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Rapid and flexible synthesis of 1-deoxy-D-xylulose-5-phosphate, the substrate for 1-deoxy-D-xylulose-5-phosphate reductoisomerase

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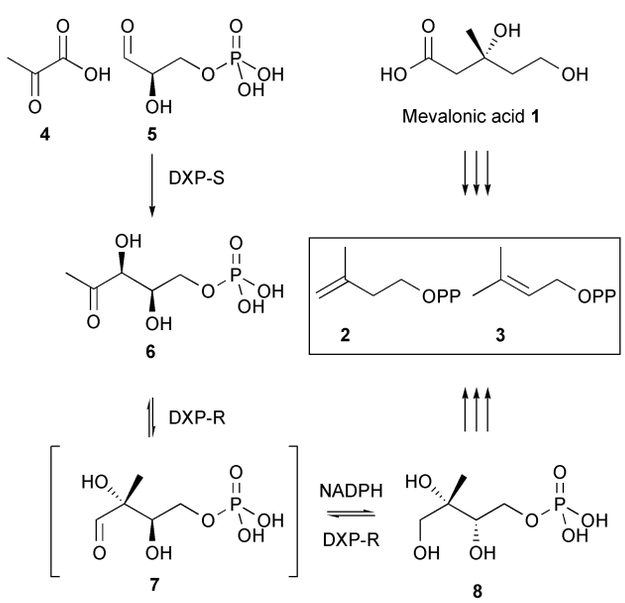
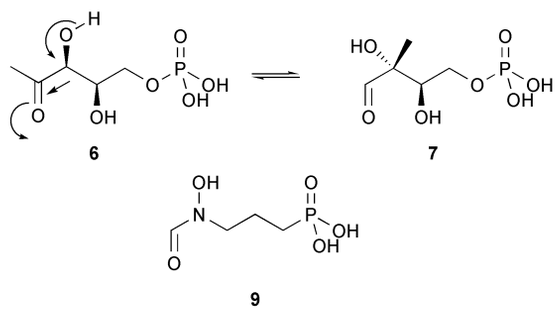
1-Deoxy-D-xylulose-5-phosphate (DXP) is a key intermediate in the non-mevalonate pathway to terpenoids in bacteria, and it is the substrate for the enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXP-R). In order to study the mechanism of DXP-R, we required a flexible synthesis of the substrate which would allow the incorporation of isotopic labels, and the variation of the two stereocentres. Thus 1,4-dihydroxypent-2-yne was selectively reduced to give the *E*-olefin, and selective phosphorylation of the primary alcohol followed by oxidation of the secondary alcohol gave a substrate suitable for dihydroxylation. Dihydroxylation using stoichiometric OsO_4 in the presence of chiral ligands gave protected DXP in high ee. Final hydrogenolysis gave DXP in quantitative yield and high purity. DXP-R was produced by rapid cloning of the *dxr* gene from *Escherichia coli* through controlled expression and ion exchange chromatography. The synthetic DXP was fully active in enzyme assays catalysed by recombinant DXP-R.

Introduction

For many years the accepted pathway for terpenoid biosynthesis was known to proceed *via* mevalonic acid **1** to the terpene precursors isopentenyldiphosphate **2** and dimethylallyldiphosphate **3** (Scheme 1). However, in the last decade it has become clear that there is another parallel biosynthetic pathway which is operative in bacteria and the plastids of plants.[1] This *non-mevalonate* pathway uses pyruvate **4** and glyceraldehyde-3-phosphate **5** to form the linear C_5 compound 1-deoxy-D-xylulose-5-phosphate **6** (DXP, Scheme 1) catalysed by the enzyme DXP synthase (DXP-S). In order to introduce the branched structure of **2** and **3**, DXP must be isomerised and this is achieved by the enzyme DXP reductoisomerase (DXP-R) *via* an intriguing α-ketol rearrangement (Scheme 2). The aldehyde product of this reaction, **7**, is then reduced, using hydride from NADPH, again catalysed by DXP-R, yielding 2-*C*-methyl-D-erythritol 4-phosphate **8** (MEP).



Scheme 1 Biosynthetic routes to the terpene precursors **2** and **3**.



Scheme 2 Rearrangement catalysed by DXP-R.

