initial rates were determined by linear squares fits of the progress curves and were used to determine inhibition constants.

To measure enzyme-bound NADH formation during enzyme inhibition, a 0.8 mL solution of MOPS buffer (50 mM, pH 7.7) containing $CoCl_2$ (50 μM), NAD^+ (10 μM), and DHQ synthase (10 μM) was incubated at rt in a quartz cuvette. Spectra (190−820 nm) were measured to establish the base-line absorbance. A solution containing a known concentration of an inhibitor was added to the cuvette, and the change in absorbance at 340 nm was monitored.

Protected DHQ Intermediate 19. In the following order, H_2O (300 mL), KIO_4 (43.0 g, 187 mmol), K_2CO_3 (3.38 g, 24.5 mmol), and finally $RuCl_3$ (0.50 g, 1.9 mmol) were added to a solution of **18**[9] (30.2 g, 94.2 mmol) in $CHCl_3$ (300 mL). Vigorous stirring was continued at rt until the reaction was complete. The mixture was filtered through Celite, and the aqueous and organic phases were separated. After saturation with NaCl, the aqueous layer was extracted with EtOAc (3×). The combined organic layers were dried and concentrated to give protected DHQ intermediate **19** as a white solid (23.0 g, 77%): ^{1}H NMR ($CDCl_3$) δ 4.43 (dd, J = 10, 1 Hz, 1 H), 4.26 (ddd, J = 13, 10, 4 Hz, 1 H), 3.85 (s, 3 H), 3.27 (s, 3 H), 3.24 (s, 3 H), 2.91 (dd, J = 14, 1 Hz, 1 H), 2.52 (dd, J = 14, 3 Hz, 1 H), 2.36 (dd, J = 13, 13 Hz, 1 H), 2.13 (ddd, J = 13, 4, 3 Hz, 1 H), 1.41 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 199.4, 174.0, 100.4, 99.5, 77.1, 74.0, 66.9, 53.5, 48.9, 48.2, 47.9, 37.7, 17.6, 17.4. Anal. Calcd for $C_{14}H_{22}O_8$: C, 52.82; H, 6.97. Found: C, 52.65; H, 6.93.

Carbaacetate Intermediate 22. To a vigorously stirred mixture of carbaacetate intermediate **21**[3b] (3.33 g, 11.0 mmol) in $CH_3CN/CCl_4/H_2O$ (16 mL/16 mL/24 mL) were added $NaIO_4$ (9.89 g, 46.2 mmol) and $RuCl_3 \cdot 7H_2O$ (0.063 g, 0.24 mmol). Stirring was continued overnight at rt. The biphasic reaction mixture was filtered through Celite to remove suspended solids and allow organic and aqueous layers to separate. After extraction of the aqueous layer with CH_2Cl_2 (3×), the combined organic layers were dried, concentrated, redissolved in CH_3OH (20 mL), and treated with an ethereal CH_2N_2 solution at −20 °C. The solvent was evaporated and the residue was purified by flash chromatography (EtOAc/hexane, 1:1, v/v). Carbaacetate intermediate **22** was obtained as a yellow oil (2.39 g, 65%): ^{1}H NMR ($CDCl_3$) δ 8.02−8.05 (m, 2 H), 7.58−7.64 (m, 1 H), 7.45−7.50 (m, 2 H), 5.14 −5.16 (m, 1 H), 4.91−4.93 (m, 1 H), 3.83 (br, 1 H), 3.66 (s, 3 H), 2.82−2.88 (m, 1 H), 2.39−2.75 (m, 5 H), 1.87 (d, J = 16 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 178.5, 171.9, 165.0, 133.6, 129.6, 129.5, 129.0, 128.6, 128.5, 75.3, 71.7, 70.4, 51.6, 38.4, 36.6, 36.5, 33.2; HRMS (FAB) calcd for $C_{17}H_{18}O_7$ ($M + H^+$) 335.1130, found 335.1133.

Carbaacetate 2. Carbaacetate intermediate **22** (0.23 g, 0.69 mmol) was stirred in a mixture of THF (10 mL) and aqueous NaOH (0.2 N, 10 mL) for 24 h. The aqueous layer was washed with EtOAc (1×), passed down a Dowex 50 (H^+) column, and concentrated in vacuo to yield carbaacetate **2** as a colorless film (100% yield based on NMR analysis): ^{1}H NMR (D_2O, pH 8.1) δ 3.77 (ddd, J = 12, 10, 5 Hz, 1 H), 3.23 (dd, J = 10, 10 Hz, 1 H), 2.08−2.34 (m, 4 H), 1.71−1.94 (m, 3 H); ^{13}C NMR (D_2O, pH 8.1) δ 181.1, 180.2, 80.1, 76.8, 73.1, 42.4, 40.5, 39.5, 37.7; HRMS (FAB) calcd for $C_9H_{12}O_7Na_2$ ($M + H^+$) 279.0457, found 279.0450.

