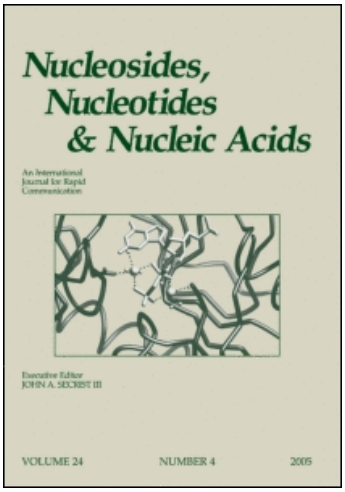

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SYNTHESIS OF ACYCLONUCLEOSIDE PHOSPHONATES AS ANTIVIRAL AGENTS AGAINST CYTOMEGALOVIRUS[1]

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ABSTRACT

Phosphonic acid analogs of ACV and DHPG that are isosteric with ACV phosphate and DHPG phosphate have been synthesized and evaluated for antiviral activity against human, murine, and guinea pig strains of cytomegalovirus. The phosphonates showed high activity against all of the strains. They were also evaluated against a DHPG resistant strain of human cytomegalovirus. Although the activity dropped considerably, significant antiviral activity was still evident.

INTRODUCTION

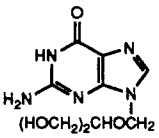
Cytomegalovirus (CMV) infection occurs worldwide and can be responsible for numerous clinical disorders in immunosuppressed patients.[2] Currently, two drugs have been approved by the FDA for treatment of CMV retinitis—DHPG (ganciclovir, Cytovene, **1**) and foscarnet (phosphonoformic acid trisodium salt Foscavir, **2**). Both drugs are clinically effective against CMV and play a useful role in the treatment of CMV infections. Unfortunately neither compound is as innocuous as acyclovir, the outstanding antiviral agent used for the treatment of herpes simplex virus-1 and -2. DHPG antagonizes the anti-HIV activity of zidovudine *in vitro*[3] and synergistic cytotoxic effects between them have been observed.[4] Its primary toxic effect *in vivo* is granulocytopenia, making it difficult to administer with AZT, the primary anti-AIDS therapy.[5] It is interesting to note that Freitas et al.[6] did not observe antagonism between DHPG and AZT in combination therapy for treatment of murine CMV in Swiss-Webster mice. Toxic effects of foscarnet include seizures, renal dysfunction, hypokalemia, hypocalcemia, anemia, fever, and rash.[7] Toxic effects caused 22 patients to be switched from foscarnet to DHPG, whereas only 1 patient did the reverse.[7]

Obviously, additional therapeutic approaches to the treatment of CMV are needed, both from the standpoint of lessening the toxic side effects inherent in the present therapies and to help counter the inevitable development of resistance to available therapy.[8]

Dedicated to the memory of the late Professor Roland K. Robins

Acyclonucleosides have been shown to possess high antiviral activity against members of the herpes family of viruses. The first member of this class to be discovered was acyclovir (9-[2-hydroxyethoxymethyl]guanine, ACV, **3**) which has excellent activity against HSV-1 and HSV-2, *in vitro* and *in vivo*.[9] Although it shows activity against HCMV *in vitro*, this result is not translated to the *in vivo* situation and acyclovir is ineffective against HCMV *in vivo*.

The mechanism by which ACV manifests its antiviral activity against HSV is centered on an initial phosphorylation of the nucleoside by a viral thymidine kinase to give the monophosphate. Cellular kinases carry out further phosphorylations on the monophosphate to give the ultimate antiviral agent ACV triphosphate which inhibits viral DNA polymerase while having minimal effect on the cellular DNA polymerases. This is believed to be the reason for its lack of adequate activity against HCMV, since HCMV does not possess the viral thymidine kinase that is necessary to initiate the above sequence. DHPG also must be phosphorylated to the triphosphate to manifest its activity by inhibition of the viral DNA polymerase. In contrast to ACV, DHPG shows good *in vivo* activity against HCMV. There are data that suggest that a protein kinase homolog, UL97 controls phosphorylation of DHPG in HCMV-infected cells.[10] This kinase phosphorylates DHPG to the triphosphate to levels at least 10-fold higher than those measured for the conversion of ACV to the triphosphate and up to 100-fold higher than the levels found in uninfected cells.[11]



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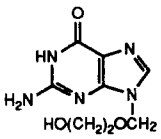

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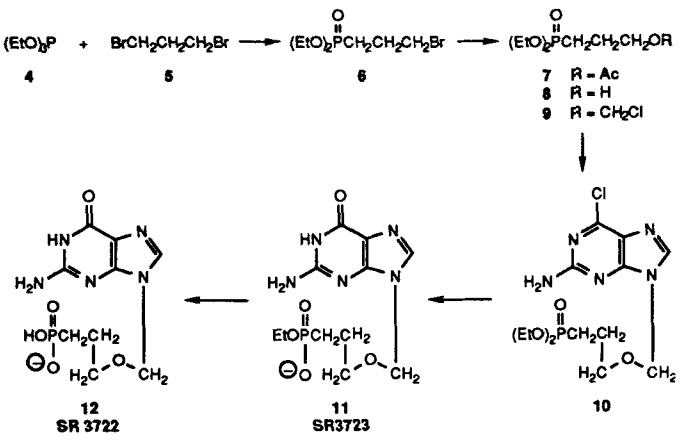
Thus both ACV and DHPG depend on phosphorylation to the monophosphate and subsequent further phosphorylation by cellular kinases to form the ultimate antiviral acyclonucleoside triphosphate. Any mutation of the kinase responsible for the initial phosphorylation can result in a virus resistant to the nucleoside. Resistance is beginning to appear in the clinic[8] and could well be due to such a mutation.

