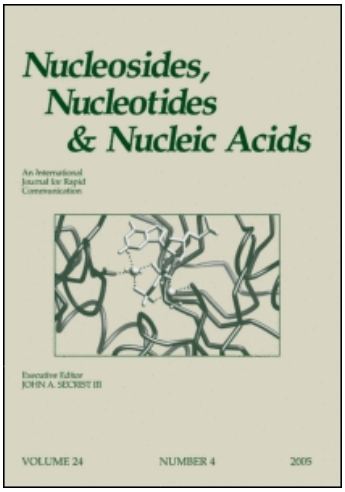


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Syntheses of Phosphonate Analogues of Dideoxyadenosine (DDA)-, Dideoxycytidine (DDC)-, Dideoxyinosine (DDI)-, and Deoxythymidine (DDT)-5'-Monophosphates

John A. Secrist III[a]; Robert M. Riggs[a]; Robert N. Comber[a]; John A. Montgomery[a]
[a] Organic Chemistry Research Department, Southern Research Institute, Alabama, Birmingham

To cite this Article Secrist III, John A. , Riggs, Robert M. , Comber, Robert N. and Montgomery, John A.(1992) 'Syntheses of Phosphonate Analogues of Dideoxyadenosine (DDA)-, Dideoxycytidine (DDC)-, Dideoxyinosine (DDI)-, and Deoxythymidine (DDT)-5'-Monophosphates', Nucleosides, Nucleotides and Nucleic Acids, 11: 2, 947 — 956
To link to this Article: DOI: 10.1080/07328319208021749
URL: http://dx.doi.org/10.1080/07328319208021749

NUCLEOSIDES AND NUCLEOTIDES, 11(2-4), 947-956 (1992)

SYNTHESES OF PHOSPHONATE ANALOGUES OF DIDEOXYADENOSINE (DDA)-, DIDEOXYCYTIDINE (DDC)-, DIDEOXYINOSINE (DDI)-, AND DEOXYTHYMIDINE (DDT)-5'-MONOPHOSPHATES

John A. Secrist III,* Robert M. Riggs, Robert N. Comber, and John A. Montgomery*

Southern Research Institute, Organic Chemistry Research Department, Birmingham, Alabama 35255-5305

Abstract: Phosphonate derivatives of ddA, ddC, ddI and ddT (**5f, 5e, 5c,** and **5a**) were prepared by condensing the 5'-aldehydes with diphenyl triphenylphosphoranylidenemethylphosphonate, reducing the resultant olefins and hydrolyzing the phosphonate phenyl esters, sequentially, with base and then *C. atrox* phosphodiesterase.

INTRODUCTION

Presently 3'-azido-3'-deoxythymidine (AZT) and dideoxyinosine (ddI) have been approved by the FDA for the treatment of AIDS. Both compounds inhibit HIV replication at the level of reverse transcriptase by competing with the normal substrates, 2'-deoxynucleoside-5'-*O*-triphosphates. The result is incorporation of dideoxynucleoside monophosphates into the primer template causing chain termination of the developing viral DNA. For biological activity, the dideoxynucleosides require a stepwise conversion to their respective 5'-*O*-triphosphates. It is known that initial conversion to the 5'-*O*-monophosphates, mediated by cellular kinases, is efficient for AZT.[1] This conversion is slower and less efficient for dT,[2] ddC,[3] ddI[4] and ddA,[5] the latter compound being much more rapidly deaminated to ddI than phosphorylated. All of the above dideoxynucleosides, including AZT, are inefficiently converted to their triphosphates, with ddI-monophosphate utilizing an indirect route via conversion first to ddA-monophosphate.[4] Therefore, compounds that serve as better substrates for the kinases, while still possessing features that would result in chain termination, should possess interesting activity.

Dedicated to the memory of Professor Tohru Ueda.

As previously noted,[6] attempts to design better substrates have mainly resulted in manipulations either at the 3′-position or the base portion of 2′,3′-dideoxynucleosides. The phosphonate isostere of AZT 5′-phosphate, 1-(3-azido-2,3,4,6-tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-2,4-dioxo-5-methylpyrimidine,[6,7] is clearly one of the few exceptions. 5′-*C*-Phosphonomethyl compounds such as the latter mimic the naturally occuring nucleotides maintaining the proper spacing between the base and phosphorous atom. Several acyclic phosphonate analogues, including S-HMPA,[8] PMEA[9] and others[10] have been synthesized and evaluated for antiviral activity. These are all presumably converted to triphosphates intracellularly to exert their antiviral effect.

