# Diastereoselection and in Vivo Inhibition of 3-Dehydroquinate Synthase

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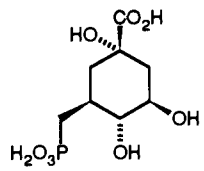
**Abstract:** Epimeric carbaphosphonate [1*R*-(1α,3α,4β,5α)]-1,3,4-trihydroxy-5-(phosphonomethyl)cyclohexane-1-carboxylic acid and carbaphosphonate [1*S*-(1α,3β,4α,5β)]-1,3,4-trihydroxy-5-(phosphonomethyl)cyclohexane-1-carboxylic acid are potent inhibitors of purified 3-dehydroquinate (DHQ) synthase. Can either of these carbocyclic diastereomers inhibit DHQ synthase in intact plants? Obtaining sufficient amounts of both diastereomers for in vivo inhibition studies required the development of efficient synthetic routes to both molecules. Carbaphosphonate and epimeric carbaphosphonate were synthesized from a single epoxy alcohol derived from quinic acid. Payne rearrangement of the epoxy alcohol provided an equilibrium mixture of two diastereomeric epoxides which could then be converted into carbaphosphonate and epimeric carbaphosphonate. An essential step in these conversions entailed introduction of a phosphonomethyl functionality via regioselective, nucleophilic attack on the diastereomeric epoxides with lithium diisopropyl methanephosphonate. Enzyme inhibition in whole plants was measured by the accumulation of 3-deoxy-D-*arabino*-heptulosonic acid (DAH), the dephosphorylated substrate of DHQ synthase, in plant tissue subsequent to foliar application of the carbocyclic inhibitors. Accumulations of DAH of up to 40-fold increases over control DAH levels were observed when carbaphosphonate was applied to *Pisum sativum*, *Echinochloa crusgalli*, *Setaria viridis*, *Sorghum halepense*, and *Avena fatua*. Application of epimeric carbaphosphonate to the same range of plant species resulted in a maximum increase of only 2-fold in DAH concentration. This diastereoselection contrasts sharply with the comparable levels of in vitro inhibition of purified DHQ synthase observed for both carbaphosphonate and epimeric carbaphosphonate.

An important factor determining whether in vivo inhibition of a biosynthetic enzyme will be herbicidal[1] is the nature of the molecular linkage between enzyme inhibition and plant death. Depletion of metabolites derived from a biosynthetic pathway disrupted by inhibition of a pathway enzyme may be the underlying cause of herbicidal activity. Alternatively, plant death might be determined by whether the substrate (or related metabolite) which accumulates due to enzyme inhibition is toxic to the plant.[2] Metabolites which at elevated levels are toxic to plants may define a very small subset of biosynthetic enzymes.

One approach to delineating the molecular linkage between enzyme inhibition and plant death requires that comparable levels of disruption of a biosynthetic pathway be achieved with in vivo inhibition of two separate pathway enzymes.[3] Association of herbicidal activity with in vivo inhibition of only one of the two targeted enzymes would establish in a plant system that factors other than depletion of biosynthetic end products are essential to herbicidal activity. The focus of this study is the in vivo inhibition of 3-dehydroquinate (DHQ) synthase[4] which is one of the enzymes of the common pathway of aromatic amino acid biosynthesis (Scheme I).[5] Another of this pathway's enzymes, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, is the target of the commercial herbicide *N*-phosphonomethylglycine (glyphosate).[6]

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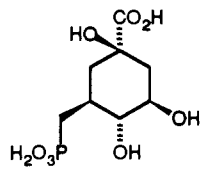


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Two diastereomers, carbaphosphonate **5** and epimeric carbaphosphonate **6**, have recently been identified as potent inhibitors of DHQ synthase.[4b,d,j] Although in vitro studies of enzyme inhibition require only a few milligrams of inhibitor, determination of in vivo inhibition of the targeted enzyme in a range of plant species demands access to substantially larger quantities of inhibitor. Efficient syntheses of both carbocyclic, diastereomeric inhibitors of DHQ synthase are now detailed. Carbaphosphonate **5** and epimeric carbaphosphonate **6** were synthesized from a common intermediate readily derived from commercially available quinic acid.

Each of the synthesized diastereomers has been separately applied to a range of plant species. Accumulation in plant tissue of 3-deoxy-D-*arabino*-heptulosonic acid DAH (**1b**, Scheme I) is used as the basis for comparing in vivo inhibition of DHQ synthase by carbaphosphonate **5** and epimeric carbaphosphonate **6**. Although in vitro inhibition of DHQ synthase has been previously determined not to be diastereoselective,[4j] in vivo inhibition of DHQ

(1) For some reviews covering herbicides associated with disruption of plant biosynthetic cascades, see: (a) Kishore, G. M.; Shah, D. M. *Ann. Rev. Biochem.* **1988**, *57*, 627. (b) *Target Sites of Herbicide Action*; Böger, P., Sandmann, G., Ed.: CRC: Boca Raton, FL, 1989.

(2) (a) LaRossa. R. A.; Van Dyk, T. K. *BioEssays* **1987**, *7*, 125. (b) Fischer, R. S.; Berry, A.; Gaines, C. G.; Jensen, R. A. *J. Bacteriol.* **1986**, *168*, 1147.

(3) For instances where herbicidal activity has been appraised for a molecule which inhibits a biosynthetic pathway enzyme which is remote to a pathway enzyme precedented to be the target of a commercial herbicide, see: (a) Aulabaugh, A.; Schloss, J. V. *Biochemistry* **1990**, *29*, 2824. (b) Pompliano, D. L.; Reimer, L. M.; Myrvold, S.; Frost, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 1866. (c) Schulz, A.; Spönemann, P.; Köcher. H.; Wengenmayer, F. *FEBS Lett.* **1988**, *238*, 375. (d) Holländer, H.; Kiltz, H.-H.; Amrhein, N. *Z. Naturforsch.* **1979**, *34c*, 1162. (e) Amrhein, N.; Holländer, H. *Planta* **1979**, *144*, 385. (f) Baillie. A. C.; Corbett, J. R.; Dowsett. J. R.; McCloskey. P. *Pestic. Sci.* **1972**, *3*, 113. (g) Wittenbach, V. A.; Aulabaugh, A.; Schloss, J. V. In *Pesticide Chemistry*; Frehse, H., Ed.; VCH: 1990, p 151.

(4) For recent research dealing with in vitro inhibition or delineation of the mechanism of action of DHQ synthase, see: (a) Widlanski, T.; Bender. S. L.; Knowles. J. R. *J. Am. Chem. Soc.* **1987**, *109*, 1873. (b) Widlanski, T.; Bender, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 2299. (c) Bender, S. L.; Mehdi, S.; Knowles. J. R. *Biochemistry* **1989**, *28*, 7555. (d) Bender, S. L.; Widlanski, T.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7560. (e) Widlanski. T.; Bender. S. L.; Knowles, J. R. *Biochemistry* **1989**, *28*, 7572. (f) Bartlett. P. A.; Satake, K. *J. Am. Chem. Soc.* **1988**, *110*, 1628. (g) Bartlett, P. A.; McLaren, K. L.; Alberg, D. G.; Fässler. A.; Nyfeler. R.; Lauhon. C. T.; Grissom, C. B. *BCPC Monogr.* **1989**, *2*, 155. (h) Nikolaides, N.; Ganem. B. *Tetrahedron Lett.* **1989**, *30*, 1461. (i) Myrvold, S.; Reimer, L. M.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1989**, *111*, 1861. (j) Piehler, L. T.; Montchamp, J.-L.; Frost, J. W.; Manly, C. J. *Tetrahedron* **1991**, *47*, 2423. (k) Montchamp, J.-L.; Frost, J. W. *J. Am. Chem. Soc.* **1991**, *113*, 6296.

(5) A selective sampling of this extensively reviewed subject includes the following: (a) Haslam, E. *The Shikimate Pathway* Wiley; New York, 1974. (b) Ganem, B. *Tetrahedron* **1978**, *34*, 3353. (c) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley: New York, 1980. (d) Herrmann. K. M. In *Amino Acids: Biosynthesis and Genetic Regulation*; Herrmann, K. M., Somerville, R. L., Ed.; Addison-Wesley: Reading, 1983; p 301. (e) Dewick, P. M. *Nat. Prod. Rep.* **1984**, *1*, 451. (f) Dewick, P. M. *Nat. Prod. Rep.* **1985**, *2*, 495. (g) Dewick, P. M. *Nat. Prod. Rep.* **1986**, *3*, 565. (h) *The Shikimate Pathway*; Conn, E. E., Ed.; Plenum: New York, 1986. (i) Pittard, A. J. In *Escherichia coli and Salmonella typhimurium*; Neidhardt. F. C., Ed.; American Society for Microbiology: Washington, DC, 1987; Vol. 1, p 368. (j) Dewick, P. M. *Nat. Prod. Rep.* **1988**, *5*, 73. (k) Dewick, P. M. *Nat. Prod. Rep.* **1989**, *6*, 263. (l) Dewick, P. M. *Nat. Prod. Rep.* **1990**, *7*, 165.