As well as serving as a terpene precursor, DXP also acts as a precursor to the enzyme cofactor pyridoxal-5′-phosphate.[2]

The enzyme DXP-R is of current interest for two reasons. Firstly its catalysed α-ketol rearrangement is rather unusual and the mechanism has not been investigated in depth. Secondly, DXP-R is a potential target for the development of new antibacterial agents. It is known that deletion of the DXP-R gene, *dxr*, is lethal to bacteria,[3] and the known potent DXP-R inhibitor fosmidomycin **9** shows good clinical antibacterial activity, as well as *in vitro* and *in vivo* antimalarial activity.[4]

We wished to investigate the mechanism of DXP-R through the use of isotopically labelled substrates. To this end we required a synthetic route which would allow incorporation of ^{2}H, ^{3}H, ^{13}C and ^{14}C as flexibly as possible. One biochemical and two chemical synthetic routes to DXP **6** have recently been exploited. Biochemically, DXP **6** can be made using the enzyme DXP-S with the substrates pyruvate **4** and glyceraldehyde 3-phosphate **5**.[5] Although this method is efficient, there is little scope for isotope incorporation and no option for variation of stereochemistry. Poulter has synthesised DXP **6** in eight steps from protected D-threitol in good overall yield, but again there is little scope for isotope incorporation or stereochemical variation.[6] Finally, Begley and coworkers reported a seven step synthesis starting from D-diethyltartrate, but the overall yield of 5% makes its use for isotope incorporation cumbersome.[7]

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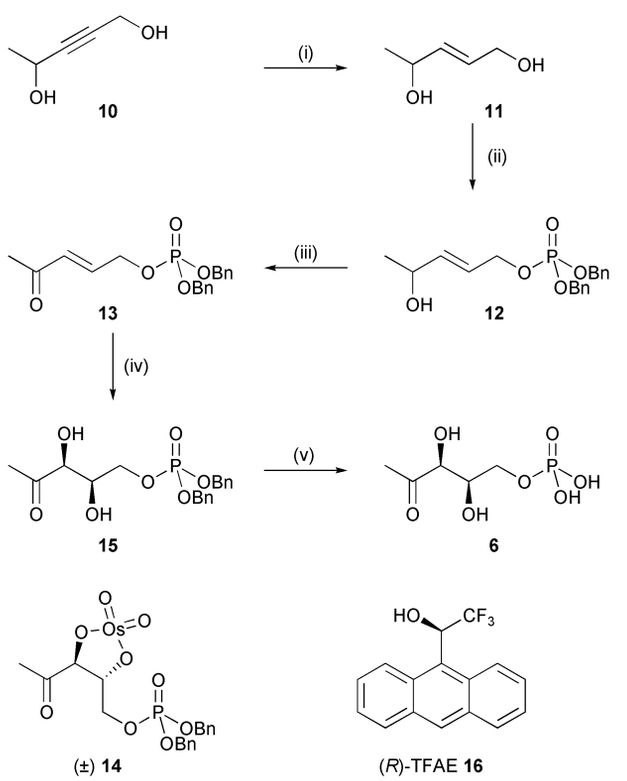
Results

We envisaged a shorter synthesis starting from the known acetylenic diol **10**.[8] Selective reduction using REDAL-H yielded the *E*-olefin **11** in high yield, with no observable production of the *Z*-isomer (Scheme 3). Selective activation of the primary alcohol to form the benzylphosphate **12** was achieved in good yield using the methods of Bolte and coworkers[9] and Widlanski and Stowell[10] involving treatment of tribenzylphosphite (generated by treatment of PCl_3 with BnOH) with one equivalent of iodine (forming the phosphoryliodide *in situ*), followed by addition of the diol **11**. Dess–Martin oxidation[11] of the secondary alcohol **12** then gave the unsaturated ketone **13**, in good yield. These methods contrasted with attempts to phosphorylate the diol **10** first, followed by reduction. In this case phosphorylation proceeded in low yield, and REDAL-H promoted reduction was unsuccessful. A further route involving attempted yneone reduction also failed.



Scheme 3 Synthesis of **6**. *Reagents and conditions*: (i) REDAL, THF, RT, 85%; (ii) PCl_3, BnOH, Et_3N, then I_2, then **11**, 48%; (iii) Dess–Martin periodinane, CH_2Cl_2, RT, 83%; (iv) OsO_4, ligand, CH_2Cl_2, −78 °C, 70–90%; (v) H_2, Pd/C, MeOH, RT, quant.

We then attempted asymmetric dihydroxylation methods to form the two stereogenic centres. Standard Sharpless asymmetric dihydroxylation conditions have proved successful in similar circumstances. For example, Kumar and Fernandes have dihydroxylated ethyl dodec-2-enoate in 94% yield and 99% ee,[12] while Lerner and Barbas *et al*. have used AD-mix-α to dihydroxylate a benzyl ether anologue of **13** in *ca*. 80% ee.[13] However, these conditions were unsuccessful, as were all modifications of methods involving catalytic amounts of OsO_4. Isolation of starting material **13** in high yield indicated lack of catalytic turnover.

Attempts to use stoichiometric amounts of OsO_4 showed that in CH_2Cl_2 at −78 °C, the osmate ester **14** was formed rapidly and in quantitative yield in the presence of the achiral ligand TMEDA. However, all attempts to hydrolyse this osmate ester under basic conditions were unsuccessful, explaining the failure of the catalytic Sharpless conditions. Vigorous acid hydrolysis, using HCl in refluxing methanol, was more successful, yielding the diol (±)-**15** in 90% yield.