Use of a preformed nucleoside monophosphate is one strategy that could circumvent this type of resistance if a suitable phosphate derivative can be obtained. In general, polar molecules such as nucleoside phosphates are poorly absorbed into cells because of the lipid nature of the cell membrane. It has been reported[12] that the cell membrane of cells infected by virus are more permeable to polar molecules so that nucleoside phosphate derivatives may be able to cross into an infected cell.

Preparation of phosphonic acid analogs of ACV and DHPG offers intriguing possibilities for the development of useful drugs.[13] Isosteric phosphonate analogs have similar steric bulk to the phosphate ester. They can be further phosphorylated to the equivalent of the triphosphate that inhibits the viral DNA polymerase. They cannot be readily cleaved by normal esterases that cleave phosphate esters. In addition a phosphonate is somewhat less polar than phosphate, so it is possible that a nucleoside phosphonate could have some degree of selective absorption into a virally infected cell and thus be concentrated in the cells that need it.



Scheme 1

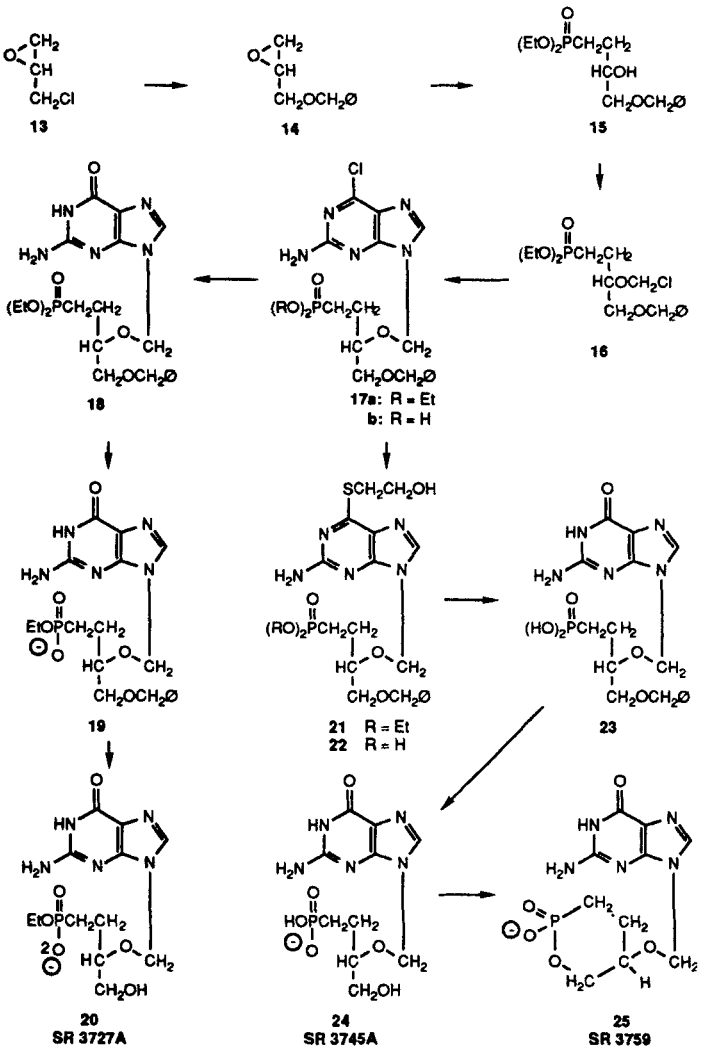
CHEMISTRY

The syntheses of ACV analogs was accomplished as outlined in Scheme 1 starting from the reaction of triethylphosphite (**4**) with 1,3-dibromopropane to give diethyl 3-bromopropyl phosphonate (**6**) in 69% yield. Replacement of the 3-bromo by acetate was accomplished using sodium acetate in DMF. Acid catalyzed hydrolysis of the O-acetate (**7**) using Dowex 50(H^+) gave diethyl 3-hydroxypropylphosphonate (**8**) in 40% overall yield from the bromide (**6**). Chloromethylation of **8** was accomplished using paraformaldehyde and hydrogen chloride to give diethyl 3-chloromethoxy-propyl phosphonate (**9**) as an oil that was suitable for coupling with an appropriate purine.

Reaction of **9** with the sodium salt of 2-amino-6-chloropurine gave (**10**) in 44% yield. Treatment of **10** with aqueous sodium hydroxide yielded 45% of **11** as a crystalline solid. Complete deesterification of **11** with bromotrimethylsilane gave a 61% yield of **12**.

Phosphonic acid analogs of DHPG were prepared starting from epichlorohydrin (**13**) by the sequence outlined in Scheme 2. In contrast to the acyclovir analogs prepared in Scheme 1, the DHPG analogs of Scheme 2 have a chiral center and occur as R and S enantiomers. In this report, racemic epichlorohydrin was used and the resulting acyclonucleosides (**17-25**) are R,S-enantiomeric mixtures.

