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New EPSP Synthase Inhibitors: Synthesis and Evaluation of an Aromatic Tetrahedral Intermediate Mimic Containing a 3-Malonate Ether as a 3-Phosphate Surrogate

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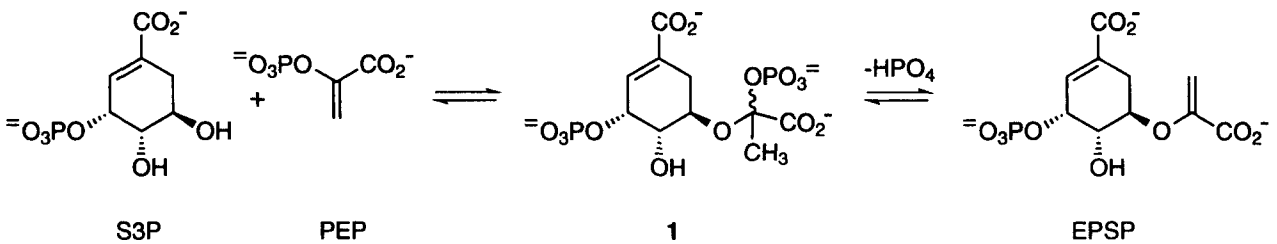
Abstract—A new analog of the EPSP synthase enzyme reaction intermediate **1**, containing a 3-malonate ether moiety in place of the usual 3-phosphate group, was synthesized from 3,5-dihydroxybenzoic acid. This simple, synthetically accessible aromatic compound (**5**) is an effective competitive inhibitor versus S3P with an apparent K_i of 1.3 ± 0.22 μM. This result demonstrates that a simple benzene ring can be a suitable achiral substitute for the more complex shikimate ring in the design of EPSP synthase inhibitors. Furthermore, the greater potency of **5** versus the phenol **6**, glycolate **7** and the gallic acid analog **8** demonstrates the requirement for multiple anionic charges at the dihydroxybenzoate 5-position in order to attain effective inhibition of this enzyme. However, this 3-malonate ether substituted compound was at least 10-fold less effective as a bisubstrate inhibitor than the corresponding 3-phosphate. This suggests that tetrahedral intermediate mimics possessing a 3-malonate ether moiety are less effective than their corresponding 3-phosphates in accessing the optimal enzyme conformation stabilizing **1**.

Introduction

The enzyme 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase (EPSPS, EC 2.5.1.19) is a key component of aromatic amino acid biosynthesis[1] and as such represents an important target for rational inhibitor design.[2] EPSPS catalyzes the unusual transfer of the carboxyvinyl moiety from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), via the single, kinetically competent, tightly bound, tetrahedral intermediate **1**[3] (Scheme 1). Various shikimate-based analogs of **1** have been identified as highly potent EPSPS bisubstrate inhibitors.[4] The potency of these inhibitors is a direct consequence of their ability to simultaneously occupy both the S3P and PEP substrate sites. Spectroscopic investigations conducted in these laboratories have also determined that the shikimate ring in enzyme-bound S3P and EPSP adopts an unusually flattened conformation.[5] As a result, the aromatic tetrahedral intermediate mimic **2** has recently been identified as a potent EPSPS inhibitor, where the simplified benzene ring functions as a suitable substitute for the more highly functionalized shikimate ring.[6]

These inhibitors contain either allylic or phenolic 3-phosphate groups, which are quite susceptible to hydrolytic cleavage. The 3-phosphate group in S3P contributes more than 8 kcal mol^{-1} in binding energy to EPSPS.[7] Thus, inhibitors lacking this important group usually exhibit little significant interaction with this enzyme.[8–10] A search for a more stable 3-phosphate replacement moiety unexpectedly uncovered the 3-malonate ether group, which serves as a suitable 3-phosphate mimic in 4,5-dideoxy-S3P inhibitors.[8] Similarly, compound **3**, the corresponding analog of S3P in which the 3-phosphate is replaced by a 3-malonate ether, undergoes enzyme-catalyzed conversion to an EPSP-like product. While the kinetic and mechanistic details of this conversion have yet to be investigated, one might assume that this conversion proceeds through the tetrahedral intermediate **4** and that close structural analogs of **4** would also be potent EPSPS inhibitors. Here we report the synthesis and biochemical evaluation of the 3,5-dihydroxybenzoate analog **5** as a new



Scheme 1. The reaction catalyzed by EPSP synthase.

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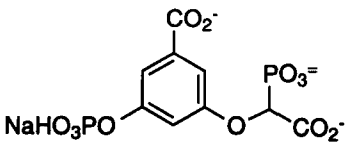

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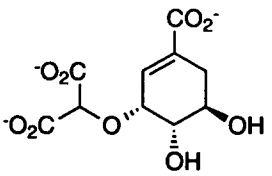

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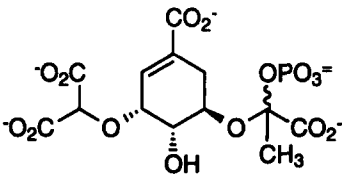

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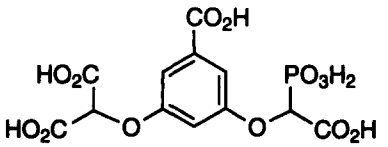

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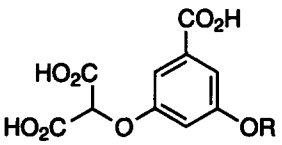
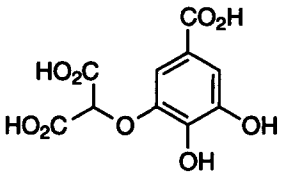


6, R = H
7, R = CH_2CO_2H



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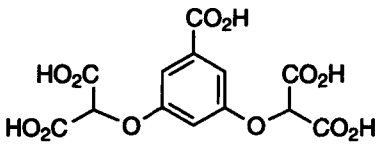

