Synthesis and Biological Evaluation of 1′,2′-Seconucleo-5′-phosphonates

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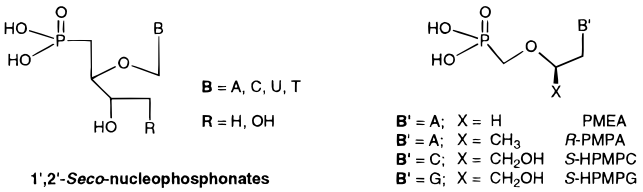
A series of 1′,2′-seconucleophosphonate analogues were prepared containing adenine, cytosine, thymine, and uracil as the nucleobase. The synthetic methodology is efficient and uses chloromethyl ethers derived from the chirons diethyl (3*S*)-(benzyloxy)-(2*R*)-hydroxybutanephosphonate (**1**) and diethyl (3*S*),4-bis(benzyloxy)-(2*R*)-hydroxybutanephosphonate (**2**). Selected deblocked derivatives, *i.e.*, two monoesters (**13** and **14**), four phosphonic acids (**15**−**18**), and one cyclic phosphonate (**23**), were screened for *in vitro* activity against certain RNA, adeno, and HIV viruses. All of them were found to be devoid of activity.

There is considerable interest in phosphonates as biologically active surrogates of naturally occurring phosphates.[1] Phosphonate replacement is attractive since the carbon−phosphorus bond in phosphonates is not susceptible to enzymatic degradation by phosphatases, thus enhancing physiological stability. In addition, phosphonate esters are less polar, and this feature gives rise to better cell permeability.[2]

With this in mind, we explored the synthesis of 5′-phosphonate analogues of 1′,2′-seconucleotides. In preparing the title acyclic analogues, we focused on several structural features. First, we patterned the spatial relationship of the *C*3′- and *C*4′-positions of our 1′,2′-seconucleo-5′-phosphonates[3] with those of arabino/ribo-



nucleotides, and second, we took into account the distance between the phosphonate moiety and the nucleobase ring nitrogen in order to examine what influence it had on antiviral activity. Regarding the latter issue, PMEA, (*R*)-PMPA, (*S*)-HPMPC, and (*S*)-HPMPG, acyclic phosphonate analogues which have the same distance between the phosphorous and nitrogen atoms as our title compounds, exhibit potent and selective antiviral activity.[4] Prior to starting our work, several published accounts described the syntheses of purine acyclophosphonate nucleotide analogues[2,5] using Arbuzov[2] and Wittig[5] conditions. Our initial attempt at preparing 1′,2′-seconucleophosphonates involved the Arbuzov reaction[6] (see Scheme 4) on suitably functionalized 1′,2′-seconucleosides, *e.g.*, **21**.[7] We soon realized that this procedure was cumbersome, time consuming, and cost inefficient; thus we sought an alternative route. We envisaged that chiral β- and γ-hydroxybutanephosphonate esters would serve as ideal synthons of the desired chloromethyl ethers which, in turn, could then be condensed with a variety of nucleobases. This methodology appeared more attractive, efficient, and conducive to scale up than our original approach which attached the phosphonate at a late stage in the synthesis[8] to individual functionalized 1′,2′-seconucleosides. The chiral phosphonate synthons have now been prepared[9] in good yields using a regiospecific and nucleophilic ring opening of chiral epoxides.[10] This paper discloses their application in the synthesis of the title 1′,2′-seconucleophosphonates and reports on the biological evaluation of selected acyclophosphonate analogues.

Chemistry

The synthetic strategy developed for the preparation of the title 1′,2′-seconucleophosphonates is depicted in Scheme 1. This practical, convergent approach uses the chirons diethyl (3*S*)-(benzyloxy)-(2*R*)-hydroxybutanephosphonate (**1**)[9] and diethyl (3*S*),4-bis(benzyloxy)-(2*R*)-hydroxybutanephosphonate (**2**).[9] Chloromethylation[11] of either **1** or **2** followed by condensation with the desired, functionalized heterocycle furnished the blocked phosphonate esters **3**−**6** and **8**. In a typical reaction, **1** was chloromethylated with paraformaldehyde and hydrogen chloride gas in dry methylene chloride (CH_2Cl_2) at 0 °C. The isolated chloromethyl ether of **1** was then used immediately with either persilylated cytosine, thymine, or uracil in the presence of a catalytic amount of tetraethylammonium iodide. After workup, the pure, blocked 1′,2′-seconucleophosphates **3**−**5** were obtained in good yields.

The synthesis of **6** relied on a different approach and involved the alkylation of the sodium salt of 6-chloropurine[12] (generated with sodium hydride) with the chloromethyl ether of chiron **1** in dry acetonitrile at room temperature. Thin layer chromatography of the reaction mixture indicated the presence of two products presumed to be the *N*7- and *N*9-alkylated isomers of 6-chloropurine, with the *N*9-isomer predominating. The site of alkylation of the major and faster running isomer was subsequently confirmed by converting it to the adenine 1′,2′-seconucleo-5′-phosphonate **11** and examining the UV spectra of this analogue. Phosphonate **11** exhibited the characteristic spectral features of an *N*9-substituted adenine [λ_{max} (pH 1) 257 nm and (pH 11) 259 nm]. Compound **6** was converted to **7** using methanolic ammonia at 90 °C in a sealed reaction vessel.

