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Synthesis and Evaluation of 1-Deoxy-D-xylulose 5-Phosphoric Acid Analogues as Alternate Substrates for Methylerythritol Phosphate Synthase

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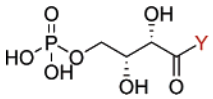

Y = Et, CH_2F, CHF_2, CF_3

Four deoxyxylulose phosphate (DXP) analogues were synthesized and evaluated as substrates/inhibitors for methylerythritol phosphate (MEP) synthase. In analogues CF_3-DXP (**1**), CF_2-DXP (**2**), and CF-DXP (**3**), the three methyl hydrogens at C1 of DXP were sequentially replaced by fluorine. In the fourth analogue, Et-DXP (**4**), the methyl group in DXP was replaced by an ethyl moiety. Analogues **1**, **2**, and **4** were not substrates for MEP synthase under normal catalytic conditions and were instead modest inhibitors with IC_{50} values of 2.0, 3.4, and 6.2 mM, respectively. In contrast, **3** was a good substrate (k_{cat} = 38 s^{-1}, K_m = 227 μM) with a turnover rate similar to that of the natural substrate. These results are consistent with a retro-aldol/aldol mechanism rather than an α-ketol rearrangement for the enzyme-catalyzed conversion of DXP to MEP.

Introduction

With greater than 30000 representative compounds, the isoprenoid family is the largest and most chemically diverse group of natural products.[1] These molecules are assembled from the simple five-carbon precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). For several decades it was thought that isoprenoid compounds were derived from acetyl-CoA by the mevalonate biosynthetic pathway.[2] Recently, an alternative pathway based on pyruvate and D-glyceraldehyde 3-phosphate (GAP) was discovered in eubacteria, algae, and plant chloroplasts.[3−9] In this pathway, 1-deoxy-D-xylulose 5-phosphate (DXP) is synthesized from D-glyceraldehyde 3-phosphate (GAP) and pyruvate in a thiamine diphosphate-dependent reaction catalyzed by DXP synthase (DXS).[10,11] The branched carbon skeleton typical of isoprenoid compounds is then constructed from the linear deoxysugar by a reversible rearrangement−NADPH-dependent reduction catalyzed by 2-*C*-methyl-D-erythritol 4-phosphate (MEP) synthase.[12,13] Since DXP is also required for synthesis of vitamins B_1 and B_6,[14,15] the rearrangement of DXP to MEP represents the first committed step in the pathway for the biosynthesis of isoprenoids. MEP is ultimately converted to both IPP and DMAPP by the action of five enzymes (see Scheme 1).[16]

MEP synthase catalyzes both the rearrangement and reduction of DXP. In the first step, the C3−C4 bond in DXP is broken with concomitant formation of a new bond between C2 and C4 of the substrate to produce a putative aldehyde intermediate, 2-*C*-methyl-D-erythrose 4-phosphate, which is then reduced by NADPH. The stereo-

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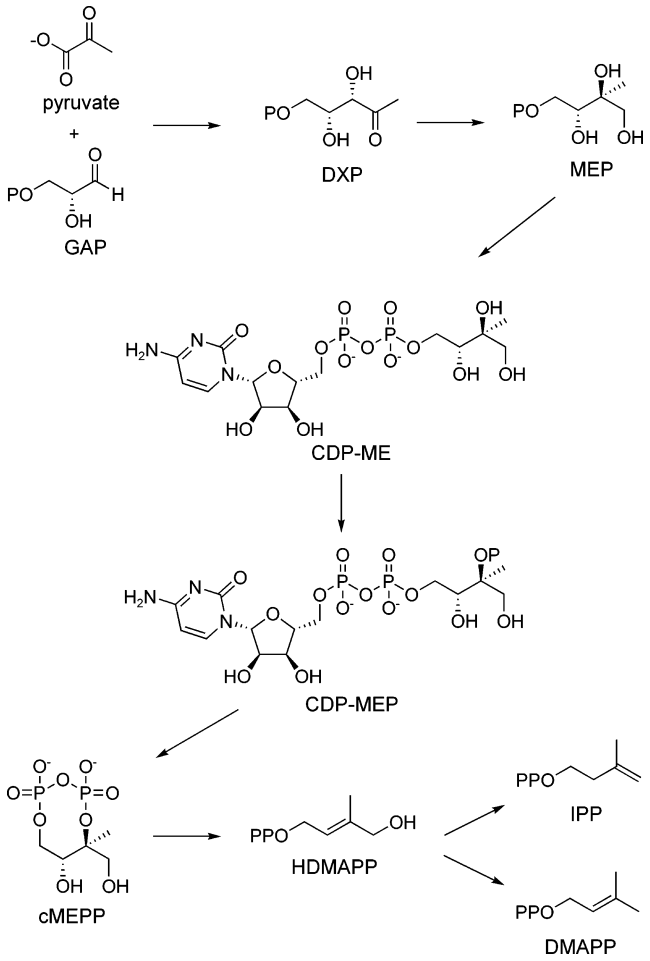
SCHEME 1. Methylerythritol Phosphate Pathway for Isoprenoid Biosynthesis



chemistry for the reduction was studied by Proteau et al.[17] and Arigoni et al.,[18] who found that the *pro-S*-hydride from NADPH was delivered to the *re* face of the aldehyde. Thus, MEP synthase can be classified as a class B dehydrogenase.[17,19] The coupled reactions catalyzed by MEP synthase, while uncommon, are not unprecedented. Ketol-acid reductoisomerase catalyzes a similar carbon skeletal rearrangement and NADPH-dependent reduction of either 2-acetolactate or 2-acetohydroxybutyrate to yield 2,3-dihydroxy-3-isovalerate or 2,3-dihydroxy-3-methylvalerate, respectively, during biosynthesis of valine, isoleucine, and leucine.[20−24] Although the two reactions mediated by MEP synthase and ketol-acid reductoisomerase are similar, the enzymes do not share similar

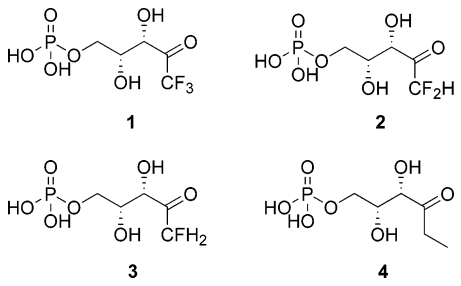


FIGURE 1. Structures of DXP analogues **1**−**4**.

amino acid sequences. In addition, the X-ray crystal structures of both MEP synthase and ketol-acid reductoisomerase are quite dissimilar, although both enzymes appear to follow an induced fit model.[25] Ketol-acid reductoisomerase and MEP synthase bind NADPH and divalent cation, which causes a slight conformational change, followed by binding of the substrate to cause a more drastic conformational change prior to the enzymatic reaction. Due to similarities in the overall reactions catalyzed by MEP synthase and the ketol-acid reductoisomerase, it was generally accepted that the mechanism was also similar. However, Rohmer et al.[26] recently suggested that the aldehyde intermediate could be formed by a retro-aldol/aldol mechanism. As part of an effort to distinguish between the ketol-acid and retro-aldol/aldol mechanisms, we synthesized four DXP analogues, CF_3-DXP (**1**), CF_2-DXP (**2**), and CF-DXP (**3**), where hydrogen atoms at C1 were replaced with fluorine, and Et-DXP (**4**), where the methyl group was replaced by an ethyl moiety (Figure 1). While this paper was being prepared, related studies were reported by Liu et al.[27] and Proteau et al.[28]

