Pergamon

Tetrahedron 56 (2000) 1485–1489

TETRAHEDRON

Chemical Synthesis of Enantiopure 2-*C*-Methyl-d-Erythritol 4-Phosphate, the Key Intermediate in the Mevalonate-Independent Pathway for Isoprenoid Biosynthesis

Jean-François Hoeffler, C. Pale-Grosdemange and Michel Rohmer*

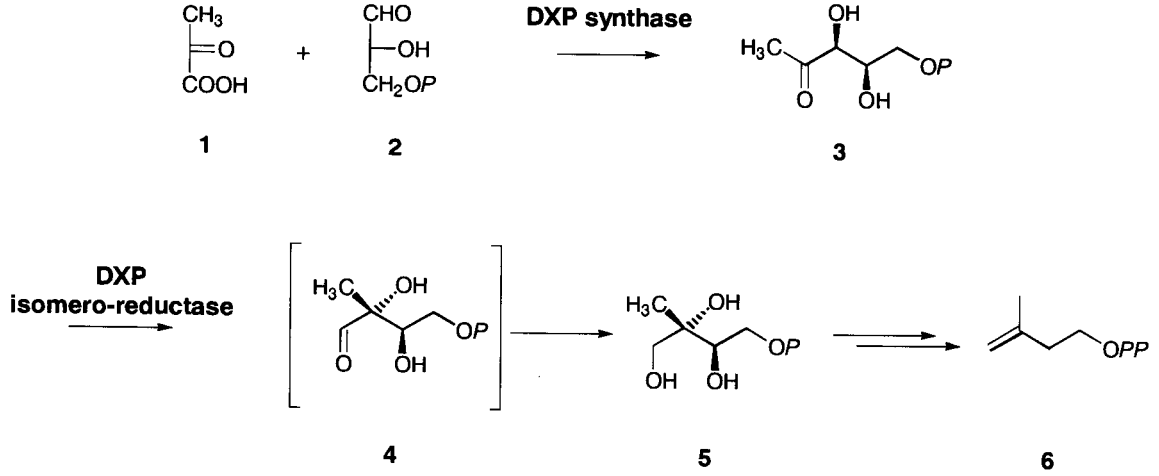
Laboratoire de Chimie et Biochimie des Microorganismes, Université Louis Pasteur, Institut Le Bel, 4 rue Blaise Pascal, 67043 Strasbourg Cedex, France

Received 31 August 1999; accepted 24 January 2000

Abstract—Optically pure 2-*C*-methyl-d-erythritol 4-phosphate, a key intermediate in the mevalonate-independent route for isoprenoid biosynthesis, is conveniently synthesized from 1,2-*O*-isopropylidene-α-d-xylofuranose, including the possibility of radiolabeling of this substrate.

Introduction

Two biosynthetic pathways are leading to isopentenyl diphosphate **6** (IPP), the universal precursor of isoprenoids: the well known mevalonate (MVA) pathway and the long overlooked 2-*C*-methyl-d-erythritol 4-phosphate **5** (MEP) route starting from triose phosphate derivatives,[1] recently discovered in eubacteria and later found in the plastids of phototrophic eukaryotes (Scheme 1).[1,2] Only two C_5 intermediates are known: 1-deoxy-d-xylulose 5-phosphate **3** (DXP) is formed from the condensation of (hydroxyethyl)thiamin diphosphate on glyceraldehyde 3-phosphate **2**,[3] and 2-*C*-methyl-d-erythritol 4-phosphate **5** is obtained by an intramolecular rearrangement of DXP followed by the reduction of the resulting aldehyde **4**.[4] The key role of 2-*C*-methyl-d-erythritol derivatives was first evidenced by the incorporation of the free tetrol into the prenyl chain of ubiquinone and menaquinone from *E. coli*.[5] The corresponding monophosphate **5** (MEP) is however the precursor of IPP, as it is directly formed from DXP by the DXP isomero-reductase.[4] Due to the probable absence of an efficient kinase, free ME is only poorly incorporated into isoprenoids by the wild-type *E. coli* and not incorporated at all by all other tested organisms.[5b] An efficient synthesis of MEP is therefore required in order to investigate its conversion into IPP in cell-free systems.



Scheme 1. 2-*C*-Methyl-d-erythritol 4-phosphate pathway for isoprenoid biosynthesis.