Reaction of **13** with benzyl alcohol gave an 86% yield of benzyl glycidyl ether (**14**). Treatment of **14** with the lithium salt of diethyl methylphosphonate yielded diethyl 4-O-benzyl-3,4-dihydroxybutylphosphonate (**15**) in 95% yield. Chloromethylation of **15** and reaction of the resulting chloromethyl ether (**16**) with the sodium salt of 2-amino-6-chloro purine gave a 50% yield of 2-amino-6-chloro-9-[(1-benzyloxymethyl-3-diethylphosphono)-1-propyloxymethyl]9H-purine (**17**) after chromotography. Conversion of the 2-amino-6-chloropurine derivative (**17**) to the guanine analog (**18**) was accomplished in 65% yield. Partial saponification of the diethyl ester to the monoethyl ester (**19**) and hydrogenolysis of the benzyl ether gave a 95% yield of 9-[(3-ethylphosphono-1-hydroxymethyl)-1-propyloxymethyl]-guanine (**20**) in 95% overall yield



Scheme 2

from **18**. The preparation of **24** from **17** could be accomplished in 63% overall yield by the sequence outlined in **17** → **21** → **22** → **23** → **24**. Cyclization of the δ-hydroxy phosphonic acid (**24**) using DCC yielded 67% of the cyclic phosphonate ester (phostonate) (**25**). An alternative synthesis of **24** is described by Prisbe et al.[14]

VIROLOGY

The antiviral activity of the phosphonic acid analogs of acyclovir and ganciclovir was measured against human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV) and guinea pig cytomegalovirus (GPCMV). Studies with HCMV utilized plaque reduction assay.

Table 1

***IN VITRO* CYTOMEGALOVIRUS INHIBITORY ACTIVITY[a] OF PHOSPHONIC ACID ANALOGS OF ACYCLOVIR AND GANCICLOVIR**

Compound	HCMV[b]		HCMV-R[c]		MCMV[d]		GPCMV[e]	
	EC_{50}[a]	T.I.[f]	EC_{50}[a]	T.I.[f]	EC_{50}[a]	T.I.[f]	EC_{50}[a]	T.I.[f]
12, SR3722	11.9	84	—	—	—	—	—	—
11, SR3723	16	94	82	18	10	300	50	30
20, SR3727A	2.5	610	32	47	6.1	246	20	75
24, SR3745A	2	750	15	100	10	>100	20	>50
25, SR3759	5	>200	—	—	—	—	8	14
ACV	30	60	—	—	32	32	75	13
DHPG	2	500	30	33	0.9	17	75	4.3

[a]μg/ml.

[b]Plaque reduction test run in MRC-5 cells using AD169 strain. See also Huffman et al.[15]

[c]Plaque reduction test run in MRC-5 cells using C8704 strain.

[d]CPE inhibition tests run in 3T3 cells using Smith MSGV strain.

[e]CPE inhibition tests run in GPE cells using 22122 strain.

[f]T.I. = CD_{50}/EC_{50}.

Studies with MCMV and GPCMV utilized inhibition of viral cytopathic effect (CPE). All the acyclonucleoside phosphonates described show significant activity against HCMV, MCMV, and GPCMV. The ACV phosphonates appear to be more active than ACV itself. The DHPG phosphonates are equal to or possibly better than DHPG.

Against a DHPG resistant strain of HCMV, all showed less activity, however SR3745A still showed a better therapeutic index (T.I.) and lower ED_{50} than did DHPG thus lending some support to the notion that nucleoside phosphonates may be able to circumvent at least some of the resistance that arises when using DHPG.

Viruses: The CMV used included AD-169, C8704, the Smith MSGV strain of MCMV and the 22122 strain of GPCMV. The AD-169 was obtained from the American Type Culture Collection (ATCC), Rockville, MD; the other human virus strains, recent clinical isolates, were provided by Dr. Karen Biron of Burroughs Wellcome Co. C8704 is resistant to DHPG. The MCMV was obtained from the ATCC and the GPCMV was provided by Brigette Griffith, Veteran's Administration Medical Center (West Haven, CT). The human virus stocks were prepared in human diploid embryonic lung cells (MRC-5). The murine virus was prepared in Swiss albino mouse fibroblast (3T3) cells and the guinea pig virus was grown in guinea pig embryonic (GPE) cells. All were stored at -70°C in sealed ampules until used.

Cells: The cells used included MRC-5 cells described above, 3T3 and GPE cells; all were obtained from the ATCC. The MRC-5 cells were grown in Eagles Basal Medium with 10% fetal bovine serum (FBS) without antibiotics. Dulbecco's medium with 10% FBS and 0.1%

$NaHCO_3$ was used for the 3T3 cells. The GPE cells were grown in minimum essential medium with 10% FBS and 0.1% $NaHCO_3$.

EXPERIMENTAL

^1H-NMR spectra were obtained with Varian 300 MHz or 400 MHz spectrometers. UV absorption spectra were recorded with a Perkin Elmer Model 575 UV/visible double beam. TLC was carried out using E. Merck nondusting silica gel plates. Plates were visualized by UV or by charring with H_2SO_4.

