ARTICLE

OBC
www.rsc.org/obc

Isoprenoid biosynthesis *via* the MEP pathway. Synthesis of (3*R*,4*S*)-3,4-dihydroxy-5-oxohexylphosphonic acid, an isosteric analogue of 1-deoxy-D-xylulose 5-phosphate, the substrate of the 1-deoxy-D-xylulose 5-phosphate reducto-isomerase

Odile Meyer, Catherine Grosdemange-Billiard, Denis Tritsch and Michel Rohmer *
Université Louis Pasteur, CNRS UMR 7123, Institut Le Bel, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France. E-mail: mirohmer@chimie.u-strasbg.fr; Fax: +33 3 90 24 13 45; Tel: +33 3 90 24 13 46

Received 1st October 2003, Accepted 21st October 2003
First published as an Advance Article on the web 6th November 2003

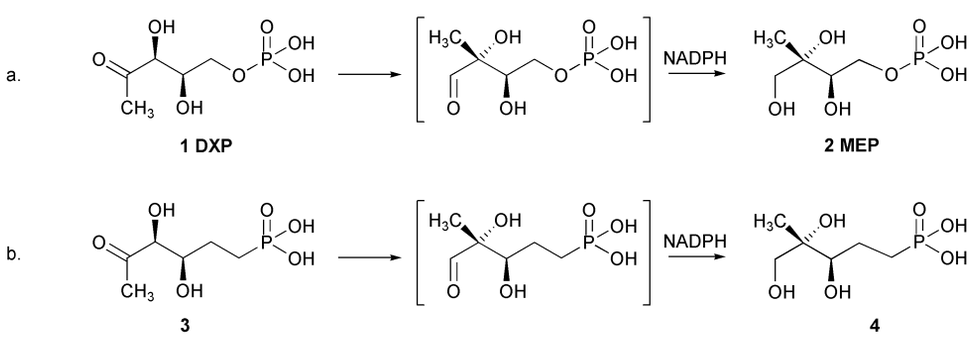
(3*R*,4*S*)-3,4-Dihydroxy-5-oxohexylphosphonic acid, an isosteric analogue of 1-deoxy-D-xylulose 5-phosphate (DXP), was obtained in enantiomerically pure form from (+)-2,3-*O*-benzylidene-D-threitol by a seven-step sequence. This phosphonate did not affect the growth of *Escherichia coli*. It did not inhibit the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), but was converted by this enzyme into (3*R*,4*R*)-3,4,5-trihydroxy-3-methylpentylphosphonic acid, an isosteric analogue of 2-*C*-methyl-D-erythritol 4-phosphate. The enzyme was, however, less efficient with the methylene phosphonate analogue than with the natural substrate.

Introduction

Isoprenoids are synthesized by all living organisms and include essential compounds (*e.g.* sterols as membrane stabilizers or steroid precursors, carotenoids of the plant chloroplasts, prenyl chains of ubiquinones, plastoquinone or prenylated proteins) as well as a wealth of secondary metabolites (*e.g.* mono- and sesquiterpenes from essential oils). They are synthesized from two precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Two different metabolic routes leading to IPP have been identified. In animals, fungi, plant cytoplasm, archaebacteria and some eubacteria, IPP is synthesized through the mevalonate pathway,[1] while the recently discovered methylerythritol phosphate (MEP) pathway is present in most bacteria, in some unicellular eukaryotes, including *Plasmodium falciparum*, the parasite responsible for malaria, and in the chloroplasts of all phototrophic organisms.[2] As the inhibition of the biosynthesis of essential isoprenoids is lethal, each enzyme of the MEP pathway is a potential target for antibacterial or antiparasitic drugs.[3] The MEP pathway is now nearly fully elucidated. The initial step of this pathway is the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) **1** (Scheme 1) by condensation of hydroxyethylthiamine diphosphate derived from pyruvate decarboxylation and D-glyceraldehyde 3-phosphate mediated by the DXP synthase.[4] An intramolecular rearrangement of DXP followed by a NADPH dependent reduction, catalysed by the DXP reductoisomerase (DXR), affords 2-*C*-methyl-D-erythritol 4-phosphate (MEP) **2** (Scheme 1(a)).[5] Further steps include the formation of methylerythritol 4-diphosphocytidine, its 2-phosphate derivative, methylerythritol 2,4-cyclodiphosphate,[6] and 4-hydroxy-3-methylbut-2-enyl diphosphate,[7] which is converted by a single enzyme (LytB) either into IPP or into DMAPP.[8]

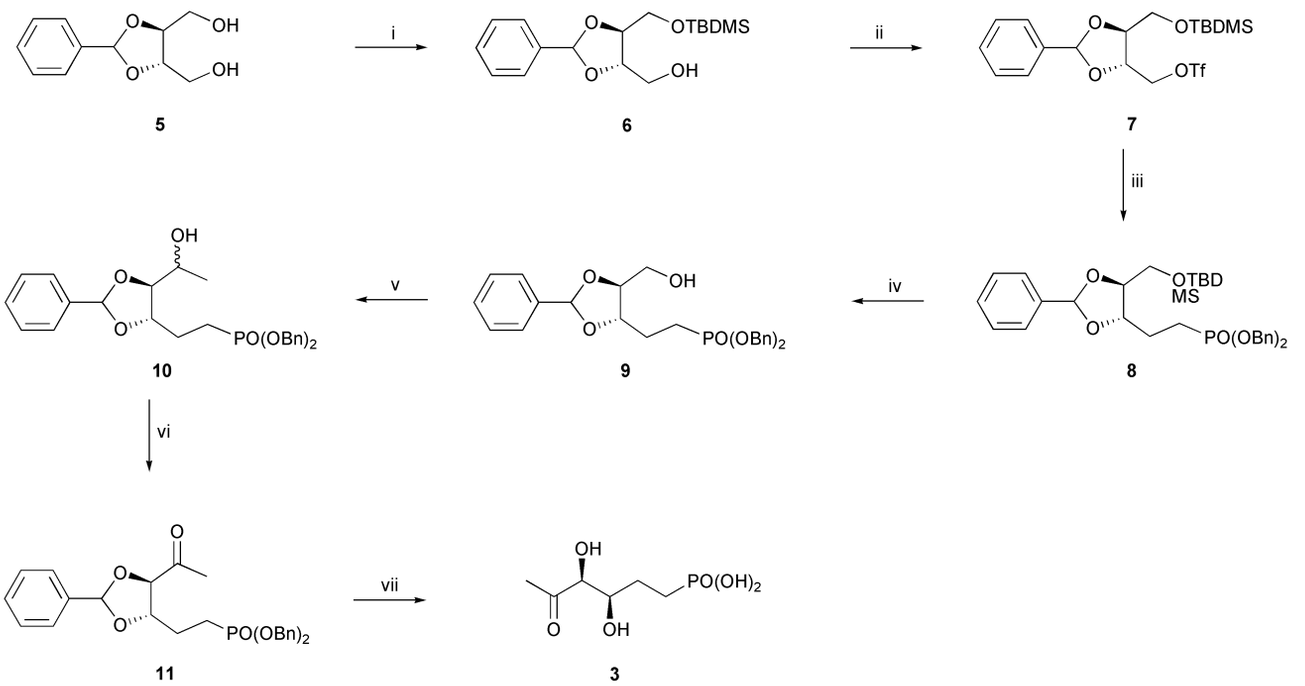
Due to the rather recent elucidation of the MEP pathway, only few inhibitors of its enzymes were reported. Fosmidomycin (3-(*N*-formyl-*N*-hydroxyamino)propylphosphonate) and the related FR900098 specifically inhibit the DXP reductoisomerase (DXR) from the bacteria *E. coli*[9] and *Zymomonas mobilis*[10] and led to the inhibition of the MEP pathway in higher plants[11] and in *Plasmodium falciparum*.[12] Its efficiency as an antimalarial drug was verified.[13] The recently determined crystal structure of the *E. coli* DXR complexing manganese and fosmidomycin may contribute to the development of specific inhibitors of this enzyme.[14] Two DXP analogues respectively lacking the hydroxyl group at C(4) and C(3) were found to be reversible mixed type inhibitor of the DXR.[15] Concerning other enzymes of the MEP pathway, fluoropyruvate[16] and 5-ketoclomazone, a breakdown product of the herbicide clomazone,[17] were reported to inhibit DXP synthase.