Removal of the 3′-*O*-benzyl protecting group, as well as the 2′-*O*-benzyl protecting group in the case of **8**, was carried out smoothly by catalytic transfer hydrogena-

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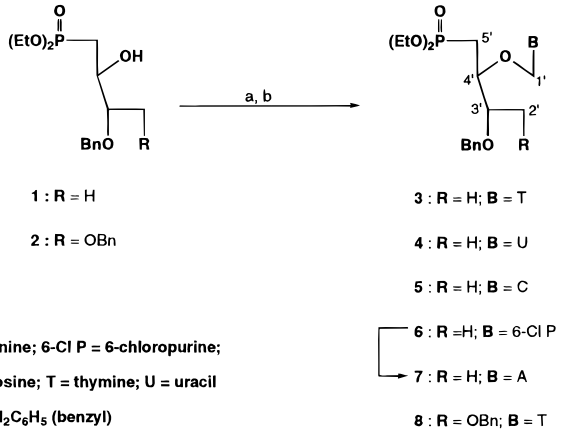
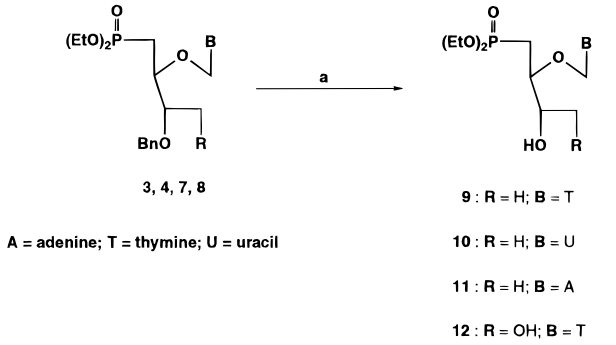
Scheme 1[a]



[a] Reagents: (a) $(CH_2O)_n$, HCl_g, CH_2Cl_2, 0 °C; (b) B-TMS, CH_2Cl_2, TEAI or B-Na, CH_3CN.

Scheme 2[a]

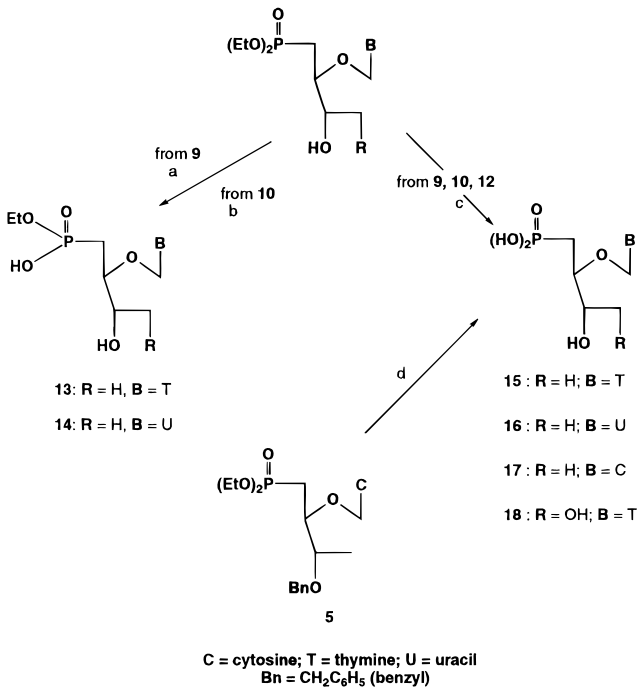


[a] Reagents: (a) 20% $Pd(OH)_2$/C, EtOH, cyclohexene, reflux (Δ).

tion[11] using 20% palladium hydroxide on carbon (Pearlman's catalyst) and cyclohexene (Scheme 2). This debenzylation procedure provided the diethyl phosphonate esters **9**−**12** in excellent yield. Deesterification of the phosphonate moiety was conducted in several ways (Scheme 3). Reaction of **9** with 4 equiv of bromotrimethylsilane (TMS-Br) for 1 h at 0 °C under nitrogen gave the monoethyl ester **13** in 94% yield, while **10** was subjected to an aqueous ethanolic solution of sodium hydroxide at room temperature to furnish a quantitative yield of the monoethyl ester **14**. When the reaction time of bromotrimethylsilane deesterfication was increased to *ca*. 16 h, while keeping the ratio of TMS-Br to diethyl phosphonate 4:1, complete deesterification of the phosphonate moiety was realized. It is worth mentioning that **5** was deblocked and deesterified in one step to **17** using iodotrimethylsilane.[13] The phosphonic acids **15**−**18** were easily purified using gravity flow C_{18} reverse phase silica gel column chromatography and isolated in near-quantitative yields.