Results and Discussion

Synthesis of CF_3-DXP (1). The route used to synthesize 1,1,1-trifluoro-1-deoxy-D-xylulose 5-phosphoric acid (CF_3-DXP, **1**) is outlined in Scheme 2. Aldehyde **7** was obtained from (−)-2,3-*O*-isopropylidene-D-threitol (**5**) according to the sequence reported by Blagg et al.[29] The trifluoromethyl moiety was introduced using (trifluoromethyl)trimethylsilane (TMS-CF_3) and a catalytic amount of potassium *tert*-butoxide, following the procedure of Olah et al.,[30] to give **8** as a 3:1 mixture of diastereomers. The trifluoromethylated bissilyl ether **8** was unstable to silica gel chromatography and therefore was treated directly with 2 equiv of tetra(*n*-butyl)ammonium fluoride (TBAF) to afford diol **9** as a mixture of diastereomers. Regioselective phosphorylation of the primary alcohol was accomplished using dibenzyl phosphoroiodidate (DBPI), prepared in situ from tribenzyl phosphite and

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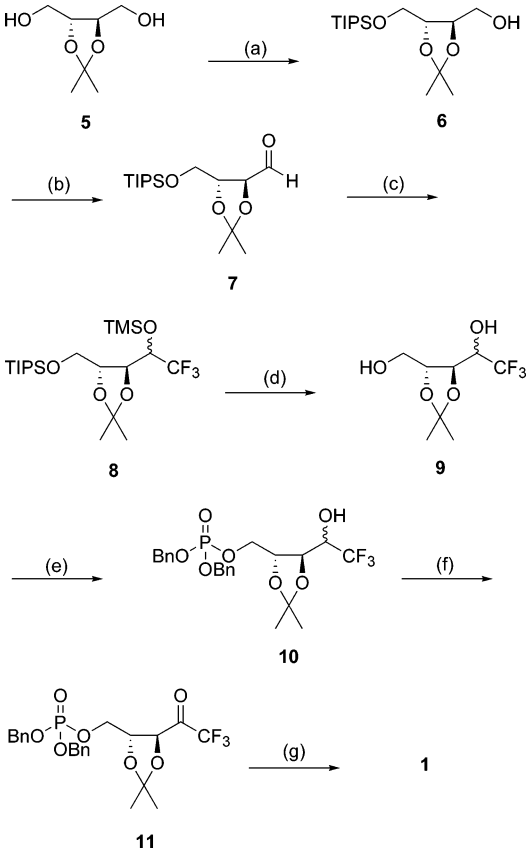
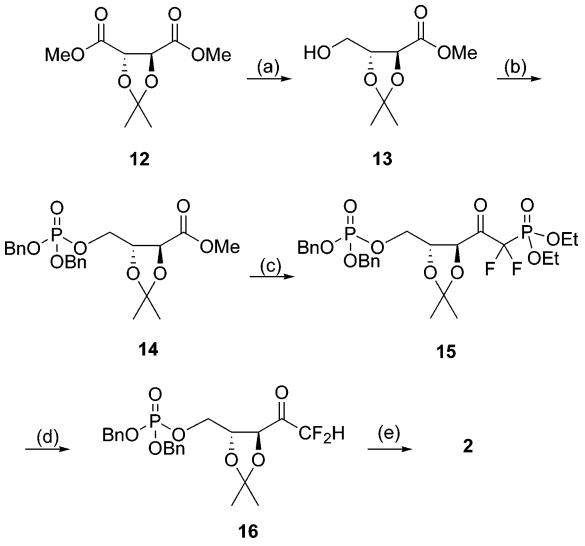
SCHEME 2. Synthesis of CF_3-DXP[a]



[a] Reagents and conditions: (a) (TIPS)Cl, NaH, THF, 0 °C to rt, 90%; (b) oxalyl chloride, DMSO, NEt_3, CH_2Cl_2, −78 °C, 95%; (c) 0.5 M CF_3-TMS in THF, cat. *t*-BuOK, THF, 0 °C; (d) 1.0 M TBAF in THF, THF, 0 °C, 80% (two steps); (e) $P(OBn)_3$, I_2, pyridine, CH_2Cl_2, −40 °C to rt, 77%; (f) Dess−Martin reagent, CH_2Cl_2, nonbasic workup, 90%; (g) (i) H_2/10 wt % Pd/C, *t*-BuOH, (ii) H_2O, 5 d, 96%.

iodine,[31,32] to give phosphorus triester **10**. Both Swern[33] conditions and TPAP/NMO[34] were not suitable for the oxidation of alcohol **10**; however, Dess−Martin[35] oxidation followed by a nonbasic workup afforded protected CF_3-DXP **11**. The oxidation was monitored by ^{19}F NMR spectroscopy, and only one product, corresponding to the protected trifluoromethyl ketone, was seen. Following workup and silica gel flash chromatography, **11** existed almost exclusively in the hydrate form (90:10) as judged by both ^{19}F and ^{13}C NMR spectroscopy. Removal of the protecting groups from **11** proved to be difficult. We found that the isopropylidene moiety was unexpectedly stable to acid (TFA/THF/H_2O and AcOH/THF/H_2O) even under refluxing conditions. This problem was circumvented by catalytic hydrogenation to deprotect the phosphate ester, followed by hydrolysis of the isopropylidene group by the resulting phosphoric acid moiety. Use of methanol/water as the solvent for hydrogenation, as described by Bacher et al. in their synthesis of 2-*C*-methyl-D-erythritol 4-phosphoric acid (MEP),[36] resulted in the formation of a stable methoxy hemiketal. However, a hemiketal did not form when methanol was replaced by *tert*-butyl alcohol in the hydrogenation step, presumably because of the bulky *tert*-butyl group. After removal of palladium, the solution was carefully concentrated with a stream of nitrogen. The fluorinated analogues partially degraded if concentrated by either rotory evaporation or lyophilization, giving a complex mixture of products as judged by ^{19}F NMR spectroscopy. The isopropylidene group was then removed by stirring in the water/*tert*-butyl alcohol system for several days. The solution was then again carefully concentrated with a stream of nitrogen to a final concentration of ∼25−75 mM to give **1** as the free acid. Trifluoromethyl analogue **1**, >95% pure as judged by ^{19}F NMR spectroscopy, was obtained in five steps with a 53% overall yield from known aldehdye **7**. The concentration of a solution of **1** was determined by comparison of the intensity of the ^{19}F resonance of the trifluoromethyl group with that of an internal standard of trifluoroacetic acid (TFA). The fluorinated analogues were found to be stable for approximately six months when stored as an aqueous solution (pH ≈ 1) at −20 °C.

