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Synthesis and antiviral evaluation of alkoxyalkyl esters of acyclic purine and pyrimidine nucleoside phosphonates against HIV-1 in vitro

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Abstract

Alkoxyalkyl esters of cidofovir, an acyclic nucleoside phosphonate, have been shown to have antiviral activities several orders of magnitude greater than unmodified cidofovir against cytomegalovirus, herpes simplex virus, vaccinia, cowpox, ectromelia and adenoviruses in vitro. Hexadecyloxypropyl-cidofovir is orally bioavailable and active in lethal animal models of vaccinia, cowpox, ectromelia and cytomegalovirus. To see if this strategy is also applicable to other acyclic nucleoside phosphonates, we have converted several phosophonomethoxyethyl purines and pyrimidines to their hexadecyloxypropyl, octadecyloxyethyl and oleyloxyethyl esters and compared their activity against HIV-1 with the activity of the respective unmodified acyclic nucleoside phosphonates. The hexadecyloxypropyl esters of phosphonomethoxyethyl-adenine, phosphonomethoxyethyl-2,6-diaminopurine and phosphonomethoxyethyl-N^6-cyclopropyl-diaminopurine were 3–5 orders of magnitude more active against HIV-1 in vitro than the parent nucleotides. The EC_{50} values for these compounds were in the 10–20 pM range with selective indexes of 1250 to >4000. The acyclic pyrimidine phosphonates were generally inactive against HIV-1 in vitro. Phosphonomethoxyethyl-cytosine and phosphonomethoxyethyl-5-fluorocytosine were inactive against HIV-1. Surprisingly, hexadecyloxypropyl-phosphonomethoxyethyl-5-fluorocytosine was active against HIV-1 with a submicromolar EC_{50} and a selective index of 174. Esterification of acyclic nucleoside phosphonates with alkoxyalkyl moieties may represent a general approach for increasing antiviral activity and selectivity of this class of antivirals.

Keywords: HIV; Acyclic nucleoside phosphonates; Prodrugs; Alkoxyalkyl esters

1. Introduction

Acyclic nucleoside phosphonates are becoming widely used for treatment of viral diseases. Currently licensed compounds of this class include cidofovir ((*S*)-1-(3-hydroxy-2-phosphonomethoxypropyl)cytosine, Vistide®) for cytomegalovirus, adefovir dipivoxil (9-(2-phosphono-methoxyethyl)adenine dipivoxil, Hepsera®) for HBV and tenofovir disoproxil fumarate (9-[2-(*R*)-(phosphonomethoxy)propyl]adenine disoproxil fumarate, Viread®) for HIV (De Clercq, 2004; De Clercq and Holy, 2005).

Cidofovir (CDV) is active against herpes group viruses including herpes simplex virus, types 1 and 2 and human cytomegalovirus (De Clercq et al., 1986, 1987; De Clercq, 1993). It is approved for treatment of CMV retinitis in AIDS patients but must be given intravenously because of poor oral absorption. The dose limiting toxicity with CDV is nephrotoxicity caused by concentration of the drug in the proximal tubule (Jacobson, 1997; Cundy, 1999). To circumvent these problems, we synthesized alkoxyalkyl ester prodrugs of CDV and found that they exhibit enhanced activity against HSV, CMV, and orthopoxviruses in vitro. Hexadecyloxypropyl-CDV (HDP-CDV) was >100-fold more active against HCMV and 30- to 40-fold more active against cowpox and vaccinia than CDV itself (Beadle et al., 2002; Kern et al., 2002). This appears to be due to enhanced cellular uptake and conversion of HDP-CDV to CDV diphosphate (Ciesla et al., 2003). HDP-CDV was orally active in mice against cowpox and vaccinia (Quenelle et al., 2004) and in ectromelia infection (Buller et al., 2004). HDP-CDV was also orally active in SCID mice with human fetal retina or thyroid/liver implants infected with HCMV (Bidanset et al., 2004).

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To see if this design paradigm could be effective for antiviral phosphonates with activity against HIV-1 infection, we synthesized a series of alkoxyalkyl esters of purine and pyrimidine based nucleoside phosphonates and evaluated their antiviral activity and selective index in MT-2 cells infected with wild type HIV-1.