(6) *The Herbicide Glyphosate*; Grossbard, E., Atkinson. D., Ed.; Butterworths: Boston, 1985.

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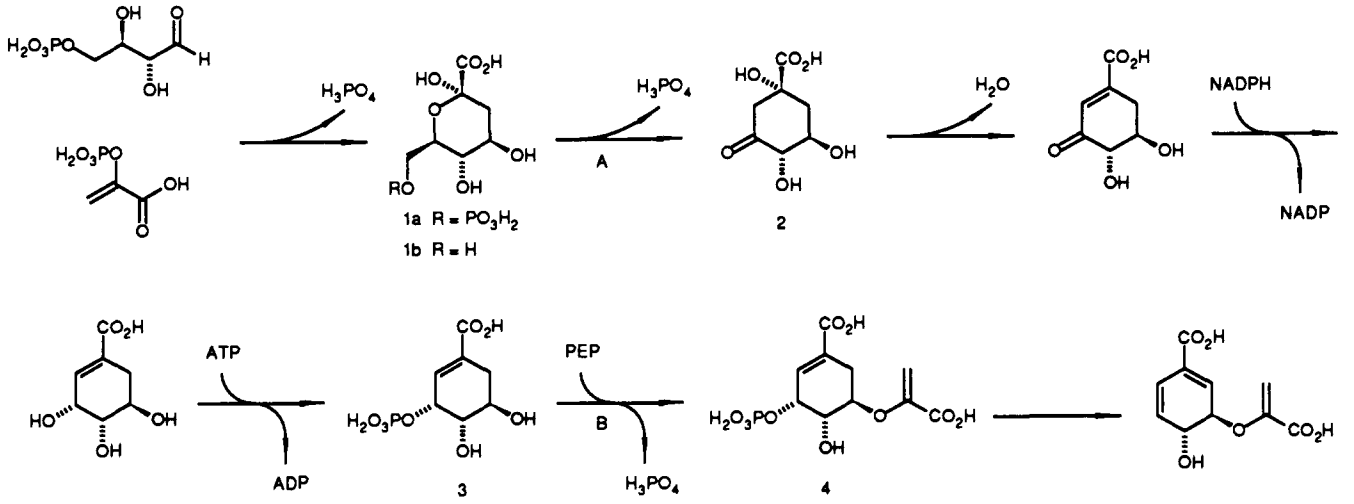
Scheme I

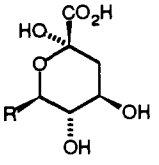



A. DHQ synthase
B. EPSP synthase

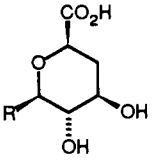
synthase has been discovered to be very diastereoselective. Foliar application of carbaphosphonate **5**, but not epimeric carbaphosphonate **6**, leads to substantial accumulation of DAH in plant tissue.

## Results and Discussion

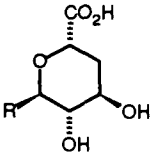
Approaches to inhibition of DHQ synthase have proceeded past several distinct milestones with the first being the synthesis of the nonisosteric substrate analogue 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphonate **7**.[7] This organophosphonate was a micromolar, competitive inhibitor relative to the binding of substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate **1a** (Scheme I). As opposed to nonisosteric **7**, isosteric 3-deoxy-D-*arabino*-heptulosonic acid 7-homophosphonate **8** was a poor enzyme inhibitor.[7] The phosphonomethyl moiety of **7** has become a dominant feature of many inhibitors of DHQ synthase. A tremendous step forward was taken with the discovery of a new class of inhibitory substrate analogues where the pyranosyl ring oxygen was replaced with a methylene group.[4b,d] Carbaphosphonate [1*S*-(1$\alpha$,3$\beta$,4$\alpha$,5$\beta$)]-1,3,4-trihydroxy-5-(phosphonomethyl)cyclohexane-1-carboxylic acid **5** was a nanomolar, slow-binding inhibitor.[4b,d] Other carbocyclic analogues have been efficiently synthesized in an attempt to generate a Michael acceptor at the enzyme active site which was capable of suicide inactivation of DHQ synthase.[4h]



**7** R=$CH_2PO_3H_2$
**8** R=$(CH_2)_2PO_3H_2$



**9** R=$CH_2PO_3H_2$
**10** R=$CH_2OPO_3H_2$



**11** R=$CH_2PO_3H_2$
**12** R=$CH_2OPO_3H_2$

While examining substrate analogues which did not rearrange to furans, 2,6-anhydro analogues **11** and **12** of substrate DAHP possessing $\alpha$-carboxylates at the C-2 carbon were discovered to be inhibitors of DHQ synthase.[4i] This observation was surprising given that DHQ synthase presumably binds the diastereomer of DAHP characterized by a $\beta$-carboxylate at the anomeric, C-2 carbon. 2,6-Anhydro analogues **9** and **10** with $\beta$-carboxylates at the C-2 carbon were much poorer inhibitors of DHQ synthase.[4i] This curious stereochemical promiscuity exhibited by DHQ synthase has been ascribed to potential interaction of the $\alpha$-carboxylate with a basic amino acid residue at the active site which might catalyze the intramolecular aldol condensation mediated by the enzyme.[4j] Given the inhibitory pattern of 2,6-anhydro analogues, the potential existed for epimeric carbaphosphonate **6** to be an even more potent inhibitor of DHQ synthase than carbaphosphonate **5**.

**Synthesis of Carbaphosphonate and Epimeric Carbaphosphonate.** Quinic acid **13** was the starting material for synthesis of epimeric carbaphosphonate **6**. Two different strategies were envisioned (Schemes II and III). The strategy followed in Scheme II entailed initial destruction of the stereochemistry at the C-1 carbon of quinic acid to give exocyclic olefin **16**. This olefinic intermediate was precedented to undergo preferential attack by electrophilic reagents at the $\beta$-face of the olefin.[8] Dihydroxylation of olefin **16** should provide intermediate **18** which could be converted to epimeric carbaphosphonate **6**. A second strategy (Scheme III) avoided any destruction of asymmetric carbons in quinic acid by exploiting a unique stereochemical feature of quinic acid. Specifically, inversion of the asymmetric center at C-4 is equivalent to inverting the stereochemistry at C-1.[9]

Synthesis of epimeric carbaphosphonate **6** (Scheme II) began with the acid-catalyzed reaction of benzaldehyde and quinic acid **13**.[10] Resulting intermediate **14** was characterized by benzylidene protection of the C-3 and C-4 alcohols along with lactonization of the C-1 carboxylate and C-5 alcohol. Both possible diastereomeric products epimeric at the benzylidene methine proton were generated during the protection although only one diastereomer was obtained after recrystallization. Lactone **14** was subsequently reduced to *vic*-diol **15** with sodium borohydride. The vicinal diol provided the handle necessary for removal of the C-1 asymmetric center. Deoxygenation by the Eastwood procedure gave the exocyclic olefin **16**.[11,8] Treatment of the olefin with catalytic osmium

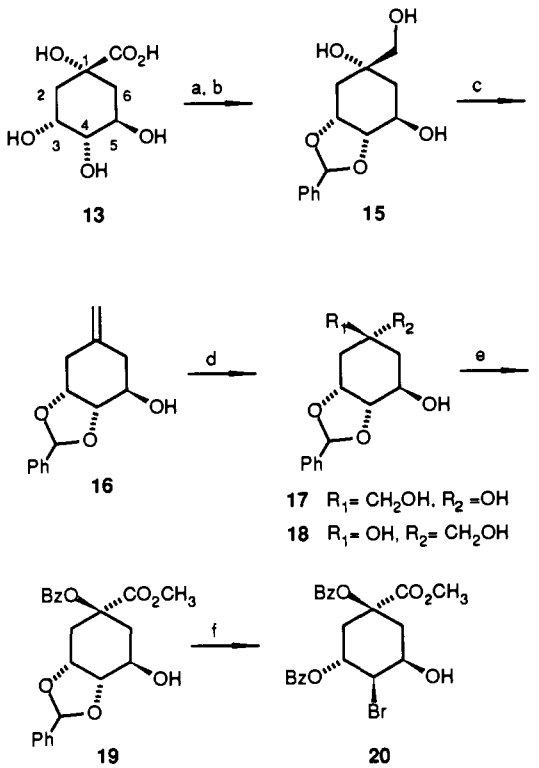
(7) (a) Le Maréchal, P.; Froussios, C.; Level, M.; Azerad, R. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1104. (b) Le Marechal, P.; Froussios, C.; Level, M.; Azerad, R. *Carbohydr. Res.* **1981**, *94*, 1.