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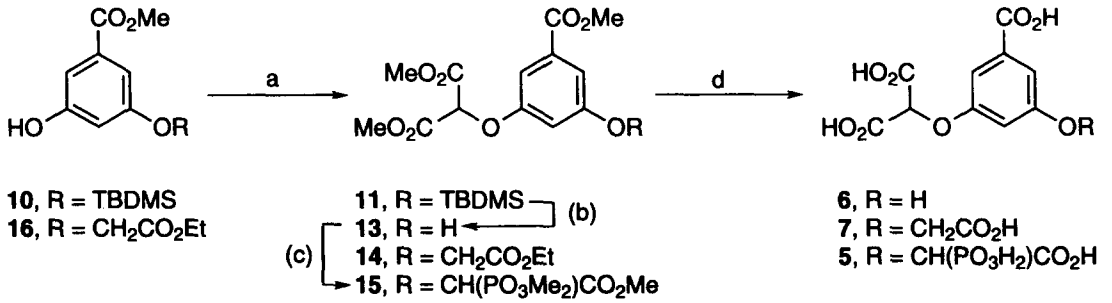
aromatic tetrahedral intermediate mimic which incorporates a 3-malonate ether in place of the 3-phosphate group. This compound represents a novel EPSPS inhibitor which helps define the utility and limitations of 3-malonate ethers as 3-phosphate replacement groups in this system. Furthermore, the greater potency of **5** versus the phenol **6**, glycolate **7**, gallic acid analog **8** and the recently identified[11] symmetrical 3,5-bis-malonate ether **9** demonstrates the requirement for multiple anionic charges at the dihydroxybenzoate 5-position in order to attain effective EPSPS inhibition. Inhibitor **5** also provides another example where the more synthetically accessible benzene ring can be used effectively in place of the chiral shikimate ring in EPSPS bisubstrate inhibitors.

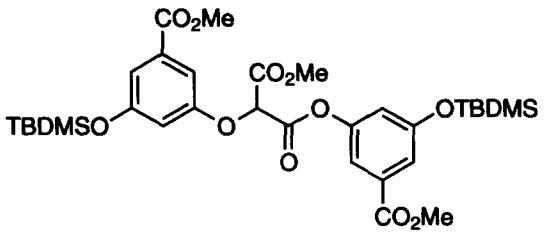
Results and Discussion

Synthesis of these benzene-based inhibitors (Scheme 2) began with commercially available methyl 3,5-dihydroxybenzoate via the known[6] mono-protected *t*-butyldimethylsilyl ether **10** or glycolate **16**. Treatment of **10** with dimethyl diazomalonate[12] using rhodium diacetate catalysis[13] gave **11**, which required chromatographic purification since it was always accompanied by a small amount of the dimeric ester **12**. Removal of the silyl protecting group from **11** with fluoride ion afforded the malonate ether phenol intermediate **13**. Subsequent reaction of **13** with trimethyl diazophosphonoacetate,[14] under rhodium diacetate catalysis,[13] provided the phosphonoacetate ether **15**. Similarly, treatment of the known[6] phenol glycolate **16** with dimethyl diazomalonate[12] under rhodium diacetate catalysis,[13] provided the glycolate ether **14**. In this case, slow addition of the diazomalonate to a more dilute solution of substrate **16** gave much cleaner conversion to the desired product **14** without any accompanying dimeric ester by-product. Synthetic intermediates **13** and **14** were readily converted to the desired deprotected aromatic inhibitors **6** and **7** by saponification (NaOH), anion exchange chromatography (DEAE Sephadex, triethylammonium bicarbonate) and acidification with Dowex H^+ resin, which provided the fully deprotected acids. Intermediate **15** required the removal of the

phosphonate esters (TMSBr) prior to the saponification and purification steps which subsequently led to **5**.

The synthesis of the gallic acid derivative **8** began (Scheme 3) from commercially available methyl gallate via the known[15] diphenyl ketal **17**. Treatment with dimethyl diazomalonate[12] using rhodium diacetate catalysis[13] gave **18**, which was separated chromatographically from the dimeric ester **19**. Trifluoroacetic acid cleanly removed the benzophenone ketal protecting group from **18** and subsequent saponification (NaOH), anion exchange chromatography (DEAE Sephadex, triethylammonium bicarbonate) and acidification with Dowex H^+ resin provided the fully deprotected triacid **8**.





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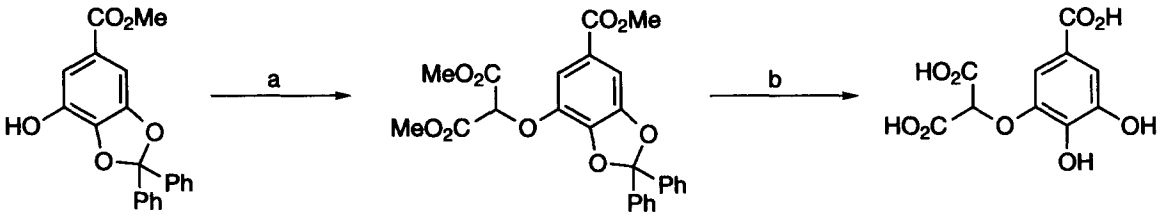
Scheme 2. Reagents and conditions: (a) Preparation of **11** and **14**: $MeO_2CC(=N_2)CO_2Me$, $Rh_2(OAc)_4$, benzene, reflux; (b) Conversion of **11** to**13**: TBAF, THF; (c) Conversion of **13** to **15**: $Me_2O_3PC(=N_2)CO_2Me$, $Rh_2(OAc)_4$, benzene, reflux; (d) TMSBr (15 only); NaOH, H_2O; AG 50W-X8 (H^+).