We first communicated[11] the anti-HIV activities of phosphonate analogues and isosteres of 5′-*O*-phosphoryl-2′,3′-dideoxyribofuranosyl adenine, cytosine, hypoxanthine and thymine (compounds **5f**, **5e**, **5c**, and **5a**) in 1989. Since then several groups have reported the syntheses and biological evaluation of both *C*- and *O*-phosphonate nucleoside analogues including the 3′,5′-dideoxy-5′-*C*-phosphonomethyl 3′-deoxynucleosides[12] and the 5′-*O*-phosphonomethyl-2′,3′-dideoxynucleosides by Herdewijn.[13] Herein we present the synthetic details for our compounds.

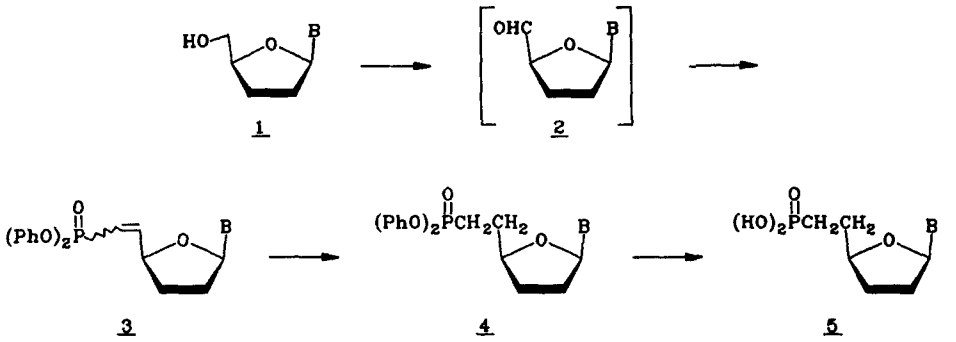
CHEMICAL DISCUSSION

The dideoxynucleoside precursors required for our synthetic work, dT (**1a**),[14] ddI (**1c**),[15] ddA (**1f**),[16] and ddC (**1e**),[17] are all available or easily resynthesized following known procedures. The amine functions of ddC and ddA were blocked with benzoyl and dimethylaminomethylene groups to give protected derivatives **1b** and **1d** (Scheme I). The initial oxidation step in our sequence gave a much lower yield with the free amines **1e** and **1f**. The Wittig reaction was chosen for carbon-carbon bond formation at C-5′, following the sequence developed by Moffatt and coworkers.[18]

Dideoxynucleosides **1a-d** were each converted to their respective 5′-aldehydes (**2**) by Moffatt oxidation[19] and then condensed, without isolation, with diphenyl triphenylphosphoranylidenemethylphosphonate.[20] The resultant olefins **3a-d** were purified by either gravity or centrifugal chromatography and then, without complete characterization, reduced over 5% or 10% Pd on carbon to the saturated phosphonates **4a-d** (in 22 to 50% overall yield).

Initially, transesterification was attempted to convert esters **4a-d** to their dibenzyl ester counterparts, which would then have been hydrogenolyzed to the free phosphonic acids **5**.[18] Although the desired dibenzyl esters could be obtained in modest yields after chromatographic purification, in our hands, the transesterification reaction also produced

Scheme I



a: B = Thymine
b: B = N^6-Benzoylcytosine
c: B = Hypoxanthine
d: B = N^6-Dimethylaminomethyleneadenine
e: B = Cytosine
f: B = Adenine

a number of decomposition products in both the pyrimidine and purine series. It should be noted that a pure sample of the dibenzyl ester derivative of **5c** was obtained and hydrogenolyzed over 10% Pd/C in 96% EtOH. This reaction was carried out in the absence of any scavenger and it was found that the resultant free phosphonic acid hydrolyzed the nucleoside to hypoxanthine and the sugar. In light of the known lability of dideoxynucleosides under acidic conditions,[21] it is not surprising that this analogue did not exhibit the same stability as its counterpart in the normal nucleoside series.[18]

Phosphonates **5** were successfully obtained by a two-step sequence also used by Moffatt.[18] First, the protected phosphonate nucleosides **4** were converted to their respective monophenyl esters *via* alkaline hydrolysis. The thymidine analogue **4a** was hydrolyzed with 0.2 *N* NaOH, followed by conversion to its triethylammonium salt by elution through a Dowex (Et_3NH^+ form) column. Analogues **4b-d** were hydrolyzed in concentrated NH_4OH. The second phenyl group was removed in each case by dissolving the monophenyl esters in 0.1M triethylammonium bicarbonate buffer (TEAB, pH = 7.4-8.5) and treating with *C. atrox* phosphodiesterase.