(8) Trost, B. M.; Romero, A. G. *J. Org. Chem.* **1986**, *51*, 2332.

(9) (a) Gorin, P. A. J. *Can. J. Chem.* **1963**, *41*, 2417. (b) Corse, J.; Lundin, R. E. *J. Org. Chem.* **1970**, *35*, 1904. (c) Snyder, C. D.; Rapoport, H. *J. Am. Chem. Soc.* **1973**, *95*, 7821.

(10) Lesuisse, D.; Berchtold, G. A. *J. Org. Chem.* **1985**, *50*, 888.

**Scheme II.**[a]



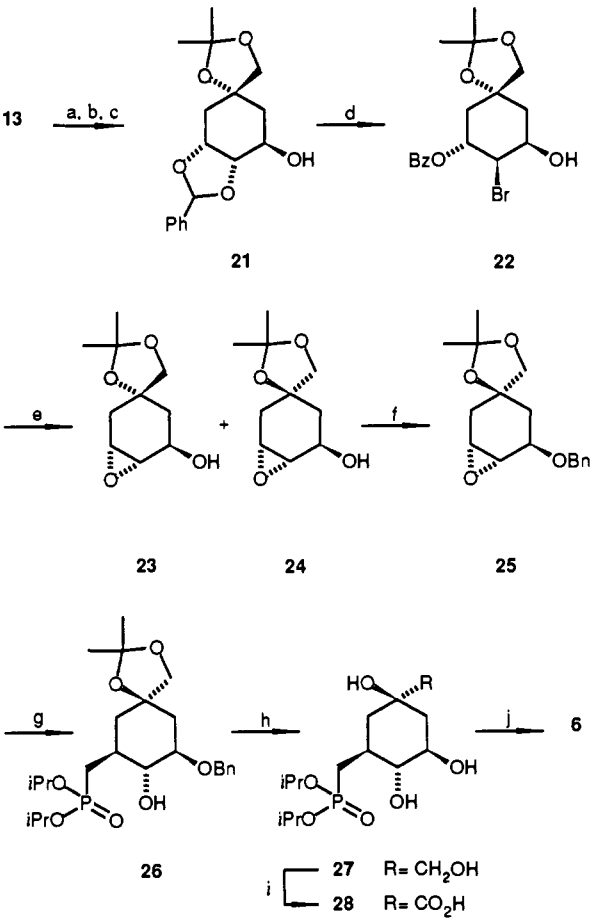

[a] (a) PhCHO, *p*-TsOH, $C_6H_6$, reflux, 85%; (b) $NaBH_4$, EtOH, 0 °C, 75%; (c) PhCOOH, $(EtO)_3CH$, 200 °C, 37%; (d) $OsO_4$, NMMO, $H_2O$/acetone (1:4), 0 °C, 74% (2:1, **18**:**17**); (e) (i) $O_2$, Pt, $NaHCO_3$, $H_2O$/acetone (5:1), 45 °C, (ii) BzCl, Pyr (iii) $CH_2N_2$, $Et_2O$/MeOH (1:1), room temperature, 70%; (f) NBS, $C_6H_6$, room temperature, 63%.

tetroxide and *N*-methylmorpholine *N*-oxide[12] gave a diastereomeric mixture of vicinal diols **17** and **18** which were readily separable by chromatography on silica gel. Osmium attack on the exocyclic olefin yielded a 2:1 ratio of **18**:**17** where diastereomer **17** was found to be identical with intermediate **15**. Selective oxidation of the primary hydroxyl group of **18**, benzoylation of the C-1 alcohol, and carboxylate esterification afforded protected *epi*-quinate **19**. During benzoylation, the C-5 hydroxyl was not protected. Conversion of intermediate **18** to protected *epi*-quinate **19** proceeded without any intervening purifications.

The previously reported strategy[4d] of radical coupling of allyltributyltin with a carbocyclic bromide was targeted as the methodology for attaching a side chain which could ultimately be transformed into the phosphonomethyl moiety. Obtaining the required bromide relied on oxidative bromination of intermediate **19**.[13] Spectroscopic characterization of **19** along with the reaction conditions required for protection of the C-5 hydroxyl were consistent with a predominance of one *epi*-quinate **19** conformer in solution with axial C-1 benzoate and axial C-5 hydroxyl substitution. Such a conformational preference along with transdiaxial attack[14] was expected to lead to regioselective bromination at C-3. Unfortunately, reaction of protected *epi*-quinate with *N*-bromosuccinimide led to a C-4 bromide instead of the desired C-3 bromide. This complete dominance of C-4 bromination precluded synthesis of epimeric carbaphosphonate **6** by the route of Scheme II.

The second strategy (Scheme III) was identical to the first part of the synthesis of Scheme II with simultaneous benzylidene

**Scheme III.**[a]




[a] (a) PhCHO, *p*-TsOH, $C_6H_6$, reflux, 85%; (b) $NaBH_4$, EtOH, 0 °C, 75%; (c) 2-methoxypropene, *p*-TsOH, DMF, room temperature, 70%; (d) NBS, $C_6H_6$, room temperature, 79%; (e) MeONa, MeOH/THF (1:2), reflux, 82% (1:1.5, **23**:**24**); (f) NaH, BnBr, THF, room temperature, 90%; (g) $(iPrO)_2P(O)CH_2Li{\cdot}BF_3$, THF, −78 °C, 86%; (h) (i) AcOH/$H_2O$/THF (2:2:1), 70 °C, 93%, (ii) $H_2$, 10% Pd on C, MeOH, 100%; (i) $O_2$, Pt, $NaHCO_3$, $H_2O$, 55 °C, 65%; (j) (i) TMSBr, $Et_3N$, $CH_2Cl_2$, room temperature, (ii) $H_2O$, room temperature, 40%.

formation and lactonization of quinic acid **13** followed by reduction of the lactone. However, the vicinal diol was protected (Scheme III) to give the isopropylidene of intermediate **21** instead of deoxygenation of the exocyclic olefin **16** (Scheme II). Reaction of intermediate **21** with *N*-bromosuccinimide yielded bromobenzoyl **22** with exclusive bromination at C-4. Such C-4 bromination, which was an insurmountable problem in the first synthetic route (Scheme II), was not an impediment in the second route to epimeric carbaphosphonate **6** (Scheme III). Subsequent reaction of the bromobenzoyl **22** with sodium methoxide in methanol resulted in removal of the benzoate followed by epoxide formation.

When the debenzoylation/epoxidation was run at lower temperatures for short reaction times, only epoxy alcohol **23** was formed. Debenzoylation/epoxidation at reflux temperatures for longer reaction times yielded a 1.5:1 ratio of diastereomeric epoxy alcohols **24**:**23** suggestive of an intervening Payne rearrangement.[15,16] Both diastereomers were readily separable by silica gel chromatography although ambiguities arose during attempts to assign the correct structure to each diastereomer. As a consequence, one of the epoxy alcohols was converted into a carbaphosphonate and then compared with carbaphosphonate **5** synthesized by literature[4d] procedure. Crystals of the epoxy alcohol

(11) (a) Josan, J. S.; Eastwood, F. W. *Aust. J. Chem.* **1968**, *21*, 2013. (b) Block, E. *Org. React.* **1984**, *30*, 457.
(12) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973.
(13) (a) Hanessian, S. *Carbohydr. Res.* **1966**, *2*, 86. (b) Hanessian, S.; Plessas, N. R. *J. Org. Chem.* **1969**, *34*, 1035.
(14) King, J. F.; Allbutt, A. D. *Can. J. Chem.* **1969**, *47*, 1445.
(15) For examples of Payne rearrangements in closely related systems, see: (a) Wood, H. B.; Ganem, B. *Tetrahedron Lett.* **1989**, *30*, 6257. (b) Wood, H. B.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 8907.
(16) Payne, G. B. *J. Org. Chem.* **1962**, *27*, 3819.

**24** suitable for crystallographic analysis (Figure 1, supplementary material) were also ultimately obtained. With structures finally assigned to the isolated epoxy alcohols, epimeric carbaphosphonate **6** was synthesized (Scheme III) from the epoxy alcohol **24**, while carbaphosphonate **5** was synthesized (Supplementary Material) via a similar route from epoxy alcohol **23**.

After protection of the C-5 hydroxyl group of epoxy alcohol **24** as the benzyl ether to yield intermediate **25**, the phosphonomethyl moiety was introduced. Relevant literature routes for introducing phosphonomethylene[4d] or phosphorylmethylene[4h] moieties at C-3 utilized cumbersome, multistep strategies. A substantially simplified route for phosphonomethylene attachment resulted from discovery that reaction of epoxide **25** with lithium diisopropyl methanephosphonate and an equivalent amount of boron trifluoride etherate yielded organophosphonate **26**.[17,18] The epoxide ring opening proceeds at −78 °C with apparent exclusive attack at C-3.