Diethyl 3-bromopropylphosphonate (6)

A solution of 202 g (101.5 ml, 1.0 mole) of 1,3-dibromopropane (**5**) and 33.2 g (34.3 ml, 0.2 mole) of triethylphosphite (**4**) under an argon atmosphere in a 500 ml flask equipped with reflux condenser was heated with stirring in an oil bath set at 150°C. A vigorous exothermic reaction began in about 10 min and resulted in a 20° temperature rise. After 2 hr at 150°, the reaction was cooled to room temperature. The condenser was removed and the bromoethane by-product, and excess dibromopropane were removed by distillation, ultimately using an oil bath temperature of 125° and pressure of 0.5 torr. The residue was transferred to a 250 ml round bottom flask equipped with an all-in-one Vigreux distillation apparatus and was distilled *in vacuo*. The desired product (**6**) was collected at b.p. 92-94° (0.2 torr.) and weighed 35.8 g (68% yield). ^1H NMR ($CDCl_3$): δ 1.28 (tr, 6H); 1.85 (m, 2H); 2.10 (m, 2H); 3.43 (tr, 2H); and 4.06 ppm (m, 4H). TLC (silica): R_f 0.45 (ethyl acetate). Eberhard and Westheimer report b.p. 74° (0.1 torr.).[16]

Diethyl 3-acetoxypropylphosphonate (7)

A mixture of 35.8 g (0.138 mole) of diethyl 3-bromopropylphosphonate (**6**) and 35.8 g (0.436 mole) of anhydrous sodium acetate in 325 ml of N,N-dimethylformamide (DMF) was stirred with heating in a heating mantle to maintain an internal temperature of 90-95°. After 5 hr, the reaction was complete as indicated by NMR and the reaction was evaporated to dryness *in vacuo*. The residue was triturated with 200 ml of water and the resulting aqueous suspension was extracted with 3 × 100 ml of ethyl acetate. The ethyl acetate solution was washed with 50 ml of saturated aqueous sodium chloride, then dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give 15.3 g (46%) of product (**7**). ^1H NMR ($CDCl_3$): δ 1.28 (tr, 6H); 1.7-1.9 (m, 4H); 2.01 (s, 3H); and 4.07 ppm (m, 6H). TLC (silica): R_f 0.30 (ethyl acetate-dichloromethane, 2:1).

Diethyl 3-hydroxypropylphosphonate (8)

To a stirring solution of 15.2 g of diethyl 3-acetoxypropylphosphonate (**7**) in 160 ml of absolute ethanol was added 70 ml of Dowex 50 X 8 (H^+, 50-100 mesh) that had previously been washed with water, then absolute ethanol. The reaction was stirred at room temperature for 5 days by which time all the starting material had reacted according to thin-layer chromatography (SiGF; ethyl acetate-methanol, 9:1). The Dowex 50 was removed by filtration through a sintered glass funnel and the filtrate was evaporated to dryness *in vacuo* to constant weight to obtain 10.9 g (87%) of product as a pale yellow oil that was satisfactory for the chloromethylation reaction. ^1H NMR ($CDCl_3$): δ 1.33 (tr, 6H); 1.8-2.0 (m, 4H); 3.50 (s, 1H); 3.69 (tr, 2H); and 4.10 ppm (m, 4H). TLC (silica): R_f 0.45 (ethyl acetate-methanol, 9:1).

Diethyl 3-chloromethoxypropylphosphonate (9)

The procedure of Kelley et al.[17] was adopted for this reaction.

A stirred solution of 9.68 g (50 mmol) of **8** and 3.92 g (120 mmol) of paraformaldehyde in 275 ml of 1,2-dichloroethane was saturated with dry HCl gas at -10°. After 1 hr at 0°, the solution was dried over $CaCl_2$, filtered and evaporated *in vacuo* to give **9** as an oil which was used directly in the coupling reaction without further purification. The NMR ($CDCl_3$) showed a singlet at 5.0 ppm indicative of the chloromethyl group.

Sodium salt of 2-amino-6-chloropurine

A mixture of 10.0 g (59 mmol) of 2-amino-6-chloropurine in dry DMF (100 ml) at 0° was treated with 2.48 g (62 mmol) of sodium hydride (60% in oil) and stirred at room temperature for 1 hr to yield the sodium salt which was used directly in the coupling reactions.

2-Amino-6-chloro-9-[(3-diethylphosphono)-1-propyloxymethyl]-9H-purine (10)

The chloromethyl ether (**9**) was dissolved in 75 ml of dry DMF and added dropwise at -50° to the solution of sodium salt of 2-amino-6-chloropurine in DMF. The reaction mixture was stirred under an argon atmosphere at -20° for 1 hr, then poured into 375 ml of dichloromethane and washed with 500 ml of saturated sodium bicarbonate solution. The aqueous washes were extracted with 3 × 200 ml of dichloromethane. The combined organic layers were dried (Na_2SO_4) and evaporated to give 23.0 g of crude product as a gummy solid.

Purification of the product was accomplished by chromatography on silica (Merck 70-230 mesh #7734, 600 ml) using methanol/dichloromethane (5:95). The resulting product was recrystallized from ethyl acetate/diethyl ether (4:1) to yield 8.2 g (44%) of **10** as white crystals, m.p. 106.5-108.5. ^1H NMR ($CDCl_3$): δ 1.31 (t, 6H); 1.7-1.9 (m, 4H); 3.58 (t, 2H); 4.07 (m, 4H); 5.48 (s, 2H); and 7.90 ppm (s, 1H). TLC (silica): R_f 0.30 (methanol-dichloromethane, 1:19).