After removal of the buffer, phosphonates **5** were treated with 10% aqueous $CaCl_2$ and precipitated as their stable Ca^{+2} salts by the addition of EtOH. These compounds were evaluated for activity against HIV induced cytopathy in CEM-4 T-cell lines. These results were presented in an earlier communication.[11] Briefly, only the ddA (**5f**) and ddC

(**5e**) analogues showed any significant activity against HIV in T-lymphocyte cell culture,[22] with RI_{50}'s of 60 μM and 150 μM, respectively. All the targets were noncytotoxic at the highest dosages tested (200 μM).

EXPERIMENTAL SECTION

Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded with a Nicolet NMC 300NB spectrometer operating at 300.635 MHz for ^1H and at 75.6 MHz for ^{13}C. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast atom bombardment (FAB) mode. Microanalyses were performed by the Molecular Spectroscopy Section of the Organic Chemistry Research Department at Southern Research Institute.

***N*^{4}-Benzoyl-2′,3′-dideoxycytidine (1b).** Dideoxycytidine (**1e**, 250 mg, 1.2 mmol), benzoic anhydride (530 mg, 2.3 mmol), and 1-hydroxybenzotriazole (70 mg, 0.52 mmol) were stirred in 20 mL of DMF for 60 h. The solvent was removed *in vacuo* and the residue was triturated with 3:1 ethyl acetate-diethyl ether (20 mL) to precipitate **1b**, which was filtered and dried to yield 217 mg (57%), mp 230 °C (dec). FABMS *m/z*: 316. ^1H NMR (DMSO-d_6) δ 1.85 (m, 2, H-3′), 2.0 (m, 1, H-2′), 2.4 (m, 1, H-2′), 3.6 (m, 1, H-5′), 3.8 (m, 1, H-5′), 4.1 (m, 1, H-4′), 5.15 (m, 1, OH), 5.95 (dd, 1, H-1′), 7.35 (d, 1, ArH), 7.5 (m, 2, ArH), 7.6 (m, 1, ArH), 8.0 (m, 2, ArH), 8.55 (d, 1, H-6), 11.2 (s, 1, NH). *Anal.* Calcd for $C_{16}H_{17}N_3O_4 \cdot 0.25H_2O$: C, 60.09; H, 5.52; N, 13.14. Found: C, 60.03; H, 5.74; N, 12.87.

***N*-[(Dimethylamino)methylene]-2′,3′-dideoxyadenosine (1d).** A solution containing dideoxyadenosine (**1f**)[16] (600 mg, 2.55 mmol) and dimethylformamide dimethylacetal (610 mg, 5.11 mmol) in dry DMF (10 mL) was stirred overnight (18 h) at room temperature. After removal of the DMF, the residue was stirred under diethyl ether for 1 h, filtered and dried to give 550 mg (74%) of **1d**, mp 105-107 °C. FABMS *m/z*: 291. ^1H NMR (DMSO-d_6) δ 2.07 (m, 2, H-3′), 2.55 (m, 2, H-2′), 3.14 (s, 3, N-CH_3), 3.20 (s, 3, N-CH_3), 3.52 (m, 1, C*H*$_2$OH), 3.65 (m, 1, C*H*$_2$-OH), 4.13 (m, 1, H-4′), 5.04 (t, 1, CH_2OH), 6.29 (t, 1, H-1′), 8.42 (s, 1, H-2), 8.49 (s, 1, H-8), 8.92 (s, 1, C*H*-NMe_2). *Anal.* Calcd for $C_{13}H_{18}N_6O_2$: C, 53.78; H, 6.25; N, 28.95. Found: C, 53.79; H, 6.67; N, 29.09.