Hydrolysis of the isopropylidene followed by hydrogenolysis unmasked the C-1 and C-5 alcohols and the C-1 hydroxymethylene substituent of intermediate **27**. Subsequent retrieval of the carboxylate was complicated by the need to oxidize a hydroxymethylene substituent which was also part of a *vic*-diol. Oxidation with chromium reagents or ruthenium tetroxide resulted in cleavage of the bond connecting the *vic*-diol and formation of a carbonyl at C-1. Fortunately, reaction of the *vic*-diol with platinum black[19] and oxygen provided smooth conversion of the hydroxymethylene group of **27** to the carboxylate of **28**. Removal[20] of the phosphonate esters from intermediate **29** then afforded epimeric carbaphosphonate **6**.

**In Vivo Inhibition of 3-Dehydroquinate Synthase.** Detection of shikimate accumulation in plant tissue treated with glyphosate was one of the key discoveries leading to recognition of EPSP synthase inhibition as the primary basis of glyphosate's herbicidal activity.[21] The substrate expected to accumulate during in vivo inhibition of EPSP synthase would be shikimate 3-phosphate. However, the action of phosphatases typically results in accumulation of shikimate. These observations suggested that inhibition of DHQ synthase should result in accumulation of 3-deoxy-D-*arabino*-heptulosonic acid, DAH (**1b**, Scheme I). The relative increase in DAH in tissue extract upon foliar application of epimeric carbaphosphonate **6** versus carbaphosphonate **5** was expected to indicate which diastereomer was the most potent in vivo inhibitor.

*Pisum sativum* (pea), *Echinochloa crusgalli* (barnyard grass), *Setaria viridis* (green foxtail), *Sorghum halepense* (johnson grass), and *Avena fatua* (wild oat) were grown in vermiculite watered every 2 days with modified Hoagland's[22] solution. Carbaphosphonate **5** and epimeric carbaphosphonate **6** were applied once after the sprouts broke the surface of the vermiculite followed by foliar application every third day. Application solution was formulated to insure effective wetting of the plant surfaces. After 14 days, the plants were harvested. The weight of freshly harvested control, untreated plants relative to treated plants and visual inspection for discoloration and desiccation were used to gauge herbicidal activity. None of the plant species suffered herbicidal effects upon exposure to epimeric carbaphosphonate **6** or carbaphosphonate **5**. By contrast, glyphosate treatment led to quite recognizable herbicidal activity.

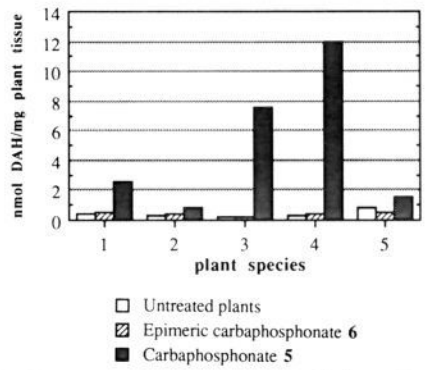



**Figure 2.** DAH concentrations (nmol per mg of desiccated plant tissue) in untreated plants (□) and DAH concentrations in plants after exposure to epimeric carbaphosphonate **6** (▨) and carbaphosphonate **5** (■). Plant species included (1) *Pisum sativum* (pea), (2) *Echinochloa crusgalli* (barnyard grass), (3) *Setaria viridis* (green foxtail), (4) *Sorghum halepense* (johnson grass), and (5) *Avena fatua* (wild oat). Growth, inhibitor application, and analysis for DAH concentrations are described in Results and Discussion and in the Experimental Section.

Treated and untreated plants were dried to a constant weight and $^{14}$C-labeled[3b] DAH added to the dried tissue. Refluxing aqueous ethanol extracted metabolites from the dried plant tissue. Extracts were then fractionated via high pressure liquid chromatography using a semipreparative strong anion exchange column. Radiolabeled fractions were collected, and the concentrations of DAH in these fractions colorimetrically determined with periodate oxidation followed by treatment with thiobarbituric acid.[23] The concentrations of inhibited enzyme substrate in treated plant tissue are summarized in Figure 2. Substantial levels of DAH accumulation (40-fold as the maximum) were detected in a number of the plant species treated with carbaphosphonate **5**. Treatment with epimeric carbaphosphonate **6** resulted in little or no increases in DAH.

Given the similar in vitro inhibition constants for epimeric carbaphosphonate **6** ($K_i = 7.3 \times 10^{-9}$ M) and carbaphosphonate **5** ($K_i = 5.4 \times 10^{-9}$ M),[4j] the tremendous difference in accumulation of DAH upon foliar application of these diastereomeric carbaphosphonates constitutes a compelling example of diastereoselection during in vivo enzyme inhibition. Part of this in vivo diastereoselection may reflect differences in the rates at which epimeric carbaphosphonate and carbaphosphonate bind to and dissociate from DHQ synthase.[34] Plants can respond to inhibition of a common pathway enzyme by increasing the flow of carbon into the common pathway.[28] Resulting buildup of DAHP and reversal of enzyme inhibition can be forestalled if the release of inhibitor from DHQ synthase is sufficiently slow. The slower rate of dissociation[4j] of epimeric carbaphosphonate **6** ($k_{off} = 1.1 \times 10^{-4}$ $s^{-1}$) would thus be more advantageous to maintaining in vivo enzyme inhibition than the faster rate[4j] for carbaphosphonate **5** ($k_{off} = 7.5 \times 10^{-4}$ $s^{-1}$). On the other hand, in vivo enzyme inhibition will be delayed by a slow rate for binding of the inhibitor to targeted enzyme. Epimeric carbaphosphonate **6** ($k_{on} = 1.5 \times 10^4$ $M^{-1}$ $s^{-1}$) binds more slowly[4j] to DHQ synthase than carbaphosphonate **5** ($k_{on} = 1.4 \times 10^5$ $M^{-1}$ $s^{-1}$). Diastereoselection during in vivo inhibition of DHQ synthase may also indicate that penetration through cellular and plastidal barriers, translocation to meristematic tissue, degradative pathways, or conjunction rates may be different for epimeric carbaphosphonate **6** and carbaphosphonate **5**.

The in vivo inhibition of DHQ synthase achieved with carbaphosphonate **5** is a significant step towards the ultimate goal of

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achieving comparable levels of disruption of aromatic amino acid biosynthesis with inhibition of two different pathway enzymes. A necessary next step must entail establishing the extent to which aromatic amino acid biosynthesis is disrupted by respective inhibition of DHQ synthase and EPSP synthase by carbaphosphonate **5** and glyphosate. One option is to quantitate the change in the size of pools of free L-phenylalanine, L-tyrosine, and L-tryptophan. The straightforward appearance of such a determination is deceptive given the rapid rate at which plants hydrolyze proteins to replenish pools of free amino acids when biosynthesis of these building blocks is disrupted by herbicide action.[24] As a consequence, the concentration of free amino acids may remain unchanged or actually increase when their biosynthesis is chemically disrupted.[25,27] Circumvention of this problem might be possible by quantitating the relative rates of $^{15}N$ incorporation into pools of free aromatic amino acids when DHQ synthase and EPSP synthase are inhibited. Although such experiments have been reported for inhibition of glutamine synthetase[26] and acetolactate synthase,[27] glyphosate-induced turnover of pools of aromatic amino acids has not been quantitated. Measurement of increases in the specific activity of DAHP synthase when DHQ synthase and EPSP synthase are inhibited may provide an alternative way to establish the extent in which aromatic amino acid biosynthesis is disrupted.[28]

The levels of in vivo inhibition of DHQ synthase currently being realized with carbaphosphonate **5** justify development of the detailed biochemical analyses needed to establish the extent aromatic amino acid biosynthesis is disrupted. It is possible that levels of in vivo inhibition of DHQ synthase have already been realized where aromatic amino acid biosynthesis is disrupted to an extent comparable to that achieved with in vivo inhibition of EPSP synthase by herbicidal concentrations of glyphosate. Alternatively, more potent in vivo inhibitors of DHQ synthase may be required to reach the necessary level of disruption of aromatic amino acid biosynthesis. In this regard, carbaphosphonate **5** constitutes an invaluable foundation for design and synthesis of future in vivo inhibitors of DHQ synthase.

