Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 519–521
Tetrahedron Letters

(3*R*,4*S*)-3,4,5-Trihydroxy-4-methylpentylphosphonic acid, an isosteric phosphonate analogue of 2-*C*-methyl-D-erythritol 4-phosphate, a key intermediate in the new pathway for isoprenoid biosynthesis

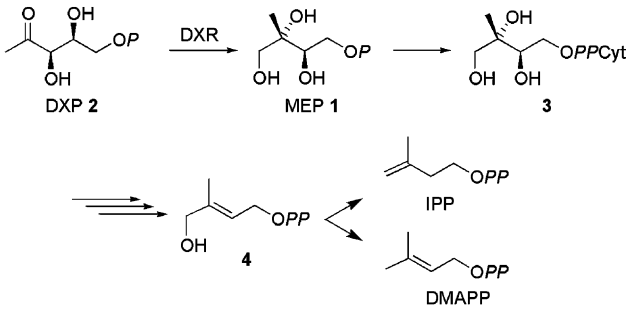
Guillaume Hirsch, Catherine Grosdemange-Billiard, Denis Tritsch and Michel Rohmer*

CNRS UMR 7123, Institut Le Bel, Université Louis Pasteur, 4 rue Blaise Pascal, 67070 Strasbourg Cedex, France

Received 17 September 2003; revised 30 October 2003; accepted 3 November 2003

Abstract—2-*C*-Methyl-D-erythritol 4-phosphate (MEP) is the first intermediate in the mevalonate-independent pathway for isoprenoid biosynthesis presenting the branched C_5 isoprene skeleton. Enantiopure (3*R*,4*S*)-3,4,5-trihydroxy-4-methylpentylphosphonic acid (MEP_N), an isosteric phosphonate analogue of MEP was synthesized from 1,2-*O*-isopropylidene-α-D-xylofuranose.

Recently an alternative metabolic route, the methylerythritol phosphate (MEP, **1**) pathway (Scheme 1), was discovered for the formation of isoprenoids in many bacteria, green algae and plastids from plants and other algal phyla.[1] The presumed first committed step in the MEP pathway is the conversion of 1-deoxy-D-xylulose 5-phosphate (DXP, **2**) to 2-*C*-methyl-D-erythritol 4-phosphate **1**.[2] In later steps, MEP is coupled with cytidine triphosphate to produce 4-diphosphocytidyl 2-*C*-methyl-D-erythritol 4-phosphate **3**,[3] which is subsequently phosphorylated,[4] cyclized and converted into (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate **4**, which is the last intermediate of the pathway.[5]



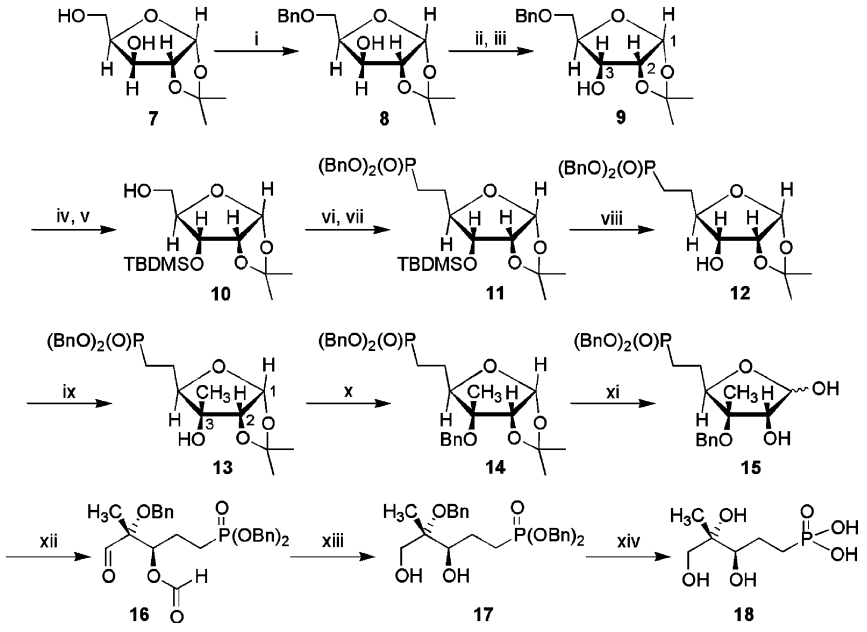
Scheme 1. Methylerythritol phosphate pathway for isoprenoid biosynthesis.