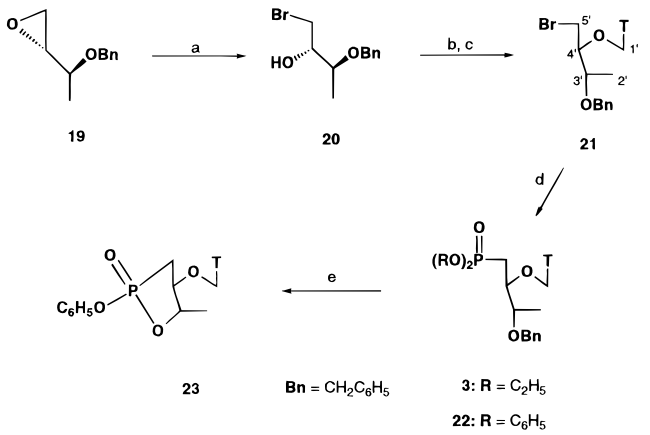
As mentioned above, our first approach to prepare the title compounds involved phosphonate formation after alkylation of the requisite heterocycle (nucleobase). This approach employed the chloromethyl ether of bromohydrin **20** (Scheme 4). Bromohydrin **20** was easily prepared by regiospecific opening of epoxide **19** with dilithium tetrabromonickelate(II).[14] Chloromethylation of **20** followed by condensation with persilylated thymine furnished **21**. Introduction of the 5′-phosphonate[3] function was accomplished by treatment of the bromothymine **21** with either triethyl or diphenyl ethyl phosphite to give esters **3** and **22**, respectively. While

Scheme 3[a]



[a] Reagents: (a) TMS-Br, N_2, 0 °C, 1 h; (b) NaOH, EtOH−H_2O (1:1), room temperature; (c) TMS-Br, N_2, 0 °C → room temperature, 16 h; (d) TMS-I, N_2, 0 °C (1 h) → room temperature (1 h).

Scheme 4[a]



[a] Reagents: (a) Li_2NiBr_4, THF, room temperature, 15 h; (b) $(CH_2O)_n$, HCl_g, CH_2Cl_2, 0 °C; (c) T-TMS, CH_2Cl_2; (d) $(EtO)_3P$, 155 °C, 28 h or $(C_6H_5O)_2POEt$, 165 °C, 18.5 h; (e) 10% Pd/C, EtOH, cyclohexene, reflux (Δ), 1.5 h.

deblocking of **3** gave the expected ester **9**, the analogous reaction of the diphenyl ester **22** gave, unexpectedly, the cyclic phosphonate ester **23**. Structure proof of this compound was derived from the correct elemental analysis and a comparison of its ^1H NMR data with those of compound **13**. While the proton spectrum of **13** in $CDCl_3$ clearly showed three D_2O exchangeable protons, that of **23** had only one corresponding to the N(3)*H* proton. Furthermore, in compound **23**, the phosphorus atom is chiral making **23** a mixture of diastereomers. This was evident from the ^1H NMR spectrum where the 2′-methyl group appeared as two separate doublets (J = 6 Hz). On the other hand, the methyl group in compound **13** appeared, as anticipated, as one doublet (J = 6 Hz) since the phosphorus atom is achiral. The formation of **23** can best be explained by initial debenzylation followed by intramolecular trans-

esterification accompanied by the loss of phenol. This reaction, expectedly, takes place only with phenyl esters since subjecting the diethyl ester **9** to the same reaction conditions resulted in the isolation of the starting material. In addition, when **3** was treated in a similar manner only debenzylation occurred to furnish the diethyl phosphonate **9**.

Biological Evaluation

Compounds **13**−**18** and **23** were screened for activity against adenovirus type 2 (AD2), human immunodeficiency virus (HIV), Japanese encephalitis (JBE) virus, pichinde (PIC) virus, punto toro (PT) virus, Rift Valley fever (RVF) virus, Sicilian sandfly fever (SFS) virus, Venezuelan equine encephalomyelitis (VEE) virus, vesicular stomatitis (VSV) virus, and yellow fever (YF) virus. None of the 1′,2′-seconucleo-5′-phosphonates exhibited any *in vitro* activity up to 320 μg/mL. It is interesting to note that certain thymine, cytosine, and guanine 2-(phosphonomethyl)-1,3-dioxolane nucleotides,[15] which were designed as constrained analogues of PMEA, and like our title compounds mimic the distance between the phosphonate moiety and the nucleobase ring nitrogen, were also shown to be devoid of antiviral activity. It appears that the pK_a of the phosphonate moiety and thus the spatial location of the oxygen atom in the acyclic chain, *i.e.*, it must be positioned β to the phosphorus atom, are critical factors for antiviral activity.[4d]

Experimental Section

Melting points were determined on a Buchi 535 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 141 automatic digital readout polarimeter. ^{1}H NMR spectra were recorded on either a Varian EM 390 or a Bruker AM-300 spectrometer using Me_4Si (TMS) as an internal standard. UV absorption spectra were recorded with a Beckman DU-64 spectrophotometer. All mositure-sensitive reactions were performed using flame-dried glassware. Methylene chloride (CH_2Cl_2) and acetonitrile were dried over CaH_2 and distilled. Evaporations were performed under diminished pressure using a Buchi rotary evaporator unless stated otherwise. A Harrison 7924T chromatotron was used to complete various separations as indicated. Plates (1.0 and 2.0 mm) used were coated with silica gel PF254 containing $CaSO_4$. Thin-layer chromatography was performed on precoated silica gel plates (60-F254, 0.2 mm) manufactured by EM Science, Inc., and short-wave ultraviolet light (254 nm) was used to detect the UV-absorbing compounds. Silica gel (Merck grade 60, 230−400 mesh, 60 Å) suitable for column chromatography was purchased from Aldrich. C_{18} reverse phase silica gel column chromatography was conducted on custom bonded Davisil (35−75 μm, 60 Å; Alltech Associates). All solvent proportions are by volume unless stated otherwise. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