SCHEME 3. Synthesis of CF_2-DXP[a]



[a] Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 35%; (b) $P(OBn)_3$, I_2, pyridine, CH_2Cl_2, −40 °C to rt, 86%; (c) LDA, diethyl (1,1-difluoromethyl)phosphonate, THF, −78 °C, 83%; (d) NaOMe, MeOH, 0 °C, 83%; (e) (i) H_2/10 wt % Pd/C, *t*-BuOH, (ii) H_2O, 3 d, 86%.

Synthesis of CF_2-DXP (2). 2 was prepared as illustrated in Scheme 3. Synthesis of **2** was based on a modification of the procedure reported by Bouvet and O'Hagan[37] in their synthesis of 1,1-difluoro-1-deoxy-D-xylulose (CF_2-DX). Following desymmetrization of (−)-2,3-*O*-isopropylidene-D-tartrate (**12**) with $NaBH_4$, monoalcohol **13**[38] was treated with DBPI to give phosphorus triester **14**. Monoester **14** was treated with lithiated

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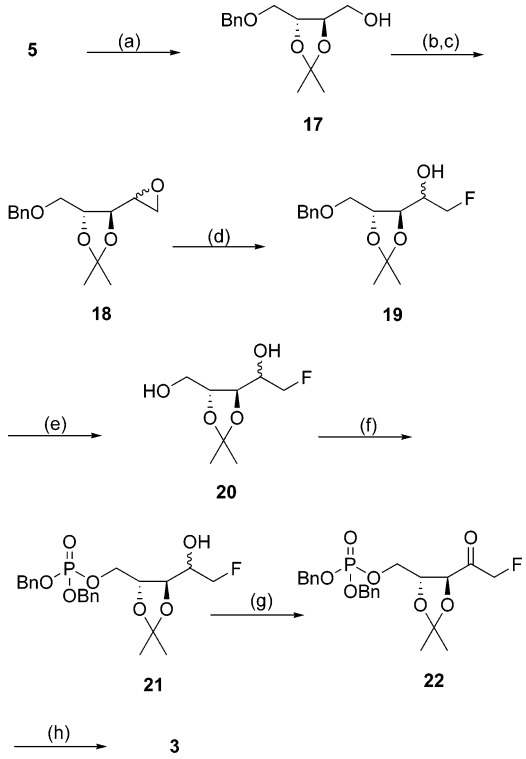
SCHEME 4. Synthesis of CF-DXP[a]



[a] Reagents and conditions: (a) BnBr, NaH, THF, 0 °C, 81%; (b) Dess−Martin reagent, CH_2Cl_2, rt; (c) NaH, trimethyloxosulfonium iodide, DMSO, rt, 50% (two steps); (d) $(i\text{-Pr})_2NH{\cdot}3HF$, neat, 100 °C, 47%; (e) H_2/Pd/C, EtOH, rt, 95%; (f) $P(OBn)_3$, I_2, pyridine, CH_2Cl_2, −40 °C to rt, 69%; (g) Dess−Martin reagent, CH_2Cl_2, rt, 89%; (h) (i) H_2/10 wt % Pd/C, *t*-BuOH, (ii) H_2O, 3 d, 90%.

diethyl (difluoromethyl)phosphonate[39] to give (difluoromethyl)phosphonate **15**, and the phosphonate was cleaved in the presence of the dibenzyl phosphate triester using NaOMe. O'Hagan et al. reported a 49% yield for this step with the base-stable *tert*-butyldimethylsiloxy (TBDMS)-protected alcohol using 0.5 equiv of sodium methoxide in dry methanol. We obtained a similar yield when **15** was used directly after silica gel chromatography. Examination of **15** by ^{19}F NMR spectroscopy revealed an 80:20 mixture of hydrate/ketone forms. Since the ketone is required for cleavage, the low yield observed by O'Hagan may be due to a slow hydrate−ketone equilibration under the conditions for cleavage. We converted the hydrate to the ketone by azeotropic removal of water from **15** with benzene and replaced benzene with dry methanol and sodium methoxide. This method routinely gave the fully protected CF_2-DXP **16** in greater than 80% yield with no detectable cleavage of the phosphate triester moiety. The free acid **2** was obtained from **16** by the procedure described for deprotection of **11**. The ratio of ketone to hydrate for **2** was ∼2:98, as judged by ^{19}F NMR spectroscopy.

Synthesis of CF-DXP (3). The synthesis of 1-fluoro-DXP (CF-DXP, **3**) from alcohol **17** is outlined in Scheme 4. After the monoprotection of **5** as the benzyl ether,[40] **17** was converted to oxirane **18** by oxidation of the alcohol

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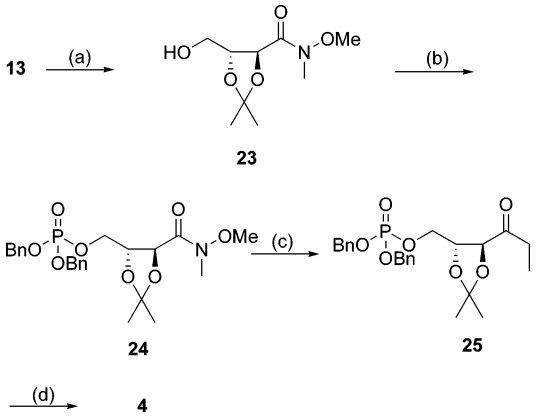
SCHEME 5. Synthesis of Et-DXP[a]



[a] Reagents and conditions: (a) 3.0 M CH_3MgBr, *N*,*O*-dimethylhydroxylamine hydrogen chloride, THF, −20 °C, 60%; (b) $P(OBn)_3$, I_2, pyridine, CH_2Cl_2, −40 °C to rt, 87%; (c) 3.0 M EtMgBr, Et_2O, −40 °C, 85%; (d) (i) H_2/10 wt % Pd/C, MeOH, (ii) H_2O, 2 d, $NaHCO_3$ to pH 7.0, 99%.

to the corresponding aldehyde followed by treatment with dimethyloxosulfonium methylide.[41] Preparations of **18** routinely gave a 4:1 mixture of diastereomers in yields of 45−55% over the two steps. Regioselective ring opening of oxirane **18** with diisopropylamine trihydrogen fluoride[42] gave 47% of the C-1-monofluorinated alcohol **19**. The benzyl group was removed by hydrogenolysis (Pd/C, H_2), and the resulting diol **20** was regioselectively phosphorylated (**21**), followed by Dess−Martin oxidation, to give fully protected CF-DXP **22**. The ketone was deprotected as described for **1** and **2** to give a 77:23 mixture of ketone/hydrate.