Carbasuccinate Intermediate 23. A solution of intermediate **19** (0.661 g, 2.08 mmol) and (carbethoxymethylene)-triphenylphosphorane (1.45 g, 4.16 mmol) in anhydrous CH_3CN (10 mL) was refluxed for 15 min. After the solution was cooled to rt, the solvent was removed and the residue was purified by flash chromatography (EtOAc/hexane, 1:1, v/v) to give carbasuccinate intermediate **23** as a yellow foam (0.727 g, 90%): ^{1}H NMR ($CDCl_3$) δ 6.25 (d, J = 2 Hz, 1 H), 4.11−4.20 (m, 3 H), 4.05 (dd, J = 14, 3 Hz, 1 H), 3.97 (ddd, J = 11, 10, 5 Hz, 1 H), 3.80 (s, 3 H), 3.25 (s, 3 H), 3.22 (s, 3 H), 3.21 (s, 1 H), 2.34 (d, J = 14 Hz, 1 H), 2.12 (dd, J = 12, 12 Hz, 1 H), 2.00 (ddd, J = 13, 5, 3 Hz, 1 H), 1.36 (s, 3 H), 1.31 (s, 3 H), 1.28 (t, J = 7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.8, 166.8, 150.6, 115.1, 100.1, 99.4, 74.8, 72.9, 68.5, 60.0, 53.0, 47.9 (2), 37.3, 36.9, 17.6 (2), 14.1; HRMS (FAB) calcd for $C_{18}H_{28}O_9$ ($M + H^+$) 389.1812, found 389.1829. Anal. Calcd for $C_{18}H_{28}O_9$: C, 55.66; H, 7.27. Found: C, 55.59; H, 7.32.

Carbasuccinate Intermediate 25. 1,1′-Carbonyldiimidazole (0.415 g, 2.56 mmol) and carbasuccinate intermediate **23** (0.497 g, 1.28 mmol) were dissolved in anhydrous $ClCH_2CH_2Cl$ (5 mL) and heated at reflux. Heating was stopped 5 h later and the mixture cooled to rt. Ether was added, and the organic layer was washed with water (1×) and brine (1×). Concentration yielded a yellow oil that was purified by flash

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chromatography (EtOAc/hexane, 1:1, v/v) to afford carbasuccinate intermediate **24** as a white foam (0.506 g, 82%): ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 1 Hz, 1 H), 7.33 (dd, J = 2, 2 Hz, 1 H), 7.05 (dd, J = 2, 1 Hz, 1 H), 6.27 (m, 1 H), 4.59 (dd, J = 15, 3 Hz, 1 H), 4.21 (dd, J = 10, 2 Hz, 1 H), 4.08−4.16 (m, 2 H), 3.82 (s, 3 H), 3.75−3.84 (m, 1 H), 3.26 (s, 3 H), 3.21 (s, 3 H), 2.68 (ddd, J = 14, 5, 3 Hz, 1 H), 2.47 (dd, J = 15, 1 Hz, 1 H), 2.26 (dd, J = 14, 12 Hz, 1 H), 1.37 (s, 3 H), 1.32 (s, 3 H), 1.22 (t, J = 7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.4, 166.0, 147.4, 137.2, 130.7, 117.1, 116.8, 100.3, 99.7, 83.5, 72.5, 68.1, 60.3, 53.3, 48.2, 48.0, 35.3, 33.9, 17.6, 14.1; HRMS (FAB) calcd for $C_{22}H_{30}N_2O_{10}$ ($M + H^+$) 483.1979, found 483.1983.

Benzeneselenol (0.26 mL, 2.4 mmol) and carbasuccinate intermediate **24** (0.567 g, 1.17 mmol) were dissolved in anhydrous $ClCH_2CH_2Cl$ (5 mL), and the resulting solution was heated at reflux overnight. Solvent removal and purification by flash chromatography (EtOAc/hexane, 5:1, v/v) yielded carbasuccinate intermediate **25** as a white solid (0.582 g, 87%): mp 174−176 °C; ^{1}H NMR ($CDCl_3$) δ 7.57−7.60 (m, 2 H), 7.30−7.38 (m, 3 H), 6.25 (s, 3 H), 4.64 (dd, J = 15, 3 Hz, 1 H), 4.10−4.22 (m, 3 H), 3.81 (ddd, J = 12, 10, 5 Hz, 1 H), 3.74 (s, 3 H), 3.23 (s, 6 H), 2.44 (ddd, J = 14, 4, 3 Hz, 1 H), 2.41 (dd, J = 14, 12 Hz, 1 H), 1.38 (s, 3 H), 1.31 (s, 3 H), 2.02 (dd, J = 14, 12 Hz, 1 H), 1.25 (t, J = 7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.8, 165.7, 165.2, 147.9, 135.4, 129.1, 129.0, 125.6, 116.1, 100.1, 99.5, 83.2, 72.3, 67.5, 59.9, 52.8, 47.9 (2), 35.8, 33.1, 17.5, 14.1; HRMS (EI) calcd for $C_{25}H_{32}O_{10}Se$ ($M + H^+$) 573.1238, found 573.1260. Anal. Calcd for $C_{25}H_{32}O_{10}Se$: C, 52.54; H, 5.65. Found: C, 52.45; H, 5.65.