## Experimental Section

**General Chemistry.** $^1H$ NMR spectra were recorded at 200 MHz on a Varian Gemini-200 spectrometer, at 300 MHz on a General Electric QE-300, or at 500 MHz on a Varian VXR-500 spectrometer. Chemical shifts for $^1H$ NMR spectra are reported (in parts per million) relative to internal tetramethylsilane ($(CH_3)_4Si$, $\delta$ = 0.0 ppm) with $CDCl_3$ or acetone-$d_6$ as solvent and to sodium 3-(trimethylsilyl)propionate-2,2,3,3-$d_4$ (TSP, $\delta$ = 0.0 ppm) when $D_2O$ was the solvent. $^{13}C$ NMR spectra were recorded at 50 MHz on a Varian Gemini-200 spectrometer or at 75 MHz on a General Electric QE-300 spectrometer. Chemical shifts for $^{13}C$ NMR spectra are reported (in parts per million) relative to $CDCl_3$ ($\delta$ = 77.0 ppm) or internal acetonitrile ($CH_3CN$, $\delta$ = 3.69 ppm) in $D_2O$ or acetone-$d_6$. $^{31}P$ NMR spectra were recorded on a Varian XL-200 spectrometer at 80 MHz, and chemical shifts are reported (in parts per million) relative to external 85% phosphoric acid (0.00 ppm) or external trimethyl phosphate (TMP, $\delta$ = 6.15 ppm). A Perkin-Elmer Model 1800 FTIR spectrometer was used for infrared spectra which were recorded in wavenumbers ($cm^{-1}$). Low resolution electron impact (EI) and chemical ionization (CI) mass spectra (MS) were recorded on a Finnigan 4000 mass spectrometer, and high resolution mass spectra (HRMS) were recorded on Kratos MS50 or MS25 mass spectrometers. Isobutane was used as the primary ion source for chemical ionization mass spectra. Fast atom bombardment (FAB) mass spectroscopy was performed on a Kratos MS50 spectrometer, employing glycerol as a matrix. Ultraviolet/visible spectra were recorded on a Perkin-Elmer Lambda 3B spectrophotometer. Phosphorus and phosphate were determined by the method of Ames.[29] Hydrogenations were carried out with a Parr hydrogenation apparatus (Parr Instrument Co.). Radial chromatography was carried out with a Harrison Associates Chromatotron using 1-, 2-, or 4-mm layers of silica gel 60 $PF_{254}$ containing gypsum (E. Merck). Silica gel 60 (40–63 $\mu$m, E. Merck) was used for flash chromatography.[30] Analytical thin-layer chromatography (TLC) utilized precoated plates of silica gel 60 F-254 (0.25 mm, E. Merck). TLC plates were visualized by immersion in anisaldehyde stain (by volume: 93% ethanol, 3.5% sulfuric acid, 1% acetic acid, and 2.5% anisaldehyde) followed by heating. *N*-Bromosuccinimide was recrystallized from water. Benzoyl chloride was diluted with benzene (1:1, v/v), washed twice with cold aqueous sodium bicarbonate (5%), dried with calcium chloride, and filtered. Benzene was subsequently removed in vacuo, and the benzoyl chloride vacuum distilled. Dimethylformamide was distilled from Linde 4A molecular sieves under reduced pressure, followed by storage over Linde 4A molecular sieves under nitrogen. Pyridine, triethylamine, and dichloromethane were distilled from calcium hydride under nitrogen. Benzene was distilled from sodium under nitrogen. Tetrahydrofuran was distilled under nitrogen from sodium benzophenone ketyl. Methanol was dried over activated 3A molecular sieves. Uniformly $^{14}C$-labeled shikimic acid was obtained from NEN. Radiolabeled [4,5,6,7-$^{14}C$]DAH was prepared according to literature procedure.[32,3b]

**[2*S*-(2$\alpha$,3a$\alpha$,5$\beta$,7$\alpha$,7a$\alpha$)]-Hexahydro-5-(hydroxymethyl)-2-phenyl-1,3-benzodioxole-5,7-diol (15).**[31] A solution of lactone **14**[10] (27.5 g, 0.105 mol) in absolute ethanol (135 mL) was slowly added to a solution of sodium borohydride in absolute ethanol (415 mL) at 0 °C. After stirring for 45 min at 0 °C, acetone (65 mL) was added with vigorous stirring, followed by acetic acid until no more gas was evolved. After removal of the solvent in vacuo, the foamy residue was dissolved in a mixture of ethyl acetate and saturated aqueous ammonium chloride. Saturated sodium bisulfate was added until the aqueous layer was about pH 5, and then the aqueous layer was saturated with sodium chloride. The aqueous layer was extracted with ethyl acetate (3 × 150 mL), and the combined organic layers were dried with magnesium sulfate and silica gel (1 g). After removal of the solvent in vacuo, the resulting hygroscopic white foam was dried under vacuum (0.2 mmHg) and over phosphorus pentoxide, affording 21.0 g (78.7 mmol, 75%) of triol **15** as a dry white foam: $^1H$ NMR ($CDCl_3$) $\delta$ 1.60 (dd, $J$ = 13, 9 Hz, 1 H), 1.92–2.09 (m, 2 H), 2.35 (dd, $J$ = 16, 2 Hz, 1 H), 2.45 (s, 1 H), 2.72 (s, 1 H), 2.97 (s, 1 H), 3.43 (d, $J$ = 14 Hz, 1 H), 3.51 (d, $J$ = 14 Hz, 1 H), 4.07–4.21 (m, 2 H), 4.50–4.59 (m, 1 H), 5.90 (s, 1 H), 7.37–7.46 (m, 3 H), 7.46–7.58 (m, 2 H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 33.1, 37.8, 68.6, 69.9, 72.0, 76.2, 79.6, 103.8, 126.6, 128.5, 129.5, 136.9; IR ($CDCl_3$) 3422 (s, br), 3066 (w), 3038 (w), 2928 (m), 2882 (m), 1460 (m), 1408 (s), 1362 (m), 1064 (s); MS, *m/e* (rel intensity) EI 266 (6, $M^+$), 107 (100), 105 (71), 95 (22), 91 (21), 85 (40), 79 (57), 77 (52), 57 (45), 55 (29); CI: 267 (97, M + $H^+$), 249 (100), 143 (29), 125 (22), 107 (31); HRMS (EI) calcd for $C_{14}H_{18}O_5$ ($M^+$) 266.1154, found 266.1151. Anal. ($C_{14}H_{18}O_5 \cdot {}^1/_2H_2O$) C, H.

**[2*S*-(2$\alpha$,3a$\alpha$,5$\beta$,7$\alpha$,7a$\alpha$)]-Hexahydro-7-hydroxy-2',2'-dimethyl-2-phenylspiro[1,3-benzodioxole-5,4'-[1,3]dioxolane] (21).** Triol **15** (5.82 g, 21.9 mmol) was dissolved in dry dimethylformamide (200 mL), and 2-methoxypropene (4.2 mL, 43.7 mmol) was added dropwise using a syringe. A catalytic amount (0.030 g, 0.16 mmol) of *p*-toluenesulfonic acid was subsequently added, and the reaction solution was stirred for 24 h at room temperature. After addition of pyridine (6 mL), the reaction solution was concentrated and purified by flash chromatography (1:1 ethyl acetate/hexane, v/v) to yield 4.69 g (15.3 mmol, 70%) of **21** as a colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (s, 6 H), 1.59 (dd, $J$ = 13, 11 Hz, 1 H), 1.97–2.04 (m, 2 H), 2.12 (ddd, $J$ = 13, 4, 1 Hz, 1 H), 2.29 (ddd, $J$ = 14, 4, 1 Hz, 1 H), 3.31 (d, $J$ = 8 Hz, 1 H), 3.98 (dd, $J$ = 7, 7 Hz, 1 H), 4.20 (ddd, $J$ = 13, 7, 5 Hz, 1 H), 4.38–4.46 (m, 1 H), 5.90 (s, 1 H), 7.35–7.42 (m, 3 H), 7.52–7.59 (m, 2 H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 27.1, 27.3, 36.0, 39.0, 69.0, 74.3, 75.2, 79.0, 80.0, 104.0, 109.6, 126.8, 128.3, 129.3, 137.4; IR ($CCl_4$) 3458 (s, br), 3070 (w), 3037 (w), 2987 (s), 2935 (s), 2869 (s), 1458 (s), 1370 (s), 1250 (s), 1215 (s), 1065 (s); MS, *m/e* (rel intensity) EI 306 (7), 291 (79), 143 (44), 125 (100), 107

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(87), 104 (64), 97 (40), 85 (58), 83 (42), 79 (88), 77 (49), 72 (76), 59 (66), 57 (44); CI 307 (100), 249 (60), 219 (17), 201 (38), 143 (20), 125 (14), 107 (15); HRMS (EI) calcd for $C_{17}H_{22}O_5$ ($M^+$) 306.1467, found 306.1458.

