Synthesis of (3R,4S)-3,4,5-Trihydroxy-4-methylpentylphosphonic Acid as a Potential Inhibitor of the Nonmevalonate Pathway

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The nonmevalonate pathway is widely found in higher plants and in many eubacteria, including pathogenic ones, but not in mammals. Identifying a nonmevalonate pathway inhibitor would greatly contribute to the search for new herbicides and antibacterial drugs to treat, for example, malaria. We describe here the synthesis of (3R,4S)-3,4,5-trihydroxy-4-methylpentylphosphonic acid as a candidate for inhibiting MEP cytidyllyltransferase, which is the third step on the nonmevalonate pathway.

Key words: nonmevalonate pathway; inhibitor; drug discovery; malaria; MEP cytidyllyltransferase

Terpenes widely exist in nature, and more than six thousand of them are already known. Terpenes are composed from some C5 isoprene units which are biosynthesized by the conjugation of isopentenyl diphosphate (IPP) and 3,3-dimethylallyl diphosphate (DMAPP). Although IPP had been thought to be produced only through the ubiquitous mevalonate pathway, Rohmer and his co-workers reported in 1996 a new IPP biosynthesis pathway, the nonmevalonate pathway.1–3) Later, this nonmevalonate pathway has also been found in bacteria, algae and higher plants.4)

The initial step on the nonmevalonate pathway is the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) by cross coupling between pyruvate and D-glyceraldehyde 3-phosphate.3) The second step is the reduction of DXP to 2-C-methyl-D-erythritol 4-phosphate (MEP). Rohmer and his co-workers have assumed that this second step involved two stages: i) a pinacol rearrangement type of reaction from DXP to 2-C-methylerythrose 4-phosphate, and ii) subsequent reduction of 2-C-methylerythrose 4-phosphate to MEP. Based on this study, Seto and his co-workers have reported that fosmidomycin 3-(N-formyl-N-hydroxyamino)propylphosphonic acid (FMM),5) a potent antibacterial compound against most Gram-negative and some Gram-positive bacteria,6–8) also had specific inhibition ability for DXP reductoisomerase.9)

This specificity would have been due to the structural similarity between FMM and 2-C-methylerythrose 4-phosphate.

The third step on the nonmevalonate pathway is the conversion from MEP to 4-(cytidine 5’-diphospho)-2-C-methyl-D-erythritol (CDP-ME).10) We think that finding specific inhibitors of the nonmevalonate pathway would lead to new herbicides or antibacterial drugs to treat, for example, malaria, because mammals do not have the nonmevalonate pathway. We describe here (3R,4S)-3,4,5-trihydroxy-4-methylpentylphosphonic acid (1),11) whose chemical structure is similar to that of MEP, as a potential specific inhibitor of MEP cytidyllyltransferase.

Results and Discussion

We planned to apply the D-glucose chirality to form the two chiral carbons of the target compound, (3R,4S)-3,4,5-trihydroxy-4-methylpentylphosphonic acid (1). As shown in Scheme 1, we used glucose derivative 2 as the key intermediate to synthesize 1. It would be possible to convert 2 from known compound 3 which has been reported to synthesize from D-glucose in four steps.12)

Synthesis of (3R,4S)-3,4,5-Trihydroxy-4-methylpentylphosphonic Acid (1)

Selective deprotection of the cyclohexylidene groups of 3 was achieved by 70% AcOH12) to give diol 5 in an 85% yield. Treatment of 5 with NaIO4 led to an aldehyde,12) and the subsequent Wittig reaction with diphenyl triphenylphosphoranylidene methyl phosphonate13) gave phosphoryl compound 6 in an 82% yield in two steps. Hydrogenation with Pd/C under a hydrogen atmosphere14) in a short time (within one hour) gave 7 in a high yield without any cleavage of the benzyl group in 6. The phenoxy groups on the phosphorus of 7 were changed to benzylxoy groups by the trans-ester reaction as 8.15,16) The remaining cyclohexylidene group was deprotected with trifluoroacetic acid in 70% acetic acid17) to give the key intermediate compound 2. Oxidation of 2 by NaIO4 and subsequent reduction with NaBH4 gave diol 9 in a 51% yield. Finally, depro-

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Biological Activities of (3R,4S)-3,4,5-Trihydroxy-4-methylpentylyphosphonic Acid (1) for the Nonmevalonate Pathway

A preliminary examination of the growth-inhibiting
effect on *E. coli* by (3R,4S)-3,4,5-trihydroxy-4-methylpentylphosphonic acid (1) did not give a satisfactory result.* We are planning to conduct a more detailed biological assay as an inhibitor of the nonmevalonate pathway. However, we have designed the method and started to synthesize other derivatives to find those with good inhibition, details of which will be reported in due course.