Osmate ester formation was also rapid in the presence of the chiral ligand $(DHQD)_2$-PHAL, and hydrolysis was achieved under the same acidic conditions, albeit in lower yield, to give the enantioenriched diol (+)-**15**. Enantiopurity was assessed by ^{1}H NMR at 500 MHz in the presence of the chiral alcohol (*R*)-TFAE **16**. Concentrations of **16** of 140 mg mL^{-1} in $CDCl_3$ shifted the methyl resonance of **15** by 0.01 ppm – sufficient to give baseline resolution at 500 MHz. A similar effect was observed in the ^{31}P NMR spectrum with a 0.016 ppm separation of enantiomers. Integration of the resonances for the individual enantiomers then allowed determination of the ee. In the presence of 2.2 equivalents of $(DHQD)_2$PHAL, (+)-**15** was formed in 84% ee and 70% yield, while 1.0 equivalent of the chiral ligand in the dihydroxylation gave similar yields, but lower enantioenrichment (74% ee).

Deprotection of (+)-**15** was achieved in quantitative yield by catalytic hydrogenation under 1.0 atm of H_2. Both ^{1}H and ^{13}C NMR of **6** showed the intact phosphate ester through coupling of ^{31}P to the diastereotopic 5-methylene protons and C-5 and C-4. In the same way, the proton coupled ^{31}P NMR spectrum showed the expected proton coupling at P.

DXP-R was obtained by PCR of the *dxr* gene from boiled *Escherichia coli* cells (Fig. 1). The PCR product was confirmed by sequencing and then cloned into the *E. coli* expression vector pET15b. IPTG mediated induction afforded exceptional protein expression (37.5 mg L^{-1}), and DXP-R was rapidly purified by his_6-Ni^{2+} ion affinity chromatography followed by chromatographic desalting.

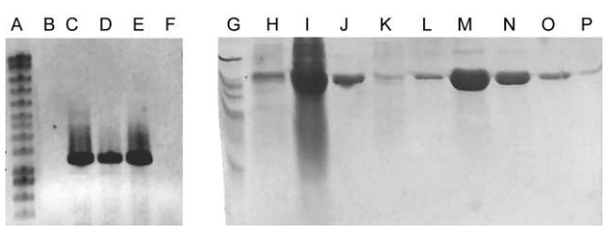


Fig. 1 Lanes A–F: 0.7% agarose TAE gel developed with ethidium bromide and visualised under uv light showing PCR products after amplification of *E. coli dxs*. **A**, Hyperladder markers (Bioline); **B**, 2 mM Mg_2^+; **C**, 2.5 mM Mg_2^+; **D**, 3.0 mM Mg_2^+ **E**, 3.5 mM Mg_2^+, **F**, 4.0 mM Mg_2^+. Lanes G–P: 12% SDS-PAGE gel developed with coomassie Blue, showing DXP-R protein purification. **G**, Sigma low range Mw markers; **H**, soluble cell-free extract; **I**, insoluble protein; **J**, desalted protein; **K**, nickel column void protein; **L**–**P**, imidazole eluted fractions.

ESMS analysis confirmed the the expected addition of the his_6-tag and the loss of the *N*-terminal methionine (Fig. 2, calculated 45417.7, found 45426.8 ± 8.7). In preliminary assays this purified protein rapidly turned-over **6**, using NADPH (observed at 340 nm). No significant reaction was observed in the absence of protein or **6**, and very slow reaction was observed without the addition of Mn^{2+} (Fig. 3). Quantitative assays revealed that 92% of the substrate was consumed at the end of the reaction, consistent with the measured 84% ee of (+)-**15**.

Discussion

The synthesis of DXP **6** has been achieved in five steps. The route allows for the introduction of isotopic labels in a number of ways as the starting material **10** is made *via* condensation of acetaldehyde with propargyl alcohol. Both of these compounds can be formed with isotopic labels in selective positions. Further labels could be introduced selectively through the use of REDAL-D, and D_2O work-up. The route offers stereochemical flexibility as the enantiomer of **15** should be available by use of the ligand $(DHQ)_2$-PHAL.[12] Diastereomers of **15** should be available by epoxidation of **13** followed by hydrolysis. Attempts to improve the enantioselectivity of the dihydroxylation reaction are currently underway.

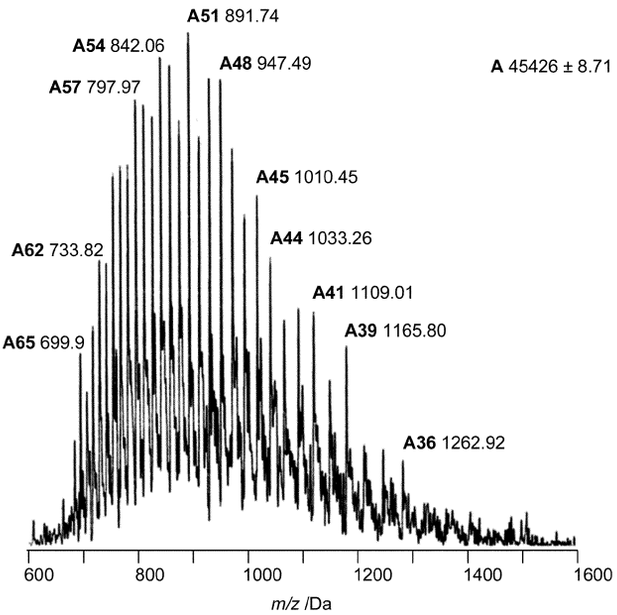


Fig. 2 ESMS spectrum of DXP-R, showing selected charge states.