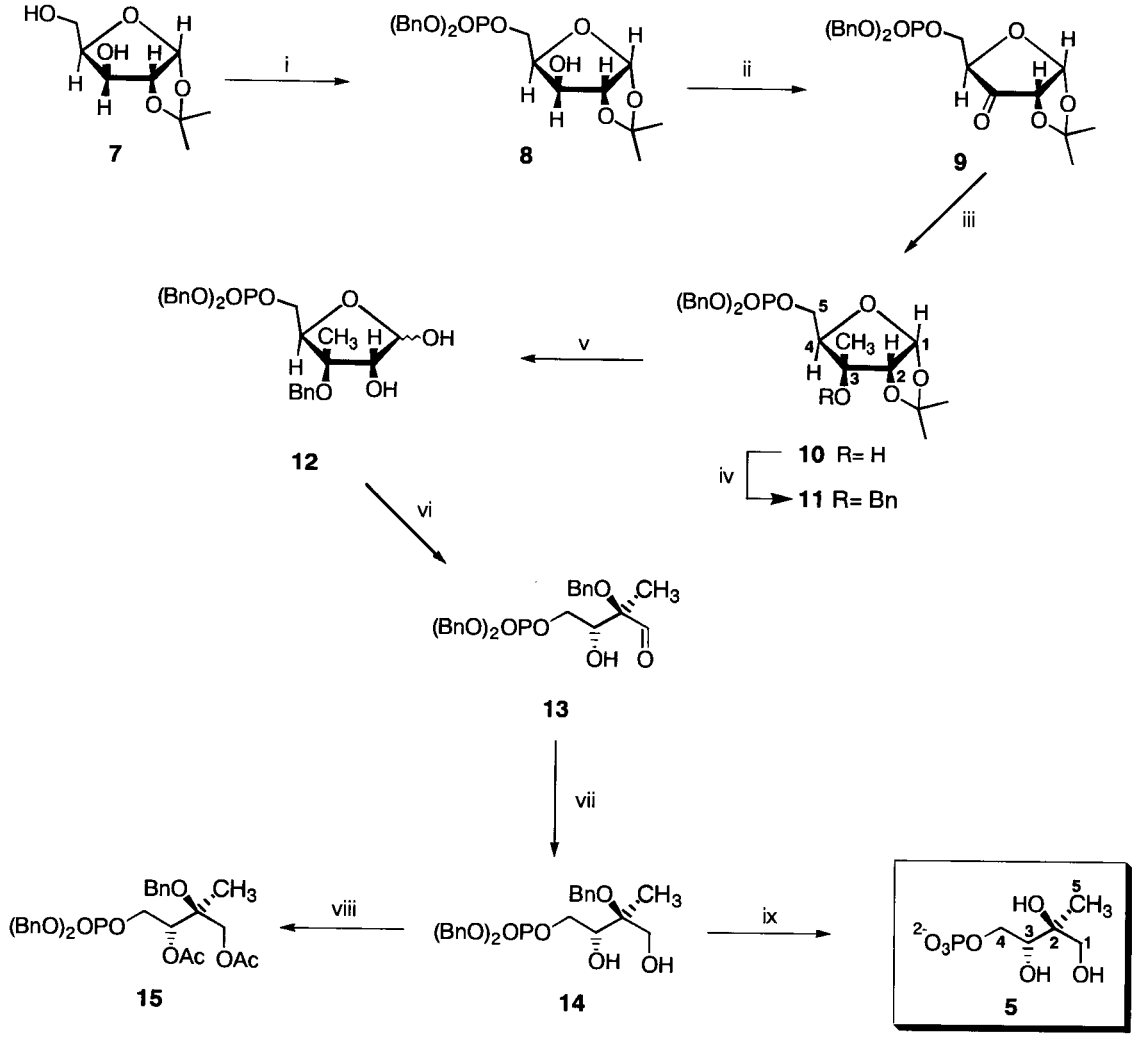
Keywords: biosynthesis; isoprenoids; 2-*C*-methyl-d-erythritol 4-phosphate.
* Corresponding author. Tel.: 33-(0)3-88-41-61-02; fax: 33-(0)3-88-41-61-01; e-mail: mirohmer@chimie.u-strasbg.fr

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00065-X

Results and Discussion

The 3-oxo group of the ketone obtained from protected 1,2-*O*-isopropylidene-α-d-xylofuranose exhibits an excellent stereoselectivity in addition reactions to yield 3-*C*-alkyl-ribofuranoses with the alkyl group on the β-face of the carbohydrate ring.[6,7] The configurations of these 3-alkyl ribofuranosides at C-3 and C-4 are respectively identical with those at C-3 and C-2 of 2-*C*-methyl-d-erythritol 4-phosphate. The synthesis of 2-*C*-methyl-d-erythritol 4-phosphate from commercially available 1,2-*O*-isopropylidene-α-d-xylofuranose **7** was therefore attempted using a methodology based on these literature data (Scheme 2).

The first step of the synthetic route is the selective protection of the primary alcohol **7** with dibenzylphosphorochloridate[8] in pyridine to give 5-dibenzylphosphate-1,2-*O*-isopropylidene-α-d-xylofuranose **8** in 95% yield. Oxidation of the free alcohol with pyridinium dichromate (PDC) in the presence of acetic acid[9] gave the corresponding ketone **9**, best isolated and stored as a hydrate. The *gem*-diol had to be dehydrated back to the ketone **9**: this could be conveniently carried out by azeotropic distillation of the water with toluene. The required configuration for the 2-*C*-methyl-d-erythritol was expected to be obtained by introducing a methyl group at the C-3 carbon atom of the 1,2-*O*-isopropylidene-α-d-xylofuranose **1**. Consequently, the key step of this approach was the stereoselective nucleophilic addition of methylmagnesium chloride on the carbonyl group of ketone **9**. Treatment of ketone **9** with methylmagnesium chloride resulted in the addition of the methyl group from the less hindered β-face and yielded the tertiary alcohol **10** with the desired stereochemistry.[10] The structure assigned to product **10** was verified using ^{1}H-^{1}H COSY and NOESY NMR experiments. The NOEs between H-1 and H-2 confirmed the anomeric α configuration. The position of the methyl group at C-3 on the β-face of the furanose ring was supported on the one hand by strong NOEs between H-2 and the protons from the C-3-methyl group, and on the other hand by medium NOEs between the protons of the C-3 methyl protons and H-4 was very weak. Benzylation of the tertiary alcohol **10** could only be performed using benzyl 2,2,2-trichloroacetimidate in acidic catalytic conditions.[11] The acetonide protection in **11** was removed in the presence