Synthesis of Et-DXP (4). **4** was prepared as shown in Scheme 5. Monoester **13** was converted to amide **23** in 60% yield, along with ∼10−15% of the corresponding methyl ketone and ∼5% tertiary alcohol, by a modification of the Weinreb[43] protocol using methylmagnesium bromide to prepare the *N*-methoxy-*N*-methylamide.[44] The methyl ketone, *O*-isopropylidene-protected deoxyxylulose (DX), was isolated, phosphorylated, and deprotected to give DXP. Phosphorylation of **23** followed by addition of ethylmagnesium bromide to **24** gave protected Et-DXP **25**. Deprotection of **25** and purification over cellulose gave the disodium salt of **4**.

Enzymatic Activity of DXP Analogues 1−4. Recombinant MEP synthase was obtained from an *Escherichia coli* clone as previously reported.[13] Details of the enzyme assays are outlined in the Supporting Information. **1** and **2** were not alternate substrates for DXP under catalytic conditions. Since both analogues exist predominantly in the hydrate form (>99.9% for **1** and ∼98% for **2**), we expected they might bind to the enzyme but perhaps not serve as substrates. However, **1** and **2** were relatively poor inhibitors, with IC_{50} values of 2.0 and 3.4 mM, respectively. **4** was also studied in an effort to sort

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whether the lack of turnover and poor inhibition were due to the presence of the hydrate or an increase in steric bulk at the methyl position upon substitution of hydrogen with fluorine. The ethyl analogue was not turned over under catalytic conditions and was a worse inhibitor, IC_{50} = 6.2 mM, than either **1** or **2**. Proteau et al.[28] reported K_i = 630 μM for Et-DXP for MEP synthase from *Synechocystis* PCC6803, which is also considerably higher than K_m for DXP. We conclude that steric interactions in the active site are primarily responsible for the lack of turnover and poor inhibition observed for Et-DXP and are probably responsible for similar behavior by the CF_3 and CF_2 analogues. Thus, it appears that the active site pocket in MEP synthase has strict size requirements with respect to the C-1 position of DXP and related analogues.[45]

In contrast, **3** was an excellent substrate for the enzyme, with k_{cat} = 38 s^{-1}, K_m = 227 μM, and k_{cat}/K_m = 0.17 s^{-1} μM^{-1}. These values are similar to those for DXP, with k_{cat} = 29 s^{-1}, K_m = 50 μM, and k_{cat}/K_m = 0.58 s^{-1} μM^{-1}. Some of the increase in K_m^{CF-DXP} might be due to the presence of the hydrated form (~25%), which may not bind as tightly as the ketone, and the increase in the steric bulk at C-1. Liu et al.[27] reported similar results for CF-DXP and the *E. coli* enzyme, with k_{cat} = 4.5 s^{-1} and K_m = 100 μM.

Mechanistic Considerations. Only a few inhibitors have been reported for enzymes in the MEP pathway. Fosmidomycin and the related analogue FR-900098 specifically inhibit MEP synthase in *E. coli*[46] and *Zymomonas mobilis*,[47] and fosmidomycin has been used to treat parasitic infections by *Plasmodium falciparum* and *Plasmodium vinckei* in mice.[48] A recent crystal structure of *E. coli* MEP synthase complexed with manganese and fosmidomycin has provided some insight about the architecture of the active site of the enzyme.[49] However, an understanding of the detailed reaction mechanism can also be important for the rational design of novel inhibitors. An α-ketol rearrangement (mechanistically related to acetoin and pinacol rearrangements) has been commonly favored for the rearrangement of DXP to MEP (see Figure 2) and is similar to the mechanism proposed for ketol-acid reductoisomerase.

A retro-aldolization/aldolization mechanism also gives the same aldehyde intermediate. This mechanism is also well-documented by the reactions catalyzed by ribulose-5-phosphate 4-epimerase[50,51] and bacterial class II aldolases.[52,53] In the retro-aldolization mechanism, initial

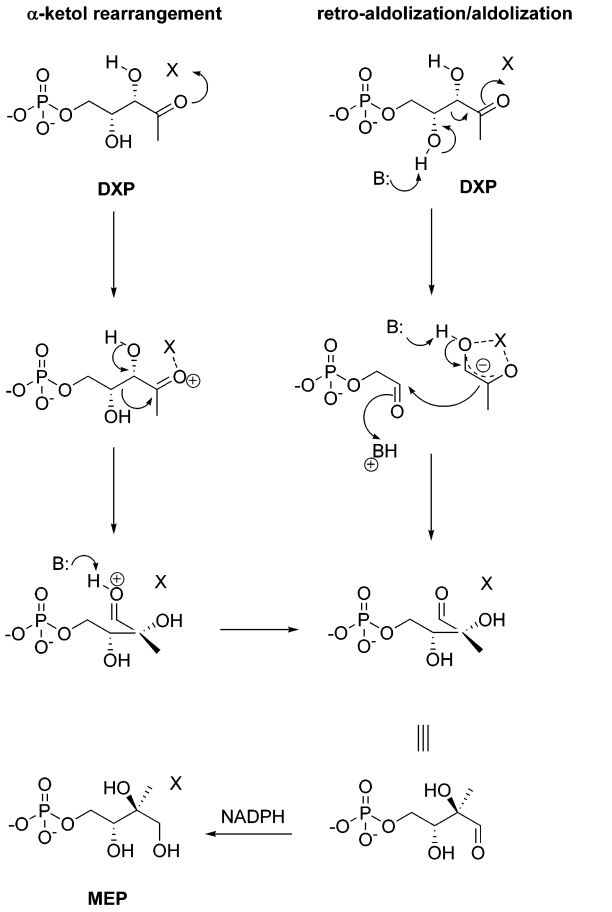

FIGURE 2. Conversion of DXP to MEP catalyzed by MEP synthase, α-ketol rearrangement vs retro-aldolization/aldolization (X = H^+ or divalent metal).

deprotonation occurs at the C-4-hydroxyl group, followed by cleavage of the C-3−C-4 bond to give glycoaldehyde phosphate and the enol(ate) of hydroxyacetone (Figure 2). Subsequent aldolization then gives 2-*C*-methyl-D-erythrose 4-phosphate, identical to the aldehyde intermediate generated by an α-ketol rearrangement. Both mechanisms utilize NADPH to reduce the aldehdye to the primary alcohol to generate MEP.

Fluorine has been used to great advantage for mechanistic studies and development of inhibitors in biological and medicinal chemistry.[54] In general, the development of positive charge at a β-position is destabilized by the powerful electron-withdrawing effect of a fluorine atom. Conversely, a carbanionic center β to a fluorine atom is stabilized.[55] The similar values for k_{cat} for DXP and CF-DXP found by us and Liu et al.[27] are more consistent with the retro-aldol/aldol mechanism for the rearrangement step catalyzed by MEP synthase. One would anticipate that the rearrangement would be slower for the CF analogue for an α-ketol mechanism because of the development of positive charge at C-2 upon activation of

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the ketone by protonation. Thus, the mechanism of the rearrangement catalyzed by MEP synthase appears to be related to those of ribulose phosphate epimerase and bacterial class II aldolases.