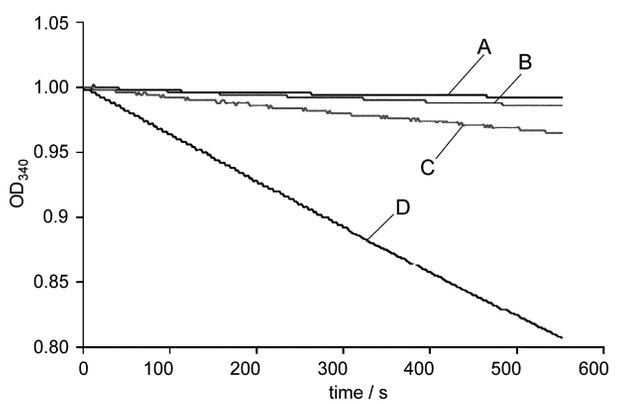


Fig. 3 Assays of DXP-R: A, no substrate; B, no enzyme; C, no Mn^{2+}; D, all components present.

Experimental

All reagents and solvents were obtained from the Sigma-Aldrich chemical company and were of ACS grade and not further purified unless otherwise stated. All anhydrous solvents were purchased from Fluka and were transferred under dried N_2 gas. NMR spectra were obtained using JEOL Λ-300, Δ-270 and Δ-400 spectrometers operating at 300, 270 and 400 MHz (^{1}H) and 75.5, 67.9 and 100.7 MHz (^{13}C) respectively. Chemical shifts are quoted in ppm relative to TMS. Coupling constants (*J*) are quoted in Hz. ^{31}P NMR experiments were proton-coupled unless ortherwise noted ({^{1}H}). IR spectra were obtained using a Perkin Elmer 1600 FTIR spectrometer, using KBr discs for solids and thin film between NaCl plates for oils. Melting points were obtained using a Reichert hot-stage apparatus equipped with microscope and Comark digital thermometer. Mass spectra were obtained in the indicated mode using a VG analytical autospec instrument (EI, CI, FAB, accurate mass) or Fisons VG Quattro spectrometer (ESMS). Optical rotations were obtained using a Perkin Elmer 141 polarimeter using a 1dm cell of 1mL capacity and are measured in units of 10^{-1} deg cm^2 g^{-1}. Flash chromatography was performed according to the method of Still using Merck silica gel 60 (0.040–0.063 mm). Tlc analysis was performed using Merck glass backed 0.2 mm silica plates (F_{254}) developed with phosphomolybdic acid or $KMnO_4$ when necessary.

Cloning DXP-R

Two oligonucleotide primers were synthesised (University of Bristol, School of Biological Sciences) with the following sequences: DXPR1, 5′-TGTCATATGAAGCAACTCAC-CATTCTGGGCTCG-3′; DXPR2, 5′-GGAGGATCCT-CAGCTTGCGAGACGCATC-ACCTC-3′. *E. coli XL1-Blue* (Stratagene) cells were boiled for 2 min, the cell solids precipitated by centrifugation and 1 μL of the supernatant used in the following PCR reaction: Taq DNA polymerase (2.5 units, Roche Long Template PCR enzyme mix); 10 × PCR buffer (5 μL, Promega); DXPR1 (25 μM); DXPR2 (25 μM); in a total volume of 50 μL. PCR was optimised with varying Mg^{2+} concentrations ranging from 1.75 mM to 3.75 mM. Samples were overlaid with mineral oil to prevent evaporation. The following temperature cycle yielded a 1197 bp product: 32 cycles with denaturation at 94 °C for 25 seconds, annealing at 62 °C for 30 seconds and extension at 68 °C for 1 min 10 seconds. After the first 11 cycles the extension time was increased 5 seconds each cycle and a total of 20 cycles were run in this way. A final elongation cycle at 68 °C for 10 min was performed.