Carbasuccinate Intermediate 26. A solution of AIBN (0.035 g, 0.21 mmol) and Bu_3SnH (0.86 mL, 3.2 mmol) in benzene (50 mL) was slowly added via syringe pump (0.10 mmol/h) to a refluxing solution of carbasuccinate intermediate **25** (1.21 g, 2.12 mmol) in benzene (25 mL). After completion of the addition, heating at reflux was continued for another 2 h. Solvent was removed under reduced pressure, and the residue was purified by radial chromatography (2 mm thickness, EtOAc/hexane, 5:1, v/v) to give carbasuccinate intermediate **26** as a white solid (0.23 g, 27%): mp 180−181 °C; ^{1}H NMR ($CDCl_3$) δ 4.07−4.19 (m, 2 H), 3.90−3.96 (m, 2 H), 3.83 (s, 3 H), 3.21 (s, 3 H), 3.18 (s, 3 H), 2.89 (d, J = 18 Hz, 1 H), 2.80 (d, J = 18 Hz, 1 H), 2.76 (dd, J = 12, 2 Hz, 1 H), 2.42−2.49 (m, 1 H), 2.34 (d, J = 12 Hz, 1 H), 1.98−2.06 (m, 1 H), 1.28 (s, 6 H), 1.26 (t, J = 7 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.2, 170.0, 169.0, 101.1, 100.3, 81.6, 70.8, 66.8, 60.7, 52.9, 48.0, 47.8, 47.0, 42.9, 34.4, 31.7, 17.6, 17.5, 14.0; HRMS (FAB) calcd for $C_{19}H_{28}O_{10}$ ($M - H^+$) 415.1604, found 415.1600. Anal. Calcd for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.70; H, 6.73.

Carbasuccinate 3. Carbasuccinate intermediate **26** (0.152 g, 0.360 mmol) was stirred in CF_3CO_2H/H_2O (20:1, v/v, 3 mL) for 20 min. Water and CF_3CO_2H were removed in vacuo. The brown residue was stirred in a mixture of THF (10 mL) and aqueous NaOH (0.2 N, 10 mL) for 24 h. The aqueous layer was washed with EtOAc (1×), passed down a Dowex 50 (H^+) column, and concentrated to afford carbasuccinate **3** as a colorless film (100% yield based on NMR analysis): ^{1}H NMR (D_2O, pH 7.5) δ 3.87 (m, 1 H), 3.47 (d, J = 10 Hz, 1 H), 2.67 (d, J = 14 Hz, 1 H), 2.56 (d, J = 14 Hz, 1 H), 2.36 (dd, J = 15, 2 Hz, 1 H), 1.82−2.03 (m, 3 H); ^{13}C NMR (D_2O, pH 7.5) δ 185.5, 184.9, 182.1, 80.8, 78.1, 71.7, 52.4, 49.3, 44.3, 42.8; HRMS (FAB) calcd for $C_{10}H_{11}O_9Na_3$ ($M + H^+$) 345.0174, found 345.0171.

Carbamalonate Ether Intermediate 27. Intermediate **19** (2.01 g, 6.31 mmol) and $NaBH(OAc)_3$ (5.35 g, 25.3 mmol) were dissolved in CH_3CN (10 mL) and HOAc (10 mL). The mixture was stirred at rt for 8 h. Solvents were removed in vacuo, and the residue was dissolved in ether and washed with aqueous $NaHSO_4$ (0.1 N). The aqueous layer was back-extracted with ether (3×). The combined organic layers were then washed with aqueous phosphate buffer (2 N, pH 7), dried, and concentrated to a yellow solid. Purification by flash chromatography (EtOAc/hexane, 2:1, v/v) gave white, crystalline diol **27** (1.74 g, 86%): m.p. 164−166 °C; ^{1}H NMR ($CDCl_3$) δ 3.90−4.07 (m, 2 H), 3.80 (s, 3 H), 3.48 (dd, J = 10, 10 Hz, 1 H), 3.30 (s, 3 H), 3.26 (s, 3 H), 3.24 (s, 1 H), 2.58 (d, J = 2 Hz, 1 H), 1.99−2.06 (m, 1 H), 1.98 (dd, J = 12, 12 Hz, 1 H), 1.82−1.90 (m, 2 H), 1.35 (s, 3 H), 1.30 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 175.7, 99.6, 99.5, 76.3, 73.3, 66.9, 65.1, 53.2, 47.9, 47.8, 40.5, 37.9, 17.7 (2). Anal. Calcd for $C_{14}H_{24}O_8$: C, 52.49; H, 7.55. Found: C, 52.43; H, 7.51.