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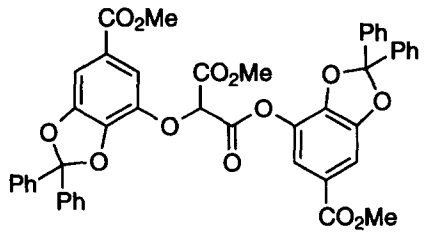

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Scheme 3. Reagents and conditions: (a) $MeO_2CC(=N_2)CO_2Me$, $Rh_2(OAc)_4$, benzene, reflux; (b) NaOH, H_2O; AG 50W-X8 (H^+).

Compounds **5** to **8** were evaluated for inhibition of *Escherichia coli* EPSP synthase[16] by monitoring the conversion of ^{14}C PEP or ^{14}C S3P to ^{14}C EPSP.[7,17] As a first approximation, IC_{50} values were determined and are shown in Table 1. For comparison purposes, the IC_{50} for EPSP-mediated product inhibition of the forward reaction of EPSP synthase is also shown in Table 1. Our results demonstrate that the aromatic malonate ethers display significant inhibition of EPSP synthase, with an increase in enzyme affinity proceeding from the S3P analog **6**, to the glycolate analog **7** and finally to the tetrahedral intermediate mimic **5**. This increase in binding parallels the trend observed previously for the analogous shikimate and aromatic 3-phosphate series of compounds[4,6,11] and demonstrates that appropriate ionic functional groups incorporated at the C-5 position can access the enzymatic PEP binding site and thus markedly enhance inhibition. A comparison of the activity of the gallic acid malonate ether **8** versus **6** demonstrates that the 4-hydroxyl group in this aromatic series contributes little to the overall potency. This effect has also been observed previously in various deoxy shikimate 3-phosphate inhibitors.[18]

Table 1. Inhibition of *E. coli* EPSP synthase

Compound	IC_{50} (mM)*	$K_{i,\,apparent}$ (μM)†
2	0.02‡	0.16 ± 0.04‡
5	0.07	1.3 ± 0.22
6	10	
7	2	
8	3	
9	0.21§	2.5 ± 0.40§
EPSP	0.18	(K_d = 1.0 ± 0.01)¶

*Concentration of inhibitor necessary to provide 50% inhibition at fixed concentrations of S3P and PEP of 100 μM at 30 °C in 100 mM HEPES:KOH, 50 mM KCl, pH 7.0.
†Apparent K_is versus S3P were determined at [PEP] fixed at 100 μM.
‡Ref. 6.
§Ref. 11.
¶Ref. 16b.

A more complete kinetic characterization of the most active aromatic compound **5** was conducted at varying S3P concentrations demonstrating competitive inhibition versus S3P (Fig. 1) with an apparent K_i of 1.3 ± 0.20 μM.[19] The inhibition potency observed for **5** is comparable to that recently reported for the symmetrical 3,5-bis-malonate ether **9**.[11] This result demonstrates that a simple benzene ring is an acceptable substitute for the more complex shikimate ring in the design of EPSP synthase inhibitors containing a 3-malonate ether as a 3-phosphate replacement group.

However, the apparent K_i obtained for the 3-malonate **5** is about an order of magnitude weaker than that reported for the corresponding 3-phosphate **2**. Similarly, the micromolar potency observed for **5** is considerably weaker than the low nanomolar potency obtained with various shikimate-based[4] bisubstrate inhibitors possessing a 3-phosphate group. Since the enzymatic product EPSP also has affinity for free enzyme in the low micromolar range,[16b] it is tempting to speculate that these bisubstrate inhibitors **5** and **9** containing a 3-malonate ether moiety simply function as substrate-analog inhibitors and are consequently not able to access the optimum enzyme conformation which stabilizes **1**. The limitation of these 3-malonate ethers to function as effective 3-phosphate replacements in this system is more likely to be due to their overall larger size than any charge effects, since the measured pK_a of **3** indicates that the malonate ether group will be fully ionized under physiological conditions.[8] Molecular modeling experiments indicate that a malonate ether occupies nearly 20% more volume than a phosphate group.[20] The added volume required by the 3-malonate functionality must, therefore, be sufficient to prevent optimal binding at the EPSPS active site.

In conclusion, we have now shown in two series of aromatic inhibitors that the structural requirements for effective inhibition of EPSP synthase can be dramati-

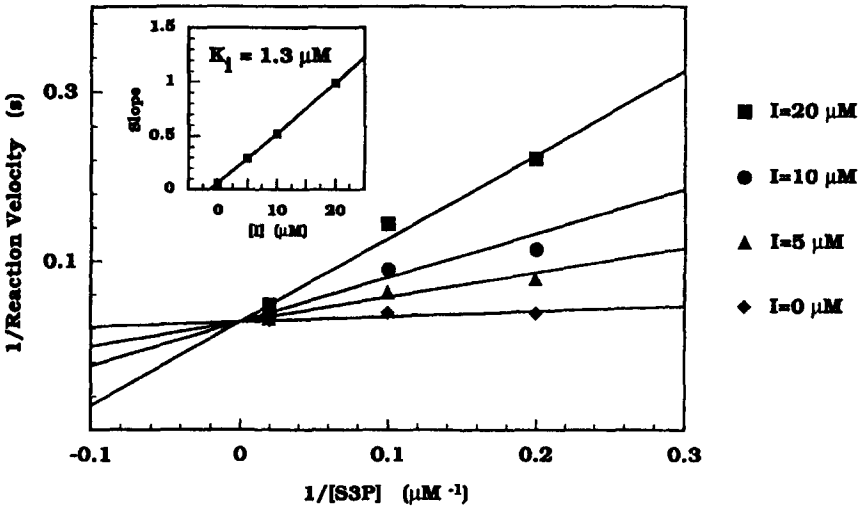

Figure 1. Inhibition of EPSP synthase by compound **5**. Double reciprocal plot of reaction velocity versus S3P concentration obtained at varying concentration of inhibitor **5**. The lines drawn were obtained from a nonlinear least squares fit to the observed data using equation (1), which corresponds to a kinetic model for competitive inhibition. Fitting to equation (2), corresponding to a kinetic model for mixed inhibition, gave a very poor fit. The data, therefore, is best interpreted in terms of competitive inhibition versus S3P.

cally simplified by taking advantage of information about the conformation of the reaction substrate and product. Reasonable potency can be maintained in aromatic inhibitors streamlined by elimination of all three stereocenters from the shikimate ring. We have also more clearly defined the scope and limitations of using the 3-malonate ether group as a 3-phosphate replacement in these aromatic bisubstrate inhibitors of EPSP synthase. Our results suggest that the 3-malonate ether moiety represents a less than ideal solution as a spatial mimic of the more labile 3-phosphate group in this system. Our efforts continue to identify a more spatially effective 3-phosphate mimic which would be more generally applicable to EPSP synthase inhibitors.