The PCR product was ligated into pGEM-T Easy vector (pre-cut with 3′-T overhangs) and the vector cloned into *E. coli* TAM1 F′. DNA sequencing (LARK Technologies) confirmed that the correct clone had been isolated. The *dxr* gene was excised from the cloning vector using Nde I and Bam HI and cloned into pre-cut pET15b (Novagen). Successful clones were selected and transformed into the expression host *E. coli* BL21 (DE3). A single colony was picked, grown overnight in LB media (3 mL) containing carbenicillin (100 μgmL^{-1}). A 2 mL aliquot of this was transferred to LB media (200 mL) containing carbenicillin (100 μg mL^{-1}) and grown until an OD_{600} of 0.8 was reached and then induced by the addition of IPTG to a final concentration of 1 mM. After 2 h, the cells were obtained by centrifugation, lysed in his-tag binding buffer by sonication at 0 °C and cell solids were removed by centrifugation. Purification of this cell free extract was performed on a Ni^{2+}-chelating column (Pharmacia) according to manufacturers instructions. Fractions eluted with imidazole were analysed by SDS PAGE. Product containing fractions were combined and desalted into 20 mM Tris buffer pH 8.0. A total of 7.5 mg protein was eluted in 10 mL buffer. This protein was used for all further manipulations.

E-Pent-2-en-1,4-diol 11[8]

Red-Al® (2 mL of a 70% w/w solution in toluene, 3.6 mmol, 1.8 eq.) was added dropwise to a stirred solution of pent-2-yne-1,4-diol (200.0 mg, 2.0 mmol, 1.0 eq.) in dry THF (13.3 mL) at RT under nitrogen. The reaction mixture was stirred at RT for 1 h, then quenched by dropwise addition of water resulting in a small amount of gum formation. Solid $NaHCO_3$ (9.0 mg) was added, the mixture was vigorously stirred for 30 min and filtered under *vacuum*. The filter residues were exhaustively washed with ethyl acetate, the layers separated and the organic phase dried ($MgSO_4$) and concentrated. The product was dried under *vacuum* overnight, to give the desired diol (0.2 g, 85%); R_f (ethyl acetate–hexane, 70 : 30) 0.12; ν_{max} (film)/cm^{-1} 3310 (O–H), 1738 (C=C trans, stretching), 967 (C=C trans, bending out of plane); δ_H (270 MHz; $CDCl_3$) 5.90–5.81 (1 H, dt, $^3J_{HH\ (d\ trans)}$ 15.2 and $^3J_{HH\ (t)}$ 4.3, HC=CH–CH_2), 5.82–5.74 (1 H, dd, $^3J_{HH\ (d\ trans)}$ 15.5 and $^3J_{HH\ (d)}$ 4.6, (OH)HC–*H*C=CH), 4.39–4.31 (1 H, qd, $^3J_{HH\ (q)}$ 6.3 and $^3J_{HH\ (d)}$ 4.6, CH_3–(OH)*H*C–HC=), 4.17–4.15 (2 H, d, $^3J_{HH}$ 4.3, =CH–CH_2–OH), 1.77 (2 H, br s, 2 × O*H*) and 1.29 (3 H, d, $^3J_{HH}$ 6.6, CH_3); δ_C (67.9 MHz; $CDCl_3$) 135.6 and 128.9 (2-C and 3-C), 68.2 (4-C), 62.9 (1-C) and 23.4 (5-C); *m/z* (CI) 85 (30%, [M − H_2O]H$^+$), 71 (28%, [CH_3CH(OH)-C≡CH]H$^+$), 57 (33%, [H–C≡CH_2OH]H$^+$), 55 (100%, [C≡CCH_2OH]$^+$).

(1-*O*-Dibenzylphosphoryl)-1,4-dihydroxypent-2-ene 12

Preparation of tribenzyl phosphite. A freshly distilled solution of PCl_3 (6.1 mL, 0.07 mol, 1.0 eq.) in dry Et_2O (330 mL) was vigorously stirred at −10 °C. Triethylamine (30.7 mL, 0.22 mol, 3.1 eq.) in dry Et_2O (30 mL) and benzyl alcohol (21.8 mL, 0.21 mol, 3.0 eq.) in Et_2O (25 mL) were successively added at −10 °C, resulting in the formation of a thick suspension of Et_3NHCl. The homogeneous suspension was further stirred for 30 min at −10 °C, then for 24 h at RT. The solvent was removed *in vacuo* and the resultant solid was extracted with anhydrous hexane (3 × 250 mL). The hexane solution was transferred *via* a fritted cannula into a dry flask. The transparent solution was then concentrated *in vacuo* to yield tribenzyl phosphite (18.5 g, 75%) as a colourless liquid. The product was stored in the absence of oxygen and used in the next ransformation without any further purification; δ_H (300 MHz; no solvent) 7.05–6.80 (15 H, m, 3 × Ar), 4.58 (6 H, d, $^3J_{HP}$ 7.9, 3 × CH_2–Ar); δ_C (75.4 MHz; neat) 138.3 (d, $^3J_{CP}$ 4.6, 3 × 1-ArC), 128.2 (s, 6 × 2-ArC or 3-ArC), 127.5 (s, 3 × 4-ArC), 127.3 (s, 6 × 3-ArC or 2-ArC) and 64.1 (d, $^2J_{CP}$ 10.9, 3 × CH_2–Ar); δ_P (121.4 MHz; neat; {^{1}H}) 139.5 (s); δ_P (121.4 MHz; neat) 139.5 (septet, $^3J_{HP}$ 8.4).