9-[(3-Ethylphosphono)-1-propyloxymethyl]guanine (11), monosodium salt

A mixture of 8.1 g (21.4 mmol) of **10** in 350 ml of 1 N aqueous sodium hydroxide was gently refluxed for 1 hr. The reaction was cooled, neutralized to pH 7 and desalted using Dowex 50 X 8 (pyridinium form). The resin was removed by filtration and rinsed with water. The aqueous fractions were evaporated *in vacuo* to give 4.61 g of product. This material was chromatographed on 150 g of silica gel using acetonitrile/water (7:3) to obtain 2.98 g of solid that was recrystallized by dissolving in 850 ml of hot absolute ethanol, filtering and concentrating to 300 ml. The solution was cooled and 2.3 g of product was collected in 2 crops. UV (H_2O): λ_{max} 250.9 nm, (12,770); 271.8 (sh), (8829). ^1H NMR (D_2O): δ 1.21 (t, 3H); 1.53 (m, 2H); 1.75 (m, 2H); 3.64 (t, 2H); 3.83 (q, 2H); 5.51 (s, 2H); and 7.97 ppm (s, 1H). TLC (silica): R_f 0.28 (acetonitrile/0.1 N ammonium chloride, 7:3). Calcd for $C_{11}H_{17}N_5O_5PNa \cdot 0.5 H_2O$: C, 36.47; H, 5.01; N, 19.33. Found, C, 36.34; H, 4.95; N, 19.34.

9-[(3-Phosphono)-1-propyloxymethyl]guanine (12), monosodium salt

To a stirring mixture of 1.13 g (3.41 mmol) of 9-[(3-ethylphosphono)-1-propyloxymethyl]guanine (**11**) in chloroform (47 ml) and hexamethyldisilazane (47 ml) at room

temperature was added 3.45 ml (26 mmol) of bromotrimethylsilane. After 72 hr, the mixture was evaporated to dryness, slurried with water, filtered, and rinsed with acetone. A white solid (1.24 g) was obtained, which was treated with 1 eq. sodium hydroxide and evaporated to dryness. This was recrystallized from 1 ml water and then 6 ml 50% ethanol to give 627 mg (61%) of product (**12**). ^{1}H NMR (D_2O): δ 1.50 (m, 2H); 1.76 (m, 2H), 3.61 (t, 2H); 5.48 (s, 2H); and 7.93 ppm (s, 1H). UV (H_2O) λ_{max} 251.3 (ε 12,647) 270.1 (sh) (8877). TLC (silica): R_f 0.20 (acetonitrile/0.1 N ammonium chloride, 7:3). Calcd for $C_9H_{13}N_5O_5PNa$•0.2 EtOH: C, 33.76; H, 4.28; N, 20.94. Found: C, 33.41; H, 4.18; N, 20.68.

Benzylglycidyl ether (14)

To a stirred mixture of 50% w/w aqueous sodium hydroxide (600 ml), epichlorohydrin (**13**) (250 ml) and tetrabutylammonium hydrogen sulfate (8.4 g, 0.025 mole), benzyl alcohol (62 ml, 64.8 g, 0.6 mole) was gradually added over 20 min with cooling in ice so that the temperature did not exceed 25°C. The reaction mixture was stirred for 4 hr at 25° and then poured onto ice/water (2 l). The aqueous phase was extracted with diethyl ether (3 × 400 ml). The combined ether extracts were washed with brine (5 × 200 ml) to neutrality, dried (Na_2SO_4), evaporated to dryness and distilled under vacuum to yield 84.5 g (86%); b.p 75°-77°C/0.2 mm Hg. ^{1}H NMR ($CDCl_3$): δ 7.34 (m, 5H); 4.58 (dd, 2H); 3.75 (dd, 1H); 3.45 (dd, 1H); 3.20 (m, 1H); 2.80 (t, 1H); and 2.62 ppm (t, 1H). The nmr was compatible to the literature values for **14** prepared by an alternative procedure.[18]

Diethyl 4-O-benzyl-3,4-dihydroxybutylphosphonate (15)

n-Butyllithium (1.6 M in hexane, 33.75 ml, 54 mmol) was added dropwise to a solution of diethyl methylphosphonate (7.9 ml, 54 mmol) in dry tetrahydrofuran (THF) (60 ml) at -78°C. After 30 min of stirring at -78°C, the resulting white suspension was added in a slow stream through a cannula to a stirred solution of BF_3•Et_2O (6.64 ml, 54 mmol) in THF (120 ml) at -78°C. After 10 min, neat benzylglycidyl ether (3 g, 18 mmol) was added quickly to this suspension. The reaction was stirred 30 min at -78°C, then was quenched by adding saturated aqueous $NaHCO_3$ (30 ml) and warmed to room temperature. The reaction was extracted three times with ether. The ether layers were dried ($MgSO_4$) and evaporated to dryness. The residue was pumped at 90° under 1 mm Hg for 2 hours to remove most of the excess diethyl methylphosphonate. The product obtained at this point consisted of **15** contaminated with 5-10% diethyl methylphosphonate. The product had R_f 0.46 (hexane/ethyl acetate: 30:70); ^{1}H NMR (300 MHz), ($CDCl_3$) δ = 7.29-7.3 (m, 5H); 4.55 (s, 2H); 4.0-4.15 (m, 4H); 3.8 (m, 1H); 3.35-3.5 (m, 2H); 3.0 (broad s, 1H); 1.65-2.0 (m, 4H); 1.3 (t, 6H). Extra nmr absorption at 4.0, 1.45, and 1.3 ppm indicated the presence of about 10% diethyl methylphosphonate.