Experimental

EPSP synthase kinetic assays

EPSP synthase was isolated[16a] from over-expressing *E. coli* strain pMON6001 and was purified[16b] by a procedure described previously. Enzyme inhibition assays were conducted as previously described.[7,17] Inhibition was measured by monitoring the conversion of ^{14}C S3P or ^{14}C PEP to ^{14}C EPSP, as determined by HPLC with radioactive flow detection.

Data obtained from the examination of enzyme activity as a function of substrate concentration in the presence of fixed concentrations of inhibitor were analyzed using the commercial software GraFit.[19] Fitting was performed using the following steady-state rate equations corresponding to competitive and mixed inhibition, respectively, in a single substrate system:

$$\upsilon = V_{max} A / [K_m (1 + I/K_{is}) + A] \quad (1)$$

$$\upsilon = V_{max} A / [K_m (1 + I/K_{is}) + A (1 + I/K_{ii})], \quad (2)$$

where υ represents the observed reaction velocity expressed as Turnover Number with units of reciprocal seconds, V_{max} represents the theoretical maximal velocity, A represents the concentration of S3P, K_m represents the apparent Michaelis constant for S3P at a fixed concentration of 100 μM PEP, I represents the concentration of the inhibitor, and K_{is} and K_{ii} represent the apparent inhibition constants for competitive and uncompetitive contributions, respectively, to the overall inhibition versus S3P (i.e. slope and intercept effects in reciprocal Lineweaver–Burk plots).

Synthesis: general

Anhydrous CH_2Cl_2 was Aldrich anhydrous grade solvent. Reactions requiring anhydrous conditions were performed in oven-dried glassware under a positive pressure of nitrogen. Benzene for rhodium-catalyzed diazo coupling reactions was generally dried in the reaction flask immediately prior to use by distillation of a few milliliters of benzene:water azeotrope. Purified H_2O for ion exchange chromatography, hydrolysis reactions and manipulation of ionic compounds was obtained from a Barnstead Nanopure II purification unit.

Melting points were obtained in unsealed capillaries and are uncorrected. Elemental analyses for carbon and hydrogen were performed by Atlantic Microlabs, Inc.

^{1}H NMR spectra were recorded at 400, 360 or 300 MHz. Chemical shifts are reported in ppm (δ) using either internal TMS or the residual protons of the deuterated solvent as standard. ^{13}C NMR spectra (proton decoupled) were recorded at 100, 90 or 75 MHz. Chemical shifts are reported in ppm downfield from TMS using internal TMS, the deuterated solvent, or, for D_2O as solvent, the internal instrument lock as standard. ^{31}P NMR spectra (proton decoupled) were recorded at 121.4 MHz and are referenced to external 85% H_3PO_4 or to the internal deuterium lock reference.

Chromatography

Preparative normal phase chromatography was performed on a Waters Associates Prep 500A chromatograph using Prep–Pak 500 silica gel cartridges or on a medium pressure system (MPLC) using Lichroprep Si60 columns (size C) at a 15 mL min^{-1} flow rate. Flash chromatography was performed using Merck Kieselgel 60 (no. 9385), 230–400 mesh. Preparative reverse phase chromatography was performed on a medium pressure system (RPMPLC) using either Lichroprep RP-18 columns (size B) at a 7.5 mL min^{-1} flow rate or Lichroprep RP-8 columns (size C) at a 15 mL min^{-1} flow rate.

Preparative ion exchange chromatography was performed at 4 °C in a 5.5 × 60 cm glass column packed with DEAE Sephadex A-25 anion exchange resin (Pharmacia). Columns were eluted at 3–4 mL min^{-1} with a 5 to 8 L linear gradient of triethylammonium bicarbonate (TEAB) buffer. Fractions containing pure product (determined by analytical ion exchange chromatography, UV detection at 254 nm for aromatics, 220 nm for shikimates) were combined and evaporated, and the residue was concentrated twice from EtOH to remove traces of TEAB.

Dimethyl 2-[3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-(methoxycarbonyl)phenoxy]propanedioate (11)

A flask fitted with a reflux condenser containing 175 mL of azeotropically-dried benzene was charged with the known[6] silyl phenol **10** (10.04 g, 35.6 mmol) and $[Rh(OAc)_2]_2$ (56 mg, catalytic). The mixture was heated to reflux and dimethyl diazomalonate[12] (5.9 g, 37.3 mmol) was added slowly over 5 min by syringe. After 40 min of reflux, an additional 1.0 g (6.3 mmol, total of 43.6 mmol) of dimethyl diazomalonate was added and reflux was maintained for an additional 45 min. The mixture was then cooled to rt, filtered through a 0.75 in pad of silica gel with 50:50 EtOAC:cyclohexane and evaporated to an oil. Purification by silica gel chromatography (Prep 500A, 10% EtOAc:cyclohexane) gave 8.53 g (58%) of **11** as a colorless oil which very

slowly crystallized to a white solid, mp 38–39.5 °C: ^{1}H NMR ($CDCl_3$) δ 7.19 (*d*, 2H, *J* = 2.3 Hz), 6.68 (*dd*, 1H, *J* = 2.3, 2.3 Hz), 5.26 (*s*, 1H), 3.88 (*s*, 3H), 3.85 (*s*, 6H), 0.97 (*s*, 9H), 0.20 (*s*, 6H); ^{13}C NMR ($CDCl_3$) δ 166.20, 165.56, 157.50, 156.88, 132.24, 115.96, 112.73, 108.62, 76.52, 53.28, 52.25, 25.54, 18.12, –4.51. Anal. calcd for $C_{19}H_{28}O_8Si$: C, 55.32; H, 6.84; found: C, 55.23; H, 6.83.