Scheme 2. Chemical synthesis of 2-*C*-methyl-d-erythritol 4-phosphate. (i) $(BnO)_2P(O)Cl$, pyridine (95%); (ii) PDC, AcOH, 3 Å molecular sieves, CH_2Cl_2 (90%); (iii) MeMgCl, THF (84%); (iv) benzyl 2,2,2-trichloroacetimidate, CF_3SO_3H (cat), cyclohexane/CH_2Cl_2 2/1 (70%); (v) 90% aq. TFA, −10°C (80%); (vi) $NaIO_4$, MeOH–H_2O (93%); (vii) $NaBH_4$, MeOH (92%); (viii) Ac_2O, TEA, DMAP, CH_2Cl_2 (95%); (ix) H_2, 10% Pd/C, EtOH (95%).

of 90% aqueous trifluoroacetic acid. The resulting mixture of the two anomers of hemicetal **12** underwent a sodium metaperiodate mediated glycol oxidative cleavage into the tribenzyl derivative **13** of 2-*C*-methyl-d-erythrose 4-phosphate. Sodium borohydride reduction of aldehyde **13** followed by hydrogenolysis of the benzyl groups[12] yielded free 2-*C*-methyl-d-erythritol-4-phosphate **5**, which was characterized either directly[4a] or via its tribenzyl derivative **14** and the corresponding diacetate **15**.

This straightforward synthesis easily affords optically pure 2-*C*-methyl-d-erythritol 4-phosphate **5** in satisfactory yields. It can be extended to the obtention of free methyl-erythritol. Radiolabeling can be performed with ^{14}C at the level of the addition of the Grignard reagent using [^{14}C]methylmagnesium halide, and more easily with ^{3}H by reduction of the tribenzylated 2-*C*-methyl-d-erythrose 4-phosphate **13** using tritiated sodium borohydride.

Experimental

General methods and materials

Most analytical techniques were as previously described.[13] All non-aqueous reactions were run in dry solvents under an argon atmosphere. 'Dried and concentrated' refers to removal of residual amounts of water with anhydrous Na_2SO_4 followed by evaporation of solvent on a rotary evaporator. Flash chromatography was performed on Merck silica gel (40–63 μm) with the indicated solvent system.[14] Thin-layer plates were developed with an ethanol solution of *p*-anisaldehyde (2.5%), sulfuric acid (3.5%) and acetic acid (1.6%) by heating up to 100°C. NMR spectra were recorded on Bruker AC200, AV400 or ARX500 spectrometers at 200 and 400 MHz for ^{1}H NMR, 50.32 MHz and 125.77 MHz for ^{13}C NMR and 162 MHz for ^{31}P NMR. NMR experiments were carried out in $CDCl_3$ or D_2O using as internal standard $CHCl_3$ (δ=7.26 ppm) or DHO (δ=4.65 ppm) for ^{1}H NMR and $CDCl_3$ (δ=77.03 ppm) or *t*-butanol (δ=31.60 ppm) for ^{13}C NMR. ^{31}P NMR spectra were calibrated against an external H_3PO_4 standard (δ=0.00 ppm). Negative mode electrospray mass spectrometry was performed on a Hewlett Packard 1100MS spectrometer using acetonitrile/water (1:1) as solvent, and high-resolution mass spectrometry on a ZAB-HF spectrometer with an acceleration potential of 8 keV using *m*-nitrobenzyl alcohol as matrix and xenon as ionization gas. All intermediates as well as the final 2-*C*-methyl-d-erythritol 4-phosphate **(5)** were found to be pure by the ^{1}H-, ^{13}C- and ^{31}P NMR (in the latter case after H-decoupling) and TLC criteria.