In the search of novel drugs acting on the MEP pathway, we synthesized (3*R*,4*S*)-3,4-dihydroxy-5-oxohexylphosphonic acid (DXP_N) **3** (Scheme 1(b)), an isosteric analogue of 1-deoxy-D-xylulose 5-phosphate and checked its action on the *E. coli* DXR.



Scheme 1 DXP reducto-isomerase catalysed reactions: (a) in the MEP pathway, (b) for the conversions of the phosphonate isostere **3** of DXP **1**.

DOI: 10.1039/b312193c



Scheme 2 Synthesis of (3*R*,4*S*)-3,4-dihydroxy-5-oxohexylphosphonic acid **3**. (i) TBDMSCl, NaH, DME (96%); (ii) Tf_2O, NEt_3, CH_2Cl_2 (95%); (iii) $MePO(OBn)_2$, *n*-BuLi, HMPA, THF (70%); (iv) Bu_4NF, THF, (98%); (v) $(COCl)_2$, DMSO, NEt_3, MeMgCl, THF, (86%); (vi) TPAP, NMO, 3 Å molecular sieves, CH_2Cl_2 (80%), (vii) H_2, 10% Pd/C, MeOH–H_2O (9 : 1) (quantitative).

Results and discussion

Synthesis of DXP_N

DXP_N was synthesized following the reaction sequence shown in Scheme 2. The commercially available D-threitol benzylidene derivative **5** (Scheme 2) was a starting material of choice because it possesses the required configurations of the carbon atoms bearing hydroxyl groups. The benzylidene protection of the vicinal diol was preferred, despite the resulting complication of the NMR spectra. Indeed, this benzylidene group was simultaneously removed with the phosphonate benzyl protecting group by one single catalytic hydrogenation, requiring no further purification of the free phosphonate **3**.

The first step of the synthesis was the protection of one of the two primary alcohol groups by an *O*-silyl group. This was completed with *tert*-butyldimethylsilyl chloride in dimethoxyethane, after formation of the alcoholate using sodium hydride. The remaining hydroxyl group was then activated by conversion into a triflate by action of triflic anhydride in the presence of triethylamine. The triflate **7** was displaced by the methylphosphonate anion, formed by action of *n*-butyl lithium on freshly prepared methylphosphonate[18] to give **8** in 95% yield. After deprotection of the *O*-silyl protective group with tetrabutylammonium fluoride, the resulting alcohol **9** was oxidized using the Swern oxidation modified by Ireland and Norbeck.[19] In these conditions, the methyl group was directly introduced by addition of methyl magnesium chloride without isolation of the aldehyde intermediate. The new C_6 carbon framework **10** was obtained in 86% yield. The following mild oxidation with TPAP/NMO gave rapidly after silica gel chromatography the ketone **11** in 80% yield.[20] Finally, the phosphonate **3** was quantitatively obtained by catalytic hydrogenation with palladium over charcoal in a mixture of methanol and water. This step required no purification. The benzylidene protection of D-threitol and the dibenzyl protection of methylphosphonate were chosen for this reason.

This synthesis afforded optically pure phosphonate **3** in satisfactory yields. It can be extended to the synthesis of α-halogenated phosphonate.

Table 1 Kinetic constants of DXR for DXP **1** and DXP_N **3**

Substrate	K_m/μM	k_{cat}/min^{-1}	(k_{cat}/K_m)/min^{-1} μM^{-1}
DXP **1**	30	740	24.7
DXP_N **3**	120	74	0.6

Kinetic studies

Incubation of DXP_N and NADPH in the presence of DXR led to the formation of $NADP^+$, as evidenced by the decrease of the NADPH absorbance at 340 nm. The enzyme seemed thus to catalyse the conversion of DXP_N **3** into MEP_N **4**, the phosphonate isostere of MEP (Scheme 1(b)). To confirm this hypothesis, the enzymatic reaction was followed by ^{31}P NMR in the presence of Na_2HPO_4 as an internal standard (Fig. 1). The ^{31}P NMR spectrum of the DXP_N **3** in the conditions of the enzymatic reaction displays a signal at 24.7 ppm (Fig. 1(a), $t = 0$). The enzyme was incubated for 24 h in the presence of DXP_N and NADPH. Bovine serum albumin was added to the reaction medium to stabilize the enzyme[21] as well as a NADPH recycling system.[22] The decrease in the intensity of the signal of the DXP_N **3** (δ 24.7 ppm) was accompanied by the concomitant increase of a novel signal at 25.8 ppm corresponding to the MEP_N **4** (Fig. 1(a), $t = 12$ h). The structural assignment for the novel reaction product was supported by the addition of synthetic MEP_N in the NMR tube, which lead to an increase of the intensity of the signal at 25.8 ppm (Fig. 1(b)). DXR catalysed not only the rearrangement of DXP_N to the phosphonate analogue of methylerythrose phosphate, but also the reduction of the latter aldehyde intermediate to MEP_N (Scheme 1). The kinetic constants (K_m and k_{cat}) of DXR for DXP and DXP_N were compared (Table 1). The increase of the K_m for DXP_N and the diminution of the rate of the enzymatic reaction led to a considerable decrease of the catalytic efficiency (40-fold) of DXR towards DXP_N.