Phosphorylation procedure. I_2 (5.9 mmol, 1.8 eq.) was added to a solution of tribenzyl phosphite (6.9 mmol, 2.1 eq.) in dry CH_2Cl_2 (15 mL) at −20 °C. The red, clear solution was allowed to warm to 25 °C over 20 min. The solution was then added dropwise to a solution of **11** (3.3 mmol, 1.0 eq.) and pyridine (13.2 mmol, 4.0 eq.) in dry CH_2Cl_2 (15 mL) at −30 °C over a period of 35 min. The resultant solution was then allowed to reach RT over 1h, filtered and concentrated *in vacuo* to give a dark oil. This residue was diluted with Et_2O : H_2O (27 : 7 mL), the phases were separated and the aqueous phase was extracted with Et_2O. The combined organic phases were washed with aqueous $KHSO_4$ (0.3 M), aqueous saturated $NaHCO_3$ and brine and then dried ($MgSO_4$). After concentration, the crude compound was chromatographed, eluting with 80 : 20 ethyl acetate : hexane, to give the *monophosphorylated alcohol* **12** (0.6 g, 48%) as a pale yellow oil, R_f (ethyl acetate–hexane, 80 : 20) 0.25; ν_{max} (film)/cm^{-1} 3406 (O–H), 1671 (C=C trans, stretching), 1256 (P=O), 995 (P–O-alkyl); δ_H (400 MHz; $CDCl_3$) 7.38–7.31 (10 H, m, 2 × Ar), 5.77 (1 H, dd, $^3J_{HH\ (d\ trans)}$ 15.4 and $^3J_{HH\ (d)}$ 5.1, (OH)HC–*H*C=CH), 5.69 (1 H, dt, $^3J_{HH\ (d\ trans)}$ 15.4 and $^3J_{HH\ (t)}$ 5.5, HC=C*H*–CH_2), 5.04 (4 H, 2 × dd, $^3J_{HP}$ 8.4 and $^2J_{HH}$ 11.7, 2 × CH_2–Ar), 4.46 (2 H, dd, $^3J_{HP}$ 8.4 and $^3J_{HH}$ 5.5, =CH–CH_2–OP), 4.30–4.24 (1 H, qd, $^3J_{HH\ (q)}$ 6.2 and $^3J_{HH\ (d)}$ 5.1, CH_3–(OH)*H*C–HC=), 1.82 (1 H, br s, O*H*) and 1.23 (3 H, d, $^3J_{HH}$ 6.2, CH_3); δ_C (100.6 MHz; $CDCl_3$) 138.7 (3-C), 135.9 (d, $^3J_{CP}$ 6.9, 1-ArC), 128.6 and 127.9 (2-ArC and 3-ArC), 128.6 (4-ArC), 123.7 (d, $^3J_{CP}$ 6.2, 2-C), 69.3 (d, $^2J_{CP}$ 5.4, *C*H_2–Ph), 67.7 (4-C), 67.5 (d, $^2J_{CP}$ 5.4, 1-C) and 23.1 (5-C); δ_P (121.4 MHz; $CDCl_3$; {^{1}H}) −0.23; δ_P (121.4 MHz; $CDCl_3$) −0.24 (septet, $^3J_{HP}$ 8.3); *m*/*z* (CI) 363 (18%, MH^+), 345 (50%, $[M - H_2O]H^+$), 279 (20%, $[HOP(O)(OBn)_2]H^+$), 181 (55%, $[CH_3CH(OH)$-C≡$CCH_2OP(O)(OH)_2]H^+$), 91 (100%, $[PhCH_2]^+$), 85 (10%, $[CH_3CHC{\equiv}CCH_3]H^+$); $C_{19}H_{23}O_5P^+$ requires 362.1283, found 362.1285.