3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-5-(methoxycarbonyl)phenyl methyl [3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-(methoxycarbonyl)phenoxy]propanedioate (12)

A viscous resin (4.17 g, 13%) was obtained from an earlier eluting band in the chromatography of **11**, above, and 0.9 g was further purified by reverse phase MPLC (Lichroprep RP-18, 100% CH_3CN) to give 0.63 g of **12** as a very viscous, colorless resin: ^{1}H NMR ($CDCl_3$) δ 7.31 (*m*, appears as *d*, 2H, *J* = 2.3 Hz), 7.19 (*dd*, 1H, *J* = 1.3, 2.4 Hz), 7.15 (*dd*, 1H, *J* = 1.3, 2.3 Hz), 6.81 (*dd*, 1H, *J* = 2.4, 2.4 Hz), 6.77 (*dd*, 1H, *J* = 2.4, 2.4 Hz), 5.47 (*s*, 1H), 3.94 (*s*, 3H), 3.91 (*s*, 3H), 3.90 (*s*, 3H), 0.981 (*s*, 9H), 0.978 (*s*, 9H), 0.218 (*s*, 6H), 0.213 (*s*, 6H); ^{13}C NMR ($CDCl_3$) δ 166.15, 165.75, 165.15, 163.53, 157.44, 157.03, 156.59, 150.51, 132.35, 132.28, 119.32, 117.73, 116.27, 115.15, 112.98, 108.50, 76.61, 53.56, 52.35, 52.31, 25.56, 25.54, 18.13, –4.49, –4.54. Anal. calcd for $C_{32}H_{46}O_{11}Si_2$: C, 57.98; H, 6.99; found: C, 57.98; H, 7.09.

Dimethyl [3-hydroxy-5-(methoxycarbonyl)phenoxy]propanedioate (13)

To 8.50 g (20.6 mmol) of silyl malonate **11** in 130 mL of THF at 0 °C was added 21 mL (21 mmol) of 1 M tetra-*n*-butylammonium fluoride in THF slowly by syringe. A yellow solution resulted which was stirred for 2 h at 0 °C, then was partitioned between CH_2Cl_2 and a small amount of H_2O. The aqueous layer was washed with CH_2Cl_2 and the combined organics were washed with H_2O, dried ($MgSO_4$) and evaporated to 9.01 g of light brown resin. The resin was purified by silica gel chromatography (Prep 500A, 40:60 EtOAc:cyclohexane) resulting in 4.84 g (75%) of resin, which solidified to an off-white solid (95% pure by RPHPLC) after pumping at high vacuum for several hours. The solid (0.9 g) was further purified by reverse phase MPLC (Lichroprep RP-18, 45:55 CH_3CN:H_2O) providing 0.61 g of **13** as a clear resin, which slowly crystallized to a white solid, mp 94.5–95.5 °C: ^{1}H NMR ($CDCl_3$) δ 7.24 (*dd*, 1H, *J* = 1.3, 2.3 Hz), 7.15 (*dd*, 1H, *J* = 1.3, 2.3 Hz), 6.73 (*dd*, 1H, *J* = 2.3, 2.3 Hz), 5.28 (*s*, 1H), 3.89 (*s*, 3H), 3.86 (*s*, 6H); ^{13}C NMR ($CDCl_3$) δ 166.70, 165.85, 157.58, 157.55, 132.12, 111.57, 108.26, 107.49, 76.34, 53.45, 52.43. Anal. calcd for $C_{13}H_{14}O_8$: C, 52.35; H, 4.73; found: C, 52.48; H, 4.73.

Dimethyl [3-[1-(dimethoxyphosphinyl)-2-methoxy-2-oxoethoxy]-5-(methoxycarbonyl)phenoxy]propanedioate (15)

The malonate phenol **13** (3.22 g, 10.8 mmol) was dissolved in 85 mL of azeotropically-dried benzene and $[Rh(OAc)_2]_2$ (55 mg, catalytic) was added followed by trimethyl diazophosphonoacetate (5.20 g, 25.0 mmol). The mixture was heated at reflux for 4 h, at which time additional $[Rh(OAc)_2]_2$ and trimethyl diazophosphonoacetate (1.5–2.0 g, 8 mmol, 33 mmol total) were added. Reflux was continued for 16 h, then the mixture was cooled to rt, filtered through silica gel with EtOAc and evaporated to a brown oil. Purification by normal phase MPLC (Lichroprep Si60, 100% EtOAc, two injections) followed by reverse phase MPLC (Lichroprep RP-18, 40:60 CH_3CN:H_2O) provided 1.94 g (38%) of **15** as a colorless resin: ^{1}H NMR ($CDCl_3$) δ 7.27 (*m*, 2H), 6.79 (*dd*, 1H, *J* = 2.4, 2.4 Hz), 5.26 (*s*, 1H), 5.09 (*d*, 1H, *J* = 19.1 Hz), 3.93 (*s*, 3H), 3.90 (*s*, 3H), 3.89 (*s*, 3H), 3.86 (*s*, 3H), 3.85 (*s*, 6H); ^{13}C NMR ($CDCl_3$) δ 166.14, 165.65, 165.29, 165.26, 158.48 (*d*, *J* = 13.3 Hz), 157.75, 132.70, 110.26, 109.98, 108.14, 76.42, 74.08 (*d*, *J* = 159.5 Hz), 54.48 (*d*, *J* = 6.4 Hz), 54.46 (*d*, *J* = 6.4 Hz), 53.35, 53.26, 52.42; ^{31}P NMR ($CDCl_3$) δ 11.95. Anal. calcd for $C_{18}H_{23}O_{13}P$: C, 45.20; H, 4.85; found: C, 44.92; H, 4.90.