**[5*R*-(5α,7α,8β,9β)]-7-(Benzoyloxy)-8-bromo-2,2-dimethyl-1,3-dioxaspiro[4.5]decan-9-ol (22).** *N*-Bromosuccinimide (2.66 g, 14.9 mmol) was added in one portion to a colorless solution of benzylidene **21** (4.16 g, 13.6 mmol) in dry benzene (250 mL). The reaction mixture turned red after 5 min and was then refluxed until the solution became colorless. Addition of ethyl acetate (100 mL) at room temperature was followed by removal of excess bromine with the addition of 20% aqueous sodium bisulfite (100 mL). The organic layer was washed with saturated aqueous sodium bicarbonate (2 × 100 mL) followed by brine (100 mL). After drying over magnesium sulfate, concentration of the organic layer under reduced pressure gave a yellow oil. Purification by flash chromatography (1:1 ethyl acetate/hexane, v/v) afforded 4.13 g (10.7 mmol, 79%) of **22** as a white solid: $^1$H NMR ($CDCl_3$) δ 1.34 (s, 3 H), 1.37 (s, 3 H), 1.84 (dd, $J$ = 13, 10 Hz, 1 H), 2.02–2.19 (m, 2 H), 2.31 (d, $J$ = 5 Hz, 1 H), 2.42 (ddd, $J$ = 13, 5, 1 Hz, 1 H), 3.97 (d, $J$ = 11 Hz, 1 H), 4.05 (d, $J$ = 11 Hz, 1 H), 4.28–4.35 (m, 1 H), 4.39 (dd, $J$ = 9, 3 Hz, 1 H), 7.46 (dd, $J$ = 10, 10 Hz, 2 H), 7.58 (t, $J$ = 10 Hz, 1 H), 8.06 (d, $J$ = 10 Hz, 2 H); $^{13}$C NMR ($CDCl_3$) δ 26.6, 27.4, 40.5, 59.1, 68.5, 71.7, 73.8, 79.4, 108.5, 128.3, 129.8 (2), 133.2, 165.3; IR ($CDCl_3$) 3574 (m), 3500 (m, br), 3072 (w), 2988 (s), 2956 (m), 2934 (m), 2870 (m), 1721 (s), 1604 (m), 1452 (s), 1372 (s), 1272 (s), 1110 (s); MS, *m/e* (rel intensity) EI 107 (19), 105 (100), 79 (29), 77 (40), 72 (24), 71 (8); CI: 387, 385 (73, 65, M + H$^+$), 329 (24), 327 (24), 265 (12), 263 (10), 207 (40), 205 (40), 167 (36), 123 (100), 109 (22), 107 (24), 105 (11), 79 (10), 73 (10); HRMS (CI) calcd for $C_{17}H_{21}BrO_5$ (M + H$^+$) 385.0651, found 385.0651. Anal. ($C_{17}H_{21}BrO_5$) C, H.

**[5*R*-(5α,7α,8α,9β)]-7,8-Epoxy-2,2-dimethyl-1,3-dioxaspiro[4.5]decan-9-ol (23).** Sodium (3.30 g, 145 mmol) was added under nitrogen to a solution of dry tetrahydrofuran (200 mL) and dry methanol (200 mL) at 0 °C. The bromide **22** (18.58 g, 48.26 mmol) in dry tetrahydrofuran (200 mL) was added rapidly to the reaction flask at 0 °C. After 1 h, the reaction mixture was quenched with saturated ammonium chloride, and then water added until all solid material had dissolved. The resulting homogeneous solution was concentrated under reduced pressure, and the residue obtained was extracted four times with ethyl acetate. The combined organic layers were dried over magnesium sulfate and concentrated to a white paste. Purification by flash chromatography (1:1 ethyl acetate/hexane, v/v) gave 8.42 g (87%) of epoxide **23** (free of the rearrangement product **24**) as a crystalline, white solid: $^1$H NMR ($CDCl_3$) δ 1.35 (s, 3 H), 1.42 (s, 3 H), 1.56 (ddd, $J$ = 13, 5, 1 Hz, 1 H), 1.86 (s, 1 H), 2.02, (d, $J$ = 16 Hz, 1 H), 2.12 (dd, $J$ = 13, 2 Hz, 1 H), 2.21 (ddd, $J$ = 16, 5, 1 Hz, 1 H), 3.09–3.14 (m, 1 H), 3.22 (dd, $J$ = 5 Hz, 1 H), 3.81 (d, $J$ = 10 Hz, 1 H), 3.87 (d, $J$ = 10 Hz, 1 H), 4.41–4.49 (m, 1 H); $^{13}$C NMR ($CDCl_3$) δ 26.6, 27.6, 35.1, 37.4, 51.7, 54.4, 65.5, 74.5, 77.4, 108.5; IR ($CDCl_3$) 3618 (m), 3475 (m, br), 2989 (s), 2936 (m), 2937 (m), 1372 (m), 1246 (s), 1214 (s), 1160 (s), 1086 (m), 1046 (s); MS, *m/e* (rel intensity) EI 185 (78), 125 (31), 107 (30), 97 (37), 95 (34), 86 (21), 81 (28), 79 (89), 72 (100), 71 (26), 69 (51), 67 (31), 59 (91), 58 (26), 57 (55), 55 (43), 53 (21); CI 201 (100, M + H$^+$), 185 (6), 183 (6), 183 (6), 144 (5), 143 (68), 125 (55), 107 (6), 97 (9); HRMS (CI) calcd for $C_{10}H_{16}O_4$ (M + H$^+$) 201.1127, found 201.1127.

**[5*S*-(5α,7β,8β,9α)]-7,8-Epoxy-2,2-dimethyl-1,3-dioxaspiro[4.5]decan-9-ol (24).** Dry tetrahydrofuran (200 mL) and dry methanol (200 mL) were added via syringe to a flame dried flask equipped with a reflux condenser. Sodium (1.36 g, 580 mmol) was then added in small portions under nitrogen. When all the sodium had reacted, bromide **22** (7.46 g, 19.0 mmol) in dry tetrahydrofuran (150 mL) was cannulated into the reaction flask at room temperature. The yellow reaction mixture was refluxed for 30 min and then allowed to stir at room temperature overnight. Ethyl acetate (300 mL) was then added to the concentrated reaction mixture followed by addition of aqueous ammonium chloride until the aqueous layer was neutral. Water was subsequently added to dissolve any remaining solid. This aqueous layer was then extracted with ethyl acetate several times. The combined organic layers were washed with saturated aqueous ammonium chloride and brine. After drying over magnesium sulfate, the organic solution was concentrated to a yellow liquid. Purification by flash chromatography (1:1 ethyl acetate/hexane, v/v) gave 0.420 g of **24** (2.10 mmol, 11%), 1.2 g of **23** (6 mmol, 32%), and 1.75 g of a mixture of **23** and **24**. Flash chromatography or radial chromatography of the mixture provided an additional 1.47 g (7.34 mmol, 50% overall yield) of epoxide **24**: $^1$H NMR ($CDCl_3$) δ 4.24–4.29 (m, 1 H), 3.85 (d, $J$ = 10 Hz, 1 H), 3.76 (d, $J$ = 9 Hz, 1 H), 3.68 (d, $J$ = 9 Hz, 1 H), 3.28–3.30 (m, 2 H), 2.20 (ddd, $J$ = 16, 4, 3, Hz, 1 H), 1.92 (d, $J$ = 16 Hz, 1 H), 1.85 (ddd, $J$ = 14, 3, 3 Hz, 1 H), 1.72 (dd, $J$ = 14, 4 Hz, 1 H), 1.46 (s, 3 H), 1.39 (s, 3 H); $^{13}$C NMR ($CDCl_3$) δ 110.3, 78.6, 74.0, 65.8, 54.3, 50.6, 34.8, 34.6, 27.1, 26.4; mp 64–65 °C; IR (neat, NaCl) 3386 (s, br), 2988 (s), 2916 (s), 2858 (s), 1450 (m), 1378 (m), 1344 (w), 1252 (m), 1208 (m), 1160 (m), 1082 (m), 1048 (m), 1008 (w), 966 (w), 940 (w), 886 (w), 854 (w), 830 (w), 780 (w); MS, *m/e* (rel intensity) EI 185 (100), 125 (36), 97 (37), 95 (25), 79 (65), 72 (52), 69 (33), 59 (44), 57 (29); CI 201 (M + H$^+$, 100); HRMS(CI) calcd for $C_{10}H_6O_4$ (M + H$^+$) 201.1127, found 201.1127. Anal. ($C_{10}H_6O_4 \cdot {}^1/_4H_2O$) C, H.