Some compounds with C–P bonds have attracted attention owing to their antibacterial, antiviral and herbicidal activities.[23] This is essentially valid in the case of enzymes catalysing the hydrolytic cleavage of the phosphate group. The methylene phosphonate analogues, isosteric to phosphate ester substrates,

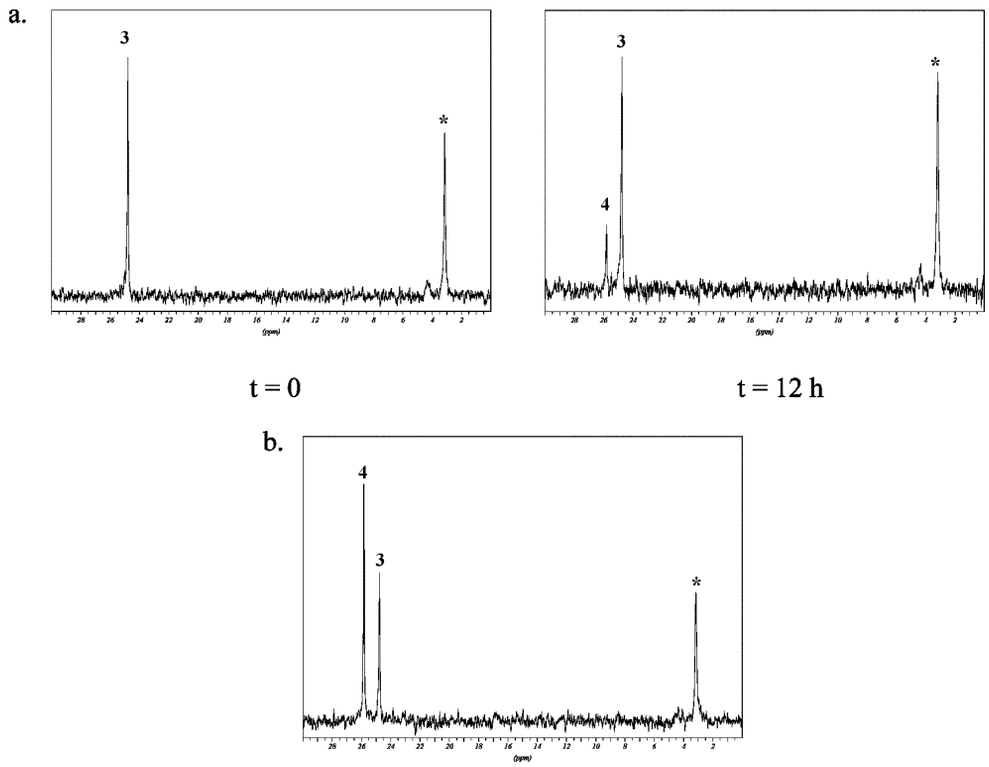


Fig. 1 ^{31}P NMR spectra of DXR enzyme assays: (a) enzymatic assays, (b) enzymatic assay + synthetic MEP_N. *Na_2HPO_4; **3**: DXP_N; **4**: MEP_N.

are often potent inhibitors, owing to the stability of the phosphonate group towards hydrolysis.[24] When the phosphate group is not directly implied in the enzymatic reaction, analogues mimicking the natural substrates are often transformed by the enzymes.[25–27] As DXP_N mimics DXP, it was not too surprising that the analogue was recognized and transformed by DXR. As reported in other studies, the efficiency of conversion is weaker with the phosphonate analogues than with the natural substrate. There are some examples where the analogue was not or almost not transformed by the parent enzyme, notably in the case of the isomerization of the phosphonomethyl analogues of glyceraldehyde 3-phosphate and of dihydroxyacetone phosphate catalysed by the triose phosphate isomerase.[26]

Several factors could contribute to the differences of the kinetic properties observed for the natural phosphate substrate and the phosphonomethyl analogues. Among them is the perturbation of the electronic properties upon the introduction of an electron-donating methylene group instead of an electronegative oxygen atom. The electronegativity loss was assessed from the pK_a value of the second deprotonation of phosphonate groups, which is one pK_a unit less acidic.[24] The non-recognition of the phosphonomethyl analogues of dihydroxyacetone phosphate by triose phosphate isomerase was attributed to the fact that the enzyme binds the substrate in the doubly ionised form and that the methylene phosphonate analogue is rather in a mono ionised form owing to the increase of the second pK_a.[25,28] To circumvent this problem, the replacement of the methylene group by a monofluoro or a difluoromethylene group was proposed to render the analogues not only isosteric but also isopolar. If ameliorations of the catalytic efficiency were reported in some cases,[26] it is not a common fact, clearly showing that factors other than phosphonate ionisation state may contribute to the differential binding of the analogues to the enzymes. For instance the bridging oxygen itself of the phosphate group may be implied in interactions with amino acid(s) of the substrate binding site. Its replacement by a methylene group could be detrimental for an ideal binding of the phosphonate analogue leading to a decrease of the rate.

The effect of DXP_N on the growth of *E. coli* was tested by the agar diffusion method. Depositing the compound on the paper discs (6 mm diameter), even at the highest amount tested (100 μg/disc) did not prevent the bacterial growth. In contrast, in the presence of fosmidomycin, a phosphonate, which is known to efficiently inhibit the DXR and to prevent the growth of *E. coli in vitro*,[9,21] multiplication of the bacteria was strongly inhibited, when only small amounts of fosmidomycin (10 μg) were applied on the paper disc (30 mm diameter growth inhibition area). Such results were not fully unexpected. Even if DXP_N is taken up by the cells, DXR has a much lower affinity for DXP_N than for DXP, and a high concentration of the phosphonate would be required to inhibit the enzyme.

Experimental

General methods

All non-aqueous reactions were run in dry solvents under an argon atmosphere. Thin-layer chromatography was performed on analytical silica gel Si 60 F_{254} silica plates (Merck) and flash chromatography on silica gel Si 60 230–400 mesh (Merck) with the indicated solvent system.[29] TLC plates were developed with an ethanol solution of *p*-anisaldehyde (2.5%), sulfuric acid (3.5%) and acetic acid (1.6%) or by an ethanol solution of phosphomolybdic acid (20%) by heating up to 100 °C. NMR spectra were recorded on a Bruker AC200, AC300 and AV300 spectrometers. NMR experiments were carried out in $CDCl_3$ or D_2O using as an internal standard $CHCl_3$ (δ = 7.26 ppm) or DHO (δ = 4.65 ppm) for ^{1}H NMR and $CDCl_3$ (δ = 77.0 ppm) for ^{13}C NMR. In the description of the NMR spectra corresponding to diastereomer mixtures, signals for different diastereomers are distinguished by a *, # or § sign added to the assignments. If only one signal is described, it is common to all diastereomers. Infrared spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer using KBr discs or in $CHCl_3$ solution in 0.2 mm pathlength NaCl cells. Optical activity was measured with a Perkin Elmer 341 polarimeter. UV spectroscopy for enzyme tests was performed on an Uvikon 933 spectrophotometer (Kontron instruments). All compounds were found to be pure by ^{1}H and ^{13}C NMR spectroscopy.

Protein concentrations were measured using the method of Bradford with bovine serum albumin as the standard.