Carbamalonate Ether Intermediate 28. A solution of diethyl diazomalonate (0.239 g, 1.29 mmol) in benzene (2 mL) was slowly added via syringe pump (2.4 mmol/h) to a solution of carbamalonate ether intermediate **27** (0.206 g, 0.643 mmol) and $Rh_2(OAc)_4$ (0.004 g, 0.01 mmol) in refluxing benzene (10 mL). After completion of the addition, the mixture was refluxed for another 2 h. Removal of the solvent gave a green residue, which was purified by flash chromatography (EtOAc/hexane, 1:1, v/v) to yield carbamalonate ether intermediate **28** as a colorless oil (0.220 g, 72%): ^{1}H NMR ($CDCl_3$) δ 4.18−4.31 (m, 4 H), 3.98 (ddd, J = 12, 10, 5 Hz, 1 H), 3.89 (ddd, J = 11, 10, 5 Hz, 1 H), 3.79 (s, 3 H), 3.71 (dd, J = 10, 10 Hz, 1 H), 3.29 (s, 3 H), 3.28 (s, 1 H), 3.25 (s, 3 H), 2.23 (ddd, J = 13, 5, 2 Hz, 1 H), 1.95−2.05 (m, 1 H), 1.93 (dd, J = 12, 12 Hz, 1 H), 1.80 (ddd, J = 13, 5, 2 Hz, 1 H), 1.32 (s, 3 H), 1.29 (s, 3 H), 1.25−1.31 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 175.4, 167.6, 166.2, 99.5, 99.3, 80.0, 76.7, 75.6, 73.1, 65.3, 61.7, 61.6, 53.1, 47.9, 47.7, 39.9, 37.3, 17.6, 14.0, 13.9 Anal. Calcd for $C_{21}H_{34}O_{12}$: C, 52.60; H, 7.15. Found: C, 52.74; H, 7.20.

Carbamalonate Ether 4. Carbamalonate ether intermediate **28** (0.22 g, 0.48 mmol) was deprotected as described for carbasuccinate intermediate **26** to give carbamalonate ether **4** as a colorless film (100% yield based on NMR analysis): ^{1}H NMR (D_2O, pH 7.7) δ 4.98 (s, 1 H), 3.70−3.83 (m, 2 H), 3.54 (dd, J = 9, 9 Hz, 1 H), 2.25−2.31 (m, 1 H), 2.08−2.15 (m, 1 H), 1.97 (dd, J = 13, 12 Hz, 1 H), 1.90 (dd, J = 13, 12 Hz, 1 H); ^{13}C NMR (D_2O, pH 7.7) δ 180.2, 173.0, 172.9, 82.2, 81.4, 81.0, 76.0, 71.8, 42.2, 40.1; HRMS (FAB) calcd for $C_{10}H_{11}O_{10}Na_3$ ($M + H^+$) 361.0123, found 361.0122.

Carbamalonate Intermediate 29. To a solution of intermediate **19** (1.03 g, 3.23 mmol) in benzene (15 mL) was added malononitrile (0.235 g, 3.56 mmol), ammonium acetate (0.025 g, 0.32 mmol), and one drop of HOAc. The resulting mixture was stirred overnight at rt. Ether was added, and the organic layer was washed with aqueous $NaHCO_3$ (1×) and brine (1×). Drying and concentration afforded a yellow oil that was purified by flash chromatography (EtOAc/hexane, 1:1, v/v) to give carbamalonate intermediate **29** as a white crystalline solid (1.07 g, 90%): mp 189−190 °C; ^{1}H NMR ($CDCl_3$) δ 4.54 (d, J = 9 Hz, 1 H), 4.16 (ddd, J = 11, 9, 5 Hz, 1 H), 3.87 (s, 3 H), 3.62 (s, 1 H), 3.31 (s, 3 H), 3.24 (s, 3 H), 3.09 (dd, J = 14, 2 Hz, 1 H), 2.81 (d, J = 14 Hz, 1 H), 2.11 (dd, J = 14, 11 Hz, 1 H), 2.02 (ddd, J = 13, 5, 2 Hz, 1 H), 1.41 (s, 3 H), 1.29 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.5, 169.9, 112.3, 110.8, 101.1, 99.8, 85.3, 74.6, 74.1, 67.9, 53.8, 48.6, 48.1, 42.5, 37.5, 17.4, 16.7; HRMS (EI) calcd for $C_{17}H_{22}N_2O_7$ ($M + H^+$) 367.1505, found 367.1502. Anal. Calcd for $C_{17}H_{22}N_2O_7$: C, 55.73; H, 6.05; N, 7.65. Found: C, 55.71; H, 6.04; N, 7.68.