1-[[(3*S*)-(Benzyloxy)-1-(diethylphosphonyl)-(2*R*)-butoxy]methyl]thymine (3). Method A: In a three-necked, flame-dried flask fitted with a gas inlet and a drying tube were added paraformaldehyde (0.285 g, 9.48 mmol), the alcohol **1** (2.0 g, 6.32 mmol), and dry CH_2Cl_2 (30 mL). The mixture was cooled to 0 °C in an ice bath, and dry HCl gas was bubbled into the solution for 6 h, maintaining the temperature at 0 °C. Calcium chloride (*ca*. 5 g) was added cautiously and the mixture stirred for 15 min. After filtration, the solution was concentrated under diminished pressure to give the corresponding chloromethyl ether, which was directly converted to the title compound, as follows. Thymine (1.59 g, 12.69 mmol) and ammonium sulfate (*ca*. 50 mg) were added to hexamethyldisilazane (HMDS; 25 mL). The reaction mixture was stirred at reflux overnight with the exclusion of moisture. After cooling to room temperature (clear solution), the excess HMDS was removed under reduced pressure and the residue dried under high vacuum. A solution of the above chloromethyl ether in dry CH_2Cl_2 (20 mL) and tetraethylammonium iodide (TEAI; *ca*. 50 mg) were added to the persilylated thymine, and the mixture was stirred at reflux overnight. The reaction mixture was then diluted with water (10 mL) and methanol (30 mL), stirred for 15 min, and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL), washed successively with water, a 10% $Na_2S_2O_3$ solution, and brine, and then dried over anhydrous $MgSO_4$. The viscous material obtained after solvent removal at reduced pressure was column chromatographed, eluting with ethyl acetate, to give **3** (2.73 g, 95%) as a gum: $[\alpha]^{25}_D$ +14.8° (*c* = 1.875, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.16 (d, *J* = 6 Hz, 3H, C*H*$_3$), 1.30 (t, *J* = 6 Hz, 6H, 2CH$_2$C*H*$_3$), 1.70−2.24 (m, 2H, C*H*$_2$P), 1.84 (s, 3H, C*H*$_3$), 3.46−4.26 (m, 6H), 4.48 (s, 2H, C*H*$_2$Ph), 5.24 (AB$_q$, *J* = 12 Hz, 2H, OC*H*$_2$N), 7.18 (br s, 6H, C(6)*H*, C$_6$*H*$_5$), 9.92 (s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{21}H_{31}N_2O_7P$) C, H, N, P.

Method B: Triethyl phosphite (6.1 mL, 35.2 mmol) was added to **21** (1.33 g, 3.34 mmol) and the mixture stirred at 155 °C for 28 h. After this period, excess triethyl phosphite was distilled off and the crude product was column chromatographed. The column was eluted with hexane−ethyl acetate (1:3) and then ethyl acetate. UV-absorbing fractions containing **3** were combined and concentrated under diminished pressure. This procedure furnished pure **3** (1.15 g, 75.6%) which was identical in all respects with **3** synthesized from method A.

1-[[(3*S*)-(Benzyloxy)-1-(diethylphosphonyl)-(2*R*)-butoxy]methyl]uracil (4). Persilylated uracil [obtained from 2.12 g (18.96 mmol) of uracil] was coupled as described for **3**, with the chloromethyl ether derived from **1** (3 g, 9.48 mmol), in dry CH_2Cl_2 (30 mL) and in the presence of TEAI (70 mg). The reaction mixture was stirred and heated at reflux for 12 h. Workup and chromatography (ethyl acetate) afforded pure **4** (3.97 g, 95%): $[\alpha]^{25}_D$ −12.4° (*c* = 1.00, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, *J* = 6 Hz, 3H, C*H*$_3$), 1.26 (t, *J* = 6 Hz, 6H, 2CH$_2$C*H*$_3$), 1.84−2.54 (m, 2H, C*H*$_2$P), 3.50−4.36 (m, 6H), 4.54 (s, 2H, C*H*$_2$-Ph), 5.22 (AB$_q$, *J* = 12 Hz, 2H, OC*H*$_2$N), 5.62 (d, *J* = 8 Hz, 1H, C(5)*H*), 7.30 (s, 5H, C$_6$*H*$_5$), 7.44 (d, *J* = 8 Hz, 1H, C(6)*H*), 9.82 (br s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{20}H_{29}N_2O_7P$) C, H, N, P.

1-[[(3*S*)-(Benzyloxy)-1-(diethylphosphonyl)-(2*R*)-butoxy]methyl]cytosine (5). Persilylated cytosine [obtained from 1.67 g (15 mmol) of cytosine] was coupled as described for **3**, with the chloromethyl ether derived from **1** (3.16 g, 10 mmol), in dry CH_2Cl_2 (75 mL) and in the presence of TEAI (75 mg). The reaction mixture was stirred and heated at reflux for 12 h. Workup and chromatography (ethyl acetate-methanol, 9:1) afforded pure **5** (4.03 g, 92%): $[\alpha]^{25}_D$ +9.63° (*c* = 0.81, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, *J* = 6 Hz, 3H, C*H*$_3$), 1.28 (t, *J* = 6 Hz, 6H, 2CH$_2$C*H*$_3$), 1.82−2.30 (m, 2H, C*H*$_2$P), 3.52−4.30 (m, 6H), 4.50 (s, 2H, C*H*$_2$Ph), 5.04−5.42 (m, 2H, OC*H*$_2$N), 5.94 (d, *J* = 8 Hz, 1H, C(5)*H*), 7.24 (s, 6H, C(6)*H*, C$_6$*H*$_5$), 9.80 (br s, 2H, N*H*$_2$, D_2O exchangeable). Anal. ($C_{20}H_{30}N_3O_6P$) C, H, N, P.