All reagents and solvents used were reagent grade. 1-Deoxy-D-xylulose 5-phosphate was prepared chemically. A sample of (3*R*,4*R*)-3,4,5-trihydroxy-3-*C*-methylpentylphosphonate (MEP_N) **4** was synthesized in the laboratory by G. Hirsch (unpublished results).

(2*R*, 3*S*)-*O*-Benzylidene-4-*O*-*tert*-butyldimethylsilyl-D-threitol (6). To a cold (0 °C) solution of (+)-2,3-*O*-benzylidene-D-threitol **5** (3.0 g, 14.3 mmol, 1 eq.) in 1,2-dimethoxyethane (35 mL) sodium hydride (0.4 g, 16.7 mmol, 1.1 eq.) was added in portions. The mixture was stirred at 0 °C for 15 min before addition of *tert*-butyldimethylsilyl chloride (2.2 g, 14.4 mmol, 1 eq.). After 3 h, the starting material was consumed, and the reaction was quenched by addition of a saturated aqueous ammonium chloride solution (40 mL). The aqueous layer was extracted with diethyl ether (3 × 40 mL), and the combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated to give an orange oil (5.9 g). The residue was purified by flash-chromatography to afford **6** as a colourless oil (4.45 g, 96%) and a 1 : 1 mixture of two diastereomers (R_f = 0.20, ethyl acetate–hexane, 20 : 80). δ_H (200 MHz, $CDCl_3$) 0.08 (1/2 of 6H, s, 2 × CH_3), 0.10 (1/2 of 6H, s, 2 × CH_3*); 0.90 (1/2 of 9H, s, *t*-Bu), 0.92 (1/2 of 9H, s, *t*-Bu*), 2.25 (1/2 of 1H, dd, J_{1a-OH} = 5.4 Hz, J_{1b-OH} = 7.3 Hz, OH), 2.32 (1/2 of 1H, t, J_{1-OH} = 6.1 Hz, OH*), 3.69–4.18 (6H, m, 1-, 2-, 3- and 4-H), 5.96 (1/2 of 1H, s, C*H*Ph), 5.97 (1/2 of 1H, s, C*H*Ph*), 7.34–7.51 (5H, m, Ph); δ_C (50 MHz, $CDCl_3$) −5.3 (2 × CH_3), 18.4 (quaternary C, *t*-Bu), 26.0 (3 × CH_3, *t*-Bu), 62.6 (CH_2, C-1), 63.0 (CH_2, C-1*), 63.6 (CH_2, C-4), 63.7 (CH_2, C-4*), 78.2 (CH, C-2), 79.3 (CH, C-2*), 80.4 (CH, C-3), 80.7 (CH, C-3*), 103.9 (*C*HPh), 104.3 (*C*HPh*), 126.1, 126.7, 128.5, 128.6, 129.1, 129.5, 137.6 and 137.8 (aromatic C). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3591, 3447, 1602, 1462, 1381, 1256, 1225, 1093, 839. MS (FAB^+) *m*/*z*: 325.3 $(M + H)^+$. HRMS (FAB^+) *m*/*z*: calc. for $C_{17}H_{29}O_4Si$ 325.1835, found 325.1822.

(2*R*,3*S*)-*O*-Benzylidene-4-*O*-*tert*-butyldimethylsilyl-1-*O*-trifluoromethanesulfonyl-D-threitol (7). To a solution of **6** (0.4 g, 1.2 mmol, 1 eq.) and triethylamine (0.48 mL, 3.4 mmol, 2.9 eq.) in CH_2Cl_2 (15 mL) at −40 °C was added dropwise triflic anhydride (0.38 mL, 2.3 mmol, 2 eq.). The reaction mixture was stirred for 1 h at −40 °C, allowed to warm up to 0 °C and treated with a saturated aqueous solution of sodium bicarbonate solution (20 mL). The organic layer was washed with water (20 mL) and with brine (20 mL). Each aqueous layer was extracted with chloroform (3 × 20 mL). The combined extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue (0.9 g) was purified by flash chromatography to afford **7** as a colourless oil (0.50 g, 95%) and a 1 : 1 mixture of two diastereomers (R_f = 0.57, ethyl acetate–hexane, 20 : 80). Due to the instability of compound **7**, only NMR analysis were performed. δ_H (200 MHz, $CDCl_3$) 0.08 (1/2 of 6H, s, 2 × CH_3), 0.11 (1/2 of 6H, s, 2 × CH_3*), 0.90 (1/2 of 9H, s, *t*-Bu), 0.92 (1/2 of 9H, s, *t*-Bu*), 3.70–4.14 (3H, m, 2,4-H), 4.38–4.78 (3H, m, 1- and 3-H), 5.97 (1/2 of 1H, s, C*H*Ph), 6.02 (1/2 of 1H, s, C*H*Ph*), 7.33–7.51 (5H, m, Ph); δ_C (50 MHz, $CDCl_3$) −5.4 (2 × CH_3), 18.3 (quaternary C, *t*-Bu), 25.9 (3 × CH_3, *t*-Bu), 63.3 (CH_2, C-4), 63.4 (CH_2, C-4*), 75.4 (CH_2, C-1), 77.1 (CH, C-2), 77.3 (CH, C-2*), 77.8 (CH, C-3), 104.5 (*C*HPh), 105.1 (*C*HPh*), 126.7, 128.5, 129.8 and 136.7 (aromatic C).

Dibenzyl methylphosphonate

To a suspension of sodium hydride (1 g, 41.7 mmol, 1.3 eq.) in THF (35 mL) at 0 °C, was slowly added dibenzyl phosphite (7 mL, 31.5 mmol, 1 eq.) and the mixture was stirred for 1 h. To this was added iodomethane (2.7 mL, 43.4 mmol, 1.3 eq.). The solution was stirred for an additional 2 h, quenched by addition of water (30 mL) and diluted with diethyl ether (25 mL). The aqueous layer was extracted with diethyl ether (4 × 30 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated. The residue (9 g) was purified by flash chromatography to afford a colourless oil (8.49 g, 98%). (R_f = 0.32, ethyl acetate–hexane, 80 : 20). δ_H (200 MHz, $CDCl_3$) 1.47 (3H, d, J_{H-P} = 17 Hz, CH_3), 4.90–5.12 (4H, m, 2 × CH_2Ph), 7.27–7.39 (10H, m, 2 × Ph); δ_C (50 MHz, $CDCl_3$) 11.7 (CH_3, d, J_{C-P} = 144 Hz), 67.1 (CH_2); 67.2 (CH_2), 127.9, 128.4, 128.6, 136.3 and 136.4 (aromatic C); δ_P (121.5 MHz, $CDCl_3$) 29.0 (s).