(1-*O*-Dibenzylphosphoryl)-1-hydroxy-4-oxo-pent-2-ene 13

A solution of **12** (6.5 g, 18.0 mmol) in dry CH_2Cl_2 (210 mL) was added dropwise under nitrogen to a solution of the Dess–Martin periodinane[11] (11.65 g, 27 mmol) in dry CH_2Cl_2 (420 mL) and the mixture was stirred vigorously at RT. The reaction, monitored by TLC and LCMS, was generally completed within 2 h. The reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (500 mL) containing $Na_2S_2O_3$ (125 g) and stirred for 20 minutes. The layers were separated and the aqueous phase was extracted with ethyl acetate (3 × 250 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$, dried (Na_2SO_4) and concentrated *in vacuo*. The pale yellow crude oil was purified by flash column chromatography to give the *ketone* **13** (5.4 g, 15.0 mmol, 83%). R_f (ethyl acetate–hexane, 80 : 20) 0.35; ν_{max} (film)/cm^{-1} 1677 (C=O), 1638 (C=C trans, stretching), 1253 (P=O), 997 (P–O-alkyl), 968 (C=C trans, bending out of plane); δ_H (400 MHz; $CDCl_3$) 7.38–7.33 (10 H, m, 2 × Ar), 6.63 (1 H, dtd, $^3J_{HH\ (d\ trans)}$ 16.1 and $^3J_{HH\ (t)}$ 4.4 and $^4J_{HP\ (d)}$ 1.8, HC=C*H*–CH_2), 6.21 (1 H, dt, $^3J_{HH\ (d\ trans)}$ 16.1 and $^4J_{HH\ (t)}$ 1.8, OC–*H*C=CH), 5.06 (4 H, 2 × dd, $^3J_{HP}$ 8.8 and $^2J_{HH}$ 11.7, 2 × CH_2–Ar), 4.59 (2 H, ddd, $^3J_{HP}$ 7.7 and $^3J_{HH}$ 4.4 and $^4J_{HH}$ 1.8, =CH–CH_2–OP) and 2.21 (3 H, s, CH_3); δ_C (100.6 MHz; $CDCl_3$) 197.4 (C=O), 139.4 (d, $^3J_{CP}$ 8.3, 2-C), 135.6 (d, $^3J_{CP}$ 6.9, 1-ArC), 130.6 (3-C), 128.7 (4-ArC), 128.6 and 128.0 (2-ArC and 3-ArC), 69.6 (d, $^2J_{CP}$ 5.5, *C*H_2-Ph), 65.6 (d, $^2J_{CP}$ 5.5, 1-C) and 27.6 (5-C); δ_P (121.4 MHz; $CDCl_3$; {^{1}H}) −0.29; δ_P (121.4 MHz; $CDCl_3$) −0.29 (quintet td, $^3J_{HP\ (q)}$ 8.7, $^3J_{HP\ (t)}$ 7.7 and $^4J_{HP\ (d)}$ 1.8); *m*/*z* (EI) 361 (1.2%, $[M]H^+$), 317 (1.6%, $[M - CH_3CO]^+$), 277 (34%, $[P(O)_2$-$(OCH_2Ph)_2]^+$), 269 (40%, $[M - Bn]^+$), 180 (34%, $[CH_3COCH$=$CHCH_2OP(O)(OH)_2]^+$), 163 (100%, $[CH_3COCH$=$CHCH_2OP$-$(O)OH]^+$), 107 (52%, $[PhCH_2O]^+$), 91 (70%, $[PhCH_2]^+$), 77 (32%, $C_6H_5^+$); $C_{19}H_{22}O_5P^+$ ($[M]H^+$, EI) requires 361.1205, found 361.1205.

(±)-(2*R*,3*S*)-(1-*O*-Dibenzylphosphoryl)-1,2,3-trihydroxy-4-oxopentane (±)-15

The olefin **13** (0.91 g, 2.5 mmol) was dissolved in dry CH_2Cl_2 (165 mL) and cooled to −78 °C under nitrogen. TMEDA (0.29 g, 2.5 mmol) followed by an ethereal solution of osmium tetroxide (0.64 g, 2.5 mmol) were added dropwise to the solution at −78 °C. The solution turned yellow after addition of the osmium solution, then a dark brown. The reaction mixture was allowed to reach RT over 2.5 h and the reaction followed by TLC until no starting material was observed. The solution was concentrated *in vacuo* to a thick black oil. This crude material was dissolved in MeOH (100 mL) and conc. HCl (aq., 20 drops) was added until pH = 2. The mixture was then stirred overnight at RT. The mixture was filtered to remove a yellow precipitate, and the filtrate was concentrated *in vacuo* to a dark brown oily solid. The crude material was purified by flash chromatography, eluting with 90 : 10 ethyl acetate–hexane, to give the *diol* (±)-**15** (0.89 g, 90%) as a pale yellow oil, R_f (ethyl acetate–hexane, 90 : 10) 0.26; ν_{max} (film)/cm^{-1} 3367 (O–H), 1716 (C=O), 1247 (P=O), 1006 (P–O-alkyl); δ_H (400 MHz; $CDCl_3$) 7.39–7.30 (10 H, m, 2 × Ar), 5.05 (4 H, 2 × dd, $^3J_{HP}$ 8.6 and $^2J_{HH}$ 11.5, 2 × CH_2–Ar), 4.18 (1 H, td, $^3J_{HH\ (t)}$ 6.4 and $^3J_{HH\ (d)}$ 2.0, HOCH–HOC*H*–CH_2), 4.10 (1H, d, $^3J_{HH\ (d)}$ 2.0, HOC*H*–HOCH–CH_2), 4.15–4.08 (2H, dd, $^3J_{HP}$ 8.5 and $^3J_{HH\ (t)}$ 6.4, HOCH–HOCH–CH_2), 3.6–3.2 (2H, br s, 2 × OH), 2.21 (3H, s, CH_3); δ_C (100.6 MHz; $CDCl_3$) 207.7 (C=O), 135.5 (dd, $^3J_{CP}$ 6.9, 2 × 1-ArC), 128.8 (s, 4-ArC), 128.7 and 128.1 (2-ArC and 3-ArC), 76.6 (s, HO*C*H–HOCH), 70.2 (d, $^3J_{CP}$ 6.9, HOCH–HO*C*H), 69.7 (dd, $^2J_{CP}$ 5.0, 2 × CH_2Ph), 68.1 (d, $^2J_{CP}$ 5.4, HOCH–*C*H_2) and 25.6 (CH_3); δ_P (121.4 MHz; $CDCl_3$; {^{1}H}) 0.31; δ_P (121.4 MHz; $CDCl_3$) 0.65 (septet, $^3J_{HP}$ 9.3); *m*/*z* (EI) 395 (28%, $[M]H^+$), 321 (26%, $[(BnO)_2P(O)CH_2CHO]H^+$), 181 (38%), 91 (100%, $[PhCH_2]^+$); $C_{19}H_{24}O_7P^+$ requires 395.1260, found 395.1269.