Animals and humans synthesize their isoprenoids only via the MVA pathway, with no evidence for the presence of the MEP pathway. Each enzyme of the MEP pathway may thus represent an attractive target for the development of new biocides, which are of major interest in the present situation of bacterial resistance towards antibiotics. A first application is pointed out by the DXP reducto-isomerase (DXR, Scheme 1) inhibitor, the antibiotic fosmidomycin, which is effective against bacteria and *Plasmodium* spp., the parasite responsible for malaria, utilizing the MEP pathway.[6] We decided to synthesize (3*R*,4*S*)-3,4,5-trihydroxy-4-methylpentylphosphonic acid (MEP_N, **18**, Scheme 2), an analogue of MEP **1** that may act as an inhibitor of some of the MEP pathway enzymes. In this analogue the phosphate group of MEP is replaced by an isosteric phosphonate group.[7] In addition, the chirality of the carbon chain and its functionalization are the same in MEP_N **18** as those in MEP, the natural intermediate in the terpenoid biosynthetic pathway.

An efficient chemical synthesis of MEP from 1,2-*O*-isopropylidene-α-D-xylofuranose **7** was developed in our laboratory.[8] The synthesis of the MEP analogue, MEP_N

Keywords: Biosynthesis; Isoprenoids; 2-*C*-Methyl-D-erythritol 4-phosphate; Phosphonate; 1-Deoxy-D-xylulose reducto-isomerase; 2-*C*-Methyl-D-erythritol 4-phosphate cytidyltransferase.
* Corresponding author. Tel.: +33-3-90-24-13-49; fax: +33-3-90-24-13-45; e-mail: mirohmer@chimie.u-strasbg.fr

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.11.001



Scheme 2. Synthesis of (3*R*,4*S*)-3,4,5-trihydroxy-4-methylphosphonic acid MEP_N **18**: (i) $(Bu_3Sn)_2O$, Dean–Stark, BnBr, TBAB, toluene, 90 °C, 3 d (70%); (ii) $(COCl)_2$, DMSO, TEA; (iii) $NaBH_4$, MeOH, 0 °C (42% over two steps); (iv) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt (90%); (v) H_2, Pd/C, AcOEt, 6 d (98%); (vi) Tf_2O, TEA, CH_2Cl_2, −40 °C; (vii) $CH_3P(O)(OBn)_2$, *n*-BuLi, HMPA, THF, −78 °C; (viii) TBAF, THF, rt (67% over three steps); (ix) $(COCl)_2$, DMSO, TEA, then MeMgCl, THF (68%); (x) $CCl_3C(NH)OBn$, CF_3SO_3H (cat.), CH_2Cl_2/cyclohexane (1/2), rt (73%); (xi) TFA 90%, −15 °C (65%); (xii) $NaIO_4$, MeOH/H_2O (1/1), rt (97%); (xiii) $NaBH_4$, MeOH, −15 °C, (69%); (xiv) H_2, Pd/C, EtOH (92%).

18, was therefore performed, partly using the strategy based on these previous results.

Our first attempts to introduce the phosphonate group on an already 3-*C*-methylated 1,2-isopropylidene-α-D-ribofuranose or a xylofuranose derivative were unsuccessful, most probably due to steric hindrance.[9] The strategy was therefore changed, and the phosphonate group was introduced to a 1,2-isopropylidene-α-D-ribofuranose derivative with an unhindered β-face and the 3-*C* position was methylated afterwards. The required 1,2-*O*-isopropylidene-α-D-ribofuranose derivative was not commercially available and was synthesized from the 1,2-*O*-isopropylidene-α-D-xylofuranose **7**.