Dibenzyl [(3*R*,4*S*)-*O*-benzylidene-5-*O*-*tert*-butyldimethylsilylpentyl]phosphonate (8). A solution of dry dibenzyl methylphosphonate (2 g, 7.2 mmol, 2.6 eq.) and HMPA (1.25 mL, 7.2 mmol, 2.6 eq.) in THF (10 mL) was deoxygenated by argon bubbling at −78 °C for 30 min. A solution of *n*-butyllithium (4.5 mL, 1.6 M in hexane, 7.2 mmol, 2.6 eq.) was added, and the solution turned to orange. After stirring for 30 min at −78 °C, a previously deoxygenated solution of dry triflate **7** (1.29 g, 2.8 mmol, 1 eq.) in THF (6 mL), was added *via* a cannula at −78 °C. When the starting material disappeared, the reaction was quenched with saturated aqueous ammonium chloride solution (25 mL) and diluted with diethyl ether (10 mL). The aqueous layer was further extracted with diethyl ether (4 × 25 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated. Flash chromatography afforded **8** as a colourless oil (1.14 g, 70%) and a 1 : 1 mixture of two diastereomers (R_f = 0.46, ethyl acetate–hexane, 50 : 50). δ_H (300 MHz, $CDCl_3$) 0.05 (1/2 of 6H, s, 2 × CH_3), 0.08 (1/2 of 6H, s, 2 × CH_3*), 0.88 (1/2 of 9H, s, *t*-Bu), 0.90 (1/2 of 9H, s, *t*-Bu*), 1.77–2.19 (4H, m, 1- and 2-H), 3.61–3.85 (3H, m, 3- and 5-H), 3.96–4.15 (1H, m, 4-H), 4.88–5.17 (4H, s, 2 × CH_2Ph), 5.83 (1/2 of 1H, s, C*H*Ph), 5.86 (1/2 of 1H, s, C*H*Ph*), 7.30–7.44 (15H, m, Ph); δ_C (75 MHz, $CDCl_3$) −5.3 (2 × CH_3), 18.4 (quaternary C, *t*-Bu), 22.5 (CH_2, d, $^1J_{C-P}$ = 143 Hz, C-1), 22.7 (CH_2, d, $^1J_{C-P}$ = 143 Hz, C-1*), 25.9 (3 × CH_3, *t*-Bu), 26.4 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2), 26.6 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2*), 63.3 (CH_2, C-5), 63.7 (CH_2, C-5*), 67.2 (2 × *C*H_2Ph), 67.4 (2 × *C*H_2Ph*), 79.3 (CH, d, $^3J_{C-P}$ = 18 Hz, C-3), 79.4 (CH, d, $^3J_{C-P}$ = 18 Hz, C-3*), 80.8 (CH, C-4), 81.9 (CH, C-4*), 102.9 (*C*HPh), 103.8 (*C*HPh*), 126.3, 126.7, 127.1, 127.6, 128.0, 128.4, 128.5, 128.7, 129.1, 129.4, 136.4, 136.5, 137.6 and 137.7 (aromatic C); δ_P (121.5 MHz, $CDCl_3$) 33.9 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1602, 1498, 1456, 1380, 1256, 1240, 1224, 1091, 1044, 998, 839. MS (FAB^+) *m*/*z*: 583.4 $(M + H)^+$. HRMS (FAB^+) *m*/*z*: calc. for $C_{32}H_{44}O_6SiP$ 583.2645, found 583.2649.

Dibenzyl [(3*R*,4*S*)-*O*-benzylidene-5-hydroxypentyl]phosphonate (9). To a solution of **8** (1.12 g, 1.9 mmol, 1 eq.) in THF (25 mL) was added Bu_4NF (0.90 g, 2.9 mmol, 1.5 eq.). After stirring overnight, the solvent was removed under vacuum and the residue was purified by flash chromatography to afford a colourless oil (0.88 g, 98%) as a 1 : 1 mixture of two diastereoisomers (R_f = 0.31, ethyl acetate). δ_H (300 MHz, $CDCl_3$) 1.77–2.12 (4H, m, 1- and 2-H), 3.60–3.89 (3H, m, 3- and 5-H), 4.01–4.17 (1H, m, 4-H), 4.89–5.12 (4H, s, 2 × CH_2Ph), 5.83 (1/2 of 1H, s, C*H*Ph), 5.86 (1/2 of 1H, s, C*H*Ph*), 7.33–7.43 (15H, m, Ph); δ_C (75 MHz, $CDCl_3$) 22.5 (CH_2, d, $^1J_{C-P}$ = 150 Hz, C-1), 22.7 (CH_2, d, $^1J_{C-P}$ = 150 Hz, C-1*), 25.9 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2), 26.1 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2*), 62.3 (CH_2, C-5), 62.6 (CH_2, C-5*), 67.4 (2 × *C*H_2Ph), 67.5 (2 × *C*H_2Ph*), 77.9 (CH, d, $^3J_{C-P}$ = 16 Hz, C-3), 78.5 (CH, d, $^3J_{C-P}$ = 16 Hz, C-3*), 81.2 (CH, C-4), 82.4 (CH, C-4*), 103.1 (*C*HPh), 103.7 (*C*HPh*), 126.4, 126.7, 127.7, 128.1, 128.6, 128.8, 129.1, 129.6, 136.3, 136.4 and 137.4 (aromatic C); δ_P (121.5 MHz, $CDCl_3$) 33.9 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3595, 3405, 1602, 1498, 1456, 1379, 1237, 1221, 1095, 1047, 998. MS (FAB^+) *m*/*z*: 469.2 $(M + H)^+$. HRMS (FAB^+) *m*/*z*: calc. for $C_{26}H_{30}O_6P$ 469.1780, found 469.1777.