The nmr was similar to that of an analytical sample of chiral **15** and was satisfactory for use in subsequent coupling reactions.

2-Amino-6-chloro-9-[(1-benzyloxymethyl-3-diethylphosphono)-1-propyloxymethyl]-9H-purine (17a)

A suspension of alcohol **15** (78 g, 248 mmol) in dry dichloroethane (1400 ml) and paraformaldehyde (19.6 g) was treated for 2 hr at -10°C with HCl gas at which time the NMR indicated complete conversion to chloromethyl ether (**16**). Argon was bubbled through the

resulting clear solution for 40 min at room temperature. The solution was dried over $CaCl_2$, then evaporated to dryness, and dissolved in dry DMF (30 ml). The solution of 2-amino-6-chloro purine sodium salt prepared from 2-amino-6-chloropurine (50 g, 295 mmol) and NaH (12.42 g, 60% in oil, 295 mmol) in 500 ml of DMF was cooled to -50°C and the DMF solution of **16** was added and then the bath temperature was raised to -20°C. After 1 hr of stirring at -20°C, the reaction mixture was poured into dichloromethane (1900 ml) and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted three times with dichloromethane (2 l), and the organic extract washed with brine, dried (Na_2SO_4) then evaporated to dryness. The crude product was purified by silica gel chromatography using dichloromethane/methanol (97.5:2.5) to give 54 g of pure **17a** (37%) as a colorless thick oil; R_f 0.55 (CH_2Cl_2/MeOH, 95:5); ^1H NMR ($CDCl_3$) δ 7.82 (s, 1H); 7.15-7.35 (m, 5H); 5.80-6.05 (broad s, 2H); 5.52 (s, 2H); 4.50 (s, 2H); 3.90-4.05 (m, 4H); 3.73-3.82 (m, 1H); 3.38 (d, J = 4.9 Hz, 2H); 1.50-1.80 (m, 4H); 1.15-1.28 (t, J = 7 Hz, 6H). In a previous run, after removal of **17a**, continued elution of the column gave 12% of product assumed to the the 7-substituted isomer of **17a**. R_f 0.33 (CH_2Cl_2/MeOH, 95:5).

De-esterification of 400 mg of **17a** using trimethylsilyl bromide by the procedure described for the preparation of **23** gave the diacid (**17b**) as a white solid.

9-[1-Benzyloxy-3-diethylphosphono)-1-propyloxymethyl]guanine (18)

A solution of pure **17a** (1 g, 2 mmol) and 516 µL of 2-mercaptoethanol in 65 µL of water and 32 ml of absolute ethanol under argon was treated with 484 mg (7 mmol) of sodium ethoxide in 32 ml of absolute ethanol. The mixture was refluxed for 3.5 hours, filtered, evaporated and chromatographed on silica gel eluting with chloroform/methanol (95:5) to yield 575 mg (65%). NMR (90 MHz, $CDCl_3$) δ 7.80 (s, 1H); 7.32 (s, 5H); 5.55 (s, 2H); 5.25 (s, 2H); 4.50 (s, 2H); 3.95 (q, 4H, J = 7 Hz); 3.45 (m, 4H); 1.75 (m, 4H) and 1.28 ppm (t, 6H, J = 7 Hz).

9-[(1-Benzyloxymethyl-3-ethylphosphono)-1-propyloxymethyl]guanine monosodium salt (19)

Compound **18** (575 mg, 1.2 mmol) was added to 50 ml of 1N NaOH (prepared from Dilut-it analytical grade NaOH concentrate, under argon). This mixture was heated at reflux for 2 hours, cooled to room temperature and neutralized to pH 6-7 using Amberlite IR-120 (pyridinium). The resin was filtered and the filtrate evaporated to dryness to give 582 mg of a gummy solid. NMR (D_2O) δ 8.3 (s); 7.8 (s, 1H); 7.0-7.3 (5H); 5.4 (s, 2H); 4.5 (s, 1H); 4.3 (s, 1H); 3.5-3.9 (m, 3H); 3.2-3.5 (m, 2H); 1.3-1.8 (m, 4H) and 1.1-1.3 ppm (dt, 3H). TLC (CH_3CN/0.1NNH_4Cl:7:3). R_f 0.45.

9-[(1-Hydroxymethyl-3-ethylphosphono)-1-propyloxymethyl]guanine monosodium salt (20)

Compound **19** (582 mg, 1.2 mmol) was combined with 90 ml of 50% aqueous ethanol, cyclohexene (6.8 ml) and $Pd(OH)_2$/C damp 20% Pd (440 mg). The resulting mixture was heated at reflux for 3 hr, then stirred at room temperature (cooling) for 2 hr. The catalyst was removed by filtration through Celite and the solvent evaporated to dryness. Trituration with 2 x 10 ml absolute ethanol and filtration gave a white solid which was dried at 100° under high vacuum to give 424 mg (95% overall yield for the last two reactions).