**Experimental**

*General information.* All reagents were used as purchased without further purification. Purchased dehydrated solvents were stored over suitable activated molecular sieves and used for anhydrous reactions. These reactions were monitored by TLC which was performed with 0.25 mm Merck 60 F254 precoated silica gel on aluminum sheets. Compounds were detected by spraying the TLC sheets with a 0.5M *p*-anisaldehyde in 2.0 M sulfuric acid-methanol solution and then heating.

Reflective index data were recorded by an Atago 1T instrument at room temperature and are corrected for data at 20 °C. Infrared absorption values were recorded by a Nicolet Impact 419 instrument. All NMR data are reported in parts per million downfield shift from tetramethylsilane. 

1H-NMR spectra were routinely recorded at 500 MHz by a Varian Unity INOVA 500 or at 600 MHz by a Varian Unity INOVA 600 instruments.

Chemical shifts are expressed relative to that of the residual proton in the NMR solvent (δ 7.26 ppm and 4.70 ppm for residual *CDCl*3 and *HOD*, respectively). 

13C-NMR spectra were routinely recorded at 126 MHz by a Varian Unity INOVA 500 or at 151 MHz on a Varian Unity INOVA 600 instruments, chemical shifts being expressed relative to that of the deuterated solvent (δ 77.0 ppm for *CDCl*3). Microanalyses were performed as an in-house analytical service by the Instrumental Analysis Center for Chemistry (Graduate School of Science, Tohoku University). ESI- and FAB-mass data were recorded with a JEOL LMS-700 instrument by the in-house mass spectrometry service.

A solution of (3R,4S)-3,4,5-trihydroxy-4-methylpentylphosphonic acid (1) in 70% acetic acid (50 ml) was stirred at 70 °C for 1 h. The reaction mixture was allowed to cool to room temperature, diluted with chloroform and water, and neutralized with NaHCO3. The aqueous layer was extracted three times with chloroform. The combined extracts were washed with brine, dried over MgSO4 and filtered. The filtrate was evaporated under reduced pressure. The resulting residue was purified by silica gel chromatography.

3-O-Benzyl-1,2-O-cyclohexyldiene-3-C-methyl-α-D-allofuranose (5). A solution of 31(1) (2.12 g, 4.78 mmol) in 70% acetic acid (50 ml) was stirred at 70 °C for 1 h. The reaction mixture was allowed to cool to room temperature, diluted with chloroform and water, and neutralized with NaHCO3. The aqueous layer was extracted three times with chloroform. The combined extracts were washed with brine, dried over MgSO4 and filtered. The filtrate was evaporated under reduced pressure. The resulting residue was purified by silica gel chromatography.

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* According to our personal communication, Dr. Rohmer, Université Louis Pasteur, France, has also tested a biological assay of 1, but with the same result as ours.
gel column chromatography. Elution with n-hexane and ethyl acetate (1:1) gave 5 (1.47 g, 85% yield), [α]D
+40.8 (c 1.01, CHCl₃), mp 111–112 °C (ether). IR νmax (KBr) cm⁻¹: 1126 (s, C–O–C–O–C), 3444 (br, s, OH). FABMS m/z (M + H)⁺: calcd. for C₃₀H₂₃O₅P·H, 365.1964; found, 365.1964. NMR δH (500 MHz, CDCl₃): 1.40 (s, 3H, CH₃), 1.44–1.89 (m, 10H, cyclohexylidene–CH₂), 2.19 (t, 1H, J = 6.3 Hz, 6-OH), 2.78 (m, 1H, H-6), 3.78 (m, 1H, H-5), 4.03 (s, 1H, J = 8.8 Hz, H-4), 4.39 (m, 1H, J = 3.9 Hz, H-2), 4.63 (m, 1H, J = 10.3 Hz, CH₂CH₃), 4.67 (m, 1H, J = 10.3 Hz, CH₂CH₃), 5.76 (m, 1H, J = 3.9 Hz, H-1), 7.26–7.40 (m, 5H, aromatic-H). NMR δC (126 MHz, CDCl₃): 16.30, 23.73, 23.93, 24.86, 35.98, 36.25, 64.37, 67.21, 69.95, 78.25, 81.98, 83.34, 103.82, 113.87, 127.76, 127.89, 128.30, 137.73. Anal. Found: C, 66.01; H, 7.72%. Calcd. for C₃₀H₂₃O₅·H: C, 65.91; H, 7.74%.