2. Materials and methods

2.1. Syntheses

^{1}H NMR spectra were recorded on a Varian HG spectrophotometer operating at 300 MHz and are reported in units of ppm relative to internal tetramethylsilane at 0.00 ppm. Analtech Silica gel-GF (250 μm) plates were used for thin layer chromatography (TLC). The products were visualized with UV light, phospray (Supelco, Bellefonte, PA, USA) and charring. Flash chromatography was performed with silica gel (E. Merck silica gel 60, 230–400 mesh). Mass spectra showing the presence of a molecular ion were done using electrospray ionization (MS-ESI) in both positive and negative modes. Combustion analyses were carried out by Atlantic Microlabs (Norcross, GA).

2-Octadecyloxy-1-ethanol and 3-hexadecyloxy-1-propanol were synthesized from octadecyl methanesulfonate and 1,2-ethanediol, and hexadecyl methanesulfonate and 1,3-propanediol as previously described by Kini et al. (1997).

*9-[2-(Phosphonomethoxy)ethyl]adenine (**12**), 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (**14**), 9-[2-(phosphonomethoxy)ethyl]guanine (**15**), and [2-(phosphonomethoxy) ethyl]cytosine (**8**)* were prepared according to Holy et al. (1999).

*5-Fluoro-1-[2-(phosphonomethoxy)ethyl]cytosine (**9**).* 5-Fluorocytosine (0.78 g, 6.0 mmol) and DBU (1.05 g, 6.9 mmol) were stirred in dry DMF (15 ml) at 80 °C for 30 min and then diisopropyl 2-chloroethoxymethylphosphonate (**1**, Holy et al., 1999) (1.56 g, 6.0 mmol) in dry DMF (5 ml) was added in one portion. The mixture was stirred at 100 °C for 17 h. DMF was evaporated; and then the residue was dissolved in chloroform (100 ml) and washed with water (3 × 10 ml). The chloroform solution was dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (chloroform-methanol 0–10%) to give diisopropyl-5-fluoro-1-[2-(phosphonomethoxy)ethyl]cytosine (**3**, 1.09 g, 51% yield).

A mixture of compound **3** (2.8 mmol, 1.00 g) and bromotrimethylsilane (3 ml) in acetonitrile (30 ml) was stirred at room temperature for 20 h. Acetonitrile was evaporated, a new portion of acetonitrile was added and evaporated (2× 10 ml), and then co-evaporated with toluene (10 ml). Water (15 ml) was added to the residue. The mixture was stirred at room temperature for 1 h. The solvents were evaporated. The residue was purified by ion-exchange chromatography (DEAE $HCOO^-$ form, eluent HCOOH-linear gradient from 0 to 1 M) to give 5-fluoro-1-[2-(phosphonomethoxy)ethyl]cytosine (**9**, 0.67 g, 89%). ^{1}H NMR (D_2O): δ 8.18 (d, J = 6 Hz, 1H); 4.04 (t, J = 5 Hz, 2H); 3.84 (t, J = 5 Hz, 2H); 3.66 (d, J = 9 Hz, 2H). ^{31}P NMR (D_2O): δ 16.91. MS-ESI (*m*/*z*) 268 $(M\text{H})^+$, 266 $(M-\text{H})^-$.

*5-Bromo-1-[2-(phosphonomethoxy)ethyl]cytosine (**11**)* was synthesized from 5-bromocytosine using the method described above for **9**. The overall yield was 42%. ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 7.98 (s, 1H); 3.89 (t, J = 5 Hz, 2H); 3.73 (t, J = 5 Hz, 2H); 3.60 (d, J = 9 Hz, 2H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 22.65 MS-ESI (*m*/*z*) 328, 330 $(M\text{H})^+$, 326, 328 $(M-\text{H})^-$.

*2,6-Diamino-9-[2-(phosphonomethoxy)ethyl]purine (**13**)* was synthesized from 2,6-diaminopurine using the method described above for **9** in overall 11% yield. ^{1}H NMR (D_2O): δ 7.64 (s, 1H); 3.99 (t, J = 5 Hz, 2H); 3.64 (t, J = 5 Hz, 2H); 3.64 (d, J = 8 Hz, 2H). ^{31}P NMR (D_2O): δ 14.16. MS-ESI (*m*/*z*) 289 $(M\text{H})^+$, 287 $(M-\text{H})^-$.