Experimental Procedures

(3*S*,4*R*)-1,1,1-Trifluoro-2-hydroxy-3,4-(*O*-isopropylidenedioxy)pentan-5-ol (9). To a stirred solution of 830 mg of aldehyde **7** (2.62 mmol, 1 equiv) and 5.77 mL of a 0.5 M solution of (trifluoromethyl)trimethylsilane (2.88 mmol, 1.1 equiv) in 5 mL of THF cooled to 0 °C was added a catalytic amount (~1 mg) of potassium *tert*-butoxide. After 2 h, THF was removed by rotory evaporation and the resulting oil dissolved in 10 mL of 1:1 diethyl ether/water. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The pale yellow oil was resuspended in 5 mL of THF, cooled to 0 °C, and then treated with 5.50 mL of a 1.0 M solution of TBAF (5.50 mmol, 2.1 equiv). After the resulting solution was stirred for 2 h, THF was removed by rotory evaporation, and the residual oil was immediately chromatographed on silica (1:1 (v/v) ethyl acetate/hexanes) to give 482 mg (80%) of a pale yellow oil as a 3:2 mixture of diasteromers. ^{1}H NMR (ppm): 1.44−1.48 (3 s, 6H), 3.70−3.91 (m, 3H), 4.08−4.25 (m, 2H). ^{13}C NMR (ppm): 26.8, 26.9, 27.2, 27.4, 61.2, 62.7, 68.3 (q, J = 31.2 Hz), 71.2 (q, J = 31.2 Hz), 73.8 (q, J = 1.5 Hz), 75.4, 78.5, 110.5, 111.1, 124.3 (q, J = 283 Hz). ^{19}F NMR (ppm): −3.49 (d, J = 6.1 Hz), −4.42 (d, J = 6.1 Hz). HRMS (CI, M + H): *m/z* calcd for $C_8H_{14}F_3O_4$ 231.0839, found 231.0844.

Dibenzyl (3*S*,4*R*)-1,1,1-Trifluoro-2-hydroxy-3,4-(*O*-isopropylidenedioxy)pent-5-yl Phosphate (10). To a stirred solution of 1.40 g of freshly prepared tribenzyl phosphite[32] (3.97 mmol, 1.16 equiv) in 10 mL of CH_2Cl_2 at −40 °C was added 916 mg of I_2 (3.61 mmol, 1.05 equiv). Once the I_2 had completely dissolved, the solution was cannulated over 10 min to a separate flask containing 790 mg of diol **9** (3.43 mmol, 1.0 equiv) and 1.17 mL of freshly distilled pyridine (14.4 mmol, 4.2 equiv) in CH_2Cl_2 precooled to −40 °C. The solution was allowed to warm to room temperature over 1 h, after which the reaction was quenched with water and concentrated under reduced pressure. The residue was resuspended into 10 mL of diethyl ether, extracted with aqueous $KHSO_4$, $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography of the residue (3:2 (v/v) ethyl acetate/hexanes) gave 1.30 g (77%) of a colorless oil as a 3:2 mixture of diastereomers. ^{1}H NMR (ppm): 1.37−1.44 (3 s, 6H), 3.30 (d, J = 9.9 Hz, OH) 3.80−4.30 (m, 5H), 5.05−5.09 (m, 4H), 7.30−7.40 (m, 10H). ^{13}C NMR (ppm): 26.8, 26.9, 27.0, 27.1, 66.4 (d, J = 5.5 Hz), 67.5 (d, J = 5.5 Hz), 68.2 (q, J = 31.2 Hz), 69.8 (d, J = 5.5 Hz), 69.9 (d, J = 4.0 Hz), 71.6 (q, J = 30.2 Hz), 73.8, 74.8 (q, J = 1.5 Hz), 75.4 (d, J = 8.6 Hz), 78.5 (d, J = 6.1 Hz), 110.8, 111.5, 124.3 (q, J = 283 Hz), 124.6 (q, J = 283 Hz), 128.2, 128.3, 128.4, 128.8, 128.9, 129.0, 135.5 (d, J = 7.0 Hz), 135.6 (d, J = 7.0 Hz). ^{19}F NMR (ppm): −3.73 (d, J = 7.1 Hz), −4.01 (d, J = 7.1 Hz). ^{31}P NMR (ppm): −2.91, −3.45. HRMS (CI, M + H): *m/z* calcd for $C_{22}H_{27}F_3O_7P$ 491.1441, found 491.1435.

Dibenzyl (3*S*,4*R*)-1,1,1-Trifluoro-1-deoxy-2-oxo-3,4-(*O*-isopropylidene)-D-xylulose Phosphate (11). To a solution of 4.22 mL (2.04 mmol, 2 equiv) of Dess−Martin reagent dissolved in 2 mL of CH_2Cl_2 was added 500 mg of alcohol **10** (1.02 mmol, 2 equiv) in 1 mL of CH_2Cl_2. Upon completion, as judged by ^{19}F NMR (~1 h, 0.13 ppm relative to external TFA, 0.00 ppm), a saturated solution of $NaHCO_3$ and a 10-fold excess of solid $Na_2S_2O_3$ were added, and the mixture was stirred until the solution cleared. The solution was extracted with diethyl ether and washed in succession with a mixture of saturated $NaHCO_3$, 2.0 g of $Na_2S_2O_3$, and brine. The organic layer was dried and concentrated under reduced pressure. Flash chromatography of the residual oil (1:1 (v/v) hexanes/ethyl acetate) gave 448 mg (90%) of a colorless oil. ^{1}H NMR (ppm): (hydrate form) 1.41 (s, 3H), 1.46 (s, 3H), 4.08−4.16 (m, 2H), 4.29−4.38 (m, 1H), 4.46−4.53 (m, 1H), 5.04 (d, J = 8.0 Hz, 4H), 7.30−7.39 (m, 10H). ^{13}C NMR (ppm): (hydrate form) 26.89, 27.3, 67.5 (d, J = 5.5 Hz), 69.9 (d, J = 5.5 Hz), 70.0 (d, J = 5.5 Hz), 75.4, 75.9 (d, J = 6.8 Hz), 91.8 (q, J = 31.8 Hz), 111.6, 123.0 (q, J = 288 Hz), 128.2, 128.8, 128.9, 135.6 (d, J = 6.5 Hz). ^{19}F NMR (ppm): −10.9 (s, 3F). ^{31}P NMR (ppm): −2.87; $[\alpha]_D^{25}$ +9.9 (c = 2.2, EtOAc). HRMS (CI, M + H): *m/z* calcd for $C_{22}H_{25}F_3O_7P$ 489.1285, found 489.1288.