Carbamalonate Intermediate 30. $NaBH(OAc)_3$ (2.31 g, 10.9 mmol) was dissolved in CH_3CN (10 mL) and HOAc (10 mL). After the mixture was stirred at rt for 10 min, carbamalonate intermediate **29** (1.00 g, 2.73 mmol) was added as a solid in one portion. Stirring was continued overnight at rt. Solvents were removed in vacuo, and the residue was dissolved in ether and washed with aqueous $NaHSO_4$ (0.1 N). The aqueous layer was back-extracted with ether (3×). The combined organic layers were then washed with aqueous phosphate buffer (2 N, pH 7), dried, and concentrated to a yellow oil. Flash chromatography (EtOAc/hexane, 1:1, v/v) yielded carbamalonate intermediate **30** as a white crystalline solid (0.925 g, 92%): mp 158−159 °C; ^{1}H NMR ($CDCl_3$) δ 4.44 (d, J = 4 Hz, 1 H), 4.04 (ddd, J = 11, 10, 5 Hz, 1 H), 3.84 (s, 3 H), 3.66 (dd, J = 11, 10 Hz, 1 H), 3.52 (s, 1 H), 3.31 (s, 3 H), 3.26 (s, 3 H), 2.60−2.71 (m, 1 H), 2.11−1.89 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 174.9, 111.7, 110.7, 100.2, 99.7, 72.9, 70.4, 66.1, 53.4, 48.4, 48.0, 37.5, 37.3, 35.8, 23.2, 17.5 (2); HRMS (EI) calcd for $C_{17}H_{24}N_2O_7$ (M^+) 368.1583, found 368.1585. Anal. Calcd for $C_{17}H_{24}N_2O_7$: C, 55.42; H, 6.57; N, 7.61. Found: C, 55.28; H, 6.53; N, 7.50.

Carbamalonate 5. Carbamalonate intermediate **30** (2.0 g, 5.4 mmol) was stirred in CF_3CO_2H/H_2O (20:1, v/v, 5 mL) for 20 min. After concentration, the residue was dissolved in

aqueous HCl (6 N, 20 mL) and heated at reflux for 2 h. After being cooled to rt, the mixture was evaporated to dryness, dissolved in water, and the solution basicified by addition of aqueous NaOH to pH 12. The resulting solution was stirred overnight. The aqueous layer was washed with EtOAc (1×), passed down Dowex 50 (H^+), and concentrated to afford carbamalonate **5** as a colorless film (95% yield based on NMR analysis): ^{1}H NMR (D_2O, pH 7.9) δ 3.86 (d, J = 5 Hz, 1 H), 3.79 (ddd, J = 12, 10, 5 Hz, 1 H), 3.47 (dd, J = 11, 10 Hz, 1 H), 2.50−2.65 (m, 1 H), 2.09−2.19 (m, 1 H), 1.88−1.99 (m, 1 H), 1.86 (dd, J = 13, 12 Hz, 1 H); ^{13}C NMR (D_2O, pH 7.9) δ 180.8, 175.4, 174.8, 78.4, 76.6, 73.3, 55.0, 42.4, 40.8, 37.7; HRMS (FAB) calcd for $C_{10}H_{14}O_9$ ($M + H^+$) 279.0716, found 279.0711.

Carbahydroxymalonate Intermediate 31. Carbamalonate intermediate **30** (1.6 g, 4.3 mmol) was stirred in CF_3CO_2H/H_2O (20:1, v/v, 5 mL) for 20 min. CF_3CO_2H and water were removed in vacuo. The residue was refluxed in aqueous HCl (6 N, 20 mL) for 2 h. After being cooled to rt, the mixture was evaporated to dryness. The residue was dissolved in CH_3OH (100 mL), treated with an ethereal CH_2N_2 solution at −20 °C, and allowed to stand overnight. Evaporation gave a brown oil that was redissolved in CH_3OH (10 mL). To this solution were added 2,2,3,3-tetramethoxybutane[9] (0.918 g, 5.15 mmol), $(CH_3O)_3CH$ (4.8 mL, 43 mmol), and (±)-10-camphorsulfonic acid (0.05 g, 0.2 mmol), and the solution was refluxed for 18 h. The resulting dark brown solution was treated with powdered $NaHCO_3$ (2 g) and concentrated. The resulting residue was purified by flash chromatography (EtOAc/hexane, 1:1, v/v) to afford carbahydroxymalonate intermediate **31** as a colorless oil (1.49 g, 80%): ^{1}H NMR ($CDCl_3$) δ 3.99 (ddd, J = 12, 10, 5 Hz, 1 H), 3.95 (d, J = 4 Hz, 1 H), 3.77 (s, 3 H), 3.73 (s, 3 H), 3.72 (s, 3 H), 3.68 (dd, J = 10, 10 Hz, 1 H), 2.69−2.80 (m, 1 H), 1.82−2.01 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 175.7, 169.1, 168.6, 99.8, 99.4, 73.4, 71.3, 67.1, 53.0, 52.3, 52.0, 49.5, 47.9, 47.8, 37.5, 36.0, 35.4, 17.7, 17.6. Anal. Calcd for $C_{19}H_{30}O_{11}$: C, 52.53; H, 6.96. Found: C, 52.72; H, 7.01.