**[5*R*-(5α,7α,8β,9β)]-7-(Benzyloxy)-8,9-epoxy-2,2-dimethyl-1,3-dioxaspiro[4.5]decane (25).** A sodium hydride dispersion in mineral oil (50% by weight, 0.982 g, 20.5 mmol) was washed with hexane and dried under a fast nitrogen flow. Dry tetrahydrofuran (50 mL) was then added at room temperature followed by the epoxide (1.37 g, 6.84 mmol) dissolved in tetrahydrofuran (30 mL). The resulting suspension was vigorously stirred for 30 min and then benzyl bromide (1.65 mL, 13.7 mmol) was added dropwise via syringe. After stirring for 12 h at room temperature, the reaction was quenched with saturated aqueous ammonium chloride. The aqueous layer was extracted three times with dichloromethane, and the combined organic layers were washed with saturated aqueous sodium bicarbonate. Drying of the organic layer with magnesium sulfate and concentration under reduced pressure gave a yellow oil. Purification by flash chromatography (hexane, 1:3 ethyl acetate/hexane, v/v) gave 1.79 g of **25** (6.15 mmol, 90%) as a yellow oil: $^1$H NMR ($CDCl_3$) δ 7.29–7.38 (m, 5 H), 4.64 (s, 2 H), 3.76 (dd, $J$ = 10, 7 Hz, 1 H), 3.72 (s, 2 H), 3.26–3.34 (m, 1 H), 3.18 (d, $J$ = 4 Hz, 1 H), 2.27 (ddd, $J$ = 15, 2, 2 Hz, 1 H), 2.06–2.17 (m, 2 H), 1.63 (dd, $J$ = 13, 10 Hz, 1 H), 1.39 (s, 3 H), 1.36 (s, 3 H); $^{13}$C NMR ($CDCl_3$) δ 137.8, 128.6, 128.0, 127.8, 107.9, 73.2, 72.5, 71.4, 54.1, 53.4, 38.0, 35.8, 27.0, 26.7; IR (neat, NaCl) 2988 (m), 2934 (m), 2868 (m), 1498 (w), 1454 (m), 1370 (s), 1250 (s), 1214 (s), 1162 (m), 1090 (s), 1060 (s), 986 (w), 886 (w), 858 (m), 830 (w), 796 (w), 780 (w), 736 (m), 698 (m); MS, *m/e* (rel intensity) EI 91 (100); CI 291 (M + H$^+$, 100), 233 (76), 215 (27), 203 (37), 125 (28), 91 (27); HRMS (CI) calcd for $C_{17}H_{22}O_4$ (M + H$^+$) 291.1596, found 291.1596. Anal. ($C_{17}H_{22}O_4 \cdot {}^1/_4H_2O$) C, H.

**[5*R*-(5α,7α,8β,9α)]-7-(Benzyloxy)-2,2-dimethyl-9-[(diisopropoxyphosphinyl)methyl]-1,3-dioxaspiro[4.5]decan-8-ol (26).** Tetrahydrofuran (50 mL) was placed in a dry flask under argon, and diisopropyl methanephosphonate (22.0 mL, 121 mmol) was added via syringe. The solution was cooled to −78 °C and 1.6 M *n*-butyllithium in hexane (75.0 mL, 121 mmol) was added dropwise via syringe. The clear yellow mixture became turbid. After 1 h at −78 °C, boron trifluoride etherate (14.8 mL, 121 mmol) was added via syringe resulting in additional turbidity. After 10 min, epoxide **25** (11.7 g, 40.2 mmol) in tetrahydrofuran (50 mL) was slowly cannulated into the reaction mixture. When the reaction was complete (usually 1 h after the end of addition), the mixture was quenched at −78 °C with saturated aqueous sodium bicarbonate. The resulting aqueous layer was extracted three times with ethyl acetate. After drying the combined organic layers with magnesium sulfate, concentration gave a yellow oil Purification by flash chromatography (ethyl acetate) yielded 16.3 g (34.6 mmol, 86%) of **26** as a yellow oil: $^1$H NMR ($CDCl_3$) δ 7.29–7.36 (m, 5 H), 4.60–4.79 (m, 3 H), 4.54 (d, $J$ = 11 Hz, 1 H), 3.83 (d, $J$ = 9 Hz, 1 H), 3.76 (d, $J$ = 9 Hz, 1 H), 3.12–3.18 (m, 2 H), 2.16–2.48 (m, 3 H), 1.10–1.69 (m, 4 H), 1.39 (s, 3 H), 1.38 (s, 3 H), 1.25 (d, $J$ = 6 Hz, 12 H); $^{13}$C NMR ($CDCl_3$) δ 138.2, 128.4, 128.3, 127.7, 108.4, 80.0 ($J$ = 16 Hz), 76.5, 72.2, 71.3, 69.9 ($J_{POC}$ = 3 Hz), 41, 39.4, 34.5 ($J$ = 4 Hz), 28.6 ($J_{PC}$ = 143 Hz), 26.6, 26.8, 23.6; IR (neat, NaCl) 3378 (s, br), 2982 (s), 2936 (m), 2870 (m), 1456 (m), 1386 (m), 1374 (m), 1312 (w), 1216 (s), 1180 (m), 1142 (m), 1106 (s), 1060 (s), 984 (s), 918 (w), 898 (w), 868 (w), 834 (w), 794 (w), 736 (m), 700 (m); MS, *m/e* (rel intensity) EI 91 (100); CI 471 (M + H$^+$, 100); HRMS (CI) calcd for $C_{17}H_{22}O_4$ (M + H$^+$) 471.2512, found 471.2512.

**[1*R*-(1α,3α,4β,5α)]-1-(Hydroxymethyl)-5-[(diisopropoxyphosphinyl)methyl]cyclohexan-1,3,4-triol (27).** A homogeneous solution of **26** (0.20 g, 0.42 mmol) in tetrahydrofuran (1.5 mL) and 50% aqueous acetic acid (6 mL) was vigorously stirred at 65–75 °C for 5 h. The reaction mixture was then concentrated under high vacuum and azeotroped three times with toluene. The white powder (0.168 g, 0.389 mmol, 93%) obtained was used directly in the next step: $^1$H NMR ($CDCl_3$) δ 7.28–7.37 (m, 5 H), 4.61–4.72 (m, 2 H), 4.68 (d, $J$ = 11 Hz, 1 H), 4.49 (d, $J$ = 11 Hz, 1 H), 3.64 (d, $J$ = 12 Hz, 1 H), 3.36 (d, $J$ = 12 Hz, 1 H), 3.16–3.30 (m, 2 H), 2.24–2.46 (m, 3 H), 1.65–1.85 (m, 2 H), 1.43–1.59 (m, 2 H), 1.32 (d, $J$ = 6 Hz, 12 H); $^{13}$C NMR ($CDCl_3$) δ 138.4, 128.6, 127.9, 79.3, 77.3, 71.9, 71.2, 70.6 ($J_{POC}$ = 7 Hz), 70.4 ($J_{POC}$ = 7 Hz), 66.5, 38.9, 37.4, 33.8 ($J$ = 4 Hz), 28.9 ($J_{PC}$ = 143 Hz), 23.8; IR (neat) 3450 (s, br), 3280 (s, br), 2976 (m), 2942 (s), 1452 (w), 1372 (m), 1320 (w), 1234 (w), 1206 (s), 1086 (m), 1072 (s), 1040 (m), 982 (s), 864 (w), 802 (w), 742 (m), 694 (s); MS, *m/e* (rel intensity) EI 306 (13), 201 (13), 91 (100); CI 431 (M + H$^+$, 100); HRMS (CI) calcd for $C_{21}H_{35}O_7P$ (M + H$^+$) 431.2199, found 431.2200. The benzylated intermediate (0.168 g, 0.39 mmol) was dissolved in methanol (6 mL) and hydrogenated over 10% palladium on carbon at 50 psi hydrogen for 2 h. Filtration of the catalyst through

Celite and removal of the solvent under reduced pressure directly afforded pure **27** (0.135 g, 0.39 mmol) in quantitative yield: $^1$H NMR ($D_2O$) $\delta$ 4.63–4.79 (m, 2 H), 3.63 (d, $J$ = 12 Hz, 1 H), 3.52 (d, $J$ = 12 Hz, 1 H), 3.41–3.56 (m, 1 H), 3.10 (dd, $J$ = 9, 9 Hz, 1 H), 2.17–2.45 (m, 3 H), 1.30–1.82 (m, 4 H), 1.35 (d, $J$ = 6 Hz, 12 H); $^{13}$C NMR ($D_2O$) $\delta$ 81.3 ($J$ = 12 Hz), 75.7 ($J$ = 7 Hz), 75.8 ($J$ = 7 Hz), 75.5, 74.0, 68.5, 43.0, 41.7, 37.0 ($J$ = 6 Hz), 31.0 ($J_{PC}$ = 144 Hz), 26.3 ($J_{POC}$ = 6 Hz); MS, $m/e$ (rel intensity) EI 123 (66), 97 (100), 79 (28); CI 341 (M + H$^+$, 100), 181 (35); HRMS (CI) calcd for $C_{14}H_{29}O_7P$ (M + H$^+$) 341.1729, found 341.1726.