1,1,1-Trifluoro-1-deoxy-D-xylulose 5-Phosphoric Acid (CF_3-DXP, 1). To a solution of 27.0 mg of phosphotriester **11** (0.053 mmol (based on the hydrate form), 1.0 equiv) in 500 μL of *tert*-butyl alcohol at room temperature was added 2.9 mg of 10 wt % Pd/C (0.0028 mmol, 0.05 equiv). The flask was evacuated under vacuum, then hydrogen was introduced under atmospheric pressure, and the reaction was stirred for 5 h. The suspension was filtered, washed with H_2O, concentrated to approximately 2 mL under a stream of nitrogen, and stirred for an additional 5−6 days. Upon completion, as determined by ^{19}F NMR, the concentration of **1** was verified by ^{19}F NMR against an internal standard of sodium trifluoroacetate prepared in D_2O (4 mM final). Integration revealed 14.7 mg (96%) of **1** was present in the aqueous solution as the hydrate. ^{1}H NMR (ppm): 3.80−4.00 (m, 3H), 4.32 (t, J = 6.5 Hz, 1H). ^{13}C NMR (ppm): 65.9 (d, J = 5.1 Hz), 68.2, 69.1 (d, J = 8.8 Hz), 94.4 (q, J = 29.8 Hz), 123.0 (q, J = 288 Hz). ^{19}F NMR (ppm): −6.8 (s, 3F). ^{31}P NMR (ppm): 0.61. $[\alpha]_D^{25}$ −11.3 (c 0.4, H_2O). HRMS (FAB, M − OH, hydrate): *m/z* calcd for $C_5H_9F_3O_7P$ 269.0033, found 269.0041.

Diethyl {Dibenzyl (3*S*,4*R*)-1,1-difluoro-2-oxo-3,4-(*O*-isopropylidenedioxy)pent-5-yl phosphate}phosphonate (15). To a solution of 1.78 mL of 1.5 M LDA (2.66 mmol, 1.2 equiv) in 10 mL of THF at −78 °C was added 399 μL of diethyl (1,1-difluoromethyl)phosphonate (2.44 mmol, 1.1 equiv) via syringe in 1 mL of THF very slowly over a 10 min period. The solution was stirred for 45 min at the same temperature, at which time 1.00 g of **14** (2.22 mmol, 1.0 equiv) was added very slowly via syringe in 1 mL of THF, and the resulting solution was stirred for 2 h at the same temperature. Note: It is important to add these reagents slowly since lithiated difluorophosphonate decomposes at temperatures >−70 °C. After the reaction was completed as determined by TLC, 1 mL of glacial acetic acid was added followed by saturated NH_4Cl. The solution was allowed to warm to room temperature over a 1 h period and then extracted into CH_2Cl_2. The combined organic extracts were dried and concentrated. Flash column chromatography (3:2 (v/v) hexanes/ethyl acetate) gave 1.11 g (83%) of a pale yellow oil (80:20 hydrate/ketone). For spectral analysis, the hydrate was converted to the ketone by azeotropic distillation with benzene. ^{1}H NMR (ppm): 1.37 (2t, J = 7.1, 1.6 Hz, 6H), 1.41 (s, 3H), 1.46 (s, 3H), 4.13−4.21 (m, 1H), 4.24−4.37 (m, 5H), 4.47−4.54 (m, 1H), 4.90 (d, J = 6.3 Hz, 1H) 5.07 (d, J = 8.1 Hz, 4H), 7.31−7.39 (m, 10H). ^{13}C NMR (ppm): 16.4, 16.5, 26.3, 27.2, 66.0 (2d, J = 6.5 Hz), 66.4 (d, J = 5.0 Hz), 76.7 (d, J = 8.6 Hz), 78.3 (d, J = 2.0 Hz), 113.1, 113.8 (dt, J = 274, 197 Hz), 128.2, 128.2, 128.8, 135.8 (d, J = 2.0 Hz), 135.9 (d, J = 2.0 Hz), 196.5 (dt, J = 24.2, 13.6 Hz). ^{19}F NMR (ppm): −43.6 (AB system, J = 326, 94.0 Hz, 2F). ^{31}P NMR (ppm): 0.01 (t, J = 94.0 Hz, 1P), −3.49 (s, 1P). $[\alpha]_D^{25}$ +7.7 (c 0.4, MeOH). HRMS (FAB, M + H): *m/z* calcd for $C_{26}H_{35}F_2O_{10}P_2$ 607.1674, found 607.1642.

Dibenzyl 1,1-Difluoro-1-deoxy-3,4-(*O*-isopropylidene)-D-xylulose Phosphate (16). Benzene was added to 1.00 g of ketophosphonate **15** (1.64 mmol, 1.0 equiv), and water was removed by distillation using a Dean−Stark trap. When the hydrate was converted to the ketone, as determined by ^{19}F NMR, dry MeOH was added, the flask was cooled to 0 °C, and 53.6 mg of sodium methoxide (0.988 mmol, 0.6 equiv) was added. The reaction was monitored by TLC until the starting material was consumed (~2−4 h), then water was added, and the mixture was extracted into CH_2Cl_2. The organic layers were combined and dried, and solvent was removed under

reduced pressure. The crude product was purified by flash chromatography (3:2 (v/v) ethyl acetate/hexanes) to give 647 mg (83%) of a colorless oil as an 80:20 mixture of the hydrate/ketone forms, respectively. ^{1}H NMR (ppm): 1.39 (s, 3H), 1.44 (s, 3H), 4.06 (dd, J = 7.9, 1.5 Hz, 0.8H), 4.09−4.25 (m, 1.2H), 4.28−4.40 (m, 1H), 4.47−4.54 (m, 0.8H), 4.56 (d, J = 6.8 Hz, 0.2H), 4.80 (br s, 0.8H, hydrate OH), 5.05 (d, J = 8.0 Hz, 3.2H), 5.06 (d, J = 8.0 Hz, 0.8H), 5.77 (t, J = 55.2 Hz, 0.8H), 6.11 (t, J = 53.0 Hz, 0.2H), 7.29−7.41 (m, 10H). ^{13}C NMR (ppm): 26.2, 26.9, 26.9, 27.3, 66.1 (d, J = 5.5 Hz), 67.7 (d, J = 5.5 Hz), 69.9 (dt, J = 5.5, 2.0 Hz), 75.4 (dd, J = 8.1, 2.0 Hz), 76.2 (d, J = 8.6 Hz), 76.5, 78.4, 92.2 (t, J = 23.7 Hz), 108.5 (t, J = 251 Hz), 111.1, 113.7 (t, J = 248 Hz), 128.2, 128.3, 128.8, 128.9, 129.0, 135.6 (d, J = 7.1 Hz), 135.7 (d, J = 7.0 Hz), no C=O observed. ^{19}F NMR (ppm): −56.4 (d, J = 55.0 Hz, 0.4F), −63.4 (AB system, J = 286, 55.0 Hz, 1.6F). ^{31}P NMR (ppm): −3.14 (s, 0.8P), −3.55 (s, 0.2P). $[\alpha]_D^{25}$ +11.0 (c 0.8, EtOAc). HRMS (FAB, M − OH): m/z calcd for $C_{22}H_{26}F_2O_7P$ (hydrate − OH) 471.1384, found 471.1387.