1-(2,3,5,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-2,4-dioxo-5-methylpyrimidine, Diphenyl Ester (4a). 3′-Deoxythymidine (**1a**) (400 mg, 1.8 mmol) and dicyclohexylcarbodimide (DCC) (1.1 g, 5.4 mmol) were dissolved in dry DMSO (6 mL)

under a N_2 atmosphere. Dichloroacetic acid (20 μL, 0.24 mmol) was added and stirring was continued for 1.5 h. Dicyclohexylurea (DCU) was removed by filtration and diphenyl triphenylphosphoranylidenemethylphosphonate (1.35 g, 2.7 mmol) was added to the filtrate. After stirring for 18 h, the solvent was removed *in vacuo* and **3a** was purified by chromatography. Compound **3a** (188 mg) was dissolved in ethanol (50 mL), 5% Pd/C (50 mg) was added and the mixture was shaken at 20 psi under H_2 for 2.5 h. After removal of the catalyst by filtration, the solvent was removed *in vacuo* to give 180 mg of **4a** (22%). FABMS *m/z*: 457. ^{1}H NMR (DMSO-d_6) δ 1.8 (m, 1, H-3′), 1.8 (s, 3, CH_3), 2.0 (m, 4, 5′-CH_2, H-2′, H-3′), 2.3 (m, 3, PCH_2, H-2′), 4.0 (m, 1, H-4′), 6.0 (m, 1, H-1′), 7.2 (m, 5, ArH), 7.4 (s, 1, H-6), 7.4 (m, 5, ArH). *Anal.* Calcd for $C_{23}H_{25}N_2O_6P \cdot 0.5H_2O$: C, 59.35; H, 5.63; N, 6.02. Found: C, 59.41; H, 5.52; N, 5.82.

1-(2,3,5,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-4-benzamido-2-oxopyrimidine, Diphenylester (4b). To N^4-Bz-ddC (**1b**) (350 mg, 1.11 mmol), dissolved in dry DMSO (8 mL), was added DCC (700 mg, 3.33 mmol) and pyridine (90 mg, 1.11 mmol), followed by dry 10% trifluoroacetic acid in DMSO solution (430 μL, 0.55 mmol). After the reaction had stirred for 1 h at 25 °C under N_2, DCU was removed by filtration and diphenyl triphenylphosphoranylidene-methylphosphonate (690 mg, 1.33 mmol) was added to the filtrate followed by stirring for 24 h. The solvent was removed and the residue was purified by centrifugal chromatography using 3:1 CH_2Cl_2-MeCN as the eluant to provide 180 mg (30%) of **3b**. Compound **3b** (180 mg) was dissolved in methanol (40 mL) containing 50 mg of 5% Pd/C and was shaken under 2 atm of H_2 for 2.5 h. The solution was filtered through Celite and the solvent removed to give **4b** (170 mg, 28% from **1b**) as a viscous gum. FABMS *m/z*: 546. ^{1}H NMR (DMSO-d_6) δ 1.6 (m, 1, H-3′), 2.1 (m, 4, H-2′, H-3′, H-5′), 2.3 (m, 3, H-2′, H-6′), 4.1 (m, 1, H-4′), 6.0 (m, 1, H-1′), 7.2-8.05 (m, 17, H-5, H-6, ArH), 11.25 (br s, 1, NH). *Anal.* Calcd for $C_{29}H_{28}N_3O_6P \cdot 0.5H_2O \cdot CH_3OH$: C, 61.43; H, 5.67; N, 7.16. Found: C, 61.42; H, 6.03; N, 7.01.

Preparation of 9-(2,3,5,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-9*H*-purin-6-one, Diphenylester (4c). Dichloroacetic acid (44 μL, 70 mg, 0.54 mmol) was added to a solution containing dideoxyinosine (**1c**)[15] (225 mg, 1.08 mmol) and DCC (669 mg, 3.24 mmol) in dry DMSO (5 mL), and the solution was stirred at room temperature under nitrogen for 2.5 h. At this point TLC (silica gel, $CHCl_3$-MeOH, 6:1) indicated that all of **1c** had been converted to aldehyde **2c**. Dry pyridine (50 μL, 49 mg, 0.62 mmol) was added, the mixture was stirred 5 min and diphenyl triphenylphosphoranylidenemethyl-