Diphenyl triphenylphosphoranylidemethyl phosphonate. To a stirred solution of methyl triphenylphosphonium bromide (20.2 g, 56.5 mmol) in THF (200 ml) was added dropwise n-butyl lithium (27 ml, a 2.5 m solution in n-hexane) at 0 °C, and the mixture was stirred for 45 min at 0 °C. This reaction mixture was diluted with diethyl ether (60 ml, 28.9 mmol), and the reaction mixture was stirred for 40 min at room temperature, before being filtered. The filtrate was diluted with ethyl acetate, and extracted with a 3 m HCl solution (30 ml). The resulting aqueous layer was neutralized with a 2 m NaOH solution, and the resulting organic oil was evaporated under reduced pressure to give a yellow solid (7.21 g, 49% yield), mp 163–164 °C (ether). IR νmax (KBr) cm⁻¹: 1126 (s, 3H, CH₃), 139–185 (m, 10H, cyclohexylidene–CH₂), 4.38 (m, 1H, J = 3.8 Hz, H-2), 4.51 (m, 1H, J = 10.6 Hz, CH₂CH₂H₃), 4.58 (m, 1H, J = 10.6 Hz, CH₂CH₂H₃), 4.72 (dd, 1H, J = 2.0 and 3.2 Hz, H-4), 5.78 (m, 1H, J = 3.8 Hz, H-1), 6.25 (dd, 1H, J = 2.0, 17.2 and 22.9 Hz, H-6), 6.94 (dd, 1H, J = 3.2, 17.2, 24.3 Hz, H-5), 7.14–7.43 (m, 15H, aromatic-H). NMR δC (151 MHz, CDCl₃): 16.74 (CH₃), 23.77, 23.99, 24.92, 36.01, 36.46, 66.90, 80.76 (d, Jc-P = 22.2 Hz, PCH=CH₂), 82.07, 83.24, 103.69, 113.92, 115.27, 116.72 (d, Jc-P = 190.2 Hz, PCH=CH₂), 120.57, 120.61, 120.65, 120.69, 125.08, 127.70, 127.93, 128.29, 129.59, 129.71, 129.72, 138.03, 149.85, 149.89. Anal. Found: C, 68.43; H, 6.27%. Calcd. for C₃₀H₂₃O₅P·H: C, 68.32; H, 6.27%.

3-O-Benzyl-1,2-O-cyclohexylidene-5,6-dideoxy-6-diphenoxophosphoryl-3-C-methyl-a-D-ribo-hex-5-enofuranose (7). A mixture of 6 (1.95 g, 3.46 mmol) and 10% Pd–C (36 mg) in MeOH (60 ml) was stirred under an H₂ atmosphere for 40 min at room temperature. The reaction mixture was filtered through Celite, the filtrate being concentrated under reduced pressure. The resulting residue was as purified by silica gel column chromatography, elution with n-hexane and ethyl acetate (3:1) giving 7 (1.80 g, 92% yield), [α]D +50.8 (c 1.02, CHCl₃). IR νmax (film) cm⁻¹: 929 (s, C=O–Ph), 1194 (s, C=O). FABMS m/z (M + H)⁺: calcd. for C₃₂H₃₇O₇P·H, 565.2355; found, 565.2361. NMR δH (500 MHz, CDCl₃): 1.22 (s, 3H, CH₃), 1.31–1.86 (m, 10H, cyclohexylidene–CH₂), 1.96 (m, 1H, H-5 or 6), 2.07 (m, 1H, H-5 or 6), 2.17 (m, 1H, H-5 or 6), 2.32 (m, 1H, H-5 or 6), 4.08 (dd, 1H, J = 4.0 and 9.0 Hz, H-4), 4.36 (m, 1H, J = 3.7 Hz, H-2), 4.56 (m, 1H, J = 10.5 Hz, CH₂CH₂H₃), 4.62 (dd, 1H, J = 10.5 Hz, CH₂CH₃), 5.75 (m, 1H, J = 3.7 Hz, H-1), 7.12–7.41 (m, 15H, aromatic-H). NMR δC (151 MHz, CDCl₃): 16.04 (CH₃), 21.63 (d, Jc-P = 4.2 Hz, PCH₂CH₂), 22.93 (d, Jc-P = 142.1 Hz, PCH₂), 23.74, 23.93, 24.91, 35.93, 36.22, 66.81, 80.59 (d, Jc-P = 19.6 Hz, PCH₂CH₂OH), 82.24, 82.53, 103.53, 113.27, 120.47, 120.49, 120.50, 120.52, 124.99, 127.52, 127.86, 128.21, 129.67, 138.40, 150.20 (d, Jc-P = 3.7 Hz, CO), 150.25 (d, Jc-P = 3.2 Hz, CO). Anal. Found: C, 68.05; H, 6.53%. Calcd. for C₃₂H₃₇O₇P·C: 68.07; H, 6.61%.