1,2-*O*-isopropylidene-α-d-xylofuranose 5-dibenzylphosphate (8). Dibenzylphosphorochloridate[8] (5.56 g, 19 mmol, 2.1 equiv.) dissolved in anhydrous CCl_4 (5 ml) was added under stirring to a solution of 1,2-*O*-isopropylidene-α-d-xylofuranose **7** (1.7 g, 8.9 mmol, 1 equiv.) in pyridine (25 ml) at 0°C. The reaction was stirred at rt for 3 h, quenched by addition of water (2 ml), and the solvents were removed under vacuum. Flash chromatography (ethyl acetate/hexane, 70:30) gave a colorless oil (3.84g, 95%) (R_f=0.52, ethyl acetate/hexane, 70:30). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.32 (3H, s, CH_3); 1.50 (3H, s, CH_3); 4.02 (1H, m); 4.24 (4H, m); 4.55 (1H, d, *J*=3.7 Hz, 3-H); 5.04 (m, 4H, 2×CH_2Ph); 5.88 (d, 1H, *J*=3.3 Hz, 1-H); 7.33–7.48 (10H, m, 2×Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=26.16 (CH_3); 26.84 (CH_3); 63.54 (CH_2, d, $^{2}J_{C,P}$=4.9 Hz); 69.80 (CH_2, d, $^{2}J_{C,P}$=4.9 Hz); 69.92 (CH_2, d, $^{2}J_{C,P}$=6.6 Hz); 73.72 (CH); 78.78 (CH, C-4, d, $^{3}J_{C,P}$=4.9 Hz); 85.03 (CH); 105.00 (CH, C-1); 111.78 (quaternary C); 127.94, 128.04, 128.63, 128.72, 135.18, 135.25 and 135.38 (aromatic C). ^{31}P NMR (162 MHz, $CDCl_3$): δ=2.09 (m). HRMS (FAB^+): $(M+H)^+$ calculated for $C_{22}H_{28}O_8P$ 451.1530, found 451.1522.

1,2-*O*-isopropylidene-α-d-*erythro*-pentofuranose-3-ulose 5-dibenzylphosphate (9). To a stirred solution of 5-dibenzylphosphate-1,2-*O*-isopropylidene-α-d-xylofuranose **8** (3.1 g, 7 mmol, 1 equiv.) in dry CH_2Cl_2 (60 ml) were slowly added at 0°C activated molecular sieves (3 Å, 3 g), pyridinium dichromate (6.6 g, 17.5 mmol, 2.5 equiv.) and acetic acid (0.72 ml, 12.6 mmol, 1.8 equiv.). The reaction was stirred at room temperature for 3 h. Chromium salts were eliminated by adding Celite (3 g), $MgSO_4$ (3 g) and diethyl ether (50 ml).[14] The mixture was stirred for additional 30 min, filtered through a bed of silica gel (about 5 cm thickness) on a sintered-glass funnel. The solid cake was washed with diethyl ether (3×30 ml), and the filtrate was concentrated in vacuum to give **9** as oil. Remaining chromium salts were removed by repeating the diethyl ether treatment. The hydrated ketone was obtained after evaporation of the solvent as colorless syrup (2.85 g, 90%). (R_f=0.42, ethyl acetate/hexane, 70:30). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.39 (3H, s, CH_3); 1.45 (3H, s, CH_3); 4.18 (1H, dd, $^{3}J_{1,2}$=4.4 Hz, $^{4}J_{2,4}$=1.1 Hz, 2-H); 4.19 (1H, ddd, $^{2}J_{5a,5b}$=11.2 Hz, $^{3}J_{5a,P}$=6.2 Hz, $^{3}J_{4,5a}$=2.6Hz, 5-H_a); 4.24 (1H, ddd, $^{2}J_{5a,5b}$=11.2 Hz, $^{3}J_{5b,P}$=2.6 Hz, $^{3}J_{4,5b}$=5.7 Hz, 5-H_b); 4.43 (1H, ddd, $^{3}J_{4,5b}$=5.5 Hz, $^{3}J_{4,5a}$=2.6 Hz, $^{4}J_{2,4}$=1.1 Hz, 4-H), 5.01 (1H, dd, ^{2}J=11.7 Hz, $J_{H,P}$=8.4 Hz, CH_2Ph), 5.02 (1H, dd, ^{2}J=11.7 Hz, $J_{H,P}$=8.4 Hz, CH_2Ph), 5.04 (1H, dd, ^{2}J=11.7 Hz, $J_{H,P}$=8.4 Hz, CH_2Ph), 5.05 (1H, dd, ^{2}J=11.7 Hz, $J_{H,P}$=8.4 Hz, CH_2Ph); 5.96 (1H, d, $^{3}J_{1,2}$=4.4 Hz, 1-H); 7.38 (10H, m, 2×Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=26.97 (CH_3); 27.40 (CH_3); 66.15 (CH_2, d, $^{2}J_{C,P}$=5.2 Hz); 69.54 (2×CH_2, d, $^{2}J_{C,P}$=5.0 Hz); 76.15 (CH, C-2); 78.00 (CH, d, $^{3}J_{C,P}$=8.2 Hz, C-4); 103.25 (CH, C-1); 114.24 (quaternary C); 127.94, 128.53, 128.63, 135.38 and 135.58 (aromatic C); 207.46 (C-3). ^{31}P NMR (162 MHz, $CDCl_3$): δ=−0.13 (m). HRMS (FAB^+): $(M+H)^+$ calculated for $C_{22}H_{26}O_8P$ 449.1365, found 449.1386. The gem-diol was dried back to the ketone **9** by evaporation with dry toluene three times.