*5-Fluoro-1-[2-(phosphonomethoxy)ethyl]uracil (**10**).* Sodium nitrite (2.9 g, 42.5 mmol) was added to a solution of compound **9** (1.13 g, 4.25 mmol) in 50% AcOH (40 ml) at 0 °C. The solution was stirred at room temperature overnight and then was lyophilized, the residue was recrystallized from EtOH/water (1:1) to give 5-fluoro-1-[2-(phosphonomethoxy)ethyl]uracil disodium salt (0.9 g, 68%). ^{1}H NMR (D_2O): δ 7.92 (d, J = 6 Hz, 1H); 3.97.04 (t, J = 5 Hz, 2H); 3.82 (t, J = 5 Hz, 2H); 3.62 (d, J = 9 Hz, 2H). ^{31}P NMR (D_2O): δ 16.19. MS-ESI (*m*/*z*) 291 $(M\text{Na})^+$, 267 $(M-\text{H})^-$.

General procedure: alkylation of (phosphonomethoxy)ethyl nucleosides with alkoxyalkyl alcohol. To a mixture of [2-(phosphonomethoxy)ethyl]nucleoside (2.32 mmol) and alkoxyalkyl alcohol (2.80 mmol) in dry pyridine (10 ml) 1,3-dicyclohexylcarbodiimide (DCC, 1.15 g, 5.6 mmol) was added in one portion. The mixture was stirred at room temperature overnight (∼16–18 h). Pyridine was evaporated, and then the residue was purified by flash column chromatography (eluent: dichloromethane:methanol 0–15%). This procedure was used to prepare compounds **12a**, **12b**, **14a**, **14b**, **8a**, **9a**, **11a** and **10a**.

*Hexadecyloxypropyl 9-[2-(phosphonomethoxy)ethyl]adenine (**12a**)* was prepared from 9-[2-(phosphonomethoxy)ethyl] adenine and 3-hexadecyloxy-1-propanol with a yield of 32%. ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 8.18 (s, 1H); 8.17 (s, 1H); 7.02 (br s, 2H); 4.37 (t, J = 5 Hz, 2H); 3.82 (t, J = 5 Hz, 2H); 3.73 (dd, J = 6 Hz, J = 12 Hz, 2H); 3.50 (d, J = 9 Hz, 2H); 3.30 (t, J = 6 Hz, 2H); 3.24 (t, J = 6 Hz, 2H); 1.62–1.69 (m, 2H); 1.41–1.47 (m, 2H); 1.15–1.35 (m, 26H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 13.53. MS-ESI (*m*/*z*) 600 $(M+2\text{Na})^{2+}$, 554 $(M-\text{H})^-$.

*Octadecyloxyethyl 9-[2-(phosphonomethoxy)ethyl]adenine (**12b**)* was prepared from 9-[2-(phosphono-methoxy)ethyl]-adenine and 2-octadecyloxy-1-ethanol in 41% yield. ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 8.17 (s, 1H); 8.16 (s, 1H); 7.04 (br s, 2H); 4.35 (t, J = 5 Hz, 2H); 3.78–3.85 (m, 4H); 3.50 (d, J = 8 Hz, 2H); 3.73 (t, J = 5 Hz, 2H); 3.30 (t, J = 7 Hz, 2H); 1.41–1.47 (m, 2H); 1.18–1.32 (m, 30H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 13.38. MS-ESI (*m*/*z*) 614 $(M+2\text{Na})^{2+}$, 568 $(M-\text{H})^-$.

*Hexadecyloxypropyl 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (**14a**)* was prepared from 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (**14**) and 3-hexadecyloxy-1-propanol with yield 47%. ^{1}H NMR (DMSO-d_6): δ 8.11 (s, 1H); 6.88 (br s, 2H); 4.19–4.30 (m, 2H); 3.78–3.89 (m, 2H); 3.65–3.78 (m, 2H); 3.52 (d, J = 6 Hz, 2H); 3.17–3.32

(m, 4H); 1.55–1.74 (m, 2H); 1.34–1.55 (m, 2H); 1.11–1.34 (m, 26H); 0.86 (t, J = 7Hz, 3H).

*Octadecyloxyethyl 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (**14b**)* was prepared from 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (**14**) and 2-octadecyloxy-1-ethanol with yield 32%. ^{1}H NMR (DMSO-d_6): δ 8.06 (s, 1H); 6.60 (br s, 2H); 4.19–4.35 (m, 2H); 3.78–3.85 (m, 4H); 3.50–3.70 (m, 2H); 3.20–3.40 (m, 4H); 1.32–1.55 (m, 2H); 1.18–1.32 (m, 30H); 0.87 (t, J = 7 Hz, 3H).