The first step of the synthetic route is a regioselective protection of the primary hydroxyl group with bistributyltin oxide and benzyl bromide[10] to give the 5-*O* benzylated species **8** in 70% yield. Swern oxidation of the free alcohol gave the corresponding ketone, which was stereoselectively reduced by sodium borohydride[10] to the corresponding ribose derivative **9**. The reduction selectively occurred on the furanose β-face, which is more accessible to the reagent than the α-face, which is hindered by the isopropylidene protecting group. NOESY experiments showed a correlation between H-1 and H-2, which are located on the β-face in α-D-xylose derivatives, and between H-3 and both H-1 and H-2, indicating that H-3 is also located on the β-face and confirming the stereochemistry. Protection of the secondary hydroxyl group using *t*-butyldimethylsilyl trifluoromethanesulfonate[11] followed by hydrogenolysis of the benzyl group yielded 1,2-*O*-isopropylidene-3-*O*-*t*-butyldimethylsilyl-α-D-ribofuranose **10**. The key step of this approach was the introduction of the phosphonate on ribofuranose derivative **10** using Berkowitz's methodology.[9] After activation of the primary hydroxyl by conversion into a triflate and displacement by the dibenzylmethylphosphonate anion,[12] the 1,2-*O*-isopropylidene-3-*O*-*t*-butyldimethylsilyl-5,6-deoxy-6-dibenzylphosphono-α-D-allofuranose **11** was generated in satisfactory yield. The silyl protecting group was removed in the presence of tetrabutylammonium fluoride. Oxidation of the secondary alcohol **12** was performed in THF using the Swern oxidation as modified by Ireland.[13] Under these conditions, the methyl group was directly introduced by addition of methylmagnesium chloride without isolation of the intermediate ketone to yield the tertiary alcohol **13**. Methylation occurred on the less hindered β-face, and the stereochemistry was verified by NOESY NMR correlations. The NOE between H-1 and H-2 confirmed the anomeric α-configuration and the position of the two protons on the β-face of the five-membered ring. The position of the methyl group at C-3 on the β-face of the furanose ring was supported by a strong NOE between H-1, H-2 and the protons of the C-3 methyl group. Benzylation with benzyl-2,2,2-trichloroacetimidate[14] yielded the protected tertiary alcohol 1,2-*O*-isopropylidene-3-*C*-methyl-*O*-benzyl-5,6-deoxy-6-dibenzylphosphono-α-D-allofuranose **14**. The acetonide protecting group was removed with 90% aqueous trifluoroacetic acid solution. The resulting mixture of the two anomers of the hemiketal **15** (α/β, 85:15, based on ^1H NMR integration of the signals of the anomeric proton H-1) underwent sodium metaperiodate oxidative glycol cleavage into the (3*R*,4*R*)-dibenzyl-3-formyloxy-4-methyl-*O*-benzyl-5-oxopentylphosphonate **16**, which was reduced using sodium borohydride to afford the

benzylated phosphonate **17**.[15] Hydrogenolysis of the benzyl protecting groups yielded free MEP_N, (3*R*,4*S*)-3,4,5-trihydroxy-4-methylpentylphosphonic acid **18**.[16]

Enantiopure MEP_N **18**, an isosteric phosphonate analogue of MEP, was synthesized in 12 steps in an overall yield of 6%. The influence of MEP_N **18** on the growth of *Escherichia coli* was tested by the agar diffusion method on LB agar plates (5 cm diameter) inoculated with a bacteria suspension (100 μL, 5×10^7 cells, exponential growth phase). No growth inhibition zone was observed around 6 mm Whatman No. 1 paper disks in the presence of MEP_N (50 or 100 μg), whereas the presence of fosmidomycin (10 μg), a strong inhibitor of the DXP reducto-isomerase, induced the formation of a clear growth inhibition zone. MEP_N, like DXP_N, also had no effect on the activity of the DXP isomero-reductase from *E. coli*. The DXP reducto-isomerase converted, however, DXP_N into MEP_N, which was identified by comparison of ^{31}P NMR spectra with the synthetic sample.[17] The reaction catalyzed by this enzyme is reversible.[18] Using the same enzyme test and ^{31}P NMR spectroscopy for the characterization of the products,[17] we showed that MEP_N is converted in DXP_N by the DXP reducto-isomerase. Additional tests with other MEP pathway enzymes, such as 2-*C*-methyl-D-erythritol 4-phosphate cytidyltransferase, will follow to investigate if MEP_N can act as an inhibitor of this biosynthetic pathway.

Acknowledgements

We thank Mr. J.-D. Sauer for the 2D NMR measurements and Mr. R. Huber for the MS analyses. This investigation was supported by a grant to M.R. from the 'Institut Universitaire de France'. G.H. thanks the 'Ministère de la Jeunesse, de l'Education Nationale et de la Recherche' for financial support.