**[1*R*-(1$\alpha$,3$\alpha$,4$\beta$,5$\alpha$)]-1,3,4-Trihydroxy-5-[(diisopropoxyphosphinyl)methyl]cyclohexan-1-carboxylic Acid (28).** Platinum oxide (0.084 g) in water (2 mL) was reduced in a Parr apparatus at 50 psi hydrogen for 30 min. The catalyst was then sonicated for a few minutes and transferred to a cylindrical flask. A mixture of **27** (0.20 g, 0.59 mmol) and sodium bicarbonate (0.148 g, 1.76 mmol) in water (13 mL) was added to the well stirred suspension of activated platinum. Oxygen was bubbled through the reaction mixture with a gas dispersion tube. The reaction mixture was then heated to 55 °C and monitored by $^1$H NMR. When the reaction was complete (typically after 8 h), the mixture was centrifuged at 50000 g for 15 min (for recovery of the platinum black) or filtered through Celite and concentrated to dryness under high vacuum. The residue was dissolved in water and passed down a short Dowex 50 (H$^+$) column at 4 °C. The column was rinsed with two column volumes of distilled water. The solution obtained was concentrated to a yellowish solid (0.136 g, 0.384 mmol, 65%): $^1$H NMR ($D_2O$) $\delta$ 4.63–4.79 (m, 2 H), 3.74 (ddd, $J$ = 12, 9, 5 Hz, 1 H), 3.11 (dd, $J$ = 9, 9 Hz, 1 H), 2.28–2.58 (m, 3 H), 1.39–1.89 (m, 4 H), 1.33 (d, $J$ = 6 Hz, 12 H); $^{13}$C NMR ($D_2O$) $\delta$ 179.6, 80.0 ($J$ = 16 Hz), 76.0, 75.4 ($J_{POC}$ = 7 Hz), 75.3 ($J_{POC}$ = 7 Hz), 73.4, 43.7, 42.1, 36.3 ($J$ = 4 Hz), 30.0 ($J_{PC}$ = 142 Hz), 25.7 ($J_{POCC}$ = 4 Hz); MS, $m/e$ (rel intensity) EI 123 (75), 97 (100), 96 (49), 79 (36), 45 (68); CI 355 (M + H$^+$, 68), 337 (100); HRMS (CI) calcd for $C_{14}H_{27}O_8P$ (M + H$^+$) 355.1522, found 355.1522.

**[1*R*-(1$\alpha$,3$\alpha$,4$\beta$,5$\alpha$)]-1,3,4-Trihydroxy-5-(phosphonomethyl)cyclohexane-1-carboxylic Acid (6).** A mixture of phosphonate **28** (0.089 g, 0.25 mmol) and triethylamine (0.25 g, 2.5 mmol) in dichloromethane (25 mL) was placed in a dry flask under nitrogen. Bromotrimethylsilane (0.77 g, 5.0 mmol) was added slowly at 0 °C. A white aerosol immediately appeared, and the reaction solution discolored. After stirring at room temperature for 12 h, solvent was removed under high vacuum and the discolored residue azeotroped three times with toluene. Water (20 mL) was subsequently added at room temperature. After 1 h, the solution was concentrated to dryness, dissolved in water, and passed down a short column of Dowex 50 (H$^+$). The colorless filtrate was concentrated to a yellow, foamy solid. This solid was then dissolved in distilled water, neutralized to pH 7.0 with aqueous sodium hydroxide, and then loaded onto AG-1 X8 anion exchange resin (20 mL) which had been equilibrated with 200 mM triethylammonium bicarbonate (pH 7.2). The column was washed with water (40 mL) and eluted with a linear gradient (300 mL + 300 mL, 200–500 mM) of triethylammonium bicarbonate (pH 7.2). Fractions containing phosphonic acid were 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$^+$) to afford a 40% yield of epimeric carbaphosphonate **6**: $^1$H NMR ($D_2O$) $\delta$ 3.72 (ddd, $J$ = 13, 9, 4 Hz, 1 H), 3.15 (dd, $J$ = 9, 9 Hz, 1 Hz, 1 H), 2.50–2.54 (m, 1 H), 2.44 (ddd, $J$ = 7, 4, 2 Hz, 1 H), 2.33 (ddd, $J$ = 20, 15, 2 Hz, 1 H), 1.89–1.95 (m, 1 H), 1.67 (ddd, $J$ = 16, 15, 10 Hz, 1 H), 1.58 (dd, $J$ = 7, 7 Hz, 1 H), 1.49 (dd, $J$ = 13, 13 Hz, 1 H); $^{13}$C NMR ($D_2O$) $\delta$ 180.0, 80.7 ($J_{PCCC}$ = 15 Hz), 76.5, 73.8 ($J_{PCCCC}$ = 3 Hz), 44.1, 42.6, 36.8 ($J_{PCC}$ = 4 Hz), 31.5 ($J_{PC}$ = 138 Hz); HRMS (FAB) calcd for $C_8H_{15}O_8P$ (M + H$^+$) 271.0583, found 271.0588.

**Evaluation of in vivo Inhibition of 3-Dehydroquinate Synthase.** The procedure of Pompliano et al.,[3b] with a few minor modifications, was used. *Pisum sativum*, *Echinochloa crusgalli*, *Setaria viridis*, *Sorghum halepense*, and *Avena fatua* seeds (1 g each) were planted 1–2 cm deep in Vermiculite-filled containers (10-cm diameter, 10-cm deep); each species was planted in four separate containers. The Vermiculite was soaked in Hoagland's plant nutrient solution[22] (No. 2 basal salt mixture) and kept at 25 °C under plant growth lights (Sylvania GRO-LUX F40 WS) with a 16-h light/8-h dark cycle. Hoagland's solution used in these experiments consisted of the following: $MgSO_4$ (1 mM), $KH_2PO_4$ (1 mM), $KNO_3$ (5 mM), $Ca(NO_3)_2$ (1.5 mM), $NH_4Cl$ (0.5 mM), $H_3BO_3$ (46 $\mu$M), $MnSO_4$ (9 $\mu$M), $ZnSO_4$ (0.77 $\mu$M), $MoO_3$ (0.32 $\mu$M), $NH_4VO_3$ (0.20 $\mu$M), EDTA (0.1 mM), $Na_2CO_3$ (42 $\mu$M), and $FeSO_4$ (90 $\mu$M). The seedlings were watered with $^1/_4$-strength Hoagland's solution every 48 h. After the plants had emerged from the Vermiculite (5–6 days), one planting of each species was sprayed every 3 days with 1.3 mL of enzyme-targeted agent (10 mM) dissolved in surfactant-containing solution (by volume: 80% water, 10% methanol, 10% acetone, 0.01% Triton X-100). The second planting of each species was sprayed every 3 days with 1.3 mL of the surfactant-containing solution lacking enzyme-targeted agent. Plants were grown for 14 days after the initial treatment.

Leaves and stems (above the cotyledon) of carbaphosphonate **5** and epimeric carbaphosphonate **6**-treated plants (from the growth described above) were vacuum desiccated over sodium hydroxide pellets for 48 h, and the desiccated material was stored at −80 °C. Desiccated plants were ground to a fine powder with a mortar and pestle, and a weighed portion of the powder was added to 4 mL of 4:1 ethanol/water (v/v). After the addition of a measured amount of [4,5,6,7-$^{14}$C]DAH,[3b,32] the mixture was refluxed for 2 h to extract metabolites.[21d] The mixture was then filtered through a Centriflo CF25 ultrafiltration membrane cone (Amicon), and metabolites in the filtrate were separated by anion-exchange high-pressure liquid chromatography[33] (semipreparative Whatman Partisil-10 SAX column; 1:4 acetonitrile/7.5 mM phosphate buffer, pH 2.4). Fractions containing $^{14}$C radiolabel were pooled and concentrated, and DAH levels were determined by colorimetric assay.[23] Values for DAH accumulation were corrected for radiolabel lost during the analysis. Replicate experiments were performed on *Pisum sativum* to ascertain the uncertainty in the determined accumulation of DAH.

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**Supplementary Material Available:** Experimental procedures and characterization of Scheme II intermediates **16**, **18**, **19**, and **20** and intermediates in the synthesis of carbaphosphonate **5** are provided along with Figure 1 (ORTEP plot) and tables of crystallographic details, final positional and thermal parameters, bond lengths, and bond angles for epoxy alcohol **24** (24 pages). Ordering information is given on any current masthead page.