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Synthesis and Evaluation of 1-Deoxy-D-xylulose 5-Phosphate Analogues as Chelation-Based Inhibitors of Methylerythritol Phosphate Synthase

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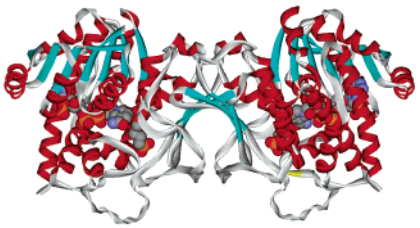
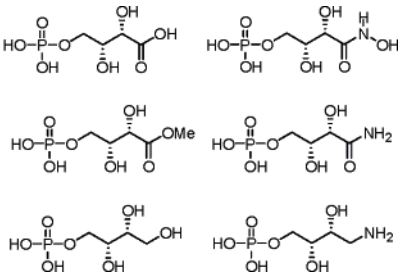
Received August 9, 2005





A series of 1-deoxy-D-xylulose 5-phosphate (DXP) analogues were synthesized and evaluated as inhibitors of *E. coli* methylerythritol phosphate (MEP) synthase. In analogues **1**−**4**, the methyl group in DXP was replaced by hydroxyl, hydroxylamino, methoxy, and amino moieties, respectively. In analogues **5** and **6**, the acetyl moiety in DXP was replaced by hydroxymethyl and aminomethyl groups. These compounds were designed to coordinate to the active site divalent metal in MEP synthase. The carboxylate (**1**), methyl ester (**3**), amide (**4**), and alcohol (**5**) analogues were inhibitors with IC_{50}'s ranging from 0.25 to 1.0 mM. The hydroxamic acid (**2**) and amino (**6**) analogues did not inhibit the enzyme.

Introduction

Isoprenoid compounds are the largest class of natural product metabolites with over 30 000 known members.[1] Although diverse in structure and function, these molecules are constructed from two simple five-carbon building blocks—isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Until the late 1980s, IPP and DMAPP were thought to originate exclusively from acetyl CoA through the mevalonate (MVA) pathway. However, labeling patterns from isotope incorporation experiments, which were inconsistent with the MVA pathway,[2−4] led to the discovery of a nonmevalonate route to isoprenoid compounds from glyceraldehyde 3-phosphate and pyruvate. The first committed step of the new route is the synthesis of 2-*C*-methyl-D-erythritol 4-phosphate (MEP) from 1-deoxy-D-xylulose 5-phosphate (DXP) by MEP synthase[5] (EC 1.1.1.267) (Figure 1). Since the discovery of MEP as a precursor for the isoprenoid pathway in the early 1990s, the remaining intermediates between MEP and IPP/DMAPP have been characterized and the genes that encode the biosynthetic enzymes have been identified.[6]

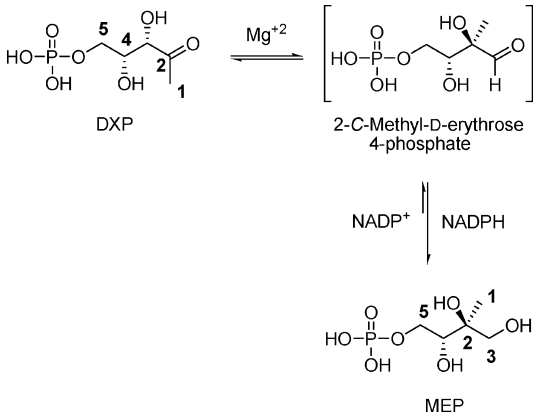


FIGURE 1. Reactions catalyzed by MEP synthase.

