

A Short Synthesis of a Novel Nucleoside Analog of Fosfomycin

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Received October 12, 2002

Key Words : Epoxyphosphonate, Fosfomycin analog, Nucleoside epoxyphosphonate

Various synthetic methods for preparation of 1,2-epoxyalkylphosphonates have been developed¹ since the discovery of the antibiotic fosfomycin [(Z)-(1R,2S)-(-)-1,2-epoxypropylphosphonic acid] originally isolated from a fermentation broth of *Streptomyces fradiae* in 1969.² Synthesis of fosfomycin analogs is highly attractive due to their potential biological significance.³ Many nucleoside and nucleotide derivatives also show antiviral activity so we are interested in the synthesis of noble compounds combining the structure of fosfomycin with a nucleoside, which can possibly show dual biological activities.

During our investigation of fosfomycin analogs we found only a few reported syntheses of 1,2-epoxyalkylphosphonates possessing a sugar⁴ or nucleoside⁵ component and none of them are similar to the structure of fosfomycin. Therefore, we now report a short synthesis of a new nucleoside epoxyphosphonate from cytidine as a nucleoside analog of fosfomycin.

Experimental Section

General. Methylene chloride and Et₃N were distilled from CaH₂ immediately prior to use. All non-aqueous reactions were conducted in flame-dried glassware, under an atmosphere of nitrogen, with magnetic stirring. NMR spectra were obtained on a Bruker AC-300 spectrometer and recorded at 300 MHz for ¹H (75 MHz for ¹³C) with CDCl₃ as solvent and (CH₃)₄Si (¹H) or CDCl₃ (¹³C, 77.0 ppm) as internal standards unless otherwise noted. All ³¹P NMR chemical shifts are reported in ppm relative to 85% H₃PO₄ (external standard). High resolution FAB mass spectra were obtained at the University of Iowa Mass Spectrometry Facility.

Diethyl [1'-(5'-hydroxy-6'-(*p*-toluenesulfonyloxy)-2',3'-*O*-isopropylidene-β-D-ribo-hexofuranosyl)-4-*N*-acetylcytosyl]-6'-phosphonate **5:** A flame-dried 100 mL round-bottom flask under N₂ was charged with diol **4** (350 mg, 0.71 mmol) and anhydrous CH₂Cl₂ (14 mL). Freshly distilled triethylamine (0.25 mL, 1.78 mmol, 2.5 eq) was then added through a syringe. After the solution was stirred for 5 min at room temperature, *p*-TsCl (148 mg, 0.78 mmol, 1.1 eq) was added quickly. The reaction mixture was allowed to stir for 2 days and quenched with distilled water. This aqueous mixture was extracted with CH₂Cl₂ and the combined organic