6-Chloro-9-[[(3*S*)-(Benzyloxy)-1-(diethylphosphonyl)-(2*R*)-butoxy]methyl]purine (6). To a suspension of sodium hydride (60%, 0.96 g, 24 mmol; prewashed with petroleum ether) in dry acetonitrile (170 mL) was added 6-chloropurine (2.85 g, 18.44 mmol), and the mixture was stirred for 2 h at room temperature. The chloromethyl ether derived from **1** (5.84 g, 18.46 mmol) was dissolved in acetonitrile (*ca*. 25 mL) and added to the above suspension, and the reaction mixture was stirred for 20 h at room temperature while under dry nitrogen. At the end of this period, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to a viscous liquid. This material was column chromatographed, using acetone−ethyl acetate (2:8) as eluant, to provide **6** (2.48 g, 28%) as a gum. A slower running material (chromatotron; acetone−ethyl acetate, 2:8) was isolated and assigned as the N7-isomer (^{1}H NMR, $CDCl_3$) but was not further characterized. Compound **6** exhibited the following physical data: $[\alpha]^{25}_D$ −13.5° (*c* = 1.15, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, *J* = 6 Hz, 3H, C*H*$_3$), 1.25 (t, *J* = 6 Hz, 6H, 2CH$_2$C*H*$_3$), 2.00 (ABq, $\Delta\nu_{AB}$ = 18

Hz, J_{AB} = 6 Hz, 2H, CH_2P), 3.50−3.65 (m, 1H), 3.80−4.10 (m, 5H), 4.40 (ABq, $\Delta\nu_{AB}$ = 15 Hz, J_{AB} = 10 Hz), 6.00 (s, 2H, OCH_2N), 7.10−7.30 (m, 5H, C$_6H_5$), 8.50 (s, 1H, C(2)*H*), 8.80 (s, 1H, C(8)*H*). Anal. ($C_{21}H_{28}O_5N_4PCl$) C, H, N, P, Cl.

9-[[(3*S*)-(Benzyloxy)-1-(diethylphosponyl)-(2*R*)-butoxy]methyl]adenine (7). Chloropurine **6** (0.360 g, 0.745 mmol) was dissolved in methanol (3 mL) and placed in a glass liner. To this solution was added liquid ammonia (7 mL), and the mixture was heated in a steel reaction vessel at 90 °C for 24 h. After cooling, excess ammonia was vented off and the remaining residue column chromatographed using ethyl acetate−methanol (95:5) as the eluant to furnish **7** (0.103 g, 30%): $[\alpha]^{25}_D$ +4.65° (*c* = 0.20, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.05 (d, *J* = 6Hz, 3H, CH_3), 1.25 (t, *J* = 6 Hz, 6H, 2CH$_2$CH_3), 2.00 (ABq, $\Delta\nu_{AB}$ = 18 Hz, J_{AB} = 6 Hz, 2H, CH_2P), 3.33−3.60 (m, 1H), 3.70−4.30 (m, 5H), 4.45 (s, 2H, OCH_2Ph), 5.70 (s, 2H, OCH_2N), 6.30−6.60 (br s, 2H, NH_2, D_2O exchangeable), 7.10−7.30 (m, 5H, C$_6H_5$), 7.95 (s, 1H, C(2)*H*), 8.30 (s, 1H, C(8)*H*). Anal. ($C_{21}H_{30}O_5N_5P$) H, N; C: calcd, 54.42; found, 54.98.

1-[[(3*S*),4-Bis(benzyloxy)-1-(diethylphosphonyl)-(2*R*)-butoxy]methyl]thymine (8). Persilylated thymine [obtained from 0.906 g (7.19 mmol) of thymine] was coupled as described for **3**, with the chloromethyl ether derived from **2** (2.02 g, 4.79 mmol), in dry CH_2Cl_2 (30 mL) and in the presence of TEAI (30 mg). The reaction mixture was stirred and heated at reflux for 12 h. Workup and chromatography (ethyl acetate−methanol, 9:1) afforded pure **8** (2.32 g, 87%): $[\alpha]^{25}_D$ +3.97° (*c* = 1.69, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 6 Hz, 6H, 2CH$_2$CH_3), 1.80 (s, 3H, CH_3), 1.92−2.38 (m, 2H, CH_2P), 3.46−4.34 (m, 8H), 4.45 (s, 2H, CH_2Ph), 4.58 (s, 2H, CH_2Ph), 5.18 (AB$_q$, *J* = 8 Hz, 2H, OCH_2N), 7.24 (br s, 11H, C(6)*H*, 2C$_6H_5$), 9.80 (br s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{28}H_{37}N_2O_8P$) C, H, N, P.