Carbahydroxymalonate Intermediate 32. To a solution of carbahydroxymalonate intermediate **31** (0.266 g, 0.613 mmol) in THF (10 mL) at 0 °C was added solid *t*-BuOK (0.151 g, 1.35 mmol) in one portion. After 20 min, the reaction mixture was cooled to −78 °C and stirring was continued at −78 °C for 15 min. A solution of 2-(benzenesulfonyl)-3-phenyloxaziridine[14] (0.320 g, 1.23 mmol) in THF (2 mL) was then cannulated into the reaction flask. The resulting mixture was stirred at −78 °C for 1 h and quenched with saturated aqueous NH_4Cl (10 mL). After addition of ether, the organic layer was separated and the aqueous layer was then extracted with ether (3×). The combined organic layers were dried, filtered, and evaporated. The resulting yellow oil was purified by flash chromatography (EtOAc/hexane, 2:1, v/v) to give carbahydroxymalonate intermediate **32** as white crystals (0.208 g, 75%): mp 206−207 °C; ^{1}H NMR ($CDCl_3$) δ 4.01 (ddd, J = 12, 10, 5 Hz, 1 H), 3.95 (s, 1 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.76 (dd, J = 11, 10 Hz, 1 H), 3.26 (s, 3 H), 3.21 (s, 3 H), 3.12 (ddd, J = 13, 11, 3 Hz, 1 H), 1.94 (dd, J = 12, 12 Hz, 1 H), 1.92 (dd, J = 13, 13 Hz, 1 H), 1.83 (ddd, J = 13, 5, 3 Hz, 1 H), 1.72 (br, 1 H), 1.55 (dt, J = 13, 3, 3 Hz, 1 H), 1.25 (s, 3 H), 1.18 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 176.1, 171.3, 170.2, 99.6, 99.4, 78.7, 73.6, 70.0, 66.7, 53.4, 53.3, 53.1, 48.5, 47.8, 40.5, 37.7, 35.0, 17.9, 17.5. Anal. Calcd for $C_{19}H_{30}O_{12}$: C, 50.66; H, 6.71. Found: C, 50.72; H, 6.73.

Carbahydroxymalonate 6. Carbahydroxymalonate intermediate **32** (0.21 g, 0.47 mmol) was deprotected as described for carbasuccinate intermediate **26** to afford carbahydroxymalonate **6** as a colorless film (100% yield based on NMR analysis): ^{1}H NMR (D_2O, pH 8.5) δ 3.84 (ddd, J = 12, 10, 5 Hz, 1 H), 3.53 (dd, J = 10, 10 Hz, 1 H), 2.93 (ddd, J = 13, 10, 3 Hz, 1 H), 2.14 (ddd, J = 13, 5, 3 Hz, 1 H), 1.89 (dd, J = 13, 13 Hz, 1 H), 1.82 (dd, J = 13, 12 Hz, 1 H), 1.72 (ddd, J = 14, 3, 3 Hz, 1 H); ^{13}C NMR (D_2O, pH 8.5) δ 180.7, 176.3, 175.4, 82.7, 76.7, 76.5, 73.2, 46.3, 42.5, 36.3; HRMS (FAB) calcd for $C_{10}H_{11}O_{10}Na_3$ ($M + H^+$) 361.0123, found 361.0129.

Carbaphosphinate Intermediate 33. Powdered P_2O_5 was added in portions to a solution of intermediate **19** (8.10 g, 25.4 mmol) and $(CH_3O)_2CH_2$ (50 mL) in $CHCl_3$ (200 mL) at rt with vigorous stirring. After completion of the reaction, the reaction mixture was poured into saturated aqueous Na_2CO_3 and then extracted with ether. The organic layer was washed with brine (1×), dried, and concentrated to a white solid (8.40 g, 91%) that could be used directly in the next experiment: ^{1}H NMR ($CDCl_3$) δ 4.76 (d, J = 7 Hz, 1 H), 4.64 (d, J = 7 Hz, 1 H), 4.41 (d, J = 10 Hz, 1 H), 4.19 (ddd, J = 10, 10, 4 Hz, 1 H), 3.78 (s, 3 H), 3.34 (s, 3 H), 3.26 (s, 3 H), 3.24 (s, 3 H), 2.96 (dd, J = 15, 3 Hz, 1 H), 2.84 (d, J = 15 Hz, 1 H), 2.51 (ddd, J = 13, 4, 3 Hz, 1 H), 2.22 (dd, J = 13, 10 Hz, 1 H), 1.39 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 199.7, 171.4, 100.2, 99.4, 92.8, 78.4, 76.7, 66.4, 56.5, 52.6, 48.1, 47.7, 45.5, 35.8, 17.4, 17.3. Anal. Calcd for $C_{16}H_{26}O_9$: C, 53.03; H, 7.23. Found: C, 53.11; H, 7.20.