3-O-Benzyl-1,2-O-cyclohexylidene-5,6-dideoxy-6-dibenzoxophosphoryl-3-C-methyl-a-D-ribo-hex-5-enofuranose (8). To a stirred mixture of NaH (160 mg, a 60% dispersion in mineral oil, 4.00 mmol) in diethyl ether (5 ml) was added dropwise benzyl alcohol (3.00 ml,
29.1 mmol) at room temperature. The mixture was stirred until H₂ gas evolution had ceased, and then concentrated under reduced pressure. To the resulting residue in benzyl alcohol (3 ml) was added 7 (210 mg, 372 μmol). The reaction mixture was stirred for 75 min at room temperature and directly purified by silica gel column chromatography. Elution with n-hexane and ethyl acetate (3:1) gave 8 (220 mg, quantitative yield), [α]D +26.7 (c 1.00, CHCl₃), mp 82–83°C (ether). IR νmax (KBr) cm⁻¹: 1009 (s, P=O–CH₂–), 1247 (s, P=O). FABMS m/z (M + H⁺): calcld. for C₃₇H₃₄O₄P⁺H+, 563.2668; found, 593.2675. NMR δ (500 MHz, CDCl₃): 1.14 (s, 3H, CH₃), 1.36–1.89 (m, 13H, two of H-5, H-6 and ten of cyclohexyldiene–CH₂–), 2.05 (m, 1H, H-5 or 6), 3.95 (dd, 1H, J = 8.8 and 9.3 Hz, H-4), 4.32 (d, 1H, J = 3.7 Hz, H-2), 4.53 (d, 1H, J = 10.5 Hz, CH₂C₂H₅), 4.59 (d, 1H, J = 10.5 Hz, CH₂C₂H₅), 4.94 (dd, 1H, J = 8.3 and 12.0 Hz, POCH₂C₂H₅), 5.00 (dd, 1H, J = 8.3 and 12.0 Hz, POCH₂C₂H₅), 5.01 (dd, 1H, J = 8.3 and 12.0 Hz, POCH₂C₂H₅), 5.70 (d, 1H, J = 3.7 Hz, H-1), 7.26–7.40 (m, 15H, aromatic-H). NMR δC (151 MHz, CDCl₃): 15.97 (CH₃), 21.50 (d, Jc,p = 4.2 Hz, PCH₂CH₂), 22.82 (d, Jc,p = 142.2 Hz, PCH₂), 23.71, 23.91, 24.90, 35.94, 36.21, 66.71, 66.91 (d, Jc,p = 6.3 Hz, POCH₂), 66.99 (d, Jc,p = 6.3 Hz, POCH₂), 80.75 (d, Jc,p = 19.6 Hz, PCH₂CH₂(OH), 82.25, 82.44, 103.34, 113.14, 127.45, 127.73, 127.74, 127.76, 127.79, 128.15, 128.16, 128.40, 128.43, 128.48, 136.31, 136.35, 138.47. Anal. Found: C, 69.09; H, 6.96%. Calcld. for C₃₄H₄₁O₈P·C, 68.90; H, 6.97%.  