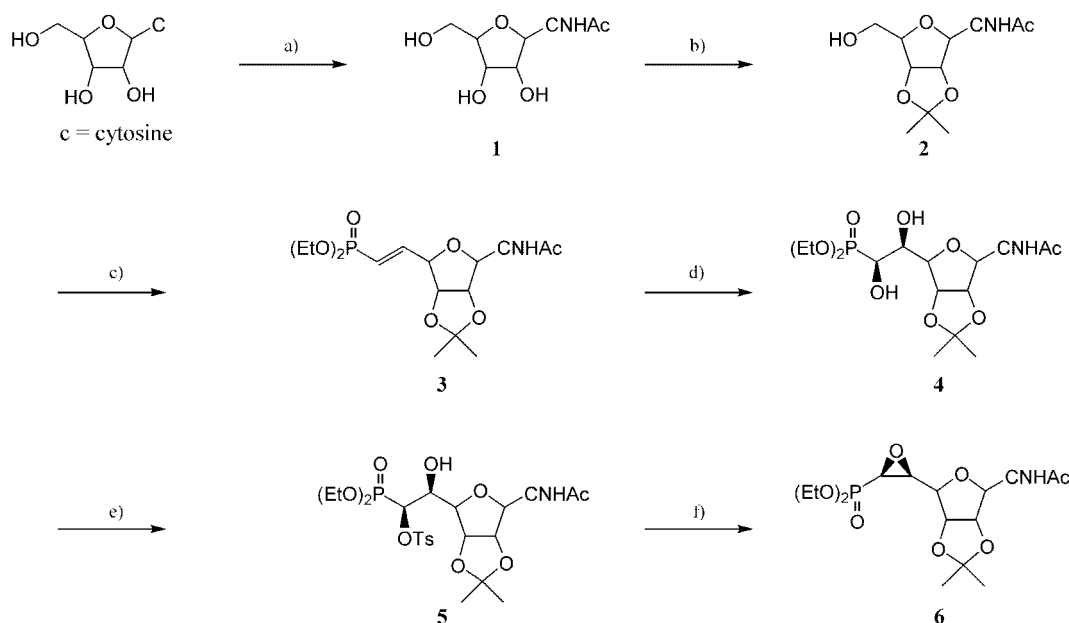
extracts were dried over anhydrous NaSO₄. After removal of the solvent under reduced pressure, the crude oil was purified by flash column chromatography with a gradient system of methylene chloride and methanol to give the desired product **5** (423 mg, 0.65 mmol, 92%). ¹H NMR δ 9.54 (bs, 1H), 7.86 (d, *J* = 8.1 Hz, 2H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.40 (d, *J* = 7.4 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 2H), 5.44 (d, *J* = 1.8 Hz, 1H), 5.16 (dd, *J* = 6.4, 3.8 Hz, 1H), 5.08 (m, 2H), 4.62 (d, *J* = 4.8 Hz, 1H), 4.43 (m, 1H), 4.26 (m, 2H), 4.12 (m, 2H), 3.78 (dd, *J* = 8.0, 3.8 Hz, 1H), 2.46 (s, 3H), 2.27 (s, 3H), 1.40 (s, 3H), 1.31 (m, 9H); ¹³C NMR δ 171.2, 163.3, 154.7, 147.1, 144.9, 133.8, 129.5 (2C), 128.0 (2C), 113.8, 96.8 (d, *J*_{C,P} = 7.8 Hz), 86.1 (d, *J*_{C,P} = 9.0 Hz), 84.3, 82.1, 77.2, 75.6 (d, *J*_{C,P} = 166.9 Hz), 69.7, 64.5 (d, *J*_{C,P} = 7.8 Hz), 63.1 (d, *J*_{C,P} = 7.6 Hz), 27.0, 25.3, 24.9, 21.6, 16.3 (d, *J*_{C,P} = 5.2 Hz), 16.2 (d, *J*_{C,P} = 6.2 Hz); ³¹P NMR δ 17.2; HRFABMS calcd for C₂₆H₃₇N₃O₁₂PS(M+1)⁺ 646.1836, found 646.1838.

Diethyl [1'-(5',6'-epoxy-2',3'-*O*-isopropylidene-β-D-ribo-hexofuranosyl)-4-*N*-acetylcytosyl]-6'-phosphonate **6:** An oven dried 50 mL one-necked round-bottom flask under N₂ was charged with tosylate **5** (90 mg, 0.14 mmol) and acetone (1.5 mL). To this solution was added activated K₂CO₃ (58 mg, 0.42 mmol, 3 eq). After the mixture was stirred for 2 days at room temperature, the reaction mixture was filtered and concentrated in vacuo to give the crude product. The oil was purified by column chromatography with a gradient system of methylene chloride and methanol to give the desired product **6** (60 mg, 0.13 mmol, 91%). ¹H NMR δ 8.87 (bs, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 5.52 (d, *J* = 1.2 Hz, 1H), 5.34 (m, 2H), 4.69 (dd, *J* = 9.0, 3.0 Hz, 1H), 4.26 (m, 4H), 3.72 (ddd, *J* = 9.0, 6.0, 4.5 Hz, 1H), 3.04 (dd, *J* = 25.8, 4.5 Hz, 1H), 2.34 (s, 3H), 1.59 (s, 3H), 1.35 (m, 9H); ¹³C NMR δ 170.1, 163.2, 154.7, 148.3, 114.0, 100.3, 96.5, 86.9, 84.8 (d, *J*_{C,P} = 3.7 Hz), 77.2, 63.1 (d, *J*_{C,P} = 6.0 Hz), 62.8 (d, *J*_{C,P} = 6.1 Hz), 56.5 (d, *J*_{C,P} = 1.9 Hz), 49.5 (d, *J*_{C,P} = 202.5 Hz), 26.9, 25.2, 25.0, 16.5 (d, *J*_{C,P} = 2.2 Hz), 16.4 (d, *J*_{C,P} = 1.7 Hz); ³¹P NMR δ 17.9; HRFABMS calcd for C₁₉H₂₉N₃O₉P(M+1)⁺ 474.1654, found 474.1641.