NMR (300 MHz, D_2O) δ 8.00 (s, 1H); 5.58 (q, 2H, N-$\underline{CH_2}$OC); 1.3-1.7 (m, 4H, $\underline{CH_2}CH_2P$) 1.16 ppm (t, 3H, $\underline{CH_3}CH_2OP$). UV (H_2O) λmax 252.1 nm (ε, 12,080); 271.3 sh

(8795). Anal. Calcd. for $C_{12}H_{19}N_5O_6Na \cdot 0.6\ C_2H_5OH \cdot 2H_2O$: C, 36.24; H, 5.72; N, 16.05. Found: C, 36.42; H, 5.13; N, 15.91.

2-Amino-6-hydroxyethylthio-9-[(1-benzyloxymethyl-3-diethylphosphono)-1-propyloxymethyl]-9H-purine (21)

A solution of **17a** (56.5 g, 113 mmol) in dry methanol (1200 ml) was treated with 2-mercaptoethanol (24 ml, 338 mmol) in the presence of sodium methoxide (18.4 g, 338 mmol) at reflux for 2 hr. The reaction mixture was cooled to room temperature, and the solvents were evaporated to dryness. The residue was dissolved in water, and the pH was adjusted to 7 with Amberlite IR-120 (pyridinium). The aqueous layer was extracted three times with chloroform (150 ml), and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvents were removed, and the residue was purified by flash chromatography over silica using dichloromethane/methanol (97.5/2.5) to give pure **21** as a colorless thick oil (the crude product may be used in the next step) (62.3 g, 96%); R_f 0.21 (blue fluorescence CH_2Cl_2/MeOH: 95/5); ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (s, 1H); 7.28-7.32 (m, 5H); 5.57 (s, 1H); 4.49 (s, 2H); 3.98-4.10 (m, 4H); 3.72 (t, J = 5.6 Hz, 2H); 3.76-3.86 (m, 1H); 3.42-3.52 (m, 4H); 1.50-1.88 (m, 4H); 1.27 (t, J = 6.6 Hz, 6H).

2-Amino-6-hydroxyethylthio-9-[(1-benzyloxymethyl-3-phosphono)-1-propyloxymethyl]-9H-purine (22)

A solution of **21** (62.3 g, 115 mmol) in a 1:1 mixture of dichloromethane (700 ml) and hexamethyldisilazane (700 ml) was treated with fresh, colorless bromotrimethylsilane (120 ml, 780 mmol) overnight at room temperature. The solvents were removed, and the residue was dissolved in a 1:1 mixture of ethanol-water (300 ml). The solvents were evaporated to dryness to give crude **22** as a white powder R_f 0.46 (blue fluorescence, CH_3CN/0.1NNH_4Cl:7:3) that was used without purification to prepare **23**; ^1H NMR (CD_3OD) δ = 8.01 (s, 1H), 7.23-7.32 (m, 5H), 5.62 (s, 2H), 4.41 (s, 2H), 3.90-3.98 (m, 1H), 3.82 (t, J = 6.7 Hz, 2H), 3.38-3.54 (m, 4H), 1.40-1.90 (m, 4H).

9-[(1-Benzyloxymethyl-3-phosphono)-1-propyloxymethyl]guanine monosodium salt (23)

A solution of the purine (**22**) (theo. 115 mmol) in methanol (2000 ml) was treated at reflux with NaOMe (50 g, 930 mmol) in the presence of water (2.7 ml) for 16 hr. The reaction mixture was cooled to room temperature, and the solvents were evaporated to give a white solid that was purified by treatment with 260 g Amberlite IR-120 (pyridinium form) in 1 l water to pH = 6-7. The mixture was filtered and the filtrate was evaporated to dryness to give 78 g of product. Trituration with 2 x 300 ml absolute ethanol gave pure **23** (39.4 g, 74% over the last 2 steps); R_f = 0.33 (CH_3CN/0.1 N NH_4Cl: 7/3); ^1H NMR (300 MHz, D_2O) δ 7.85 (s, 1H), 7.10-7.32 (m, 5H), 5.54 (q, 2H), 4.33 (s, 2H), 3.84-3.91 (m, 1H), 3.38-3.41 (m, 2H), 1.76-1.88 (m, 2H), 1.45-1.65 (m, 2H).

9-[(1-Hydroxymethyl-3-phosphono)-1-propyloxymethyl]guanine monosodium salt (24)

The benzyl ether (**23**) (20 g), in a mixture of cyclohexene (freshly distilled) (400 ml), ethanol (800 ml), and water (400 ml), was treated at reflux with Pd $(OH)_2$ (46 g, damp, Pd content 20%) for 6 hr. The hot solution was filtered through Celite and the solvents were evaporated to dryness. The solid (32 g) was recrystallized from 200 ml absolute then 100 ml

50% aqueous ethanol, to give pure **24** (8.9 g, 80%); R_f 0.22 silica (CH_3CN/0.1 N NH_4Cl: 7/3); UV λmax (H_2O), 252 nm (13,045), 271 sh (9045); ^1H NMR (300 MHz, D_2O) δ 7.96 (s, 1H); 5.59 (s, 2H); 4.80 (s, 2H); 3.53-3.68 (m, 3H); 1.30-1.75 (m, 4H). Calcd for $C_{10}H_{15}N_5O_6PNa$: C, 33.81; H, 4.26; N, 19.72. Found: C, 33.80; H, 4.38; N, 19.38.