(3R,4S)-O-Benzyl-3,5-di-hydroxy-4-methylpentylphosphonic acid (9). To a stirred solution of 2 (244 mg, 476 μmol) in methanol (15 ml) was added dropwise a solution of sodium periodate (400 mg in 10 ml of H₂O). The reaction mixture was stirred for 15 min at room temperature, and diluted with ethyl acetate and H₂O. The aqueous layer was extracted three times with ethyl acetate. The combined extracts were washed with brine, dried over MgSO₄ and filtered. The filtrate was evaporated under reduced pressure, the resulting residue being purified by column chromatography in a short silica gel. Elution with n-hexane and ethyl acetate (2:3) gave a syrup which was presumed to be an aldehyde. To a solution of this syrup in methanol (15 ml) was added sodium borohydride (25.7 mg, 679 μmol) at 0°C. The reaction mixture was stirred for 2 h at room temperature, quenched with sat. NH₄Cl, neutralized with NaHCO₃ and diluted with ethyl acetate. The aqueous layer was extracted three times with ethyl acetate. The combined extracts were washed with brine, dried over MgSO₄ and filtered. The filtrate was evaporated under reduced pressure, the resulting residue being purified by column chromatography. Elution with chloroform and methanol (43:1) gave 9 (116 mg, 51% yield), [α]D +15.8 (c 1.05, CHCl₃), IR νmax (film) cm⁻¹: 996 (s, P=O–CH₂–), 1214 (s, P=O), 3394 (br. m, OH). FABMS m/z (M + H⁺): calcld. for C₄₇H₃₇O₆P·H⁺, 845.2093; found, 845.2096. NMR δH (500 MHz, CDCl₃): 1.11 (s, 3H, CH₃), 1.63 (m, 1H, PCH₂ or PCH₂CH₂), 3.51–1.96 (m, 2H, two of PCH₂ or PCH₂CH₂), 2.07 (m, 1H, PCH₂ or PCH₂CH₂), 2.65 (t, 1H, J = 6.1 Hz, CH₂OH), 3.41 (d, 1H, J = 9.3 Hz, PCH₂CH₂(OH)), 3.65–3.74 (m, 3H, CH₂OH and PCH₂CH₂(OH)), 4.49 (s, 2H, CH₂C₂H₅), 4.95 (d, 1H, J = 12.0 Hz), 4.97 (d, 1H, J = 11.5 Hz), 5.05 (dd, 1H, J = 7.0 and 11.5 Hz), 5.06 (dd, 1H, J = 7.0 and 11.4 Hz, H-1), 7.26–7.37 (m, 15H, aromatic-H). NMR δC (600 MHz, CDCl₃): 1.64 (s, 3H, CH₃), 3.94 (dd, 1H, J = 4.2 and 9.0 Hz, H-4), 4.39 (d, 1H, J = 10.8 Hz, CH₂C₂H₅), 4.46 (d, 1H, J = 10.8 Hz, CH₂C₂H₅), 5.22 (m, 1H, H-1). NMR δ (151 MHz, CDCl₃) mixture of α-2 and β-2: 15.83 (CH₃ of α), 16.34 (CH₃ of β), 23.23 (d, Jc,p = 141.1 Hz, PCH₂), 24.26 (d, Jc,p = 4.2 Hz, PCH₂CH₂ of α), 24.85 (d, Jc,p = 4.2 Hz, PCH₂CH₂ of β), 65.74 (β), 66.09 (α), 67.15 (d, Jc,p = 6.5 Hz, POCH₂ of β), 67.16 (d, Jc,p = 6.3 Hz, POCH₂ of α), 67.23 (d, Jc,p = 6.3 Hz, POCH₂ of α), 67.30 (d, Jc,p = 6.3 Hz, POCH₂ of β), 75.26, 80.53 (d, Jc,p = 17.5 Hz, PCH₂CH₂ of α), 81.10, 82.05 (PCH₂CH₂CH₂ of β), 82.66 (d, Jc,p = 16.4 Hz, PCH₂CH₂ of β), 96.09, 102.30, 127.50, 127.66, 128.74, 129.70, 129.73, 128.07, 128.38, 128.40, 128.48, 128.55, 128.63, 136.20, 136.21, 136.23, 136.26, 137.21, 137.62. Anal. Found: C, 65.33; H, 6.49%. Calcld. for C₃₄H₄₁O₈P·C, 65.62; H, 6.49%.
References


11) A compound having the same structure as that of I was reported in the patent, Hassan, J., PCT Int. Appl. WO 00/03699 (Jan. 27, 2000).