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10.1021/jo0516786 CCC: $30.25
Published on Web 10/12/2005

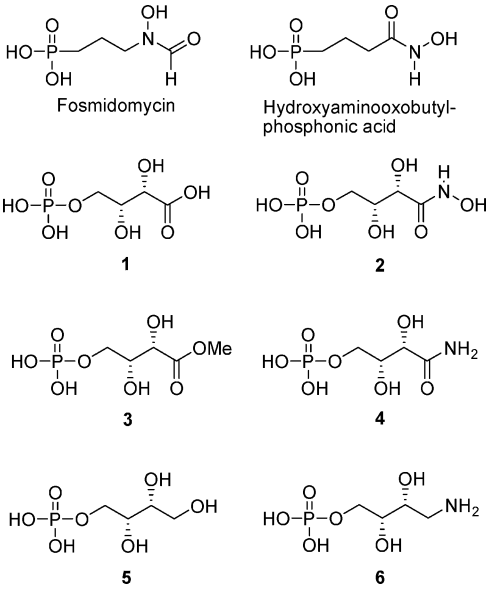
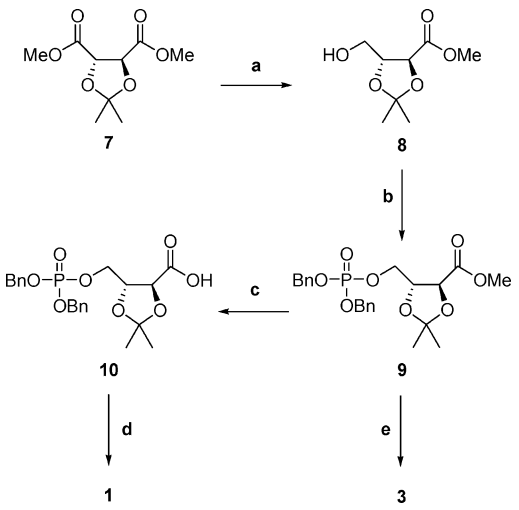


FIGURE 2. Structures of fosmidomycin and DXP analogues.

The MEP entry into the isoprenoid pathway is found in most eubacteria, green algae, and the plastids of higher plants, while the MVA entry is found in eukaryotes, including the cytosol of higher plants, and archaebacteria.[6,7] Furthermore, the MEP pathway has been found in the vestigial plastids of several pathogenic parasites, including the malarial parasite *Plasmodium falciparum*.[8] Common pathogenic bacteria that rely on the MEP root include those responsible for tuberculosis, bronchitis, and meningitis.[9] Inhibition or disruption of the enzymes in the MEP pathway is lethal and suggests a novel drug target for chemotherapy of infectious diseases.

Fosmidomycin (Figure 2) is a phosphonic acid metabolite from *Steptomyces rubellomurinus* discovered in the late 1970s.[10,11] The compound is a broad spectrum antibiotic that inhibits bacterial cell wall biosynthesis. The mechanism of action of fosmidomycin remained a mystery until after the discovery of the MEP pathway, when it was found to be a slow, tight binding inhibitor of MEP synthase with $K_I = 21$ nM.[12,13] Fosmidomycin has been used to treat malarial infections, although it has a high ED_{50} because of its poor bioavailability.[8] The hydroxamic acid isostere of fosmidomycin and its methyl derivative are also potent inhibitors of MEP synthase.[14] These results suggest that MEP synthase is a valid target for small molecules. We now report the synthesis and evaluation of six analogues of DXP.

SCHEME 1[a]



[a] Key: (a) 0.6 equiv of $NaBH_4$, MeOH, 0 °C, 2 h, 32%; (b) $P(OBn)_3$, I_2, pyridine, CH_2Cl_2, −30 °C to rt, 2 h, 79%; (c) KOH, MeOH, rt, 16 h, 99%; (d) (i) H_2, Pd/C, *t*-BuOH, rt, 2 h, (ii) H_2O, 3 d, 85%; (e) (i) H_2, Pd/C, MeOH, rt, 3 h, (ii) H_2O, 2 d, 48%.

Results

Synthesis. The structures of fosmidomycin and hydroxyaminooxobutyl phosphonic acid, known inhibitors of MEP synthase, along with the six DXP analogues reported in this study are shown in Figure 2. The syntheses of carboxylate **1** and methyl ester **3** are given in Scheme 1. (−)-Dimethyl 2,3-*O*-isopropylidene-D-tartrate (**7**) was treated with 0.6 equiv of sodium borohydride to give monoester **8**.[15] The ester was phosphorylated with dibenzyl phosphoroiodidate (DBPI) generated in situ[16] to give benzyl phosphate monoester **9**,[17] which was then saponified to provide carboxylic acid **10**. The carboxylate moiety in **10** was partially esterified when the benzyl groups were removed by hydrogenation in methanol. This side reaction was avoided by replacing methanol with *tert*-butyl alcohol. The isopropylidene moiety was removed to give **1** by allowing the debenzylated phosphate to stand in water for 2 days. Methyl ester analogue **3** was obtained from **9** by removing the benzyl groups in methanol.