phosphonate (549 mg, 1.08 mmol) was added in one portion. After stirring overnight (20 h) the DCU was filtered and the filtrate was condensed *in vacuo* on a water bath (0.1 mm Hg, 42 °C). The residue was treated with a small amount of $CHCl_3$, filtered, and the filtrate was chromatographed on silica gel (2.5 x 7.0 cm column, 70-230 mesh), eluting first with $CHCl_3$-MeOH (99:1) to remove the triphenylphosphine oxide, then with $CHCl_3$-MeOH (97:3) to give chromatographically pure **3c** (340 mg, 68%) as a glassy residue. FABMS *m/z*: 465. Without further purification this residue was placed in MeOH (30 mL) containing 10% Pd/C (300 mg) and this mixture was shaken under H_2 (3 atm) for 48 h. The solution was filtered through Celite, washed with MeOH (30 mL), and the filtrate was concentrated to give **4c** as a clear glass (254 mg, 50% for three steps). Triturating this residue with diethyl ether, decanting the supernate and pumping to dryness provided **4c** as a hygroscopic white powder. FABMS *m/z*: 467 (strong 331 peak for dideoxysugar portion). ^1H NMR (DMSO-d_6) δ 2.04 (m, 3, H-3′, H-6′), 2.17 (m, 3, H-5′, H-6′), 2.47 (m, 2, H-2′, DMSO-d_5), 4.16 (t, 1, H-4′), 6.20 (t, 1, H-1′), 7.19 (m, 3, OPh), 7.37 (m, 2, OPh), 8.04 (s, 1, H-2), 8.25 (s, 1, H-8), 12.36 (br s, 1 NH). *Anal.* Calcd for $C_{23}H_{23}N_4O_5P \cdot 0.8H_2O \cdot 0.3C_4H_{10}O$: C, 57.78; H, 5.53; N, 11.14. Found: C, 57.74; H, 5.26; N, 11.13.

1-(2,3,5,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-2,4-dioxo-5-methylpyrimidine, Ca^{2+} Salt (5a). Diphenylester **4a** (60 mg, 0.13 mmol) was stirred in 3 mL of 0.2 N NaOH (0.6 mmol) for 2 h. Dissolution was incomplete, therefore 100 μL of ethanol was added. At 3 h, another 100 μL aliquot of ethanol was added. At 3.5 h, the solution was slowly eluted through a 2.5 mL resin bed of Dowex 50W-X8 (50-100 mesh, Et_3NH^+ form) using water as the eluant. The solution (15 mL) was treated with 1 mL of 2M tetraethylammonium bicarbonate (TEAB) buffer and 1 mg *C. atrox* phosphodiesterase. After 60 h, another 500 μg portion of phosphodiesterase was added and the mixture was stirred at 37 °C for 4 h. The buffer was removed azeotropically by repeated (7 times) evaporations from water (10 mL). The residue was redissolved in 480 μL of a 10% *w/v* $CaCl_2 \cdot 2H_2O$ solution in H_2O and **5a** was precipitated by the addition of ethanol (5 mL) yielding 20 mg (46%).

A second 60-mg portion of **4a** was treated as above to give 28 mg (62%) of **5a**. The two samples were 90% pure by HPLC. The samples were combined and recrystallized from ethanol-water to give 33 mg, mp 270 °C (dec). FABMS *m/z*: 343. ^1H NMR (D_2O) δ 1.45-2.4 (m, 8, H-2′, H-3′, H-5′, H-6′), 1.85 (s, 3, 5-CH_3), 4.1 (m, 1, H-4′), 6.1 (m, 1, H-1′), 7.5 (s, 1, H-6). *Anal.* Calcd for $C_{11}CaH_{15}N_2O_6P \cdot 0.25C_2H_5OH \cdot 0.5H_2O$: C, 38.07; H, 4.86; N, 7.72. Found: C, 38.06; H, 5.06; N, 7.59.