Neat CH_2I_2 (5.30 mL, 65.6 mmol) was added to a suspension of activated Zn dust (7.72 g, 118 mmol) in THF (60 mL) at rt.[15] After 30 min, $TiCl_4$ (1.0 M in CH_2Cl_2, 14.4 mL, 14.4 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 MOM-protected ketol (4.75 g, 13.1 mmol) in THF (40 mL) was added. The mixture was vigorously stirred at rt for 2 h, ether was added, and the mixture was carefully poured into ice-cold aqueous HCl (1 N). After extraction of the aqueous layer with ether (2×), the combined ether extracts were washed with saturated aqueous $NaHCO_3$ (1×). Drying and concentration afforded a yellow oil. Purification by flash chromatography (EtOAc/hexane, 1:5, 1:1, v/v) afforded carbaphosphinate intermediate **33** as a colorless oil (3.80 g, 73%): ^{1}H NMR ($CDCl_3$) δ 5.29 (dd, J = 4, 1 Hz, 1 H), 4.95 (dd, J = 4, 1 Hz, 1 H), 4.77 (d, J = 7 Hz, 1 H), 4.62 (d, J = 7 Hz, 1 H), 4.06 (dd, J = 10, 1 Hz, 1 H), 3.86 (ddd, J = 12, 10, 4 Hz, 1 H), 3.75 (s, 3 H), 3.36 (s, 3 H), 3.26 (s, 3 H), 3.24 (s, 3 H), 2.81 (dd, J = 15, 3 Hz, 1 H), 2.50 (dd, J = 15, 1 Hz, 1 H), 2.32 (ddd, J = 14, 4, 3 Hz, 1 H) 1.94 (dd, J = 14, 12 Hz, 1 H), 1.37 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.1, 138.9, 109.8, 99.8, 99.5, 92.7, 78.7, 72.3, 67.5, 56.6, 52.3, 47.8, 47.7, 39.2, 36.0, 17.7 (2). Anal. Calcd for $C_{17}H_{28}O_8$: C, 56.65; H, 7.83. Found: C, 56.64; H, 7.78.

Carbaphosphinate 1. A solution of carbaphosphinate intermediate **33** (0.657 g, 1.82 mmol), ethyl (diethoxymethyl)-phosphinate[16] (1.07 g, 5.43 mmol), and benzoyl peroxide (0.10 g, 0.42 mmol) in anhydrous dioxane (5 mL) was heated at reflux. After 14 h, more benzoyl peroxide (0.11 g, 0.44 mmol) was added, and refluxing was continued for an additional 10 h. Concentration in vacuo afforded a yellow oil that was purified by radial chromatography (4 mm thickness, EtOAc, EtOAc/MeOH, 9:1, v/v) to afford **34** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.75−4.85 (m, 1 H), 4.60−4.70 (m, 2 H), 4.05−4.30 (m, 3 H), 3.75−3.95 (m, 3 H), 3.65−3.75 (m, 5 H), 3.41 (s, 3 H), 3.20−3.25 (m, 6 H), 2.20−2.75 (m, 5 H), 1.75−1.95 (m, 1 H), 1.35−1.65 (m, 1 H), 1.20−1.40 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 173.2, 173.1, 101.8, 99.9, 99.6, 99.2, 92.7, 92.6, 78.2, 78.1, 74.4 (2), 74.2 (2), 66.4, 65.5, 65.4, 65.2, 61.5, 61.4, 61.3, 56.4, 52.1, 47.6, 36.5, 36.1, 36.0, 35.9, 29.7, 29.6 (2), 26.3, 25.9, 25.0, 24.7, 17.6, 17.5, 16.5 (2), 16.3, 16.2, 15.0, 14.9.

Carbaphosphinate intermediate **34** was directly treated with aqueous HCl (6 N, 5 mL), and the mixture was refluxed for 3 h. After removal of the solvent in vacuo, the residue was dissolved in water and the solution was neutralized to pH 7.4. The solution was applied to AG-1 X8 anion exchange resin (20 mL) that had been equilibrated with 200 mM $Et_3NH^+HCO_3^-$ (pH 7.3). The column was washed with water (40 mL) and eluted with a linear gradient (200 mL + 200 mL, 200−500 mM) of $Et_3NH^+HCO_3^-$ (pH 7.3). Fractions containing phosphorus were identified by the methods of Avila and Ames[25] 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 filtrate was neutralized to pH 7.7 with 0.1 N aqueous NaOH. Concentration in vacuo afforded carbaphosphinate **1** as a white foam (19%, from intermediate **33**): ^{1}H NMR (D_2O, pH 7.7) δ 6.74 (d, J_{PH} = 572 Hz, 1 H), 3.73 (ddd, J = 11, 9, 5 Hz, 1 H),

(25) (a) Avila, L. Z. Ph.D. Dissertation, Stanford University, 1990. (b) Ames, B. N. *Methods Enzymol.* **1966**, *8*, 115.