1-[[1-(Diethylphosphonyl)-(3*S*)-hydroxy-(2*R*)-butoxy]methyl]thymine (9). A solution of compound **3** (0.560 g, 1.23 mmol) in ethanol (9 mL) and cyclohexene (4.6 mL) was treated with 20% palladium hydroxide on carbon [Pd(OH)$_2$/C; 50 mg]. The resulting suspension was stirred at reflux for 12 h. After cooling to room temperature, the mixture was filtered and the filtrate was concentrated at reduced pressure. The residue was column chromatographed (ethyl acetate−methanol, 9:1) to give pure **9** (0.440 g, 100%): $[\alpha]^{25}_D$ +19.5° (*c* = 1.37, CHCl$_3$); ^{1}H NMR ($CDCl_3$) δ 1.12 (d, *J* = 6 Hz, 3H, CH_3), 1.32 (t, *J* = 6 Hz, 6H, 2CH$_2$CH_3), 1.70−2.32 (m, 2H, CH_2P), 1.86 (s, 3H, CH_3), 3.54−4.36 (m, 7H, 1H exchanges with D_2O), 5.24 (AB$_q$, *J* = 8 Hz, 2H, OCH_2N), 7.32 (br s, 1H, C(6)*H*), 10.22 (s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{14}H_{25}N_2O_7P$) C, H, N, P.

1-[[1-(Diethylphosphonyl)-(3*S*)-hydroxy-(2*R*)-butoxy]methyl]uracil (10). Compound **10** was prepared from **4** (1.5 g, 3.409 mmol) by the method described for **9** in 97% yield: $[\alpha]^{25}_D$ +8.3° (*c* = 0.738, EtOH); ^{1} H NMR ($CDCl_3$) δ 1.06 (d, *J* = 6 Hz, 3H, CH_3), 1.26 (t, *J* = 6 Hz, 6H, 2CH$_2$CH_3), 1.80−2.40 (m, 2H, CH_2P), 3.53−4.39 (m, 7H, 1H exchanges with D_2O), 5.03−5.50 (m, 2H, OCH_2N), 5.70 (d, *J* = 9 Hz, 1H, C(5)*H*), 7.50 (d, *J* = 9 Hz, 1H, C(6)*H*), 10.30 (s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{13}H_{23}N_2O_7P$) C, H, N, P.

9-[[1-(Diethylphosphonyl)-(3*S*)-hydroxy-(2*R*)-butoxy]methyl]adenine (11). Adenine **11** was prepared from **7** (0.274, 0.59 mmol), by the method described for **9**, using 20% Pd(OH)$_2$/C (0.60 g), cyclohexene (4 mL), and ethanol (7.5 mL) to afford **11** (0.157 g, 71.4%) as a viscous liquid. An analytical sample was obtained using a chromatotron (ethyl acetate−methanol, 8:2): $[\alpha]^{25}_D$ −2.6° (*c* = 1.025, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, *J* = 6 Hz, 3H, CH_3), 1.90−2.20 (m, 2H, CH_2P), 3.70−4.30 (m, 7H, 1H D_2O exchangeable), 5.80 (s, 2H, OCH_2N), 6.10−6.40 (m, 2H, NH_2, D_2O exchangeable), 8.00 (s, 1H, C(2)*H*), 8.30 (s, 1H, C(8)*H*). Anal. ($C_{14}H_{24}O_5N_5P$) C, H, N, P.

1-[[1-(Diethylphosphonyl)-(3*S*),4-dihydroxy-(2*R*)-butoxy]methyl]thymine (12). Compound **12** was prepared from **8** (1.8 g, 3.2 mmol) by the method described for **9**, using 20% palladium hydroxide on carbon (0.150 g), cyclohexene (16 mL), and ethanol (31 mL), to yield 1.18 g (97%) of **12**: $[\alpha]^{25}_D$ −21.5° (*c* = 0.66, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, *J* = 6 Hz, 6H, 2CH_2), 1.72−2.54 (m, 2H, CH_2P), 1.86 (s, 3H, CH_3), 3.34−4.60 (m, 10H, 2H D_2O exchangeable), 4.98−5.62 (m, 2H, OCH_2N), 7.34 (br s, 1H, C(6)*H*), 9.98 (br s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{14}H_{25}N_2O_8P$) C, H, N.

1-[[1-(Ethoxyhydroxyphosphinyl)-(3*S*)-hydroxy-(2*R*)-butoxy]methyl]thymine (13). A solution of **9** (0.34, 0.92 mmol) and TMS-Br (0.56 g, 3.68 mmol) in dry CH_2Cl_2 (7.5 mL) was stirred at 0 °C for 1 h under nitrogen. The volatiles were removed *in vacuo*, and the residual oil was taken up in CH_2Cl_2 (5 mL) and water (5 mL) and stirred at room temperature for 10 min. The water layer was separated and evaporated *in vacuo*, and the residual solid was purified on a C_{18} reverse phase column using water as eluant to give **13** (0.29 g, 94%) as a white solid: ^{1}H NMR (D_2O)[16] δ 1.14 (d, *J* = 6 Hz, 3H, CH_3), 1.28 (t, *J* = 6 Hz, 3H, CH$_2$CH_3), 1.74−2.35 (m, 2H, CH_2P), 7.52 (s, 1H, C(6)*H*). Anal. ($C_{12}H_{21}N_2O_7P$) C, H, N, P.