1,2-*O*-isopropylidene-3-*C*-methyl-α-d-ribofuranose 5-dibenzylphosphate (10). The ketone **9** (850 mg, 1.9 mmol, 1 equiv.), dissolved in dry THF (30 ml), was treated at −10°C with a 3 M solution of methylmagnesium chloride (0.82 ml, 2.46 mmol, 1.3 equiv.) in THF. The reaction mixture was stirred at room temperature for 4 h. A saturated ammonium chloride solution (20 ml) was added to the cooled (−10°C) solution. After addition of diethyl ether (30 ml), the reaction mixture was extracted with ethyl acetate (3×50 ml). The combined extracts were washed with brine, dried, and filtered. After concentration of the filtrate,

flash chromatography of the residue (ethyl acetate/hexane, 70:30) afforded product **10** (740 mg, 84%). (R_f=0.48, ethyl acetate/hexane, 70:30). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.10 (3H, s, CH_3); 1.28 (3H, s, CH_3); 1.55 (3H, s, CH_3); 3.95 (1H, dd, $^3J_{4,5a}$=7.8 Hz, $^3J_{4,5b}$=3.0 Hz, 4-H); 4.06 (1H, ddd, $^2J_{5a,5b}$=11.1 Hz, $^3J_{4,5a}$=7.8 Hz, $^3J_{H5a,P}$= 7.8 Hz, 5-H_a); 4.09 (1H, d, $^3J_{1,2}$=3.8 Hz, 2-H); 4.19 (1H, ddd, $^2J_{5b,5a}$=11.1 Hz, $^3J_{5b,P}$=7.0 Hz, $^3J_{4,5b}$=3.0 Hz, 5-H_b); 5.04 (1H, dd, ^2J=11.8 Hz, $^3J_{H,P}$=8.0 Hz, CH_2Ph); 5.05 (1H, dd, ^2J=11.8 Hz, $^3J_{H,P}$=8.1 Hz, CH_2Ph); 5.07 (1H, dd, ^2J=11.8 Hz, $^3J_{H,P}$=8.1 Hz, CH_2Ph); 5.08 (1H, dd, ^2J= 11.8 Hz, $^3J_{H,P}$=8.0 Hz, CH_2Ph); 5.76 (1H, d, $^3J_{1,2}$=3.8 Hz, 1-H); 7.32–7.37 (10H, m, 2×Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=18.12 (CH_3); 26.52 (2×CH_3); 65.93 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 69.35 (CH_2, d, $^2J_{C,P}$=3.7 Hz); 69.45 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 76.74 (quaternary C, C-3); 80.21 (CH, d, $^3J_{C,P}$=6.6 Hz, C-4); 84.08 (CH, C-2); 103.48 (CH, C-1); 112.66 (quaternary C); 127.94, 128.00, 128.49, 135.64 and 135.77 (aromatic C). ^{31}P NMR (162 MHz, $CDCl_3$): δ=0.31 (hept, $^3J_{H,P}$=7.9 Hz). HRMS (FAB^+): $(M+H)^+$ calculated for $C_{23}H_{30}O_8P$ 465.1678, found 465.1687.

3-*O*-Benzyl-1,2-*O*-isopropylidene-3-*C*-methyl-α-d-ribofuranose 5-dibenzylphosphate (11). To a stirred solution of the tertiary alcohol **10** (450 mg, 0.97 mmol, 1 equiv.) and benzyl 2,2,2-trichloroacetimidate (0.49 g, 1.94 mmol, 2 equiv.) in cyclohexane/CH_2Cl_2 (2:1, 3 ml) under an argon atmosphere was added trifluoromethanesulfonic acid (30 μl). The mixture was stirred at room temperature and monitored by TLC (ethyl acetate/hexane, 70:30) until the starting material had completely reacted. The crystalline trichloroacetamide was removed by filtration, and the filtrate was successively washed with a saturated solution of sodium hydrogencarbonate (20 ml) and with water (20 ml). The mixture was extracted with CH_2Cl_2 (3×20 ml). The combined extracts were dried, filtered and concentrated. Finally, the residue was purified by flash chromatography to afford a colorless oil (380 mg, 70%). (R_f=0.40, ethyl acetate/hexane, 50:50). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.18 (3H, s, CH_3); 1.36 (3H, s, CH_3); 1.58 (3H, s, CH_3); 4.11 (1H, ddd, $^2J_{5a,5b}$=11.0 Hz, $^3J_{4,5a}$=7.9 Hz, $^3J_{5a,P}$=6.6 Hz, 5-H_a); 4.21 (1H, ddd, $^2J_{5a,5b}$=11.0 Hz, $^3J_{5b,P}$=6.6 Hz, $^3J_{4,5b}$=2.8 Hz, 5-H_b); 4.30 (1H, dd, $^3J_{4,5a}$=7.9 Hz, $^3J_{4,5b}$=2.8 Hz, 4-H); 4.33 (1H, d, $^3J_{1,2}$=3.9 Hz, 2-H); 4.54 (1H, d, ^2J=11.0 Hz, CH_2Ph); 4.61 (1H, d, ^2J=11.0 Hz, CH_2Ph); 5.02 (2H, 2d, ^2J=11.4 Hz, CH_2Ph); 5.04 (2H, 2d, ^2J=11.4 Hz, CH_2Ph); 5.78 (1H, d, $^3J_{1,2}$=3.9 Hz, 1-H); 7.24–7.38 (15H, m, 3×Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=16.22 (CH_3); 26.68 (CH_3); 26.84 (CH_3); 66.16 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 66.74 (CH_2); 69.25 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 69.35 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 77.26 (quaternary C, C-3); 79.67 (CH, d, $^3J_{C,P}$=8.2 Hz, C-4); 82.77 (CH, C-2); 104.34 (CH, C-1); 113.02 (quaternary C); 127.58, 127.68, 127.90, 128.00, 128.23, 128.36, 128.46, 135.77, 135.94 and 138.36 (aromatic C). ^{31}P NMR (162 MHz, $CDCl_3$) δ=0.21 (m). HRMS (FAB^+): $(M+H)^+$ calculated for $C_{30}H_{36}O_8P$ 555.2148, found 555.2158.