Analogues **2**, **4**, and **5** were obtained from methyl ester **9** as shown in Scheme 2. The methyl ester was treated with hydroxylamine, and the resulting hydroxamic acid (**11**) was deprotected as described previously to give **2**. Amide **4** was obtained by treating **9** with ammonia, followed by deprotection, while alcohol **5** was generated by reduction of **9** with lithium borohydride, followed by deprotection.

The synthesis of the amino analogue **6** is outlined in Scheme 3. C_2-symmetric (−)-2,3-*O*-isopropylidene-D-threitol (**14**) was monoprotected with TIPSCl to give **15**.[18]

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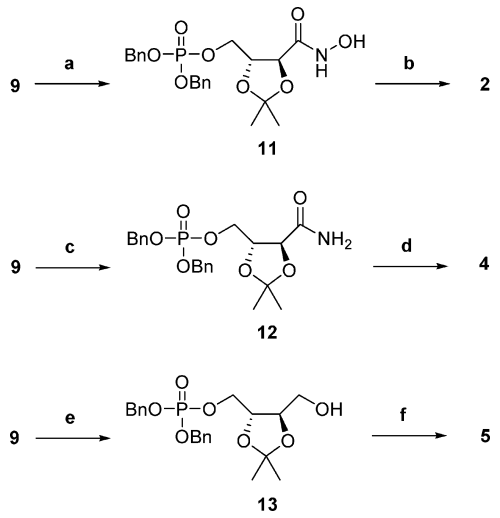
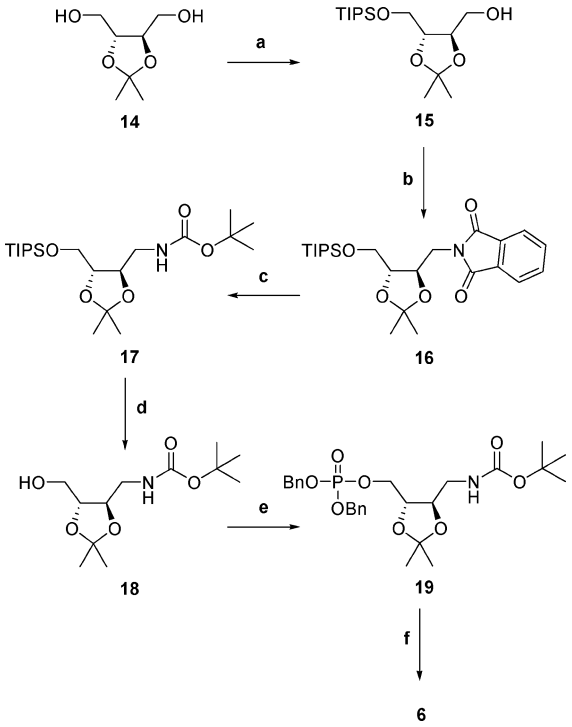
SCHEME 2[a]



[a] Key: (a) $NaOCH_3$, $NH_2OH{\cdot}HCl$, MeOH, rt, 16 h, 80%; (b) (i) H_2, Pd/C, MeOH, rt, 2 h, (ii) H_2O, 3 d, 43%; (c) 7 N NH_3, MeOH, reflux, 2 h, 99%; (d) (i) H_2, Pd/C, MeOH, rt, 2 h, (ii) H_2O, 5 d, 99%; (e) $LiBH_4$, ether, 0 °C, 1 h, 82%; (f) (i) H_2, Pd/C, MeOH, rt, 2 h, (ii) H_2O, 3 d, 94%.