1-[[1-(Ethoxyhydroxyphosphinyl)-(3*S*)-hydroxy-(2*R*)-butoxy]methyl]uracil (14). To a solution of phosphonate **10** (0.212 g, 0.606 mmol) in 1:1 water and ethanol (3.2 mL) was added NaOH (0.109 g). The mixture was stirred at room temperature for 2 h and then neutralized with Dowex 50-H$^+$ ion exchange resin and filtered. The residue obtained after concentrating the filtrate was dissolved in CH_2Cl_2 (5 mL) and water (5 mL). The aqueous layer was washed with CH_2Cl_2 (2 × 5 mL) and evaporated *in vacuo*, to afford **14** in quantitative yield: ^{1}H NMR (D_2O)[16] δ 1.13 (d, *J* = 6 Hz, 3H, CH_3), 1.26 (t, *J* = 6 Hz, 3H, CH$_2$CH_3), 1.53−2.20 (m, 2H, CH_2P), 5.76 (d, *J* = 6 Hz, 1H, C(5)*H*), 7.63 (d, *J* = 6 Hz, 1H, C(6)*H*). Anal. ($C_{11}H_{19}N_2O_7P\cdot0.5H_2O$) C, H, N, P.

1-[[(3*S*)-Hydroxy-1-phosphonyl-(2*R*)-butoxy]methyl]thymine (15). TMS-Br (0.31 g, 2 mmol) was added dropwise, *via* syringe, at 0 °C, to the phosphonate **9** (0.180 g, 0.5 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was allowed to stir for 16 h at room temperature. The volatiles were removed under vacuum, the residual oil was dissolved in CH_2Cl_2 (10 mL) and treated with water (10 mL), and the biphasic solution was stirred at room temperature for 10 min. The aqueous layer was separated and lyophylized. The residual solid was purified on a C_{18} reverse phase column using water as the eluant to furnish **15** (0.147 g, 97%) as a white solid: $[\alpha]^{25}_D$ +6.67° (*c* = 0.51, H_2O); ^{1}H NMR (D_2O)[16] δ 1.02 (d, *J* = 6 Hz, 3H, CH_3), 1.60−2.12 (m, 2H, CH_2P), 1.68 (s, 3H, CH_3), 7.36 (br s, 1H, C(6)*H*). Anal. ($C_{10}H_{17}N_2O_7P$) C, H, N.

1-[[(3*S*)-Hydroxy-1-phosphonyl-(2*R*)-butoxy]methyl]uracil (16). Compound **16** was prepared from **10** (0.175 g, 0.5 mmol) by the method described for **15**, using TMS-Br (0.306 g, 2 mmol), to provide 0.145 g (99%) of **16**: $[\alpha]^{25}_D$ −15.6° (*c* = 0.91, H_2O); ^{1}H NMR (D_2O)[16] δ 1.16 (d, *J* = 6 Hz, 3H, CH_3), 2.08 (dd, *J* = 18, 6 Hz, 2H, CH_2P), 5.86 (d, *J* = 7 Hz, 1H, C(5)-*H*), 7.74 (d, *J* = 7 Hz, 1H, C(6)*H*). Anal. ($C_9H_{15}N_2O_7P$) C, H, N, P.

1-[[(3*S*)-Hydroxy-1-phosphonyl-(2*R*)-butoxy]methyl]cytosine (17). Iodotrimethylsilane (TMS-I; 0.679 g, 3.39 mmol) was added dropwise *via* a syringe at 0 °C to the phosphonate **5** (0.481 g, 1.095 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred under N_2 at 0 °C for 1 h and then at room temperature for 1 h. The volatiles were removed under reduced pressure, the residual oil was dissolved in CH_2Cl_2 (10 mL) and treated with water (10 mL) containing a few drops of hydrochloric acid, and the biphasic solution was stirred at room temperature for 10 min. The aqueous layer was separated, washed with CH_2Cl_2 (5 × 5 mL), and lyophilized. The residual solid was purified on a C_{18} reverse phase column using water as the eluant to give **17** (0.29 g, 90%): $[\alpha]^{25}_D$ −7.3° (*c* = 0.31, H_2O); ^{1}H NMR (D_2O)[16] δ 1.20 (d, *J* = 6 Hz, 3H, CH_3), 2.03 (dd, *J* = 15, 6 Hz, 2H, CH_2P), 6.26 (d, *J* = 7.5 Hz, 1H, C(5)*H*), 7.99 (d, *J* = 7.5 Hz, 1H, C(6)*H*). Anal. ($C_9H_{16}N_3O_6P$) C, H, N, P.

1-[[(3*S*),4-Dihydroxy-1-phosphonyl-(2*R*)-butoxy]methyl]thymine (18). Compound **18** was prepared from **12** (0.256 g, 0.67 mmol) by the method described for **15**, using TMS-Br (0.618 g, 4 mmol), to furnish 0.210 g (96%) of **18**: $[\alpha]^{25}_D$ −10.7° (*c* = 0.71, H_2O); ^{1}H NMR (D_2O)[16] δ 1.88 (s, 3H, CH_3), 1.88−2.42 (m, 2H, CH_2P), 3.46−4.26 (m, 4H), 7.54 (s, 1H, C(6)*H*). Anal. ($C_{10}H_{17}N_2O_8P$) C, H, N, P.