3-*O*-Benzyl-3-*C*-methyl-d-ribofuranose 5-dibenzylphosphate (12). To a solution of **11** (380 mg, 0.68 mmol) in CCl_4 (0.5 ml) was added at −10°C a 90% aqueous solution of TFA (5 ml). After 30 min, all the starting material was consumed, and the reaction mixture was diluted with water (5 ml), neutralized with calcium carbonate and extracted with $CHCl_3$ (3×20 ml). The combined extracts were dried, filtered and concentrated to give in 80% yield a colorless oil corresponding to the two anomers of **12** in a ca. 3:1 ratio according to the integration of the quaternary methyl signals (280 mg, R_f=0.66, ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.38 (3/4 of 3H, s, CH_3); 1.43 (1/4 of 3H, s, CH_3); 2.72 (3/4 of 1H, d, ^3J=8.6 Hz, OH); 2.79 (1/4 of 1H, d, ^3J=8.4 Hz, OH); 3.72–5.20 (11H; m, 1-H, 2-H, 4-H, 5-H_a, 5-H_b and 3 benzylic CH_2); 7.28–7.36 (15H, m, 3×Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ=15.67 and 16.19 (CH_3); 65.59, 66.08, 66.66 (d, ^2J=4.9 Hz), 69.49, 69.56, 69.62 and 69.92 (CH_2 signals); 75.78, 79.73 (C-4, d, $^3J_{C\text{-}4,P}$=8.2 Hz), 81.42, 81.91, 82.27, 96.67 and 102.76 (CH signals); 127.41, 127.64, 127.97, 128.59, 135.48, 135.61 and 137.31 (aromatic C). ^{31}P NMR (162 MHz, $CDCl_3$): δ=0.18 (m); 0.46 (m). HRMS (FAB^+): $(M+H)^+$: calculated for $C_{27}H_{32}O_8P$ 515.1835, found 515.1842.

2-*O*-Benzyl-2-*C*-methyl-d-erythrose 4-dibenzylphosphate (13). To a solution of **12** (280 mg, 0.55 mmol, 1 equiv.) in methanol (3 ml) was added a solution of sodium metaperiodate (150 mg, 0.7 mmol, 1.2 equiv.) in water (3 ml). The resulting suspension was stirred for 30 min at room temperature, neutralized with sodium hydrogen carbonate and then extracted with $CHCl_3$ (3×10 ml). The combined extracts were dried, filtered and concentrated to gave a colorless oil **13** (250 mg, 93%) which was not further purified. The NMR spectra of the crude product, which corresponded to a 1:1 mixture of the aldehyde and the corresponding hydrate according to the integration of the H-1 signals, were satisfactory (R_f=0.90, ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.35 (3H, s, CH_3); 4.16 (1H, ddd, $^2J_{4a,4b}$=11.4 Hz, $^3J_{4a,P}$=7.4 Hz, $^3J_{3,4a}$=7.3 Hz, 4-H_a); 4.26 (1H, ddd, $^2J_{4b,4a}$=11.4 Hz, $^3J_{4b,P}$=6.6 Hz, $^3J_{3,4b}$=3.3 Hz, 4-H_b); 4.44 (1H, d, ^2J=11.4 Hz, CH_2Ph); 4.55 (1H, d, ^2J=11.4 Hz, CH_2Ph); 4.96–5.06 (4H, m, 2×CH_2Ph); 5.50 (1H, dd, $^3J_{3,4a}$=7.3 Hz, $^3J_{3,4b}$=3.3 Hz, 3-H); 7.3–7.5 (15H, m, 3×Ph), 7.99 (0.5 H, s, 1-H of the aldehyde hydrate); 9.55 (0.5 H, s, 1-H of the aldehyde). ^{13}C NMR (50 MHz, $CDCl_3$): δ=13.63 and 14.39 (2×CH_3); 64.95 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 66.34 and 66.57 (2×CH_2); 69.59 (CH_2, d, $^2J_{C,P}$=6.6 Hz); 72.38 (CH, d, $^3J_{C,P}$=6.6 Hz, C-3); 81.95 (quaternary C, C-2 of the aldehyde hydrate); 98.86 (quaternary C, C-1 of the aldehyde hydrate); 127.48, 128.00, 128.53, 128.63, 135.61 and 137.25 (aromatic C); 159.60 (quaternary C, C-2 of the aldehyde); 200.84 (C-1 of the aldehyde). ^{31}P NMR (162 MHz, $CDCl_3$): δ=1.66 (m). HRMS (FAB^+): $(M+H)^+$: calculated for $C_{26}H_{30}O_7P$ 485.1729, found 485.1708.