SCHEME 3[a]



[a] Key: (a) NaH, TIPSCl, THF, 0 °C to rt, 3 h, 94%; (b) phthalimide, Ph_3P, DIAD, THF, 0 °C to rt, 16 h, 99%; (c) (i) $N_2H_4{\cdot}H_2O$, EtOH, 60 °C, 1 h, (ii) di-*tert*-butyl dicarbonate, Na_2CO_3, IPA/H_2O, 0 °C to rt, 16 h, 95%; (d) 1 M TBAF, THF, 0 °C to rt, 1 h, 91%; (e) $P(OBn)_3$, I_2, pyridine, CH_2Cl_2, −30 °C to rt, 4 h, 81%; (f) (i) H_2, Pd/C, MeOH, rt, 4 h, (ii) 1 M HCl, 24 h, 52%.

The hydroxyl moiety was displaced with phthalimide using Mitsunobu conditions to generate **16**. The phthalimide moiety was replaced with *t*-BOC, and **17** was treated with TBAF to give primary alcohol **18**. The alcohol was phosphorylated and debenzylated as described previously. The isopropylidene and BOC groups were removed with HCl to give amino analogue **6**.

TABLE 1. Inhibition MEP Synthase with DXP Analogues[a]

compd	IC_{50}[b] (μM)
1	551 ± 133
2	>5000[c]
3	1024 ± 235
4	253 ± 27
5	310 ± 73
6	>5000[c]

[a] Reaction conditions: 50 mM HEPES buffer, pH 7.6, 3 mM $MgCl_2$, 1 mg/mL BSA, 8 nM MEP synthase, 150 μM NADPH, 100 μM DXP, 100−5000 μM analogue, 100 μL final volume, 37 °C, 2 min. [b] Value obtained by plotting specific activity versus concentration (n = 3). [c] 50% inhibition not observed at 5000 μM.

MEP Synthase Assays. Recombinant *E. coli* MEP synthase was obtained as previously reported.[13,19] The conversion of DXP to MEP was monitored spectrospcopically by measuring the change in absorbance at 340 nm upon oxidation of NADPH to $NADP^+$. Steady-state kinetic parameters for the recombinant his_6-tagged enzyme used in this study were k_{cat} = 33 s^{-1} and K_m^{DXP} = 81 μM. These values agree with those previously reported for the *E. coli* enzyme.[19]

IC_{50}'s for the DXP analogues were determined using the standard assay for samples where the enzyme had been preincubated with the inhibitors for 2 min. The reaction was initiated by adding DXP. The results are shown in Table 1. Two of the inhibitors, **4** and **5**, had IC_{50}'s that were slightly higher (3−4-fold) than K_m^{DXP}. The methyl ester (**3**) and carboxylic acid (**1**) analogues were less potent, and less than 50% inhibition was seen at 5 mM concentrations of the hydroxamic acid (**2**) and the amino (**6**) analogues. None of the DXP analogues were as potent as fosmidomycin.

Discussion

Several crystal structures have been reported for MEP synthase.[20−24] Overall, the structure consists of three domains: an N-terminal domain that binds NADPH; a "connective" domain that contains the active site; and a C-terminal domain also involved in cofactor binding. The active site consists of phosphate and divalent metal binding motifs separated by a hydrophobic region. The enzyme is active in buffers containing Mg^{2+} or Mn^{2+} and it is unclear which the physiologically active metal is. In the fosmidomycin−Mn^{2+} ternary complex,[22] the *N*-formyl hydroxylamine moiety engages the metal by a bidentate

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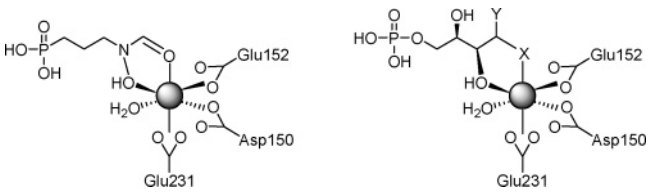

FIGURE 3. Chelation of fosmidomycin and analogues to the active site metal in MEP synthase.