References and Notes

1. Rohmer, M. In *Comprehensive Natural Product Chemistry, Isoprenoids Including Steroids and Carotenoids*; Cane, D. E., Ed.; Pergamon, 1999; Vol. 2, Chapter 2, pp 45–68.
2. Takahashi, S.; Kuzuyama, T.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9879–9884.
3. Rohdich, F.; Wungsintaweekul, J.; Fellermeister, M.; Sagner, S.; Herz, S.; Kis, K.; Eisenreich, W.; Bacher, A.; Zenk, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11758–11763.
4. Lüttgen, H.; Rohdich, F.; Herz, S.; Wungsintaweekul, J.; Hecht, S.; Schuhr, C. A.; Fellermeister, M.; Sagner, S.; Zenk, M.; Bacher, A.; Eisenreich, W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1062–1067.
5. Wolff, M.; Seemann, M.; Grosdemange-Billiard, C.; Tritsch, D.; Campos, N.; Rodríguez-Concepción, M.; Boronat, A.; Rohmer, M. *Tetrahedron Lett.* **2002**, *43*, 2555–2559.
6. Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Türbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H. K.; Soldati, D.; Beck, E. *Science* **1999**, *285*, 1573–1576.
7. Steinbacher, S.; Kaiser, J.; Eisenreich, W.; Huber, R.; Bacher, A.; Rohdich, F. *J. Biol. Chem.* **2003**, *278*, 18401–18407.
8. Hoeffler, J.-F.; Pale-Grosdemange, C.; Rohmer, M. *Tetrahedron* **2000**, *56*, 1485–1489.
9. Berkowitz, D. B.; Bhuniya, D.; Peris, G. *Tetrahedron Lett.* **1999**, *40*, 1869–1872.
10. Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C. H. *J. Am. Chem. Soc.* **1998**, *120*, 1965–1978.
11. Emery, F.; Vogel, P. *J. Org. Chem.* **1995**, *60*, 5843–5854.
12. Stamm, H.; Gerster, G.; Baumann, T. *Chem. Ber.* **1983**, *116*, 2936–2957.
13. Ireland, R. E.; Norbeck, D. W. *J. Org. Chem.* **1985**, *50*, 2198–2200.
14. Iversen, T.; Bundle, D. R. *J. Chem. Soc., Chem. Commun.* **1981**, 1240–1241.
15. ^1H NMR (300 MHz, $CDCl_3$ + 1 drop D_2O): $\delta = 1.11$ (3H, s, CH_3), 1.64 (1H, m), 1.86 (2H, m), 2.05 (1H, m), 3.66 and 3.72 (2×1H, 2d, $^2J_{Ha,Hb} = 11.9$ Hz, 4-C benzylic CH_2), 3.73 (1H, dd, $^3J_{3,H\text{-}4a} = 10.4$ Hz, $^3J_{3,H\text{-}4b} = 1.5$ Hz, 3-H), 4.49 (2H, s, 5-H), 4.96 and 5.06 (2×1H, 2dd, $^2J_{Ha,Hb} = 11.8$ Hz, $^3J_{Ha,P} = 8.2$ Hz, $^3J_{Hb,P} = 9.1$ Hz, phosphonate benzylic ester CH_2), 4.96 and 5.07 (2×1H, 2dd, $^2J_{Ha,Hb} = 11.8$ Hz, $^3J_{Ha,P} = 8.2$ Hz, $^3J_{Hb,P} = 9$ Hz, phosphonate benzylic ester CH_2), 7.32 (15H, m, 3Ph). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 16.0$ (CH_3), 23.5 (CH_2, $^1J_{C,P} = 137.5$ Hz), 24.4 (CH_2, $^2J_{C,P} = 5.5$ Hz), 64.2 (CH_2), 65.2 (CH_2), 67.4 (CH_2, $^2J_{C,P} = 6.2$ Hz), 67.5 (CH_2, $^2J_{C,P} = 6.2$ Hz), 75.4 (CH, $^1J_{C,P} = 12.9$ Hz), 79.1 (quaternary C), 127.7, 128.1, 128.6 and 128.7 (15 aromatic CH), 136.4 (2 aromatic quaternary C, $^3J_{C,P} = 6.2$ Hz), 138.9 (aromatic quaternary C). ^{31}P NMR (121 MHz, $CDCl_3$): $\delta = 34.7$ (s). HRMS (FAB^+): calcd for $C_{27}H_{34}O_6P$ $(M+H)^+$, $m/z = 485.2093$; found, $m/z = 485.2099$.
16. ^1H NMR (300 MHz, D_2O): $\delta = 1.10$ (3H, s, CH_3), 1.40 (2H, m), 1.68 (2H, m), 3.49 and 3.59 (2×1H, 2d, $^2J_{Ha,Hb} = 11.7$ Hz, 5-H). 3.50 (1H, dd, $^3J_{3,4\text{-}Ha} = 10.3$ Hz, $^3J_{3,4\text{-}Hb} = 1.6$ Hz, 3-H), ^{13}C NMR (75 MHz, D_2O): $\delta = 17.7$ (CH_3), 24.5 (CH_2), 25.3 (CH_2, $^1J_{C,P} = 131$ Hz), 66.4 (CH_2), 74.8 (quaternary C), 75.2 (CH, $^3J_{C,P} = 16$ Hz). ^{31}P NMR (121 MHz, D_2O): $\delta = 24.4$ (s). Electrospray MS: $m/z = 213$ ($M-H^+$, molecular monoanion of **18**).
17. Meyer, O.; Grosdemange-Billiard, C.; Tritsch, D.; Rohmer, M. *Org. Biomol. Chem.*, in press.
18. Hoeffler, J. F.; Tritsch, D.; Grosdemange-Billiard, C.; Rohmer, M. *Eur. J. Biochem.* **2002**, *269*, 4446–4457.