(2*R*,3*S*)-(1-*O*-Dibenzylphosphoryl)-1,2,3-trihydroxy-4-oxopentane (+)-15

$(DHQD)_2PHAL$ (0.86 g, 1.1 mmol) was dissolved under nitrogen in dry CH_2Cl_2 (15 mL), then an ethereal solution of osmium tetroxide (127 mg, 0.5 mmol) was added dropwise to the solution at RT. The bright orange mixture was cooled to −20 °C and a solution of olefin **13** (0.18 g, 0.5 mmol) in dry CH_2Cl_2 (25 mL) was added dropwise. The dark reaction mixture was allowed to reach RT over 3 h and the reaction followed by TLC until no starting material was observed. The solution was concentrated *in vacuo* to a thick black oil. This crude

material was dissolved in MeOH (20 mL) and conc. HCl (20 drops) was added (pH = 2). The mixture was then stirred overnight at RT. The solution was concentrated *in vacuo* to a dark brown oily solid, which was purified by flash chromatography, eluting with 80 : 20 ethyl acetate–hexane, to give the *diol* (+)-**15** (0.14 g, 70%) as a pale yellow oil $[\alpha]_D^{24}$ +10.5 (*c* 1.0 in CH_2Cl_2); other characterisation as (±)-**15**.

(2*R*,3*S*)-(1-*O*-Phosphoryl)-1,2,3-trihydroxy-4-oxopentane (1-deoxy-D-xylulose-5-phosphate) 6

Dibenzylphosphate (+)-**15** (0.2 g, 0.5 mmol) was dissolved in methanol (8 mL), then 10% palladium on charcoal (53.2 mg) was added at RT. The flask was sealed and the atmosphere was removed under vacuum and replaced with 1 atm of H_2. The suspension was stirred vigorously for 5.5 h, until TLC analysis indicated that the reaction was complete. The catalyst was removed by filtration through Celite®. The filter pad was successively washed with methanol and the filtrates were combined and evaporated to give the diol **6** (98.7 mg, 92%) as a transparent amorphous solid, R_f (butanol–acetic acid–water, 4 : 1 : 1) 0.18; $[\alpha]_D^{24}$ +7.0 (*c* 1.0 in MeOH), lit.[6] −1.2 (*c* 4.2 in H_2O); ν_{max} (film)/cm^{-1} 3343 (O–H), 1713 (C=O), 1122 (P=O), 997 (wide, P–O-alkyl); δ_H (400 MHz; D_2O) 4.35 (1 H, d, $^3J_{HH}$ 2.2, HOC*H*–HOCH–CH_2), 4.26 (1 H, ddd, $^3J_{HH}$ 6.6 and $^3J_{HH}$ 6.3 and $^3J_{HH}$ 2.2, HOCH–HOC*H*–CH_2), 3.80–3.95 (2 H, m, HOCH–HOCH–CH_2), 2.19 (3 H, s, CH_3); δ_H (400 MHz; D_2O, {^{31}P}) 3.80–3.95 (2H, m, HOCH–HOCH–CH_2); δ_C (100.6 MHz; D_2O) 213.3 (C=O), 77.3 (HO*C*H–HOCH–CH_2), 70.6 (d, $^3J_{CP}$ 8.4, HOCH–HO*C*H–CH_2), 66.2 (d, $^2J_{CP}$ 5.2, HOCH–HO*C*H–CH_2), 26.3 (CH_3); δ_P (121.4 MHz; $CDCl_3$; {^{1}H}) 0.79; δ_P (121.4 MHz; $CDCl_3$) 0.79 (m); *m*/*z* (ESMS) 215 (100%, $[M]H^+$), 429 (88%, $[2M]H^+$).

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