2-*O*-benzyl-2-*C*-methyl-d-erythritol 4-dibenzylphosphate (14). To an ice-cooled solution of the aldehyde **13** (250 mg, 0.52 mmol, 1 equiv.) in methanol (10 ml) was added sodium borohydride (24 mg, 0.62 mmol, 1.2 equiv.). Stirring was continued at room temperature, and after 2 h the reaction mixture was diluted with water (5 ml), treated with 0.1N HCl and extracted with $CHCl_3$ (3×10 ml). The combined extracts were dried, filtered and concentrated, and the residue afforded after flash chromatography 2-*O*-Benzyl-2-*C*-methyl-d-erythritol 4-dibenzylphosphate as a colorless oil (230 mg, 92%). (R_f=0.60,

ethyl acetate/hexane, 90:10). ^{1}H NMR (400 MHz, $CDCl_3$: δ=1.16 (3H, 1s, CH_3); 3.66 (1H, d, $^2J_{1a,1b}$=12.0 Hz, 1-H_a); 3.70 (1H, d, $^2J_{1a,1b}$=12.0 Hz, 1-H_b); 3.99–4.11 (2H, m, 4-H_a and 4-H_b); 4.33 (1H, m, 3-H); 4.47 (1H, d, ^2J=11.0 Hz, CH_2Ph); 4.52 (1H, d, ^2J=11.0 Hz, CH_2Ph); 5.00–5.11 (4H, m, 2×CH_2Ph); 7.28–7.34 (15H, m, 3×Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=15.40 (CH_3); 64.28 (CH_2); 64.80 (CH_2); 69.64 (3×CH_2, d, $^2J_{C,P}$=4.9 Hz); 72.98 (CH, d, $^3J_{C,P}$=4.9 Hz, C-3); 78.30 (quaternary C, C-2); 127.38, 127.58, 128.04, 128.43, 128.63, 135.58, 135.67 and 138.59 (aromatic C). ^{31}P NMR (162 MHz, $CDCl_3$): δ=0.11 (m). HRMS $(FAB)^+$: $(M+H)^+$ calculated for $C_{26}H_{32}O_7P$ 487.1886, found 487.1899.

Diacetate of 2-*O*-benzyl-2-*C*-methyl-d-erythritol 4-dibenzylphosphate (15). The diol **14** (30 mg, 6.22×10^{-2} mmol, 1 equiv.) dissolved in CH_2Cl_2 (2 ml) was acetylated at room temperature for 30 min with acetic anhydride (13 μl, 0.131 mmol, 2.1 equiv.) in the presence of DMAP (0.05 equiv.) and triethylamine (19 μl, 0.131 mmol, 2.1 equiv.). The reaction mixture was quenched with an aqueous solution of sodium hydrogencarbonate and extracted with CH_2Cl_2 (3×5 ml). The combined extracts were dried, filtered and concentrated. The residue was finally purified by chromatography to afford compound **15** as a colorless oil (33 mg, 95%) (R_f=0.50, ethyl acetate/hexane, 50:50). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.25 (3H, s, CH_3); 2.00 (3H, s, CH_3COO–); 2.04 (3H, s, CH_3COO–); 4.07 (1H, d, $^2J_{1a,1b}$=12.6 Hz, 1-H_a); 4.14 (1H, d, $^2J_{1a,1b}$=12.6 Hz, 1-H_b); 4.21 (1H, ddd, $^2J_{4a,4b}$=11.2 Hz, $^3J_{3,4a}$=7.9 Hz, $^3J_{4a,P}$=7.7 Hz, 4-H_a); 4.39 (1H, ddd, $^2J_{4a,4b}$=11.2 Hz, $^3J_{4b,P}$=6.6 Hz, $^3J_{3,4b}$=2.6 Hz, 4-H_b); 4.49 (2H, s, CH_2Ph); 4.97 (1H, dd, ^2J=11.7 Hz, $^3J_{H,P}$=8.1 Hz, CH_2Ph); 5.00 (1H, dd, ^2J=11.7 Hz, $^3J_{H,P}$=8.1 Hz, CH_2Ph); 5.02 (1H, dd, ^2J=11.7 Hz, $^3J_{H,P}$=8.1 Hz, CH_2Ph); 5.04 (1H, dd, ^2J=11.7 Hz, $^3J_{H,P}$=8.1 Hz, CH_2Ph); 5.43 (1H, dd, $^3J_{3,4a}$=7.9 Hz, $^3J_{3,4b}$=2.6 Hz, 3-H); 7.32–7.34 (15H, m, 3×Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ=16.52 (CH_3); 20.81 (CH_3); 20.88 (CH_3); 64.05 (CH_2); 64.64 (CH_2); 66.20 (CH_2, d, $^2J_{C,P}$=4.9 Hz); 69.39 (2×CH_2, d, $^2J_{C,P}$=6.6 Hz); 72.05 (CH, d, $^3J_{C,P}$=6.6 Hz, C-3); 76.74 (quaternary C, C-2); 127.28, 127.58, 127.94, 128.40, 128.56, 135.74, 135.90 and 138.23 (aromatic C); 169.83 (CO); 170.58 (CO). ^{31}P NMR (162 MHz, $CDCl_3$): δ=0.35 (m). HRMS (FAB^+): $(M+H)^+$: calculated for $C_{30}H_{36}O_9P$ 571.2097, found 571.2104.