interaction (Figure 3). This structure is believed to mimic substrate binding of the C2 carbonyl oxygen and the C3 hydroxyl group in DXP. These two functionalities are proposed to be in the inner coordination sphere of the metal ion. A more recent structure, where MEP synthase was cocrystallized with NADPH and fosmidomycin, revealed a large conformational change in the "connective" domain[24] to give a compact, solvent free, relatively hydrophobic active site that tightly embraces the inhibitor. The restricted volume of the pocket in the E·fosmidomycin·NADPH complex explains why larger analogues of DXP or fosmidomycin are not effective inhibitors.[25]

In the X-ray structure of the MEP synthase·fosmidomycin complex,[22] the formyl oxygen and the *N*-hydroxyl groups in fosmidomycin are coordinated to the active site Mn^{2+}. Presumably similar interactions occur between the metal ion and the corresponding carbonyl oxygens/hydroxyl groups in DXP and the rearranged aldehyde (see Figure 1). DXP analogues **1**−**6** were synthesized to explore the ability of a diol, β-amino hydroxyl, and other α-hydroxycarbonyl moieties to inhibit MEP synthase. The phosphate and diol portions of DXP were preserved in all of the analogues. In **1**−**4**, the methyl group in DXP was replaced by hydroxyl, hydroxylamino, methoxy, and amino moieties, respectively. In **5** and **6**, the acetyl moiety in DXP was replaced by hydroxymethyl and aminomethyl groups. All of these compounds should, in theory, be capable of coordinating with the active site divalent metal.

The compounds that inhibit MEP synthase have α-hydroxycarboxylate (**1**), α-hydroxyester (**3**), α-hydroxyamide (**4**), and 1,2-diol (**5**) moieties available for metal ion binding. Analogue **4** was recently synthesized and shown to inhibit (K_I = 90 μM) MEP synthase from *Synechocystis*.[26] Inhibition by **1**, **3**, and **4** in buffers containing Mg^{2+} suggests that negatively charged or neutral donor atoms are accepted as chelators of the active site Mg^{2+}. Inhibition by analogue **5** demonstrates that a carbonyl group is not required for inhibition. Interestingly, amine **6** does not inhibit the enzyme. Since Mg^{2+} forms complexes with amines, the lack of inhibition may reflect that the amino group in **6** is protonated. Also hydroxylamine analogue **2** is not an inhibitor. The lack of inhibition in this case might be a result of the extremely confined volume of the active site after the enzyme binds its substrates. The chain of analogue **2** is one atom longer than that of the potent hydroxamic acid isostere of fosmidomycin (Figure 2). In addition, a DXP analogue, where the methyl moiety is replaced with an ethyl group, that approximates the chain length of **2** is a poor inhibitor for MEP synthase with IC_{50} = 6.2 mM.[17]

In conclusion, a series of DXP analogues were synthesized and evaluated as inhibitors of MEP synthase. The most potent members of the group closely approximated the size of DXP and contained functional groups that could bind to the divalent metal in the active site. Changes that compromise either of these two features result a substantial decrease in potency.

Experimental Section

(2*S*,3*R*)-5-(Bis-benzyloxyphosphoryloxymethyl)-2,2-dimethyl[1,3]dioxolane-4-carboxylic Acid (10). To a solution of **9** (203 mg, 0.45 mmol) in methanol (60 mL) was added 1 M potassium hydroxide (2.3 mL, 2.3 mmol). After 16 h, the solution was acidified to pH 3 with 1 M HCl and diluted with ethyl acetate. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried ($MgSO_4$) and concentrated to give 195 mg (99%) of an oil: $[\alpha]_D$ +8.66 (*c* 5.8, acetone); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 6H), 4.13−4.20 (m, 1H), 4.31−4.37 (m, 3H), 5.06 (d, 2H, *J* = 3.9 Hz), 5.09 (d, 2H, *J* = 3.9 Hz), 7.33 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 25.7, 26.7, 66.6, 69.9 (d, *J* = 6.0 Hz), 74.5, 77.4 (d, *J* = 6.0 Hz), 111.9, 128.1, 128.1, 128.6, 128.6, 128.7, 128.7, 135.3, 171.5; ^{31}P NMR ($CDCl_3$) δ −0.32; HRMS (CI) calcd for $C_{21}H_{25}O_8P$ (M + H) 437.1365, found 437.1363.