(3*R*,4*R*)-1,2-Epoxy-3,4-(*O*-isopropylidenedioxy)pentan-5-ol Benzyl Ether (18). To a solution of 1.00 g of **17** (3.96 mmol, 1.0 equiv) in 20 mL of CH_2Cl_2 at room temperature was added 2.52 g of Dess−Martin reagent (5.94 mmol, 1.5 equiv). The reaction was stirred for 45 min, then saturated $NaHCO_3$ and 5.0 g of $Na_2S_2O_3$ were added in succession, and the suspension was stirred until the solution cleared. The organic layer was decanted, and the aqueous layer was extracted with diethyl ether. The combined organic extracts were washed with a mixture of saturated $NaHCO_3$ and 10.0 g of $Na_2S_2O_3$ and then brine and dried. Concentration under reduced pressure gave 940 mg of the crude aldehyde, which was used in the next step without further purification.

To 86.0 mg of a 50% NaH dispersion in mineral oil (2.14 mmol, 1.2 equiv), previously washed with hexanes, and 471 mg of trimethyloxosulfonium iodide[41] (2.14 mmol, 1.2 equiv) was added 5 mL of dry DMSO. The solution was stirred for 2 h or until H_2 evolution ceased, at which time the crude aldehyde was added in 2 mL of DMSO. The mixture was stirred until the starting material was consumed as judged by TLC (∼2 h). The reaction was quenched with water and extracted into diethyl ether. The combined organic layers were washed with water and then brine, dried, and concentrated under reduced pressure. Flash chromatography of the residue (7:3 (v/v) hexanes/ethyl acetate) gave 510 mg (49%, two steps) of a 4:1 ratio of diastereomers as a colorless oil. ^{1}H NMR (ppm): 1.43−1.45 (3 s, 6H), 2.66 (dd, J = 2.4 Hz, 0.25H), 2.70 (dd, J = 2.7 Hz, 0.75H), 2.78 (t, J = 4.5 Hz, 0.25H), 2.82 (t, J = 4.2 Hz, 0.75H), 3.03−3.07 (m, 0.25H), 3.08−3.13 (m, 0.75H), 3.57−3.75 (m, 3H), 4.16−4.22 (m, 1H), 4.60 (s, 0.5H), 4.61 (s, 1.5H), 7.28−7.39 (m, 5H). ^{13}C NMR (ppm): (major diastereomer) 26.9, 27.2, 45.2, 51.9, 70.7, 73.8, 78.2, 78.3, 110.3, 127.9, 127.9, 128.6, 138.1, (minor diastereomer) 26.8, 44.3, 51.8, 70.3, 73.8, 76.9, 79.4, 110.3, 128.0, 128.7, 137.9. HRMS (CI, M + H): m/z calcd for $C_{15}H_{21}O_4$ 265.1440, found 265.1434.

(3*S*,4*R*)-1-Fluoro-2-hydroxy-3,4-(*O*-isopropylidenedioxy)pentan-5-ol Benzyl Ether (19). Diisopropylamine trihydrofluoride was prepared according to Muehlbacher.[42] To 199 mg of diisopropylamine trihydrofluoride (1.23 mmol, 2.0 equiv) was added 163 mg of oxirane **18**. The flask was tightly stoppered with a plastic cap and the mixture heated as a homogeneous melt at 100 °C. The starting material was consumed after 19 h as observed by TLC. The flask was cooled and the semisolid extracted with diethyl ether. The organic layer was washed with water, dried, and concentrated under reduced pressure. The oil was purified by flash chromatography (4:1 (v/v) hexanes/ethyl acetate) to yield 81.6 mg (47%) of a 72:28 mixture of diastereomers as a colorless oil. ^{1}H NMR (ppm): 1.40 (s, 4.5 H), 1.43 (s, 0.75H), 1.45 (s, 0.75H), 2.55 (br s, J = 8.3 Hz, 0.25H, OH), 3.45 (br s, 0.75H, OH), 3.56−3.84 (m, 3.75H), 3.97 (dd, J = 8.3, 2.7 Hz, 0.25H), 4.13 (dt, J = 6.8, 4.9 Hz, 0.75H), 4.24 (dt, J = 8.0, 6.1 Hz, 0.25H), 4.34−4.71 (m, 4H), 7.28−7.41 (m, 5H). ^{13}C NMR (ppm): (major diastereomer) 27.0, 27.0, 70.6, 72.1 (d, J = 18.1 Hz), 74.0, 78.3 (d, J = 6.5 Hz), 78.8, 84.6 (d, J = 170 Hz), 109.8, 128.2, 128.2, 128.8, 137.2, (minor diastereomer) 27.0, 27.2, 68.7 (d, J = 19.6 Hz), 70.2, 73.8, 75.8, 78.2 (d, J = 5.0 Hz), 84.3 (d, J = 171 Hz), 110.0, 127.9, 128.0, 128.7, 137.8. ^{19}F NMR (ppm): −155.0 (dt, J = 45.8, 18.3 Hz, 0.25F), −162.3 (dt, J = 45.8, 24.4 Hz, 0.75F). HRMS (CI, M + H): m/z calcd for $C_{15}H_{22}FO_4$ 285.1502, found 285.1517.

(3*S*,4*R*)-1-Fluoro-2-hydroxy-3,4-(*O*-isopropylidenedioxy)pentan-5-ol (20). To a solution of 80.0 mg of **19** (0.281 mmol, 1.0 equiv) in 1 mL of absolute EtOH at room temperature was added 15.0 mg of 10 wt % Pd/C (0.014 mmol, 0.05 equiv). The flask was evacuated under vacuum, hydrogen was introduced, and the reaction was stirred for 3 h. The suspension was filtered, and the filtrate was concentrated under reduced pressure, which gave 52.0 mg (95%) of a colorless oil as a mixture of diastereomers. ^{1}H NMR (ppm): 1.38 (s, 2.25H), 1.39 (s, 2.25H), 1.42 (s, 0.75H), 1.43 (s, 0.75H), 3.78−3.90 (m, 4H), 4.05−4.15 (m, 1H), 4.40−4.72 (m, 2H). ^{13}C NMR (ppm): (major diastereomer) 27.0, 27.1, 63.0, 72.2 (d, J = 18.1 Hz), 77.0 (d, J = 6.6 Hz), 80.6, 84.9 (d, J = 169 Hz), 109.8, (minor diastereomer) 27.2, 61.9, 68.7 (d, J = 19.6 Hz), 77.0 (d, J = 5.5 Hz), 77.3, 84.4 (d, J = 171 Hz), 109.9. ^{19}F NMR (ppm): −156.1 (dt, J = 48.8, 18.3 Hz, 0.25F), −161.8 (dt, J = 48.8, 18.3 Hz, 0.75F). HRMS (CI, M + H): m/z calcd for $C_8H_{16}FO_4$ 195.1033, found 195.1010.