Results and Discussion

A new nucleoside 5',6'-epoxyphosphonate **6** was prepared from cytidine using the conditions shown in Scheme 1. As previously reported⁶ diol **4** was prepared from the reaction of

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Scheme 1. (a) Ac_2O , CH_3OH . (b) HClO_4 , 2,2-dimethoxypropane, Acetone. (c) i. CrO_3 , Pyridine, Ac_2O , DMF, ii. $(\text{EtO})_2\text{P}(\text{O})\text{CH}=\text{PPh}_3$, DMSO. (d) AD mix- α . (e) *p*-TsCl, Et_3N , CH_2Cl_2 . (f) K_2CO_3 , Acetone.

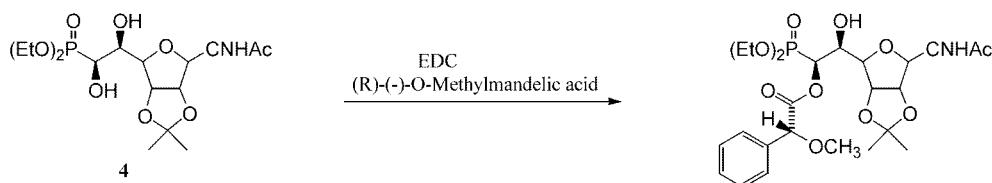


Figure 1. (R)-Mandelate ester from Diol 4.

the corresponding vinylphosphonate **3** and AD mix- α in good yield. In order to assign the stereochemistry of diol **4**, mandelate esters were prepared from the reaction of diol **4**, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) with (R)-(-)-O-methylmandelic acid or (S)-(-)-O-methylmandelic acid. The stereochemistry of diol **4** was tentatively assigned as the 5'S,6'S-diastereomer based on NMR data comparison of (S) and (R)-mandelate esters.^{6,7}

However, in this study, we were able to confirm the stereochemistry of diol **4** by the crystal structure^{8,9} of the mandelate ester obtained from the reaction of (R)-(-)-O-methylmandelic acid, EDC, and the corresponding diol **4**. (Figure 1 and 2)

With the support of ^1H NMR HOMO decoupling studies (Bruker DRX-400) of diol **4** and tosylate **5**, we have assigned each hydrogen resonance. First, the phosphorus coupled ^1H NMR spectrum of diol **4** showed resonances at C_1 (d. at 5.62 ppm), C_2 (bd. at 5.15 ppm, overlapped with OH on C_5), C_3 (dd. at 5.28 ppm), C_4 (dd. at 4.39 ppm), C_5 (m. at 4.35 ppm), C_6 (overlapped with CH_2 on the P. at 4.26 ppm), and OH on C_6 (t. at 3.99 ppm). Using this information, HOMO decoupling studies were studies on the possible positions of all the hydrogens. Irradiation of the signal at C_3 collapsed resonances at C_2 and C_4 to give a broad singlet and a doublet respectively. Irradiation of the signal at C_2 collapsed resonances at C_1 and C_3 to give a