(2*S*,3*R*)-2,3-Dihydroxy-4-phosphonooxybutyric Acid (1). To a solution of **10** (156 mg, 0.36 mmol) in *tert*-butyl alcohol (20 mL) was added 10% Pd/C (5 mg). After 2 h under hydrogen (1 atm) the suspension was filtered. The filtrate was concentrated and dissolved in water (10 mL). The mixture was allowed to stir for 3 days at rt. Lyophilization gave 66 mg (85%) of foam: $[\alpha]_D$ −2.27 (*c* 1.1, H_2O); ^{1}H NMR (D_2O) δ 3.88−4.01 (m, 2H), 4.25 (td, 1H, *J* = 2.3, 6.5 Hz), 4.41 (d, 1H, *J* = 2.3 Hz); ^{13}C NMR (D_2O) δ 65.8 (d, *J* = 5.0 Hz), 70.3, 70.7 (d, *J* = 8.0 Hz), 175.8; ^{31}P NMR (D_2O) δ 1.00; HRMS (FAB) calcd for $C_4H_9O_8P$ (M − H) 214.9915, found 214.9957.

(2*S*,3*R*)-Methyl 2,3-Dihydroxy-4-phosphonooxybutyrate (3). To a solution of **9** (200 mg, 0.44 mmol) in methanol (15 mL) was added 10% Pd/C (20 mg). After 3 h under hydrogen (1 atm), the suspension was filtered. The filtrate was concentrated and dissolved in water (10 mL). The mixture was allowed to stir for 2 days at rt. Lyophilization, followed by cellulose chromatography (7:3 2-propanol/water, 50 mM NH_4HCO_3), gave 53 mg (48%) of glassy foam: $[\alpha]_D$ −9.13 (*c* 1.9, H_2O); ^{1}H NMR (D_2O) δ 3.77 (s, 3H), 3.86 (td, 2H, *J* = 2.1, 6.8 Hz), 4.19 (td, 1H, *J* = 2.1, 6.8 Hz), 4.47 (d, 1H, *J* = 2.1 Hz); ^{13}C NMR (D_2O) δ 64.5 (d, *J* = 4.6 Hz), 70.8, 71.3 (d, *J* = 8.0 Hz), 174.7; ^{31}P NMR (D_2O) δ 3.05; HRMS (FAB) calcd for $C_5H_{11}O_8P$ (M − H) 229.0113, found 229.0094.

Dibenzyl (2*S*,3*R*)-5-Hydroxycarbamoyl-2,2-dimethyl-[1,3]dioxolan-4-ylmethyl Phosphate (11). To a solution of hydroxylamine hydrochloride (62 mg, 0.88 mmol) in methanol (25 mL) was added 30 wt % sodium methoxide (246 mg, 1.3 mmol). A solution of **9** (200 mg, 0.44 mmol) in methanol (5 mL) was cannulated into the reaction. After 16 h, the solution was acidified to pH 3 using 0.1 M HCl, diluted with ethyl acetate, and then washed with water and brine. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried ($MgSO_4$) and concentrated. The residue was chromatographed (95:5 CH_2Cl_2/MeOH) to give 159 mg (80%) of oil: $[\alpha]_D$ +2.48 (*c* 5.9, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 6H), 4.14−4.40 (m, 4H), 5.06 (s, 2H), 5.09 (s, 2H), 7.35 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 25.9, 26.7, 66.4 (d, *J* = 5.5 Hz), 69.5 (d, *J* = 4.0 Hz), 74.3, 77.8 (d, *J* = 7.0 Hz), 111.7, 128.0, 128.6, 135.5 (d, *J* = 5.0 Hz), 167.2; ^{31}P NMR ($CDCl_3$) δ −0.12; HRMS (CI) calcd for $C_{21}H_{26}NO_8P$ (M + H) 452.1474, found 452.1465.