*Hexadecyloxypropyl 1-[2-(phosphonomethoxy)ethyl]cytosine (**8a**)* was prepared from 1-[2-(phosphono-methoxy) ethyl]cytosine (**8**) and 3-hexadecyloxy-1-propanol with yield 31%. ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 7.38 (d, J = 8, 1H); 5.78 (d, J = 8, 1H); 4.07–4.18 (m, 2H); 3.82–3.99 (m, 2H); 3.77–3.3.85 (m, 2H); 3.75 (d, J = 9 Hz, 2H); 3.46 (t, J = 6 Hz, 2H); 3.37 (t, J = 6 Hz, 2H); 1.76–1.96 (m, 2H); 1.41–1.57 (m, 2H); 1.10–1.35 (m, 26H); 0.86 (t, J = 7 Hz, 3H). ^{31}P NMR ($CDCl_3$ + MeOD-d_4): δ 21.97.

*Hexadecyloxypropyl 5-fluoro-1-[2-(phosphonomethoxy) ethyl]cytosine (**9a**)* was prepared from 5-fluoro-1-[2-(phosphonomethoxy)ethyl]cytosine (**9**) and 3-hexadecyloxy-1-propanol with yield 50%. ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 7.93 (d, J = 6, 1H); 3.89 (t, J = 5 Hz, 2H); 3.79 (dd, J = 6 Hz, J = 12 Hz, 2H); 3.67 (t, J = 5 Hz, 2H); 3.46 (d, J = 8 Hz, 2H); 3.45 (t, J = 6 Hz, 2H); 3.32 (t, J = 6 Hz, 2H); 1.70–1.75 (m, 2H); 1.45–1.50 (m, 2H); 1.18–1.38 (m, 26H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 18.38. MS-ESI (m/z) 550 (MH)$^+$, 548 (M − H)$^-$.

*Hexadecyloxypropyl 5-bromo-1-[2-(phosphonomethoxy)ethyl]cytosine (**11a**)* was prepared from 5-bromo-1-[2-(phosphono-methoxy)ethyl]cytosine (**11**) and 3-hexadecyloxy-1-propanol with yield 27%. ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 8.09 (d, J = 6, 1H); 3.91–4.10(m, 4H); 3.77 (t, J = 5 Hz, 2H); 3.67 (d, J = 9 Hz, 2H); 3.52 (t, J = 6 Hz, 2H); 3.41 (t, J = 7 Hz, 2H); 3.12–3.20 (m, 4H); 1.85–1.95 (m, 2H); 1.50–1.60 (m, 2H); 1.20–1.38 (m, 26H); 0.89 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 20.76. MS-ESI (m/z) 610, 612 (MH)$^+$, 632, 635 (M + Na)$^+$, 609, 611 (M − H)$^-$.

*Hexadecyloxypropyl 5-fluoro-1-[2-(phosphonomethoxy) ethyl]uracil (**10a**)* was prepared from 5-fluoro-1-[2-(phosphonomethoxy)ethyl]uracil (**10**) and 3-hexadecyloxy-1-propanol with yield 24%. ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 8.01 (d, J = 6, 1H); 3.80–3.95 (m, 4H); 3.68–3.87 (m, 2H); 3.58 (d, J = 8 Hz, 2H); 3.41 (t, J = 6 Hz, 2H); 3.32 (t, J = 6 Hz, 2H); 1.72–1.80 (m, 2H); 1.44–1.55 (m, 2H); 1.15–1.35 (m, 26H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 13.43. MS-ESI (m/z) 573 (M + Na)$^+$, 589 (M + K)$^+$, 549 (M − H)$^-$.

*Hexadecyloxypropyl 9-[2-(phosphonomethoxy)ethyl] guanine (**15a**)*. Hexadecyloxypropyl 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine (**14a**, 0.65 g) was stirred with 1 M HCl (10 ml) and dioxane (7 ml) at reflux for 1 h. The solution was neutralized with ammonium hydroxide (pH ∼ 3–4). Solvent was evaporated. Product was crystallized with ethyl alcohol and then purified by flash column chromatography (eluent: chloroform–methanol–water–ammonium hydroxide 80:20:1:1, chloroform–methanol–water–ammonium hydroxide 70:30:3:3) to give **15a** (0.45 g, 75%). ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 7.83 (s, 1H); 6.44 (br s, 2H); 4.18 (t, J = 5 Hz, 2H); 3.97–4.00 (m, 2H); 3.86 (t, J = 5Hz, 2H); 3.73 (d, J = 8 Hz, 2H); 3.42 (t, J = 6 Hz, 2H); 3.34 (t, J = 6 Hz, 2H); 1.80–1.84 (m, 2H); 1.46–1.52 (m, 2H); 1.13–1.38 (m, 26H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 17.90. MS-ESI (m/z) 572 (M + H)$^+$, 570 (M − H)$^-$. Anal. Calcd for $C_{27}H_{49}N_5NaO_6P \times 0.5H_2O$: C, 53.81; H, 8.35; N, 11.62. Found: C, 53.58; H, 8.28; N, 11.40.