[3-Carboxy-5-(carboxyphosphonomethoxy)phenoxy]propanedioic acid (5)

To the phosphonate **15** (1.15 g, 2.40 mmol) in 25 mL of dry CH_2Cl_2 at 0 °C was added TMSBr (1.1 mL, 1.28 g, 8.3 mmol) over 5 min. The slightly cloudy solution was stirred for 3 h at rt, then rotary evaporated to an oil. The oil was dissolved in THF containing a small amount of H_2O and was evaporated again. The resulting yellowish oil in 30 mL of THF was treated at 0 °C with 1 N NaOH (23 mL, 23 mmol) and 20 mL of H_2O to make a homogeneous solution. This solution was stirred for 18 h and was then diluted with 75 mL of H_2O and adjusted to pH 7.5 by addition of AG 50W-X8 (H^+) resin. Anion exchange chromatography (DEAE Sephadex A-25, 0.3–1.2 M TEAB, 7 L linear gradient) gave a major band that was collected, providing 1.47 g of a very hygroscopic triethylammonium salt. The salt was converted to the free penta-acid by stirring an aqueous solution over 30 mL of AG 50W-X8 (H^+) resin and filtration through an additional 20 mL of resin. Evaporation and exposure to high vacuum for 1 day gave 0.61 g (58%) of **5** as an off-white glass, mp > 90 °C (dec., gas evolution): ^{1}H NMR (DMSO-d_6) δ 7.07 (*dd*, 1H, *J* = 1.1, 2.3 Hz), 7.05 (*dd*, 1H, *J* = 1.1, 2.3 Hz), 6.73 (*dd*, 1H, *J* = 2.3, 2.3 Hz), 5.44 (*s*, 1H), 4.94 (*d*, 1H, *J* = 19.1 Hz); ^{13}C NMR (DMSO-d_6) δ 168.02, 166.78, 166.76, 166.46, 159.10 (*d*, *J* = 13.3 Hz), 157.72, 133.03, 108.68, 106.85, 76.16, 74.87 (*d*, *J* = 148.2 Hz); ^{31}P NMR (DMSO-d_6) δ 10.77. Anal. calcd for $C_{12}H_{11}O_{13}P{\cdot}2.3\ H_2O$: C, 33.09; H, 3.61; found: C, 33.14; H, 3.69.

(3-Carboxy-5-hydroxyphenoxy)propanedioic acid (6)

To the triester **13** (1.10 g, 3.69 mmol) in a mixture of 35 mL of THF and 20 mL of H_2O at 0 °C was added 20 mL (20 mmol) of 1 N NaOH. The solution was allowed to warm to rt and was stirred for 28 h. The resulting solution was stirred over 45 mL of AG50W-X8 (H^+) resin for a few minutes, filtered and evaporated. Half of the

resulting solid was triturated twice with CH_3CN to give pure **6** as a cream-colored solid (0.37 g, 79%), mp 245–251 °C (dec., begins to darken above 100 °C): ^1H NMR (DMSO-d_6) δ 9.9 (*br s*), 6.99 (*dd*, 1H, *J* = 1.3, 2.2 Hz), 6.89 (*dd*, 1H, *J* = 1.3, 2.4 Hz), 6.55 (*dd*, appears as *t*, 1H, *J* = 2.3 Hz), 5.31 (*s*, 1H); ^{13}C NMR (DMSO-d_6) δ 166.9, 166.8, 158.5, 157.7, 132.8, 110.0, 106.8, 106.1, 76.0. Anal. calcd for $C_{10}H_8O_8 \cdot 0.1H_2O$: C, 46.56; H, 3.20; found: C, 46.43; H, 3.31.

Dimethyl [3-(2-ethoxy-2-oxoethoxy)-5-(methoxycarbonyl)-phenoxy]propanedioate (14)

The phenol **16**[6] (2.60 g, 10 mmol) was slurried in 300 mL of benzene and water was removed by azeotropic distillation. To the cooled solution was added $[Rh(OAc)_2]_2$ (80 mg, catalytic). The mixture was heated to reflux and a mixture of dimethyl diazomalonate (2.2 g, 14 mmol) in 100 mL of benzene was added dropwise over 1 h. The mixture was refluxed for 19 h, cooled to rt and filtered through silica gel with 25% EtOAC:hexane to afford a pale yellow liquid. The liquid was purified by silica gel chromatography (25% EtOAc:hexane) to provide **14** (2.7 g, 71%) as a tan oil: ^1H NMR ($CDCl_3$) δ 7.13 (*m*, 2H), 6.68 (*m*, 1H), 5.15 (*s*, 1H), 4.52 (*s*, 2H), 4.15 (*q*, 2H, *J* = 7 Hz), 3.78 (*s*, 3H), 3.72 (*s*, 6H), 1.20 (*t*, 3H, *J* = 7 Hz). Anal. calcd for $C_{17}H_{20}O_{10}$: C, 53.13; H, 5.24; found: C, 53.14; H, 5.29.