3-*O*-Benzyl-1-bromo-(2*S*,3*R*)-butanediol (20). To a stirred solution of the epoxide **19** (6.59 g, 0.037 mol) in 20 mL of dry THF was added 50 mL of 0.4 N Li_2NiBr_4 in THF. The reaction was monitored by TLC, and after 20 min (60 mL) and 15 h (40 mL), additional quantities of the reagent were added to the reaction mixture. The reaction mixture was allowed to stir for another 6 h at room temperature and then poured into 250 mL of pH 7 phosphate buffer. This mixture was extracted with methylene chloride (8 × 40 mL), and the organic layer was dried over anhydrous Na_2SO_4. Evaporation of the organic layer gave 8.91 g (93%) of **20**: ^{1}H NMR ($CDCl_3$) δ 1.20 (d, J = 6 Hz, 3H, CH_3), 2.75 (br s, 1H, OH), 3.30−4.12 (m, 4H), 4.47 (ABq, J = 12 Hz, 2H, OCH_2N), 7.25 (s, 5H, C_6H_5). Anal ($C_{11}H_{15}BrO_2$) C, H, Br.

1-[[(3*S*)-(Benzyloxy)-1-bromo-(2*R*)-butoxy]methyl]-thymine (21). Coupling of persilylated thymine [obtained from 3.0 g (0.0024 mol) of thymine] was accomplished in a similar manner as reported for **3** with 7.10 g (81.7% pure, 0.019 mol) of (3*S*)-(benzyloxy)-1-bromo-(2*R*)-(chloromethoxy)butane, all of which was dissolved in 60 mL of dry methylene chloride and in the presence of a catalytic amount of TBAI. This procedure afforded **21** in quantitative yield after column chromatography using ethyl acetate−hexane (1:1) as eluant. Fractions containing **21** were pooled and concentrated, and on standing the product crystallized: mp 51−56 °C; $[\alpha]^{25}_D$ +7.6 (c = 1.81, EtOH); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J = 6 Hz, 3H, CH_3), 1.88 (s, 3H, CH_3), 3.42−4.00 (m, 4H), 4.48 (d, 2H, OCH_2-Ph), 5.52 (s, 2H, OCH_2N), 7.09 (s, 1H, C(6)*H*), 7.39 (s, 5H, C_6H_5), 9.78 (br s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{17}H_{21}BrN_2O_4$) C, H, Br, N.

Diphenyl 1-[[(3*S*)-(Benzyloxy)-(2*R*)-butoxy]methyl]-thymine-1′-phosphonate (22). Diphenyl ethyl phosphite (3.2 g, 12.21 mmol) and **21** (1.2 g, 3.02 mmol) were combined and heated at 165 °C for 18.5 h. The excess reagent was distilled off, and the residue was purified by (silica gel, 60−230 mesh) column chromatography. Elution with hexene−ethyl acetate (1:1) gave 0.28 g (23%) of starting material **21** and 0.65 g (39.1%) of the phosphonate **22**, as a viscous material: $[\alpha]^{25}_D$ +15.8 (c = 1.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, J = 6Hz, 3H, CH_3), 1.63 (s, 3H, CH_3), 2.03−2.72 (m, 2H, CH_2P), 3.55−3.90 (m, 1H), 3.93−4.35 (m, 1H), 4.48 (d, 2H, OCH_2Ph), 5.13 (ABq, J = 10.5 Hz, 2H, OCH_2N), 6.85−7.38 (m, 16H, C_6H_5, C(6)*H*), 9.10 (br s, 1H, N*H*, D_2O exchangeable). Anal. ($C_{29}H_{30}N_2O_7P$) C, H, N, P.

Phenyl 1-[[(3*S*)-Hydroxy-(2*R*)-butoxy]methyl]thymine 1′,3′-Cyclic Phosphonate (23). Catalytic transfer hydrogenation of the phosphonate **22** (0.87 g, 1.58 mmol) which was dissolved in 46.6 mL of absolute ethanol was carried out, as described earlier with the exception that 10% Pd/C (0.78 g) was used as catalyst in the presence of 23.3 mL of cyclohexene at reflux for 1.5 h. Purification of product by column chromatography (silica gel, 60−230 mesh) using ethyl acetate as eluant afforded 0.375 g (64.7%) of pure **23**, as a gummy material: ^{1}H NMR ($CDCl_3$) δ 1.23 and 1.37 (2 d, J = 6 Hz, 3H, CH_3), 1.87 (s, 3H, CH_3), 2.08−2.70 (m, 2H, CH_2P), 3.96−4.88 (m, 2H), 5.06−5.28 (2 ABq, 2H, OCH_2N), 6.90−7.47 (m, 6H, C_6H_5, (C(6)*H*), 9.13 (br d, 1H, N*H*, D_2O exchangeable). Anal. ($C_{16}H_{19}N_2O_6P$) C, H, N, P.

***In Vitro* Antiviral Assays:[17−20] (a) Inhibition of Cytopathic Effect (CPE).** Virus was absorbed for 1 h in 96-well monolayer cultures of Vero cells (PT, JBE, SFS, YF), H.Ep.2 cultures (AD2), or LLC-MK2 cells (PT), after which tissue culture medium containing various drug concentrations was added. At the day of maximum CPE in virus control wells, medium was removed and monolayers were stained with crystal violet for microscopic CPE determination.

(b) Inhibition of Virus Plaque Formation. RVF plaque reduction was determined by adding a semisolid agarose overlay containing various drug concentrations to Vero monolayers after adsorption with 40−100 pfu of RVF virus. After 96 h, the overlay was removed and plaques were visualized by crystal violet staining of the monolayers.

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