Dibenzyl (2*S*,3*R*)-5-Carbamoyl-2,2-dimethyl[1,3]dioxolan-4-ylmethyl Phosphate (12). To a solution of 7 N ammonia

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in methanol (50 mL) was added **9** (150 mg, 0.33 mmol) in methanol (5 mL). After 2 h at 50 °C, the solvent was evaporated to give 147 mg (quant) of an oil: $[\alpha]_D$ +0.68 (*c* 5.6, acetone); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H), 1.41 (s, 3H), 4.13−4.24 (m, 3H), 4.36−4.42 (m, 1H), 5.04 (d, 2H, J = 1.3 Hz), 5.07 (d, 2H, J = 1.3 Hz), 6.57 (s, 1H), 6.64 (s, 1H), 7.33 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 25.9, 26.7, 66.7 (d, J = 5.5 Hz), 69.2 (d, J = 5.1 Hz), 75.0, 77.7 (d, J = 7.5 Hz), 111.2, 127.8, 127.8, 128.4, 128.4, 135.6 (d, J = 7.0 Hz), 173.0; ^{31}P NMR ($CDCl_3$) δ −0.03; HRMS (CI) calcd for $C_{21}H_{26}NO_7P$ (M + H) 436.1525, found 436.1542.

Dibenzyl (2*R*,3*R*)-5-hydroxymethyl-2,2-dimethyl[1,3]-dioxolan-4-ylmethyl Phosphate (13). A solution of **9** (500 mg, 1.11 mmol) in ether (100 mL) was cooled to 0 °C before 2 M lithium borohydride (1.1 mL, 2.2 mmol) was added. After 1 h, the solution was quenched with NH_4Cl (satd), diluted with ether, and washed with water and brine. The aqueous layer was extracted with ether, and the combined organic layers were dried ($MgSO_4$) and concentrated. The residue was chromatographed (ethyl acetate) to give 384 mg (82%) of an oil: $[\alpha]_D$ +6.67 (*c* 4.8, acetone); ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3H), 1.41 (s, 3H), 3.60 (dd, 1H, J = 4.2, 12.0 Hz), 3.74 (dd, 1H, J = 3.9, 12.0 Hz), 3.91−3.95 (m, 1H), 4.04−4.14 (m, 3H), 5.04−5.08 (m, 4H), 7.36 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 26.8, 27.0, 61.9, 66.9 (d, J = 6.0 Hz), 69.4 (d, J = 5.6 Hz), 75.9 (d, J = 8.0 Hz), 77.8, 109.7, 127.9, 128.5, 135.5 (d, J = 6.5 Hz); ^{31}P NMR ($CDCl_3$) δ −0.27; HRMS (CI) calcd for $C_{21}H_{27}O_7P$ (M + H) 423.1573, found 423.1558.

2-{(4*R*,5*R*)-2,2-Dimethyl-5-triisopropylsilanyloxymethyl-[1,3]dioxolan-4-ylmethyl}isoindole-1,3-dione (16). To a solution of **15** (5.30 g, 16.7 mmol) in THF (250 mL) were sequentially added phthalimide (2.69 g, 18.3 mmol) and triphenylphosphine (4.81 g, 18.3 mmol). Upon cooling 0 °C, diisopropyl azodicarboxylate (3.6 mL, 18.3 mmol) was added dropwise. After the mixture was warmed to rt overnight, the solvent was evaporated and resuspended in ether. The solid was filtered and triturated with ether. The filtrate was concentrated and chromatographed (8:2 hexanes/ethyl acetate) to give 7.43 g (99%) of an oil, which solidified upon standing: mp 46−47 °C; $[\alpha]_D$ +6.95 (*c* 3.8, acetone); ^{1}H NMR ($CDCl_3$) δ 1.03 (s, 18H), 1.35 (s, 3H), 1.40 (s, 3H), 3.76−4.02 (m, 5H), 4.38 (dd, 1H, J = 6.0, 12.6 Hz), 7.71−7.72 (m, 2H), 7.84−7.85 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 11.8, 17.9, 27.1, 27.2, 40.7, 63.8, 76.2, 79.7, 109.5, 123.3, 132.1, 133.9, 168.1; HRMS (CI) calcd for $C_{24}H_{37}NO_5Si$ (M + H) 448.2519, found 448.2511.