[3-Carboxy-5-(2-hydroxy-2-oxoethoxy)phenoxy]propanedioic acid (7)

To a stirred, cooled (0 °C) solution of **14** (0.43 g, 1.1 mmol) in 14 mL of THF was added 2.7 mL (6.6 mmol) of 2.5 N NaOH. Water was added (10 mL) to render the mixture homogeneous and cooling was maintained for 1 h. On warming to rt, the mixture became cloudy whereupon 4 mL of H_2O was added to afford a clear solution. This solution was stirred for 28 h at rt, then was stirred with excess AG 50W-X8 (H^+) resin. Filtration and concentration of the solution afforded **7** (0.33 g, 96%) as a white solid: ^1H NMR (D_2O) δ 7.11 (*s*, 1H), 7.08 (*s*, 1H), 6.71 (*s*, 1H), 4.46 (*s*, 2H). Anal. calcd for $C_{12}H_{10}O_{10} \cdot 2H_2O$: C, 40.79; H, 4.09; found: C, 40.78; H, 4.01.

Methyl 7-hydroxy-2,2-diphenyl-1,3-benzodioxole-5-carboxylate (17)

This diphenyl ketal was prepared from methyl gallate by the procedure of Jurd,[15] mp 164–164.5 °C (lit. 165 °C). Anal. calcd for $C_{21}H_{16}O_5$: C, 72.41; H, 4.63; found: C, 72.50; H, 4.59.

Dimethyl [[6-(methoxycarbonyl)-2,2-diphenyl-1,3-benzodioxol-4-yl]oxy]propanedioate (18)

The phenol **17** (6.00 g, 17.2 mmol) was slurried in 150 mL of benzene and the mixture was heated to boiling distilling off approximately 50 mL of benzene:water azeotrope. To the warm solution was added $[Rh(OAc)_2]_2$ (25 mg, catalytic) and the mixture was heated at reflux while dimethyl diazomalonate (3.0 g, 19.0 mmol) was added portionwise over 30 min. After 1.0 h, and again at 2.0 h, additional dimethyl diazomalonate (0.43 g and 0.30 g, respectively, 23.6 mmol total) was added. After a total of 3 h of reflux, the mixture was allowed to cool to rt, was filtered through silica gel with 50:50 EtOAc:cyclohexane and evaporated to 9.50 g of light yellow, viscous oil. The oil was purified in two batches by reverse phase MPLC (Lichroprep RP-8, 75:25 $CH_3CN:H_2O$) to give 4.88 g (59%) of **18** as a viscous, colorless resin: ^1H NMR ($CDCl_3$) δ 7.51–7.56 (*m*, 4H), 7.43 (*d*, 1H, *J* = 1.5 Hz), 7.36–7.41 (*m*, 6H), 7.32 (*d*, 1H, *J* = 1.5 Hz), 5.54 (*s*, 1H), 3.85 (*s*, 3H), 3.80 (*s*, 6H); ^{13}C NMR ($CDCl_3$) δ 165.85, 165.45, 148.95, 139.56, 139.13, 139.11, 129.44, 128.31, 126.22, 124.71, 119.09, 115.37, 105.24, 77.83, 53.19, 52.12. Anal. calcd for $C_{26}H_{22}O_9$: C, 65.27; H, 4.63; found: C, 65.15; H, 4.66.

6-(Methoxycarbonyl)-2,2-diphenyl-1,3-benzodioxol-4-yl methyl [[6-(methoxycarbonyl)-2,2-diphenyl-1,3-benzodioxol-4-yl]oxy]propanedioate (19)

Further elution in the chromatography of **18**, above, with 100% CH_3CN provided 2.33 g (17%) of **19** as a white solid glass, which melted broadly to a resinous liquid above 88 °C: ^1H NMR ($CDCl_3$) δ 7.51–7.56 (*m*, 5H), 7.43–7.49 (*m*, 5H), 7.42 (*d*, 1H, *J* = 1.4 Hz), 7.29–7.38 (*m*, 13H), 5.82 (*s*, 1H), 3.85 (*s*, 3H), 3.84 (*s*, 3H), 3.81 (*s*, 3H); ^{13}C APT spectral multipicity assignments: Q = quaternary carbon, CH = methine carbon, CH_3 = methyl carbon; ^{13}C NMR ($CDCl_3$) δ 165.71 (Q), 165.47 (Q), 164.76 (Q), 162.21 (Q), 149.01 (Q), 142.22 (Q), 139.44 (Q), 139.39 (Q), 139.05 (Q), 138.96 (Q), 138.84 (Q), 138.78 (Q), 131.74 (Q), 129.46 (CH), 129.43 (CH), 129.40 (CH), 128.29 (CH), 128.25 (CH), 126.22 (CH), 126.19 (CH), 124.75 (Q), 124.34 (Q), 120.01 (Q), 119.35 (Q), 118.48 (CH), 115.32 (CH), 108.08 (CH), 105.59 (CH), 77.56 (CH), 53.41 (CH_3), 52.16 (CH_3), 52.09 (CH_3). Anal. calcd for $C_{46}H_{34}O_{13}$: C, 69.52; H, 4.31; found: C, 69.35; H, 4.25.

(5-Carboxy-2,3-dihydroxyphenoxy)propanedioic acid (8)

To the ketal **18** (4.00 g, 8.36 mmol) in 75 mL of CH_2Cl_2 at 0 °C was added a solution of 1.0 mL of H_2O in 13 mL of CF_3COOH. The mixture was stirred 10 min, then warmed to rt. After a total of 140 min of stirring, the solution was evaporated, then evaporated an additional two times from CH_3CN to give 4.64 g of viscous oil. Dissolution of the oil in 40 mL of 45:55 EtOAc:cyclohexane caused a white solid to precipitate. Filtration afforded 1.45 g (55%) of diol triester as a fluffy white solid: ^1H NMR ($CDCl_3$) δ 7.43 (*d*, 1H, *J* = 1.9 Hz), 7.30 (*d*, 1H, *J* = 1.9 Hz), 5.10 (*s*, 1H), 3.90 (*s*, 6H), 3.87 (*s*, 3H).