Preparation of 9-(2,3,5,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-9*H*-purin-6-one, Ca^{+2} Salt (5c). A solution containing **4c** (370 mg, 0.80 mmol) in concentrated NH_4OH (75 mL) was stirred for 48 h at room temperature. The water was removed, leaving a white powder that was dissolved in 0.1 M TEAB buffer (15 mL) and placed in a preheated oil bath (37 °C). *C. atrox* phosphodiesterase (1 mg) was added and stirring was continued for 24 h. Another 0.5 mg portion of phosphodiesterase was added and stirring was continued an additional 24 h. TLC (CH_3CN-1M NH_4OH, 4:1) showed the reaction to be complete. The buffer was removed by repeated (7 times) evaporations from 10 mL of H_2O at 30 °C. The residue was placed in 0.68 M $CaCl_2$ (10%) (1.06 mL) and precipitated as its calcium salt by the addition of EtOH (15 mL). Filtration and drying gave 125 mg (40% over 2 steps) of **5c** as a white powder. FABMS *m/z*: 315, mp 325$^+$ °C. ^1H NMR (D_2O, tBuOH as internal reference, δ = 1.236 ppm) δ 1.6 (m, 2, H-6′), 1.89 (m, H-5′, H-3′), 2.3 (m, 1, H-3′), 2.55 (m, 2, H-2′), 4.28 (m, 1, H-4′), 6.29 (dd, 1, H-1′), 8.18 (s, 1, H-2), 8.27 (s, 1, H-8). ^{13}C NMR (D_2O, dioxane as internal reference) δ 25.36 (d, $P(O)CH_2$, J_{CP} = 134.2 Hz), 29.99 (d, $P(O)CH_2CH_2$-, J_{CCP} = 3.7 Hz), 29.76 (s, C-2′ or C-3′), 32.63 (s, C-2′ or C-3′), 67.4 (s, dioxane), 84.05 (d, C-4′, J_{CCP} = 18.9 Hz), 85.80 (s, C-1′), 124.62 (s, C-5, $J_{C5,H8}$ = 10.6 Hz), 140.26 (s, C-8, $J_{C8,H8}$ = 216.4), 146.62 (s, C-2, $J_{C2,H2}$ = 208.9 Hz), 148.99 (s, C-4), 159.35 (s, C-6, $J_{C6,H2}$ = 7.1 Hz). *Anal.* Calcd for $C_{11}CaH_{13}N_4O_5P \cdot 0.3C_2H_5OH \cdot 1.5H_2O$: C, 35.44; H, 4.56; N, 14.25. Found: C, 35.53; H, 4.66; N, 14.22.

1-(2,3,4,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-4-amino-2-oxopyrimidine, Ca^{2+} Salt (5e). Diphenyl ester **4b** (80 mg, 0.15 mmol) was stirred in a solution of concentrated NH_4OH (40 mL) and ethanol (2 mL) for 24 h. The solvent was reduced to 30 mL *in vacuo* and the aqueous solution was extracted with 1:1 diethyl ether-ethyl acetate (2 x 25 mL). The aqueous solution was then concentrated to near dryness and diluted to 20 mL with 0.1M TEAB buffer. *C. atrox* phosphodiesterase (0.5 mg) was added and the solution was stirred for 4 days at 20 °C. The TEAB buffer was removed azeotropically with H_2O. The residue was redissolved in 10% $CaCl_2 \cdot 2H_2O$ (400 uL) solution and crude **5e** was precipitated by the addition of ethanol (5 mL). The white solid was recrystallized from water-ethanol to give 36 mg (67%) of **1e**, mp 280 °C (dec). ^1H NMR (D_2O) δ 1.5 (m, 3, H-3′, H-2′), 2.0 (m, 4, H-6′, H-5′), 2.4 (m, 1, H-2′), 4.15 (m, 1, H-4′), 6.05 (m, 2, ArH, H-1′), 7.8 (d, 1, ArH). *Anal.* Calcd for $C_{10}CaH_{14}N_3O_5PCa \cdot 1.5H_2O \cdot 0.25C_2H_5OH$: C, 34.48; H, 5.10; N, 11.49. Found: C, 34.31; H, 4.93; N, 11.31.