*Octadecyloxyethyl 9-[2-(phosphonomethoxy)ethyl]guanine (**15b**)* was prepared as **15a** from **14b** in 78% yield. ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 8.73 (s, 1H); 6.88 (br s, 2H); 4.33 (t, J = 5 Hz, 2H); 4.05–4.17 (m, 2H); 3.93 (t, J = 5 Hz, 2H); 3.79 (d, J = 9 Hz, 2H); 3.55 (t, J = 5 Hz, 2H); 3.41 (t, J = 7 Hz, 2H); 1.47–1.54 (m, 2H); 1.15–1.35 (m, 30H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CDCl_3$): δ 15.93. MS-ESI (m/z) 586 (M + H)$^+$, 584 (M − H)$^-$. Anal. Calcd for $C_{28}H_{51}N_5NaO_6P \times H_2O$: C, 53.75; H, 8.54; N, 11.19. Found: C, 53.66; H, 8.95; N, 10.91.

*N^4-Benzoyl-5-fluoro-1-(S)-[3-trityloxy-2-hydroxypropyl] cytosine (**17**)*. A suspension of N^4-benzoyl-5-fluorocytosine (2.2 g, 8.9 mmol) and sodium hydride (0.046 g, 1.9 mmol) in dry DMF (25 ml) was stirred at room temperature for 1 h. (*S*)-Trityl glycidyl ether (2.5 g, 8.0 mmol) was added in one portion. The mixture was stirred at 100 °C for 5 h. DMF was evaporated, the residue was purified by flash column chromatography (eluent: dichloromethane–methanol 0–20%) to give **17** (4.2 g, 93%). ^{1}H NMR ($CDCl_3$): 5 8.14 (d, J = 7 Hz, 1H); 7.10–7.50 (m, 19H); 4.00–4.10 (m, 2H); 3.48–3.70 (m, 1H); 3.07–3.28 (m, 1H); 2.42 (s, 3H).

*Hexadecyloxypropyl N^4-benzoyl-5-fluoro-1-(S)-[3-trityloxy-2-(phosphonomethoxy)propyl]-cytosine (**20**)*. Hexadecyloxypropyl *p*-toluenesulfonyloxymethylphosphonate (Beadle et al., 2006) (2.72 g, 4.98 mmol) was added to a mixture of 17 (2.55 g, 4.52 mmol) and sodium hydride (0.65 g, 4.98 mmol) in dry DMF (50 ml). The mixture was stirred at 70 °C for 24 h. The solvent was evaporated, the residue was purified by column chromatography (eluent: dichloromethane–methanol 0–20%) to give 20 (1.57 g, 37% yield). ^{1}H NMR ($CDCl_3$): δ 8.15 (d, J = 7 Hz, 1H); 6.92–7.60 (m, 19H); 4.00–4.10 (m, 2H); 3.60–3.90 (m, 3H); 2.92–3.40 (m, 8H); 1.62–1.78 (m, 2H); 1.38–1.55 (m, 2H); 0.95–1.37 (m, 26H); 0.87 (t, J = 7 Hz, 3H). ^{31}P NMR ($CDCl_3$): δ 14.03. MS-ESI (m/z) 963 (MNa)$^+$, 939 (M − H)$^-$.