The triester (0.80 g, 2.55 mmol) in 15 mL of THF was treated at 0 °C with 16.7 mL (16.7 mmol) of 1 N NaOH. The two-phase mixture was allowed to warm to rt, rendered homogeneous by addition of 6 mL of H_2O and was stirred for 20 h. The resulting dark solution was acidified by addition of AG50W-X8 (H^+) resin and was

evaporated to 0.76 g of dark brown solid. The solid was purified by anion exchange chromatography on DEAE Sephadex A-25 eluting with a 6 L linear gradient from 0.25 M to 1.0 M TEAB followed by isocratic elution with 1.5 L of 1 M TEAB. Fractions containing the product were evaporated to a light rose-colored, solid triethylammonium salt which was converted to the free triacid by dissolution in 75 mL of H_2O and stirring with 35 mL of AG 50W-X8 (H^+) resin. Filtration and evaporation of the light yellow solution afforded **8** (0.40 g, 55%) as a light brown solid, mp 265–267 °C (dec., gas evolution): ^1H NMR (DMSO-d_6) δ 9.8 (*v br s*), 7.13 (*d*, 1H, *J* = 1.8 Hz), 7.00 (*d*, 1H, *J* = 1.8 Hz), 5.17 (*s*, 1H); ^{13}C NMR (DMSO-d_6) δ 167.30, 167.02, 145.87, 145.15, 140.34, 120.44, 111.79, 109.27, 77.63. Anal. calcd for $C_{10}H_8O_9 \cdot 0.8H_2O$: C, 41.91; H, 3.38; found: C, 41.86; H, 3.37.

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References and Notes

1. Haslam, E. In *The Shikimate Pathway*; Wiley: New York, 1974.

2. Sikorski, J. A.; Anderson, K. S.; Cleary, D. G.; Miller, M. J.; Pansegrau, P. D.; Ream, J. E.; Sammons, R. D.; Johnson, K. A. *Chemical Aspects of Enzyme Biotechnology*; Baldwin, T. O.; Raushel, F. M.; Scott, A. I.; Eds.; Plenum: New York, 1990.

3. (a) Anderson, K. S.; Sikorski, J. A.; Benesi, A. J.; Johnson, K. A. *J. Am. Chem. Soc.* **1988**, *110*, 6577; (b) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 7395; (c) Anderson, K. S.; Johnson, K. A. *J. Biol. Chem.* **1990**, *265*, 5567; (d) Anderson, K. S.; Johnson, K. A. *Chem. Rev.* **1990**, *90*, 1131.

4. (a) Alberg, D. G.; Bartlett, P. A. *J. Am. Chem. Soc.* **1989**, *111*, 2337; (b) Alberg, D. G.; Lauhon, C. T.; Nyfeler, R.; Fässler, A.; Bartlett, P. A. *J. Am. Chem. Soc.* **1992**, *114*, 3535; (c) Walker, M. C.; Jones, C. R.; Somerville, R. L.; Sikorski, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 7601; (d) Pansegrau, P. D.; Miller, M. J.; Font, J. L.; Ream, J. E.; Sikorski, J. A. *Fourth Chemical Congress of North America, New York*, **1991**, Organic Division Poster Presentation No. 29.

5. Leo, G. C.; Castellino, S.; Sammons, R. D.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 151.

6. Miller, M. J.; Ream, J. E.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem.* **1994**, 2, 331.

7. Gruys, K. J.; Walker, M. C.; Sikorski, J. A. *Biochemistry* **1992**, *31*, 5534.

8. Miller, M. J.; Anderson, K. S.; Braccolino, D. S.; Cleary, D. G.; Gruys, K. J.; Han, C. Y.; Lin, K.-C.; Pansegrau, P. D.; Ream, J. E.; Sammons, R. D.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1435.

9. Marzabadi, M. R.; Font, J. L.; Gruys, K. J.; Pansegrau, P. D.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1435.

10. Corey, S. D.; Pansegrau, P. D.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2857.

11. Miller, M. J.; Braccolino, D. S.; Cleary, D. G.; Ream, J. E.; Walker, M. C.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*(21), 2605.

12. Peace, B. W.; Carman, F.; Wulfman, D. S. *Synthesis* **1971**, 658.

13. For related couplings in shikimate and chorismate systems see: (a) Ganem, B.; Ikota, N.; Muralidharan, V. B.; Wade, W. S.; Young, S. D.; Yukimoto, Y. *J. Am. Chem. Soc.* **1982**, *104*, 6787; (b) Teng, C.-Y. P.; Yukimoto, Y.; Ganem, B. *Tetrahedron Lett.* **1985**, *26*, 21.

14. Mass, G.; Regitz, M. *Chem. Ber.* **1976**, *109*, 2039.

15. Jurd, L. *J. Am. Chem. Soc.* **1959**, *81*, 4606.

16. (a) Overproducing strain pMON6001: Rogers, S. G.; Brand, L. A.; Holder, F. B.; Sharps, E. S.; Brackin, M. J. *Appl. Environ. Microbiol.* **1983**, *46*, 37; (b) Purification procedure: Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 1604.

17. Padgette, S. R.; Huynh, Q. K.; Borgmeyer, J.; Shah, D. M.; Brand, L. A.; Re, D. B.; Bishop, B. F.; Rogers, S. G.; Fraley, R. T.; Kishore, G. M. *Arch. Biochem. Biophys.* **1987**, *258*, 564.

18. In other studies with deoxy-S3P derivatives, the 4-hydroxyl group has been shown to contribute a factor of approximately 4-8 in binding affinity: Pansegrau, P. D.; Anderson, K. S.; Widlanski, T.; Ream, J. E.; Sammons, R. D.; Sikorski, J. A.; Knowles, J. R. *Tetrahedron Lett.* **1991**, 2589.

19. Data were analyzed using the GraFit software: Leatherbarrow, R. J. *GraFit*,Version 2.0, Erithacus Software Ltd, Staines, U. K., 1990.

20. Miller, M. J.; Font, J. L.; Sikorski, J. A. Unpublished results.

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