Preparation of 9-(2,3,5,6-Tetradeoxy-6-phosphono-β-D-*glycero*-hexofuranosyl)-9*H*-purin-6-amine, Ca^{+2} Salt (5f). A solution containing compound **1d** (400 mg, 1.28 mmol) and dicyclohexylcarbodiimide (DCC) (854 mg, 4.14 mmol) in dry DMSO (20 mL) was placed under N_2. Dichloroacetic acid (57 μL, 89 mg, 0.69 mmol) was syringed in and the solution was allowed to stir for 2 h with gradual precipitation of DCU. Pyridine (111 μL, 109 mg, 1.38 mmol) was added and, after 5 min, diphenyl triphenylphosphoranylidenemethylphosphonate (1.4 g, 2.76 mmol) was added in one portion. After resealing under N_2 the solution was stirred overnight (20 h). The solution was filtered and most of the DMSO was removed *in vacuo* on a 40 °C water bath. Water (25 mL) was added and the solution was extracted with ether (2 x 25 mL) to remove triphenylphosphine oxide. Chloroform (1 x 20 mL) extraction removed most of the unreacted ylide. The ether and $CHCl_3$ extracts were condensed to dryness and the residue was triturated with EtOAc to back-extract the product. The water layer was extracted with EtOAc (2 x 25 mL), which was combined with the EtOAc mentioned above and condensed *in vacuo* without drying. The residue was chromatographed on silica gel (2.5 x 14 cm column, 70-230 mesh) eluting first with $CHCl_3$-MeOH (99:1) to elute triphenylphosphine oxide. Further elution with $CHCl_3$-MeOH (97:3) and removal of the solvents from the product containing fractions gave **3d** as a glassy residue, 380 mg (53%). FABMS *m/z*: 519. Compound **3d** (180 mg, 0.35 mmol) was placed without any further purification in MeOH (20 mL) with 10% Pd/C (40 mg) and was shaken under H_2 (27 psi) overnight (20 h). The catalyst was filtered over Celite and the filtrate condensed giving 170 mg (94%) of **4d** and **4f** as a glass, FABMS *m/z*: 521, 466. Compounds **4d** and **4f** (170 mg, 0.33 mmol) were placed in concentrated NH_4OH (50 mL) and stirred with gradual dissolving for 48 h at room temperature. At this point TLC (silica gel, CH_3CN-1M NH_4OH, 4:1) showed complete conversion of the mixture to the monophenyl ester (R_f ~0.6). The solution was filtered through Celite and condensed *in vacuo* (30 °C) leaving a clear glass (150 mg, 0.33 mmol). This residue was placed in 0.1 M TEAB buffer (7 mL, pH 8.8) and placed in a 37 °C oil bath. *C. atrox* phosphodiesterase I (0.5 mg) was added and the solution was stirred 24 h. The solution was evaporated to dryness and the residue was redissolved in fresh buffer. More phosphodiesterase I (0.5 mg) was added and stirring was continued overnight (24 h). The water was removed and the residue was repeatedly dissolved in H_2O (7 x 10 mL) and evaporated (30 °C) to dryness to remove the buffer. The residue was dissolved in 10% aqueous $CaCl_2$ (0.6 mL, 0.41 mmol) and the product was precipitated as its calcium salt by adding 10 mL of EtOH. Filtering and

drying gave **5f** as a white powder, 79 mg (73% for 2 steps from **4d** and **4f**), mp 325 °C$^+$ (dec). FABMS *m/z* (after addition of HCl): 314. ^{1}H NMR (D_2O) (tBuOH internal reference) δ 1.61 (m, 2, P(O)CH_2), 1.89 (m, 3, P(O)CH$_2$CH_2, H-3′), 2.3 (m, 1, H-3′), 2.51 (m, 2, H-2′), 4.27 (m, 1, H-4′), 6.21 (m, 1, H-1′, $J_{1',2'a}$ = 6.8 Hz, $J_{1',2'b}$ = 2.5 Hz), 8.12 (s, 1, H-2), 8.23 (s, 1, H-8). ^{13}C NMR (D_2O, dioxane internal reference) δ 29.94 (d, P(O)CH_2, J_{CP} = 134.5 Hz), 29.74 (s, C-2′ or C-3′), 29.96, 29.92 (d, P(O)CH$_2$$CH_2$, J_{CCP} = 3.0 Hz), 32.77 (s, C-2′ or C-3′), 67.4 (s, dioxane), 83.61, 83.86 (d, C-4′, J_{CCCP} = 18.2 Hz), 85.45 (s, C-1′), 119.16 (s, C-5), 140.04 (s, C-8, $J_{C8,H8}$ = 214.5 Hz, $J_{C8,H\text{-}1'}$ = 2.4 Hz), 148.47 (s, C-4), 152.87 (S, C-2, $J_{C2,H2}$ = 202.3 Hz), 155.70 (s, C-6, $J_{C6,H2}$ = 11.0 Hz). *Anal.* Calcd for $C_{11}CaH_{14}N_5O_4P \cdot 0.2C_2H_5OH \cdot 0.7H_2O$: C, 36.69; H, 4.48; N, 18.77. Found: C, 36.42; H, 4.85; N, 18.89.