Dibenzyl [(3*R*,4*S*)-*O*-benzylidene-5-hydroxyhexyl]phosphonate (10). To a stirred solution of oxalyl chloride (300 μL, 3.5 mmol, 7 eq.) in THF (8 mL) at −78 °C was added dimethyl sulfoxide (300 μL, 4.2 mmol, 9 eq.). The solution was allowed to warm up to −35 °C for 5 min and was cooled again to −78 °C. A solution of alcohol **9** (0.21 g, 0.5 mmol, 1 eq.) in THF (4 mL) was then added to the reaction mixture *via* a cannula. The resulting solution was allowed to warm up to −35 °C and after 15 min was treated with triethylamine (0.5 mL, 3.6 mmol, 8 eq.). The reaction mixture was allowed to warm up to room temperature for 1 h and was then cooled to −78 °C. A 3 M diethyl ether solution of methyl magnesium chloride (0.4 mL, 1.2 mmol, 2.7 eq.) was then added dropwise. The reaction was followed by TLC (ethyl acetate) until the aldehyde had completely disappeared. The solution was diluted with ethanol (10 mL), saturated aqueous ammonium chloride solution (20 mL), water (5 mL) and diethyl ether (10 mL). The aqueous phase was extracted with diethyl ether (4 × 25 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The yellow residue (0.30 g) was purified by flash chromatography to afford **10** as a colourless oil (186 mg, 86%) and a 14 : 14 : 4 : 1 mixture of four diastereomers (R_f = 0.42, ethyl acetate). δ_H (300 MHz, $CDCl_3$) 1.18 (1/33 of 3H, d, J_{5-6} = 6.4 Hz, 6-H), 1.19 (14/33 of 3H, d, J_{5-6} = 6.4 Hz, 6-H*), 1.24 (14/33 of 3H, d, J_{5-6} = 6.4 Hz, 6-H§), 1.25 (4/33 of 3H, d, J_{5-6} = 6.4 Hz, 6-H#), 1.75–2.18 (4H, m, 1- and 2-H), 3.54–3.64 (1H, m, 5-H), 3.88–3.98 (1H, m, 4-H), 4.16–4.25 (1H, m, 3-H), 4.91–5.11 (4H, s, 2 × CH_2–Ph), 5.79 (14/33 of 1H, s, C*H*Ph), 5.81 (4/33 of 1H, s, C*H*Ph*), 5.83 (14/33 of 1H, s, C*H*Ph§), 5.86 (1/33 of 1H, s, C*H*Ph#), 7.30–7.46 (15H, m, Ph); δ_C NMR (75 MHz, $CDCl_3$) 19.2 (CH_3, C-6), 19.8 (CH_3, C-6*), 22.5 (CH_2, d, $^1J_{C-P}$ = 142 Hz, C-1), 22.8 (CH_2, d, $^1J_{C-P}$ = 142 Hz, C-1*), 27.0 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2), 27.1 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2*), 67.3 (2 × *C*H_2Ph), 67.4 (2 × *C*H_2Ph*), 67.5 (2 × *C*H_2Ph§), 67.6 (CH, C-5), 68.1 (CH, C-5*), 77.7 (CH, d, $^3J_{C-P}$ = 20 Hz, C-3), 77.8 (CH, d, $^3J_{C-P}$ = 20 Hz, C-3*), 79.0 (CH, d, $^3J_{C-P}$ = 20 Hz, C-3§), 79.1 (CH, d, $^3J_{C-P}$ = 20 Hz, C-3#), 84.3 (CH, C-4), 85.7 (CH, C-4*), 102.6 (*C*HPh), 103.7 (*C*HPh*), 126.7, 128.0, 128.1, 128.5, 128.6, 128.7, 129.6, 129.7, 136.3, 136.4, 137.2 and 137.7 (aromatic C); δ_P (121.5 MHz, $CDCl_3$) 33.8 (s), 33.9 (s), 34.3 (s) and 34.4 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3598, 3408, 1602, 1498, 1456, 1380, 1236, 1225, 1090, 1047, 998. MS (FAB^+) *m*/*z*: 483.3 $(M + H)^+$. HRMS (FAB^+) *m*/*z*: calc. for $C_{27}H_{32}O_6P$ 483.1937, found 483.1941.

Dibenzyl [(3*R*, 4*S*)-*O*-benzylidene-5-oxohexyl]phosphonate (11). To a solution of the diastereomeric alcohols **10** (75 mg, 0.16 mmol, 1 eq.) in dichloromethane (5 mL) were added activated 4 Å molecular sieves (0.17 g), *N*-methylmorpholine *N*-oxide (37 mg, 0.27 mmol, 1.7 eq.) and solid TPAP (4 mg, 0.01 mmol, 0.07 eq.). The mixture was stirred at room temperature and monitored by TLC (ethyl acetate) until the starting material completely disappeared. On completion, the reaction mixture was filtered through a pad of silica on a sintered-glass funnel. The solid cake was washed with ethyl acetate, and the filtrate was evaporated. The residue (60 mg) was purified by flash chromatography to afford **11** as a colourless oil (57 mg, 79%) and a 1 : 1 mixture of two diastereomers (R_f = 0.46, ethyl acetate). δ_H (300 MHz, $CDCl_3$) 1.18–2.15 (4H, m, 1- and 2-H), 2.21 (1/2 of 3H, s, 6-H), 2.29 (1/2 of 3H, s, 6-H*), 4.01 (1/2 of 1H, d, J_{3-4} = 7 Hz, 4-H), 4.04 (1/2 of 1H, d, J_{3-4} = 6 Hz, 4-H*), 4.05–4.17 (1H, m, 3-H), 4.92–5.11 (4H, s, 2 × CH_2Ph), 5.86 (1/2 of 1H, s, C*H*Ph), 5.89 (1/2 of 1H, s, C*H*Ph*), 7.32–7.48 (15H, m, Ph); δ_C (75 MHz, $CDCl_3$) 22.3 (CH_2, d, $^1J_{C-P}$ = 143 Hz, C-1), 22.6 (CH_2, d, $^1J_{C-P}$ = 143 Hz, C-1*), 26.6 (CH_3, C-6), 26.9 (CH_3, C-6*), 26.7 (CH_2, d, $^2J_{C-P}$ = 4 Hz, C-2), 67.4 (2 × *C*H_2Ph), 67.5 (2 × *C*H_2Ph*), 78.9 (CH, d, $^3J_{C-P}$ = 22 Hz, C-3), 79.0 (CH, d, $^3J_{C-P}$ = 22 Hz, C-3*), 84.7 (CH, C-4), 85.9 (CH, C-4*), 103.8 (*C*HPh), 104.8 (*C*HPh*), 126.7, 126.8, 128.1, 128.8, 129.8, 129.9, 136.2, 136.4, 136.5 and 136.6 (aromatic C), 207.4 (quaternary C, C-5), 208.5 (quaternary C, C-5*); δ_P (121.5 MHz, $CDCl_3$) 33.2 (s) and 33.3 (s). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1718, 1657, 1598, 1498, 1456, 1407, 1378, 1359, 1244, 1225, 1094, 998. MS (FAB^+) *m*/*z*: 481.2 $(M + H)^+$. HRMS (FAB^+) *m*/*z*: calc. for $C_{27}H_{30}O_6P$ 481.1780, found 481.1780.

(3*R*,4*S*)-3,4-Dihydroxy-5-oxohexylphosphonic acid (3). The protected DXP_N **11** (90 mg, 0.19 mmol) was hydrogenated over 15% Pd/C (14 mg) in MeOH–H_2O (95 : 5, 10 mL) for 1 h at room temperature and atmospheric pressure. The mixture was filtered through Celite and the filtrate was concentrated. The residue (39 mg) was dissolved in water (1 mL) and the solution was neutralized by addition of 1 M sodium hydroxide. The solution of the monosodium salt of DXP_N **3** was lyophilised to give a colourless vitrous solid, which decomposed at 120 °C. $[a]_D^{20}$ = + 37 (*c* 1.8, H_2O). δ_H (300 MHz, D_2O) 1.51–1.89 (4H, m, 1- and 2-H), 2.26 (3H, s, 6-H), 4.08–4.18 (1H, m, 4-H), 4.31–437 (1H, m, 3-H); δ_C (75 MHz, D_2O) 24.8 (CH_2, d, $^1J_{C-P}$ = 137 Hz, C-1), 25.7 (CH_3, C-6), 27.3 (CH_2, s, C-2), 71.9 (CH, d, $^3J_{C-P}$ = 18 Hz, C-3), 78.9 (CH, s, C-4), 213.2 (quaternary C, C-5); δ_P (121.5 MHz, D_2O) 25.9 (s). IR (KBr) ν_{max}/cm^{-1} : 3409, 1714, 1651, 1417, 1354, 1146, 1118, 1064. MS (ES^-) *m*/*z*: 211 $(M - H^+)$.

