A Short Synthesis of a Novel Nucleoside Analog of Fosfomycin

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Various synthetic methods for preparation of 1,2-epoxyalkylphosphonates have been developed[1] 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 sugar[4] or nucleoside[5] 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 Et_3N were distilled from CaH_2 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 ^{1}H (75 MHz for ^{13}C) with $CDCl_3$ as solvent and $(CH_3)_4Si$ (^{1}H) or $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards unless otherwise noted. All ^{31}P NMR chemical shifts are reported in ppm relative to 85% H_3PO_4 (external standard). High resolution FAB mass spectra were obtained at the University of Iowa Mass Spectrometry Facility.

Diethyl [1'-{5'-hydroxy-6'-(*p*-toluenesulfonyl)oxy-2',3'-*O*-isopropylidene-β-D-ribo-hexofuranosyl}-4-*N*-acetylcytosyl]-6'-phosphonate 5: A flame-dried 100 mL round-bottom flask under N_2 was charged with diol **4** (350 mg, 0.71 mmol) and anhydrous CH_2Cl_2 (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 CH_2Cl_2 and the combined organic extracts were dried over anhydrous $NaSO_4$. 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%). ^{1}H 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); ^{13}C 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, $J_{C\text{-}P}$ = 7.8 Hz), 86.1 (d, $J_{C\text{-}P}$ = 9.0 Hz), 84.3, 82.1, 77.2, 75.6 (d, $J_{C\text{-}P}$ = 166.9 Hz), 69.7, 64.5 (d, $J_{C\text{-}P}$ = 7.8 Hz), 63.1 (d, $J_{C\text{-}P}$ = 7.6 Hz), 27.0, 25.3, 24.9, 21.6, 16.3 (d, $J_{C\text{-}P}$ = 5.2 Hz), 16.2 (d, $J_{C\text{-}P}$ = 6.2 Hz); ^{31}P NMR δ 17.2; HRFABMS calcd for $C_{26}H_{37}N_3O_{12}PS(M+1)^+$ 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 N_2 was charged with tosylate **5** (90 mg, 0.14 mmol) and acetone (1.5 mL). To this solution was added activated K_2CO_3 (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%). ^{1}H 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); ^{13}C NMR δ 170.1, 163.2, 154.7, 148.3, 114.0, 100.3, 96.5, 86.9, 84.8 (d, $J_{C\text{-}P}$ = 3.7 Hz), 77.2, 63.1 (d, $J_{C\text{-}P}$ = 6.0 Hz), 62.8 (d, $J_{C\text{-}P}$ = 6.1 Hz), 56.5 (d, $J_{C\text{-}P}$ = 1.9 Hz), 49.5 (d, $J_{C\text{-}P}$ = 202.5 Hz), 26.9, 25.2, 25.0, 16.5 (d, $J_{C\text{-}P}$ = 2.2 Hz), 16.4 (d, $J_{C\text{-}P}$ = 1.7 Hz); ^{31}P NMR δ 17.9; HRFABMS calcd for $C_{19}H_{29}N_3O_9P(M+1)^+$ 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 reported[6] diol **4** was prepared from the reaction of

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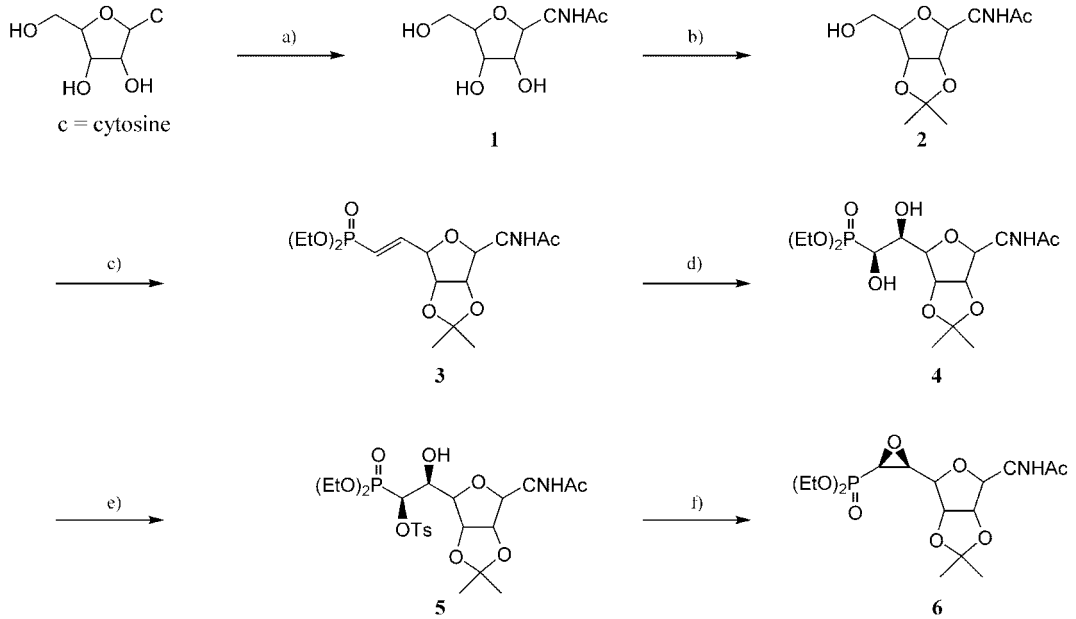