2.1.1. Hexadecyloxypropyl 5-fluoro-1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (22)

Part A. Compound 20 (1.0 g, 1.06 mmol) was added to 2 M ammonia solution in methanol (10 ml). The mixture was stirred at 70 °C overnight. Methanol was evaporated, and then the residue was purified by column chromatography (eluent: chloroform–methanol–ammonium hydroxide–water 70:58:8:8) to give hexadecyloxypropyl 5-fluoro-1-(*S*)-[3-trityloxy-2-(phosphonomethoxy)propyl]cytosine. (0.75 g, 86% yield). ^{1}H NMR (DMSO-d_6 + $CHCl_3$): δ 7.91 (d, J = 7 Hz, 1H); 7.05–7.60 (m, 15H); 4.45–4.70 (m, 2H); 4.05–4.35 (m, 3H); 3.50–3.95 (m, 6H); 3.15–3.35 (m, 3H); 1.62–1.82 (m, 2H); 1.33–1.55 (m, 2H); 1.05–1.33 (m, 26H); 0.87 (t, J = 7 Hz, 3H).

^{31}P NMR ($CDCl_3$): δ 11.83. MS-ESI (*m/z*) 861 $(M+K)^+$, 821 $(M-H)^-$.

Part B. A mixture of hexadecyloxypropyl 5-fluoro-1-(*S*)-[3-trityloxy-2-(phosphonomethoxy)propyl]-cytosine (0.75 g, 0.91 mmol) and 80% AcOH in water (20 ml) was stirred at room temperature overnight. The solvents were evaporated, and then co-evaporated with water. The residue was purified by column chromatography (eluent: chloroform–methanol–ammonium hydroxide–water 70:58:8:8) to give compound **22** (0.3 g, 69%). ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 7.61 (d, J = 6 Hz); 3.21–3.49 (m, 8H); 3.10–3.20 (m, 2H); 3.02–3.10 (m, 2H); 2.91–2.98 (m, 1H); 1.41–1.59 (m, 2H); 1.11–1.22 (m, 2H); 0.88–1.03 (m, 26H); 0.55 (t, J = 7 Hz, 3H). ^{31}P NMR ($CDCl_3$ + MeOD-d_4): δ 11.29. MS-ESI (*m/z*) 580 $(M+H)^+$, 578 $(M-H)^-$.

*5-Fluoro-1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl] cytosine (**23**)*. A mixture of **22** (0.20 g, 0.34 mmol) and bromotrimethylsilane (0.353 ml) in acetonitrile (5 ml) was stirred at room temperature for 20 h. Acetonitrile was evaporated, and a new portion of acetonitrile was added and evaporated (2× 10 ml), and then co-evaporated with toluene (10 ml). Water (15 ml) was added to the residue. The mixture was stirred at room temperature for 1 h. The solvents were evaporated. The residue was purified by ion-exchange chromatography (DEAE $HCOO^-$ form, eluent HCOOH: linear gradient from 0 to 1 M) to give **23** (0.05 g, 48%). ^{1}H NMR (D_2O): δ 8.19 (d, J = 7 Hz, 1H); 3.55–3.85 (m, 5H); 3.22–3.05 (m, 2H). ^{31}P NMR (D_2O): δ 16.35.

*Hexadecyloxypropyl 5-fluoro-1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]uracil (**21**)*. A mixture of compound 20 (0.21 g, 0.22 mmol) and 80% AcOH in water (10 ml) was stirred at room temperature overnight. The solvents were evaporated, co-evaporated with water. The residue was purified by column chromatography on silica gel (eluent: chloroform–methanol–ammonium hydroxide–water 70:58:8:8) to give **21** (0.07 g, 54%). ^{1}H NMR (DMSO-d_6 + $CHCl_3$): δ 7.85 (d, J = 6 Hz, 1H); 3.70–3.85 (m, 2H); 3.50–3.65 (m, 3H); 3.20–3.50 (m, 8H); 1.65–1.75 (m, 2H); 1.35–1.48 (m, 2H); 1.11–1.28 (m, 26H); 0.80 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CHCl_3$): δ 14.96. MS-ESI (*m/z*) 581 $(M)^+$, 603 $(M)Na^+$, 580 $(M-H)^-$.