9-[(1-Hydroxymethyl-3-phosphono)-1-propyloxymethyl]guanine, cyclic ester (25)

A dried sample of **24** (500 mg, 1.46 mmol) was suspended in 30 ml of dry pyridine and mixed with 428 mg (1.46 mmol) of N,N'-dicyclohexyl 4-morpholine carboxamidine and stirred for 15 min. To the resulting suspension was added a hot solution of 578 mg (2.8 mmol) of dicyclohexyl carbodiimide (DCC) and the reaction was refluxed for 4 hr. An additional 578 mg of DCC was added and the reaction was refluxed overnight, then evaporated to dryness *in vacuo*. The residue was treated with 200 ml of water and filtered. The aqueous filtrate was evaporated and chromatographed on 6 g of Sephadex-DEAE A 25 ion exchange resin that had been pretreated with 1 M aqueous ammonium bicarbonate. The Sephadex column was then washed with water and the phostonate, dissolved in water, was applied and the column was eluted with 0.05 M aqueous ammonium bicarbonate. Lyophilization of the eluate gave 310 mg (67%) of pure product that had one spot on TLC with R_f 0.9 (CH_3CN/0.1 N NH_4Cl, 7:3). UV (H_2O) λ_{max} 251.5 nm (ε, 12,157); 271.3 sh (8629). ^1H NMR (400 MHz, DMSO-d) 7.84 (s, 1H); 5.39 (s, 2H); 4.0 (m, 4H); 3.6 (s, 1H); 1.9 (m, 2H). Calcd. for $C_{10}H_{14}N_5O_5P \cdot 2/3\ H_2O$: C, 36.71; H, 4.71; N, 21.41. Found: C, 36.7; H, 4.81; N, 21.8.

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REFERENCES

1. Some of this work has been presented previously: (a) Reist, E. J.; and Sturm, P. A. Proceedings VIIIth International Symposium on Medicinal Chemistry, Eds. Dahlbom, R., and Nelsson, J.L.G. Swedish Pharmaceutical Press, Stockholm, 1984. (b) Reist, E. J.; Sturm, P. A. U.S. Patent 5,047,533. September 10, 1991. (c) Reist, E. J.; Ruhland-Fritsch, B. L.; Sturm, P. A.; Kern, E. R.; Huffman, J. H.; and Sidwell, R. W. 10th International Roundtable on Nucleosides, Nucleotides and their Biological Applications, Park City, Utah, September 16-20, 1992.

2. Bale Jr., J. F.; and Jordan, M. C. Handbook of Clin. Neurology 12(56) Viral Disease, R. R. McKendall, editor, Elsevier Science Publishers, B. V. Amsterdam, 1989, p. 263.

3. Medina, D. J.; Hsuing, G. D.; and Mellors, J. W. Antimicrob. Agts. Chemotherap. 1992, 36, 1127.

4. Feng, J. S.; Crouch, J. Y.; Tian, P. Y.; Lucia, H. L.; and Hsuing, G. D. Antiviral Chem. and Chemotherap. 1993, 4, 19.

5. Hirsch, M. S. N. Engl. J. Med. 1992, 326, 264.

6. Freitas, V. R.; Fraser-Smith, E. B.; Chiu, S.; Michelson, S.; and Schatzman, R. C. Antiviral Res. 1993, 21, 301.

7. Studies of Ocular Complication of AIDS Research Group. AIDS Clinical Trials Group. N. Engl. J. Med. 1992, 326, 213.

8. Drew, W. L. et al. J. Infect. Dis. 1991, 163, 716.

9. Schaeffer, H. J.; Beauchamp, L.; deMiranda, P; and Elion, G. B. Nature 1978, 272, 583.

10. Sullivan, V.; Talarico, C. L.; Stanat, S. C.; Davis, M.; Coen, D. M.; and Biron, K. K. Nature 1992, 358, 162.

11. Biron, K. K.; Stanat, S. C.; Sorrell, J. B.; Fyfe, J. A.; Keller, P. M.; Lambe, C. V.; and Nelson, D. J. Proc. Natl. Acad. Sci (USA) 1985, 82, 2473.

12. Carrasco, L. Nature 1978, 272, 694.

13. Engel, R. Chemical Reviews 1977, 77, 349.

14. Prisbe, E. J.; Martin, J. C.; McGee, D. P. C.; Barker, M. F.; Smee, D. F.; Duke, A. E.; Matthews, T. R.; and Verheyden, J. P. H. J. Med. Chem. 1986, 29, 671.

15. Huffman, J. H.; Sidwell, R. W.; Morrison, A. G.; Coombs, J.; and Reist, E. J. Nucleosides and Nucleotides 1994, 13, 0000.

16. Eberhard, A.; and Westheimer, F. H. J. Am. Chem. Soc. 1965, 87, 253.

17. Kelley, J. L.; Krochmal, M. P.; and Schaeffer, H. J. J. Med. Chem. 1981, 24, 1528.

18. Lygo, B., O'Connor, N., and Wilson, P. R. Tetrahedron 1988, 44(22), 6881.

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