Purification of 1-deoxy-D-xylulose 5-phosphate reductoisomerase

E. coli cells transformed with a plasmid bearing the *dxr* gene[15] were grown in a LB medium containing ampicillin (0.1 mg/mL). DXS expression was induced by the addition of IPTG to a final concentration of 0.4 mM at an $OD_{600\ nm}$ of 0.6. Cells were grown for an additional 3 h at 37 °C and then harvested by centrifugation (4.2 g, cell paste from a 1.5 L culture). Cells were disrupted at 0 °C by sonication (40 W, 8 × 30 s, with 30 s rest period) in a 50 mM Tris/HCl buffer (pH 7.5) containing 2 mM DTT and 0.5 mM PMSF. After centrifugation (20000 rpm, 20 min), the supernatant was applied on a Sepharose Q (Amersham Biosciences) column (2 × 10 cm) pre-equilibrated with the lysis buffer. After washing with 50 mM Tris/HCl buffer (pH 7.5) containing 50 mM NaCl, 2 mM DTT and 0.5 mM PMSF until the OD_{280nm} decreased to about 0.1, the enzyme was eluted by applying a linear gradient of NaCl (50 to 500 mM) in 50 mM Tris/HCl buffer (pH 7.5) containing 2 mM DTT and 0.5 mM PMSF (total volume 200 mL). Fractions containing DXR activity were pooled and dialysed against 2 × 1 L 25 mM imidazole, 2 mM DTT buffer pH 7.2. After centrifugation to discard precipitated proteins, the enzymatic solution was applied on a PBE94 column (2 × 11 cm) equilibrated with the dialysis buffer. After washing with the same buffer (50 mL), the pH gradient was started by applying a Polybuffer 74 solution (1/8 dilution, pH 4.0, volume 450 mL). Fractions containing DXR activity (pH fractions 4.8–5.0) were >90% pure as shown by PAGE/SDS and Coomassie Blue coloration. These fractions were pooled and dialysed against 50 mM Tris/HCl–2 mM DTT buffer (pH 8.0) by ultrafiltration on Centricon 30 units. The enzymatic solution was diluted 4-fold with buffer before the centrifugation to ensure an efficient elimination of Polybuffer. The enzymatic solution was then applied on a 2′5′ ADP Sepharose 4B column (0.8 × 2.5 cm) equilibrated with a 25 mM Tris/HCl–100 mM NaCl–DTT 2 mM buffer (pH 8.0). After washing with the equilibration buffer (8 mL), the enzyme was eluted by applying a gradient of NADPH (0 to 3 mM, total volume 16 mL). Fractions containing DXR activity were pooled, concentrated and dialyzed against 50 mM Tris/HCl–2 mM DTT buffer (pH 7.5) by ultrafiltration on Centricon 30 units. As shown by PAGE/SDS and Coomassie Blue coloration the enzyme was pure at this stage.

Determination of the DXR activity

Standard assays were routinely performed at 37 °C in 50 mM Tris/HCl buffer (pH 7.5) containing 1 mM $MgCl_2$ and 2 mM

DTT (total volume 1 mL) in the presence of 0.2 mM NADPH and 3 mM DXP. The enzymatic activity was monitored by following the decrease of the absorbance at 340 nm due to the oxidation of the cofactor. The reaction was initiated by the addition of enzyme.

To determine the kinetic constants (K_m and V_{max}) for DXP and DXP_N, assays were performed in duplicate at six different concentrations of the variable substrate, DXP (40 to 200 μM) and DXP_N (100 to 500 μM). The reaction was initiated by the addition of enzyme, 4 μg with DXP as the substrate or 6 μg with DXP_N. The graphical representation of Lineweaver–Burk was used to determine the kinetic constants.

Identification of the reaction product of DXP_N with DXR by ^{31}P NMR

The reaction was performed in a 50 mM Tris/HCl buffer (pH 7.5) containing 1 mM $MgCl_2$, 2 mM DTT in the presence of 3 mM DXP_N and 0.4 mM NADPH. The reaction volume was 600 μL. The medium contained BSA (600 μg) to increase the stability of DXR,[21] 3 mM Na_2HPO_4 as an internal standard, and D_2O (150 μL) to lock the spectrometer. The regeneration of NADPH was realized by adding 3 mM L-glutamate and glutamate dehydrogenase.[22] DXR (30 μg) was added to initiate the enzymatic reaction. A ^{31}P NMR spectrum was taken after an incubation of 12 h at 37 °C. A sample of MEP_N (3 mM final concentration) was added to the NMR tube, and the spectrum was taken again. Blank experiments were performed with DXP_N and MEP_N in the same medium but in the absence of enzymes.

Influence of DXP_N on *E. coli* growth

The susceptibility of *E. coli* cells to DXP_N was tested by the agar diffusion method. LB agar plates (5 cm diameter) were inoculated with bacteria (100 μL, OD_{600} = 0.68, 5×10^7 cells). Whatman No. 1 paper discs of 6 mm diameter were impregnated with DXP_N (50 and 100 μg). The dried discs were then placed on the agar surface. The plates were incubated overnight at 30 °C. Assays with fosmidomycin (10 to 50 μg) were performed. Antibacterial activity was indicated by the presence of clear growth inhibition zones around the discs.

Acknowledgements

We thank Mr J.-D. Sauer for all NMR measurements, Mr R. Huber for the MS analysis and Dr Eilers (Saintt Louis, Missouri) for the gift of fosmidomycin. This investigation was supported by a grant to M. R. from the 'Institut Universitaire de France'. O. M. was a recipient of a grant from the 'Ministère de la Jeunesse, de l'Education Nationale et de la Recherche'.