ACKNOWLEDGEMENT

Support for this research was provided by the National Institutes of Health through the National Cooperative Drug Discovery Group (NCDDG) program, Grant Number U01-AI26054. Spectral data was supplied by Dr. William C. Coburn, Dr. James R. Riordan, Marion C. Kirk, Christine Richards, and M. Diane Ochs.

REFERENCES

(1) Furman, P. A.; Fyfe, J. A.; St. Clair, M.; Weinhold, K.; Rideout, J.; Freeman, G. A.; Lehrman, S. N.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl. Acad. Sci. USA* **1989**, *83*, 8333.

(2) Waqar, M. A.; Evans, M. J.; Manly, K. F.; Hughes, R. G.; Huberman, J. A. *J. Cell. Phys.* **1984**, *121*, 402.

(3) Cooney, D. A.; Dalal, M.; Mitsuya, H.; McMahon, J. B.; Nadkarni, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1986**, *35*, 2065.

(4) Ahluwalia, G.; Cooney, D. A.; Mitsuya, H.; Fridland, A.; Flora, K. P.; Hao, Z.; Dalal, M.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1987**, *36*, 3797.

(5) Cooney, D. A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D. G. *Biochem. Pharmacol.* **1987**, *36*, 1765.

(6) Tanaka, H.; Fukui, M.; Haraguchi, K.; Masaki, M.; Miyasaka, T. *Tetrahedron Lett.* **1989**, *30*, 2567.

(7) Barton, D. H. R.; Géro, S. D.; Quiclet-Sire, B.; Samadi, M. *Tetrahedron Lett.* **1989**, *30*, 4969.

(8) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464.

(9) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 332.

(10) Pauwels, R.; Balzarini, J.; Schols, D.; Baba, M.; Desmyter, J.; Rosenberg, I.; Holy, A.; De Clercq, E. *Antimicrob. Agents Chemother.* **1988**, *32*, 1025.

(11) A preliminary report of this work was given at the Proceedings of the VIIIth International Round Table of Nucleosides and Nucleotides, Orange Beach, AL, U.S.A., October 2-5, 1988. See Riggs, R. M.; Comber, R. N.; Montgomery, J. A.; Secrist III, J. A. *Nucleosides, Nucleotides* **1989**, *8*, 1119.

(12) Ioannidis, P.; Classon, B.; Samuelsson, B.; Kvarnström, I. *Acta Chem. Scand.* **1991**, *45*, 746.

(13) Jie, L.; Van Aerschot, A.; Balzarini, J.; Janssen, G.; Busson, R.; Hoogmartens, J.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1990**, *33*, 2481.

(14) Prisbe, E. J.; Martin, J. C. *Synth. Commun.* **1985**, *15*, 401.

(15) Webb, II, R. R.; Wos, J. A.; Martin, J. C.; Brodfuehrer, P. R. *Nucleosides, Nucleotides* **1988**, *7*, 147.

(16) Robins, M. J.; Hansskke, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.* **1984**, *25*, 367.

(17) ddC was generously provided by the Drug Synthesis and Chemistry Branch, DTP, DCT of the National Cancer Institute, Bethesda, MD. See Motawia, M. S.; Pedersen, E. B. *Liebigs Ann. Chem.* **1990**, 599, for recent preparations.

(18) Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1968**, *90*, 5337.

(19) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661.

(20) Jones, G.; Hamamura, E.; Moffatt, J. *Tetrahedron Lett.* **1968**, *55*, 5731.

(21) York, J. L. *J. Org. Chem.* **1981**, *46*, 2171.

(22) Chaffee, S.; Leeds, J. M.; Matthews, T. J.; Weinhold, K. J.; Skinner, M.; Bolognesi, D. P.; Hershfield, M. S. *J. Exptl. Med.* **1988**, *168*, 605.