2-*C*-methyl-d-erythritol 4-phosphate (5). The diol **14** obtained after the reduction of aldehyde **13** was hydrogenated (200 mg, 0.42 mmol) over 10% Pd/C (20 mg) in EtOH (20 ml) for 2 h at room temperature and atmospheric pressure. The mixture was filtered, and the filtrate concentrated. The residue was diluted in water (5 ml), treated with a NaOH solution (1 M) until the pH 9 was reached and neutralized with HCl (0.5 M) to pH 7.5. The mixture was lyophilized to give the di-sodium salt of **5** (102 mg, 95%). ^{1}H NMR (200 MHz, D_2O): δ=0.98 (3H, s, CH_3); 3.32 (1H, d, $^2J_{1a,1b}$=11.8 Hz, 1-H_a); 3.43 (1H, d, $^2J_{1a,1b}$=11.8 Hz, 1-H_b); 3.87 (2H, m, 4-H_a and 4-H_b); 4.07 (1H, ddd, J=2.4 Hz, 6.9 and 9.3 Hz, 3-H). ^{13}C NMR (125 MHz, D_2O containing traces of *t*-BuOH): δ=21.48 (CH_3); 67.67 (CH_2, $^2J_{C-4,P}$=4.7 Hz, C-4); 72.33 (CH_2, C-1); 76.88 (CH, $^3J_{C-3,P}$=6.2 Hz, C-3); 77.15 (quaternary C, C-2). ^{31}P NMR (162 MHz, $CDCl_3$): δ=5.50 (s). Electrospray MS: m/z=215 (M-H, molecular ion of the MEP mono-anion).

Acknowledgements

We thank Mr J.-D. Sauer for all NMR measurements and Mr R. Huber for the FAB MS analyses. This investigation was supported by a grant to M.R. from the 'Institut Universitaire de France'.

References

1. (a) Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.* **1993**, *295*, 517–524. (b) Rohmer, M.; Seemann, M.; Horbach, S.; Bringer-Meyer, B.; Sahm, H. *J. Am. Chem. Soc.* **1996**, *118*, 2564–2566.

2. (a) Rohmer, M. In *Comprehensive Natural Product Chemistry*; Cane, D. E., Ed.; Pergamon: Oxford, UK, 1999; Vol. 2, pp 45–47. (b) Schwarz, M., Arigoni, D. In *Comprehensive Natural Product Chemistry*; Cane, D. E., Ed.; Pergamon: Oxford, UK, 1999; Vol. 2, pp 367–400. (c) Eisenreich, W.; Schwarz, M.; Cartayrade, A.; Arigoni, D.; Zenk, M. H.; Bacher, A. *Chem. Biol.* **1998**, *5*, R221–R233. (d) Lichtenthaler, H. K. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 47–65.

3. (a) Sprenger, G. A.; Schörken, U.; Wiegert, T.; Grolle, S.; Sahm, H. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12857–12862. (b) Lange, B.; Wildung, M. R.; McCaskill, D.; Croteau, R. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2100–2104. (c) Lois, L.-M.; Campos, N.; Rosa Putra, S.; Danielsen, K.; Rohmer, M.; Boronat, A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2105–2110.

4. (a) Kuzuyama, T.; Takahashi, S.; Watanabe, H.; Seto, H. *Tetrahedron Lett.* **1998**, *39*, 4509–4512. (b) Takahashi, S.; Kuzuyama, H.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9879–9884.

5. (a) Duvold, T.; Bravo, J.-M.; Pale-Grosdemange, C.; Rohmer, M. *Tetrahedron Lett.* **1997**, *38*, 4769–4772. (b) Duvold, T.; Cali, P.; Bravo, J.-M.; Rohmer, M. *Tetrahedron Lett.* **1997**, *38*, 6181–6184.

6. Nakatani, K.; Arai, K.; Terashima, S. *Tetrahedron* **1993**, *49*, 1901–1912.

7. Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423–3433.

8. Hall, R. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1954**, *76*, 5056–5060.

9. Czernecki, S.; Ezzitouni, A.; Krausz, P. *Synthesis* **1990**, *651*, 653.

10. Brimacombe, J. S.; Rollins, A. J.; Thompson, S. W. *Carbohydr. Res.* **1973**, *31*, 108–113.

11. (a) Iversen, T.; Bundle, D. R. *J. Chem. Soc. Chem. Commun.* **1981**, 1240–1241. (b) Widmer, U. *Synthesis* **1987**, 568–570.

12. Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260–2267.

13. Duvold, T.; Rohmer, M. *Tetrahedron* **1999**, *55*, 9847–9858.

14. Still, W. C.; Khan, M.; Mitra, M. *J. Org. Chem.* **1978**, *43*, 2923–2925.