3.15 (dd, J = 9, 9 Hz, 1 H), 1.65−2.05 (m, 6 H), 1.35−1.50 (m, 1 H); ^{13}C NMR (D_2O, pH 7.75) δ 184.7, 81.2 (J_{PCCC} = 10 Hz), 78.3, 73.8, 43.3, 42.6 (J_{PCC} = 5 Hz), 37.2 (J_{PC} = 10 Hz), 36.1; ^{31}P NMR (D_2O, pH 7.7) δ 29.3; HRMS (FAB) calcd for $C_8H_{13}O_7Na_2P$ ($M + H^+$) 299.0272, found 299.0267.

Carbaphosphonate Intermediate 35. Tetramethyl methylenediphosphonate (2.12 g, 9.15 mmol) in THF (20 mL) was deprotonated with *n*-BuLi (1.6 M in hexane, 5.8 mL, 9.3 mmol) at −78 °C. The resulting solution was cannulated into a solution of intermediate **19** (2.92 g, 9.17 mmol) in THF (20 mL) at −78 °C. After 10 min at −78 °C the reaction mixture was warmed to rt and was stirred at rt overnight. Aqueous NH_4Cl was added, and the aqueous layer was saturated with NaCl and extracted with EtOAc (3×). The combined organic layers were dried and concentrated to an off-white foam. Purification by radial chromatography (4 mm thickness, EtOAc, EtOAc/MeOH, 9:1, v/v) afforded carbaphosphonate intermediate **35** as a white foam (2.18 g, 56%): ^{1}H NMR ($CDCl_3$) δ 5.99 (d, J_{PCH} = 18 Hz), 4.18 (d, J = 10 Hz, 1 H), 3.95 (ddd, J = 11, 10, 5 Hz, 1 H), 3.65−3.80 (m, 9 H), 3.58 (d, J = 14 Hz, 1 H), 3.24 (s, 3 H), 3.23 (s, 3 H), 2.51 (dd, J = 14, 3 Hz, 1 H), 2.00−2.15 (m, 2 H), 1.36 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 174.5, 155.7 (J_{PCC} = 8 Hz), 109.6 (J_{PC} = 189 Hz), 100.1, 99.3, 74.4, 73.3 (J_{PCCC} = 18 Hz), 68.5, 52.8, 52.3 (J_{POC} = 6 Hz), 51.9 (J_{POC} = 6 Hz), 47.9, 47.8, 38.9 (J_{PCCC} = 8 Hz), 37.4, 17.5 (2). Anal. Calcd for $C_{17}H_{29}O_{10}P$: C, 48.11; H, 6.89. Found: C, 48.13; H, 6.83.

Carbaphosphonate Intermediate 36. Carbaphosphonate intermediate **35** (0.940 g, 2.21 mmol) was dissolved in EtOAc (13 mL) and hydrogenolyzed over 10% Pd on C (0.250 g) at 50 psi H_2 pressure for 54 h. After 30 and 48 h, the catalyst was replaced with fresh 10% Pd on C (0.250 g). The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. Carbaphosphonate intermediate **36** was obtained as a white foam (0.776 g, 82%): ^{1}H NMR ($CDCl_3$) δ 3.98 (ddd, J = 10, 10, 6 Hz, 1 H), 3.70−3.85 (m, 1 H), 3.65−3.80 (m, 9 H), 3.25 (s, 3 H), 3.23 (s, 3 H), 2.40−2.60 (m, 1 H), 2.20−2.40 (m, 2 H), 1.85−2.00 (m, 2 H), 1.59 (dd, J = 13, 13 Hz, 1 H), 1.30−1.50 (m, 1 H), 1.30 (s, 3 H), 1.28 (s, 3 H); ^{13}C NMR (CDCl3) δ 175.7, 99.7, 99.2, 74.3 (J_{PCCC} = 17 Hz), 73.4, 66.6, 52.7, 52.4 (J_{POC} = 7 Hz), 52.1 (J_{POC} = 7 Hz), 47.7, 47.6, 39.3, 37.5, 30.3 (J_{PCC} = 3 Hz), 25.1 (J_{PC} = 141 Hz), 17.6 (2). Anal. Calcd for $C_{17}H_{31}O_{10}P$: C, 47.88; H, 7.33. Found: C, 47.74; H, 7.36. Carbaphosphonate intermediate **36** was deprotected under acidic conditions as described for carbaphosphinate **1** to give carbaphosphonate **11** which was spectroscopically identical to previously synthesized material.[1a,5a]

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