Dibenzyl (3*S*,4*R*)-1-Fluoro-1-deoxy-3,4-(*O*-isopropylidene)-D-xylulose Phosphate (22). To a solution of 62.8 mg of Dess−Martin reagent (0.148 mmol, 1.5 equiv) in 1 mL of CH_2Cl_2 at room temperature was added 45.0 mg of alcohol **21** (0.099 mmol, 1.0 equiv). The reaction was monitored by ^{19}F NMR until completion (t, −162.58 ppm, ∼1 h), at which time saturated $NaHCO_3$ and a 10-fold excess of solid $Na_2S_2O_3$ were added, and the mixture was stirred until the solution cleared. The solution was extracted with diethyl ether and washed in succession with a mixture of saturated $NaHCO_3$ and 2.0 g of $Na_2S_2O_3$ and then brine. The organic layer was dried and concentrated under reduced pressure. Flash chromatography of the oil (1:1 (v/v) hexanes/ethyl acetate) gave 42.8 mg (89%) of a colorless oil as a 90:10 mixture of ketone/hydrate forms. ^{1}H NMR (ppm): (ketone only) 1.38 (s, 3H), 1.42 (s, 3H), 4.11 (m, 1H), 4.15−4.40 (m, 3H), 5.06 (d, J = 8.3 Hz, 4H), 5.19 (AB system, J = 47.1, 10.0 Hz, 2H), 7.36 (m, 10H); ^{13}C NMR (ppm): (ketone only) 26.3, 26.8, 66.3 (d, J = 5.5 Hz), 69.7 (d, J = 5.5 Hz), 76.4 (d, J = 8.0 Hz), 79.3, 83.6 (d, J = 184 Hz), 112.1. 128.2, 128.8, 135.8 (d, J = 2.2 Hz), 203.0 (d, J = 5.0 Hz). ^{19}F NMR (ppm): (hydrate) −158.5 (t, J = 48.8 Hz), (ketone) −162.5 (dd, J = 48.8 Hz). ^{31}P NMR (ppm): (hydrate) −2.84, (ketone) −3.55. $[\alpha]_D^{25}$ −6.7 (c 1.0, EtOAc). HRMS (CI, M + H): m/z calcd for $C_{22}H_{27}FO_7P$ 453.1478, found 453.1434.

(2*R*,3*S*)-1-Hydroxy-2,3-(*O*-isopropylidenedioxy)-*N*-methoxy-*N*-methylbutanamide (23). To a cooled (−20 °C) mixture of 276 mg of *N*,*O*-dimethylhydroxylamine hydrogen chloride (2.83 mmol, 3.0 equiv) and 180 mg of monoester **13** (0.946 mmol, 1.0 equiv) in 2 mL of THF was added 1.89 mL of a 3.0 M solution of CH_3MgBr (5.67 mmol, 6.0 equiv) over 20 min. The reaction was stirred for 2 h at the same temperature and then at room temperature overnight. The reaction was quenched by addition of saturated NH_4Cl and the aqueous layer extracted with diethyl ether and then CH_2Cl_2. The combined organic layers were dried and concentrated under reduced pressure. Flash chromatography of the residue (100% diethyl ether) gave 124 mg (60%) of a colorless oil. ^{1}H NMR (ppm): 1.47 (s, 3H), 1.49 (s, 3H), 2.15 (br s, 1H, OH), 3.23 (br s, 3H), 3.67−3.73 (m, 1H), 3.75 (s, 3H), 3.90 (dt, J = 12.2, 3.4 Hz, 1H), 4.46 (br s, 1H), 4.77 (br d, J = 5.9 Hz, 1H). ^{13}C NMR (ppm): 26.2, 27.3, 32.6, 61.9, 73.9, 77.4, 79.0, 111.2, 170.7. $[\alpha]_D^{25}$ +11.0 (c 18.4, EtOAc). HRMS (CI, M + H): m/z calcd for $C_9H_{18}NO_5$ 220.1185, found 220.1191.

Dibenzyl (4*S*,5*R*)-3-Oxo-4,5-(*O*-isopropylidenedioxy)-hexan-6-yl Phosphate (25). To a solution of 360 mg of amide **24** (0.750 mmol, 1.0 equiv) in 5 mL of diethyl ether at −40 °C

was added 300 μL of a 3.0 M solution of EtMgBr (0.900 mmol, 1.2 equiv). The reaction was stirred at the same temperature for 1 h, quenched with saturated NH_4Cl, and extracted into diethyl ether. The organic layer was washed with brine, dried, and concentrated under reduced pressure. Flash chromatography of the residue (7:3 (v/v) ethyl acetate/hexanes) gave 286 mg (85%) of a colorless oil. ^{1}H NMR (ppm): 1.06 (t, J = 7.3 Hz, 3H), 1.38 (s, 3H), 1.43 (s, 3H), 2.64 (ABX_3 system, J = 7.3, 4.4 Hz, 2H), 4.07−4.30 (m, 4H), 5.07 (d, J = 8.1 Hz, 4H), 7.29−7.40 (m, 10H). ^{13}C NMR (ppm): 7.1, 26.5, 26.9, 32.4, 67.0 (d, J = 5.8 Hz), 69.6 (d, J = 5.5 Hz), 76.5 (d, J = 7.9 Hz), 81.0, 111.3, 128.2, 128.7, 128.8, 135.9 (d, J = 7.0 Hz), 210.37. ^{31}P NMR (ppm): −3.50. $[\alpha]_D^{25}$ −8.5 (*c* 2.9, EtOAc). HRMS (CI, M + H): *m/z* calcd for $C_{23}H_{30}O_7P$ 449.1729, found 449.1722.

(4*S*,5*R*)-3-Oxo-4,5-dihydroxyhex-6-yl Phosphate (Et-DXP, 4). To a solution of 100 mg of **25** (0.223 mmol, 1.0 equiv) in 1 mL of freshly distilled MeOH at room temperature was added 12.0 mg of 10 wt % Pd/C (0.011 mmol, 0.05 equiv). The flask was evacuated under vacuum, hydrogen was introduced under atmospheric pressure, and the suspension was stirred for 6 h. The suspension was filtered and the filtrate concentrated under reduced pressure. The residue was resuspended in 2 mL of water and the suspension stirred for 2 days. The free acid was converted to the sodium form by addition of solid $NaHCO_3$ to pH 7.0 and lyophilized. Cellulose column chromatography (15−50% (v/v) 0.1% TFA in water/THF) of the residue gave 55.0 mg (99%) of a white powder. ^{1}H NMR (ppm): 1.01 (t, J = 7.1 Hz, 3H), 2.54−2.74 (m, 2H), 3.82 (t, J = 7.4 Hz, 2H), 4.28 (dt, J = 6.6, 1.6 Hz, 1H), 4.48 (d, J = 1.6 Hz, 1H). ^{13}C NMR (ppm): 7.6, 32.6, 65.5 (d, J = 5.5 Hz), 71.9 (d, J = 8.4 Hz), 77.6, 216.9. ^{31}P NMR (ppm): 4.40. $[\alpha]_D^{25}$ +1.4 (*c* 1.0, H_2O). HRMS (FAB, M − H): *m/z* calcd for $C_6H_{12}O_7P$ 227.0326, found 227.0321.

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Supporting Information Available: General methods, experimental protocols for synthesis of **2**, **3**, **14**, **17**, **21**, and **24**, and ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra for compounds **1**−**4**, **9**−**11**, **14**−**16**, **18**−**22**, and **23**−**25** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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