***tert*-Butyl{(4*R*,5*R*)-2,2-Dimethyl-5-triisopropylsilanyloxymethyl[1,3]dioxolan-4-ylmethyl}carbamate (17).** To a solution of **16** (1.0 g, 2.23 mmol) in absolute ethanol (50 mL) was added hydrazine hydrate (1.4 mL, 45 mmol) via syringe. After being warmed to 60 °C for 1 h, the reaction mixture was cooled and the precipitate filtered. The filtrate was concentrated, dissolved in CH_2Cl_2, and washed with brine. The aqueous layer was extracted with CH_2Cl_2, and the organic layers were concentrated. The residue was dissolved in 3:1 IPA/water (40 mL). After the mixture was cooled to 0 °C, sodium carbonate (1.0 g) was added, followed by addition of di-*tert*-butyl dicarbonate (0.73 g, 3.35 mmol) in IPA (10 mL). The reaction mixture warmed to rt overnight, acidified to pH 2 with 1 M HCl, diluted with ethyl acetate, and washed with brine. The aqueous layers were extracted with ethyl acetate, and the combined organic layers were dried ($MgSO_4$) and concentrated. The residue was chromatographed (9:1 hexanes/ethyl acetate) to give 0.90 g (95%) of an oil: $[\alpha]_D$ +5.46 (*c* 4.3, acetone); ^{1}H NMR ($CDCl_3$) δ 1.05−1.07 (m, 21H), 1.38 (s, 3H), 1.40 (s, 3H), 1.43 (s, 9H), 3.37−3.41 (m, 2H), 3.75−3.86 (m, 2H), 3.88−3.90 (m, 1H), 4.03 (dd, 1H, J = 5.1, 12.3 Hz), 5.04 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.8, 17.9, 26.9, 27.1, 27.4, 28.3, 42.3, 63.6, 77.9, 79.3, 94.1, 108.9, 156.4; HRMS (CI) calcd for $C_{21}H_{43}NO_5Si$ (M + H) 418.2989, found 448.2953.

***tert*-Butyl{(4*R*,5*R*)-5-Hydroxymethyl-2,2-dimethyl[1,3]-dioxolan-4-ylmethyl}carbamate (18).** A solution of **17** (5.32 g, 12.74 mmol) in THF (250 mL) was cooled to 0 °C before 1 M TBAF (15.3 mL, 15.3 mmol) was added via syringe. After 30 min, the reaction mixture was stirred at rt for 30 min. The mixture was diluted with ethyl acetate and washed with water and brine. The aqueous layers were extracted with ethyl acetate, dried ($MgSO_4$), and concentrated. The residue was chromatographed (6:4 hexanes/ethyl acetate) to give 3.00 g (91%) of an oil, which solidified upon standing: mp 69−71 °C; $[\alpha]_D$ +0.94 (*c* 2.9, acetone); ^{1}H NMR (acetone-d_6) δ 1.33 (s, 3H), 1.34 (s, 3H), 1.41 (s, 9H), 3.31 (dd, 2H, J = 4.8, 11.3 Hz), 3.66 (d, 2H, J = 4.8 Hz), 3.79−3.85 (m, 1H), 3.90−3.96 (m, 1H), 6.10 (br s, 1H); ^{13}C NMR (acetone-d_6) δ 27.4, 27.5, 28.7, 43.3, 63.0, 78.3, 79.0, 80.5, 109.3, 156.9, 206.5; HRMS (CI) calcd for $C_{12}H_{23}NO_5$ (M + H) 262.1654, found 262.1630.

Acknowledgment. We thank Dr. D. Fox for supplying recombinant enzyme. This work was supported by NIH Grant No. GM22521.

Supporting Information Available: General methods; experimental protocols for the syntheses of **2**, **4**−**6**, **8**, **9**, **15**, and **19**; ^{1}H, ^{13}C, and ^{31}P NMR spectra for compounds **1**−**6**; ^{1}H and ^{13}C NMR spectra for compounds **10**−**13** and **16**−**19**; and protocols for enzyme assays. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0516786