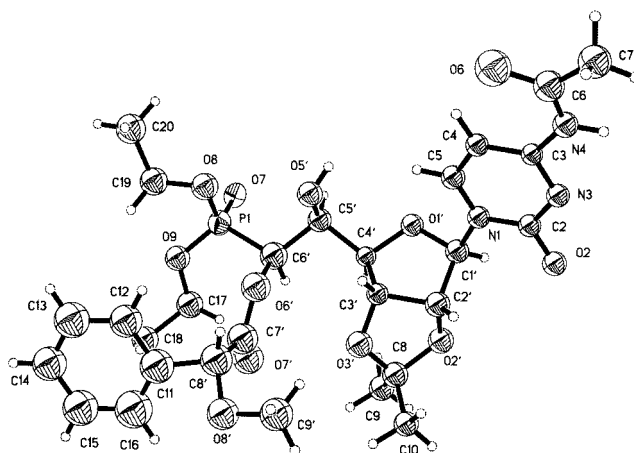


Figure 2. Thermal Ellipsoid Drawing of Mandelate Ester of Diol 4 with Atomic Labeling.

singlet and a doublet as expected.

If the resonance for C_6 overlapped with other signals in the spectrum of diol **4**, it was assumed that it would shift downfield dramatically if the tosyl group were introduced on the OH group of C_6 , due to its deshielding effect.

Based on this assumption and decoupling studies on the 5'-hydroxy-6'-tosylate **5** obtained from regioselective tosylation of diol **4** with *p*-TsCl (*p*-toluenesulfonyl chloride), we were

able to confirm that the hydrogen peak for C₆ shifted from 4.26 to 5.08 ppm (overlapped with C₂). Irradiation of the resonance at C₅ (4.43 ppm) collapsed signals at C₄ and C₆ to give a doublet for C₄ and a simplified peak for C₆ as expected. Irradiation of the resonance at C₄ (3.78 ppm) collapsed signals at C₅ and C₃ to give a broad singlet for C₅ and a doublet for C₃. Finally irradiation of the hydrogens at C₂ and C₆ (5.08 ppm) collapsed signals at C₁, C₃, and C₅ to give a singlet, a doublet, and a broad quintet respectively.

Intramolecular S_N2 reaction of the 5'(S)-hydroxy-6'(S)-tosylate phosphonate **5** with K₂CO₃ in acetone gave the desired 5'(S)-6'(R)-epoxyphosphonate **6** in excellent yield. Chemical shifts for hydrogens at C₄, C₅, and C₆ changed noticeably from chemical shifts of 3.78, 4.43, and 5.08 ppm to 4.69, 3.72, and 3.04 ppm respectively.

In summary, a new nucleoside epoxyphosphonate has been prepared from cytidine as an analog of the antibiotic fosfomycin in six steps in good yield. Its assignment and stereochemistry have been confirmed by NMR studies of compounds **4**, **5**, and **6**, as well as a crystal structure of the mandelate ester shown in Figure 1. This synthetic 5'(S)-6'(R)-epoxyphosphonate **6** will be studied for its biological activity.

Acknowledgment. The authors are indebted to Prof. David F. Wiemer (University of Iowa, USA) for helpful discussion and experimental assistance. The authors also wish to thank Dr. Dale Swenson (University of Iowa, USA) for his help in carrying out the X-ray crystallographic analysis. This work has been supported by a grant from the Korea Science and Engineering Foundation (Grant R01-2001-000-00214-0).

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- This mandelate ester was crystallized from CHCl₃ very slowly (about 2 months).
- Crystal data for mandelate ester of diol **4**: Empirical formula = C₂₈H₃₃N₃O₁₂P, M.W. = 758.95, Crystal system = monoclinic, colorless plate, Volume = 1759.6(6) Å³, Crystal size = 0.25 × 0.13 × 0.03 mm, F(000) = 792, Density = 1.432 Mg·m⁻³, space group = P2(1). Unit cell dimensions: a = 9.1150(14), b = 12.603(3), c = 15.438(3), α = 90°, β = 97.174(7)°, γ = 90°, Theta range = 2.1-20°. Absorption coefficient = 0.369 mm⁻¹. Reflections collected = 13716. Independent reflections = 3716. Final R indices [I > 2σ(I)]: R(Rw) = 0.0788(0.1630). Data was collected on a Nonius Kappa CCD diffractometer at 190(2) K and refined with a full matrix least-squares on F².