References

1 K. Bloch, *Steroids*, 1992, **57**, 378–383; D. A. Bochar, J. A. Friesen, C. V. Stauffacher and V. W. Rodwell, in *Comprehensive Natural Product Chemistry. Isoprenoids Including Carotenoids and Steroids*, ed. D. E. Cane, Pergamon, Oxford UK, 1999, vol. 2, pp. 15–44.
2 M. Rohmer, *Nat. Prod. Rep.*, 1999, **16**, 565–574; H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Türbachova, M. Eberl, J. Zeidler, H. K. Lichtenthaler, D. Soldati and E. Beck, *Science*, 1990, **285**, 1573–1576; H. K. Lichtenthaler, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1999, **50**, 47–65; W. Eisenreich, F. Rohdich and A. Bacher, *Trends Plant Sci.*, 2001, **6**, 78–84.
3 M. Rohmer, *Prog. Drug. Res.*, 1998, **5**, 135–154; M. Rohmer, C. Grosdemange-Billiard, M. Seemann and D. Tritsch, *Curr. Opin. Invest. Drugs*, in press.
4 G. A. Sprenger, W. T. Schörken, S. Grolle, A. de Graaf, S. V. Taylor, T. P. Begley, S. Bringer-Meyer and H. Sahm, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 12857–12862; B. M. Lange, M. R. Wildung, D. McCaskill and R. Croteau, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 2100–2104; L. M. Lois, N. Campos, S. Rosa-Putra, K. Danielsen, M. Rohmer and A. Boronat, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 2105–2110.
5 S. Takahashi, T. Kuzuyama, W. Watanabe and H. Seto, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 9879–9884.
6 F. Rohdich, J. Wungsintaweekul, M. Fellermeier, S. Sagner, S. Hertz, K. Kis, W. Eisenreich and A. Bacher, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 11758–11763; T. Kuzuyama, M. Takagi, K. Kaneda, T. W. Dairi and H. Seto, *Tetrahedron Lett.*, 2000, **41**, 703–706; H. Lüttgen, F. Rohdich, S. Herz, J. Wungsintaweekul, S. Hecht, C. A. Schuhr, M. Fellermeier, S. Sagner, M. H. Zenk, A. Bacher and W. Eisenreich, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 1062–1067; S. Hertz, J. Wungsintaweekul, S. Hecht, H. Lüttgen, M. Fellermeier, S. Sagner, M. H. Zenk, A. Bacher and W. Eisenreich, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2486–2490.
7 S. Hecht, W. Eisenreich, P. Adam, S. Amslinger, K. Kis, A. Bacher, D. Arigoni and F. Rohdich, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 14837–17842; M. Wolff, M. Seemann, C. Grosdemange-Billiard, D. Tritsch, N. Campos, M. Rodriguez-Conception, A. Boronat and M. Rohmer, *Tetrahedron Lett.*, 2002, **43**, 2555–2559; M. Hintz, A. Reichenberg, B. Altincicek, U. Bahr, R. M. Gschwind, A. K. Kollas, E. Beck, J. Wiesner, M. Eberl and H. Jomaa, *FEBS Lett.*, 2001, **509**, 317–322; F. Rohdich, S. Hecht, K. Gärtner, P. Adam, C. Krieger, S. Amslinger, D. Arigoni, A. Bacher and W. Eisenreich, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 1158–1163; M. Seemann, B. Tse Sum Bui, M. Wolff, D. Tritsch, N. Campos, A. Boronat, A. Marquet and M. Rohmer, *Angew. Chem., Int. Ed.*, 2002, **41**, 4337–4339.
8 B. Altincicek, E. C. Duin, A. Reichenberg, R. Hedderich, A. K. Kollas, M. Hintz, S. Wagner, J. Wiesner, E. Beck and H. Jomaa, *FEBS Lett.*, 2002, **532**, 432–436; F. Rohdich, F. Zepeck, P. Adam, S. Hecht, J. Kaiser, R. Laupitz, T. Gräwert, S. Amslinger, W. Eisenreich, A. Bacher and D. Arigoni, *Proc. Natl. Acad. Sci. USA*, 2003, **1007**, 1586–1591; M. Wolff, M. Seemann, B. Tse Sum Bui, Y. Frapart, D. Tritsch, A. Garcia Estrabot, M. Rodríguez-Concepción, A. Boronat, A. Marquet and M. Rohmer, *FEBS Lett.*, 2003, **541**, 115–120.
9 T. Kuzuyama, T. Shimizu, S. Takahashi and H. Seto, *Tetrahedron Lett.*, 1998, **39**, 7913–7916.
10 S. Grolle, S. Bringer-Meyer and H. Sahm, *FEMS Microbiol. Lett.*, 2000, **191**, 131–137.
11 J. Zeidler, J. Schwender, C. Müller, J. Wiesner, C. Weidemeyer, E. Beck, H. Jomaa and H. K. Lichtenthaler, *Z. Naturforsch., Teil C*, 1998, **53**, 980–986; M. Fellermeier, K. Kis, S. Sagner, U. Maier, A. Bacher and M. Zenk, *Tetrahedron Lett.*, 1999, **40**, 2743–2746.
12 A. Reichenberg, J. Wiesner, C. Weidemeyer, E. Dreiseidler, S. Sanderbrand, B. Altincicek, E. Beck, M. Schlitzer and H. Jomaa, *Bioorg. Med. Chem Lett.*, 2001, **11**, 833–835.
13 M. A. Missinou, S. Borrmann, A. Schindler, S. Issifou, A. A. Adegnika, P.-B. Matsiegui, R. Binder, B. Lell, J. Wiesner, T. Baranek, H. Jomaa and P. G. Kremsner, *Lancet*, 2002, **360**, 1941–1942.
14 S. Steinbacher, J. Kaiser, W. Eisenreich, R. Huber, A. Bacher and F. Rohdich, *J. Biol. Chem.*, 2003, **278**, 18401–18407.
15 J. F. Hoeffler, D. Tritsch, C. Grosdemange-Billiard and M. Rohmer, *Eur. J. Biochem.*, 2002, **269**, 4446–4457.
16 B. Altincicek, M. Hintz, S. Sanderbrand, J. Wiesner, E. Beck and H. Jomaa, *FEMS Microbiol. Lett.*, 2000, **190**, 329–333.
17 C. Mueller, J. Schwender, J. Zeidler and H. K. Lichtenthaler, *Biochem. Soc. Trans.*, 2000, **28**, 792–793.
18 D. B. Berkowitz, M. Bose, T. J. Pfannenstiel and T. Doukov, *J. Org. Chem.*, 2000, **65**, 4498–4508.
19 R. Ireland and D. W. Norbeck, *J. Org. Chem.*, 1985, **50**, 2198–2000.
20 W. P. Griffith and S. V. Ley, *Aldrichim. Acta*, 1990, **23**, 13–19.
21 A. T. Koppisch, D. T. Fox, B. S. J. Blagg and C. D. Poulter, *Biochemistry*, 2002, **41**, 236–243.
22 L. G. Lee and G. M. Whitesides, *J. Am. Chem. Soc.*, 1985, **107**, 6999–7008.
23 R. L. Hilderbrand and T. O. Henderson, in *The Role of Phosphonates in Living Systems*, ed. R. L. Hilderbrand, CRC Press Inc., Boca Raton, FL, 1983, pp. 5–29.
24 R. Engel, *Chem. Rev.*, 1977, **77**, 349–367.
25 J. Nieschalk, A. S. Batsanov, D. O'Hagan and J. A. K. Howard, *Tetrahedron*, 1995, **52**, 165–176.
26 D. B. Berkowitz and M. Bose, *J. Fluorine Chem.*, 2001, **112**, 13–33.
27 H.-L. Arth, G. Sinerius and W.-D. Fessner, *Liebigs Ann.*, 1995, 2037–2042.
28 H. B. F. Dixon and M. J. Sparkes, *Biochem. J.*, 1974, **141**, 715–719; J. G. Belasco, J. M. Herlihy and J. R. Knowles, *Biochemistry*, 1978, **17**, 2971–2978.
29 W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.