Scheme 1. (a) Ac_2O, CH_3OH. (b) $HClO_4$, 2,2-dimethoxypropane, Acetone. (c) i. CrO_3, Pyridine, Ac_2O, DMF, ii. $(EtO)_2P(O)CH=PPh_3$, DMSO. (d) AD mix-α. (e) *p*-TsCl, Et_3N, CH_2Cl_2. (f) K_2CO_3, Acetone.

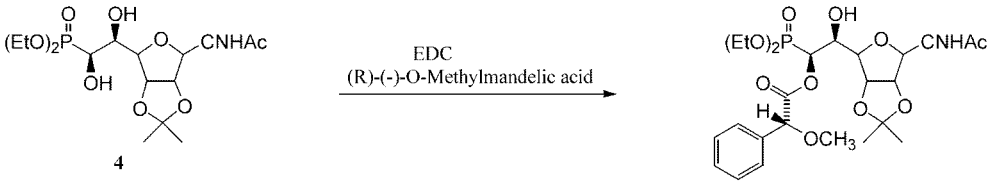

Figure 1. (R)-Mandelate ester from Diol **4**.

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 ^{1}H NMR HOMO decoupling studies (Bruker DRX-400) of diol **4** and tosylate **5**, we have assigned each hydrogen resonance. First, the phosphorus coupled ^{1}H 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 singlet and a doublet as expected.

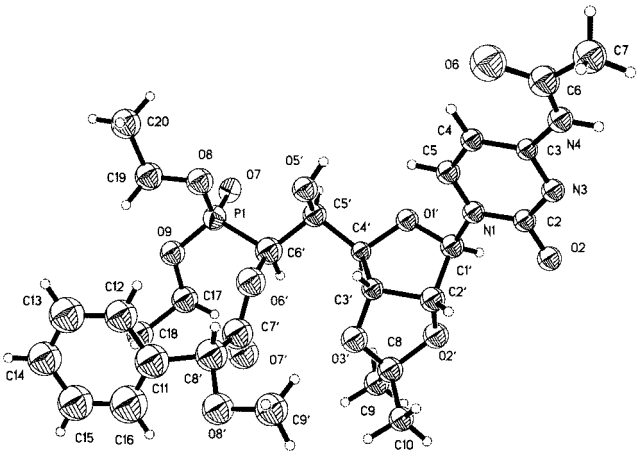

Figure 2. Thermal Ellipsoid Drawing of Mandelate Ester of Diol **4** with Atomic Labeling.

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 $C_{6'}$ shifted from 4.26 to 5.08 ppm (overlapped with $C_{2'}$). Irradiation of the resonance at $C_{5'}$ (4.43 ppm) collapsed signals at $C_{4'}$ and $C_{6'}$ to give a doublet for $C_{4'}$ and a simplified peak for $C_{6'}$ as expected. Irradiation of the resonance at $C_{4'}$ (3.78 ppm) collapsed signals at $C_{5'}$ and $C_{3'}$ to give a broad singlet for $C_{5'}$ and a doublet for $C_{3'}$. Finally irradiation of the hydrogens at $C_{2'}$ and $C_{6'}$ (5.08 ppm) collapsed signals at $C_{1'}$, $C_{3'}$, and $C_{5'}$ 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 K_2CO_3 in acetone gave the desired 5'(S)-6'(R)-epoxyphosphonate **6** in excellent yield. Chemical shifts for hydrogens at $C_{4'}$, $C_{5'}$, and $C_{6'}$ 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.

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8. This mandelate ester was crystallized from $CHCl_3$ very slowly (about 2 months).
9. Crystal data for mandelate ester of diol **4**: Empirical formula = $C_{28}H_{38}N_3O_{12}P$, M.W. = 758.95, Crystal system = monoclinic, colorless plate, Volume = 1759.6(6) $Å^3$, 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), $\alpha = 90^o$, $\beta = 97.174(7)^o$, $\gamma = 90^o$, Theta range = 2.1-20°, Absorption coefficient = 0.369 mm^{-1}, Reflections collected = 13716, Independent reflections = 3716, Final R indices [$I > 2\sigma(I)$]; 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 F^2.