*5-Fluoro-1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl] uracil (**19**)*. Diethyl *p*-toluenesulfonyloxymethyl-phosphonate (0.34 g, 1.05 mmol) was added to a mixture of compound **17** (0.56 g, 1.00 mmol) and sodium hydride (0.14 g, 6.00 mmol) in dry DMF (10 ml). The mixture was stirred at 50 °C overnight, and then DMF was evaporated. The residue was dissolved in chloroform (50 ml), washed with water (3× 15 ml). Chloroform solution was dried over $MgSO_4$ and evaporated. The residue was dissolved in acetonitrile (25 ml), and bromotrimethylsilane (1.0 ml) was added to the solution that was stirred at room temperature for 20 h. Acetonitrile was evaporated, new portion of acetonitrile was added and evaporated (2× 10 ml), and then co-evaporated with toluene (10 ml). Water (15 ml) was added to the residue. The mixture was stirred at room temperature for 1 h. The solvents were evaporated. The residue was dissolved in 80% AcOH in water (10 ml). The mixture was stirred at 50 °C overnight. The solvents were evaporated, the residue was recrystallized from ethanol–water (1:1) to give **19** (0.19 g, 63%). ^{1}H NMR (D_2O): δ 7.89 (d, J = 7 Hz, 1H); 3.70–3.85 (m, 3H); 3.55–3.65 (m, 2H); 3.22–2.95 (m, 2H). ^{31}P NMR (D_2O): δ 18.39. MS-ESI (*m/z*) 298 $(M)H^+$.

*2,6-Diamino-9-(S)-[3-trityloxy-2-hydroxypropyl]purine (**25**)* was synthesized from 2,4-diaminopurine using the same procedure as **17** in 75% yield. ^{1}H NMR ($CDCl_3$): δ 7.35–7.50 (m, 7H); 7.15–7.35 (m, 9H); 5.83 (br s, 2H); 4.91 (br s, 2H); 4.05–4.25 (m, 3H); 3.22–3.33 (m, 1H); 2.93–3.05 (m, 1H).

*Hexadecyloxypropyl 2,6-diamino-9-(S)-[3-trityloxy-2-(phosphonomethoxy)propyl]purine (**26**)* was synthesized from **25** using the same procedure described for 20 above. The yield was 32%. ^{1}H NMR ($CDCl_3$): δ 8.06 (s, 1H); 7.35–7.50 (m, 7H); 7.55–7.75 (m, 9H); 4.11–4.20 (m, 1H); 3.80–3.97 (m, 2H); 3.55–3.65 (m, 4H); 3.40–3.55 (m, 4H); 3.30–3.40 (m, 2H); 1.72–1.85 (m, 2H); 1.40–1.58 (m, 2H); 1.20–1.39 (m, 26H); 0.83 (t, J = 7 Hz, 3H). ^{31}P NMR ($CDCl_3$): δ 21.07. MS-ESI (*m/z*) 844 $(M)H^+$, 842 $(M-H)^-$.

*Hexadecyloxypropyl 2,6-diamino-9-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]purine (**27**)* was synthesized from **26** using the procedure described for **22** (part B). The yield was 31%. ^{1}H NMR (DMSO-d_6 + $CHCl_3$): δ 7.89 (s, 1H); 3.75–3.80 (m, 2H); 3.45–3.60 (m, 3H); 3.30–3.45 (m, 2H); 3.20–3.30 (m, 2H); 3.07–3.20 (m, 4H); 1.60–1.80 (m, 2H); 1.38–1.45 (m, 2H); 0.92–1.38 (m, 26H); 0.79 (t, J = 7 Hz, 3H). ^{31}P NMR (DMSO-d_6 + $CHCl_3$): δ 16.16. MS-ESI (*m/z*) 601 $(M)H^+$, 599 $(M-H)^-$.

*2,6-Diamino-9-(2-hydroxyethyl)purine (**29**)*. A mixture of 2,6-diaminopurine hydrate (0.9 g, 5.3 mmol), ethylene carbonate (0.52 g, 5.9 mmol) and sodium hydride (0.06 g, 2.65 mmol) in dry DMF (5 ml) was stirred at reflux for 1 h, and then was cooled to room temperature. Toluene (20 ml) was added to the mixture. The solid was filtered, washed with toluene, acetone, and dried under vacuum to give **29** (0.78 g, 76%). ^{1}H NMR (D_2O): δ 7.46 (s, 1H); 3.86 (t, J = 5 Hz, 2H); 3.66 (t, J = 5 Hz, 2H). MS-ESI (*m/z*) 195 $(M)H^+$, 217 $(M)Na^+$, 193 $(M-H)^-$.

*Hexadecyloxypropyl 2,6-diamino-9-[2-(phosphonomethoxy) ethyl]purine (**31**)* was synthesized from **29** using the same procedure as **20** in 33% yield. ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 7.86 (s, 1H); 4.20–4.30 (m, 2H); 3.92–4.08 (m, 4H); 3.82 (d, J = 7 Hz, 2H); 3.53 (t, J = 6Hz, 2H); 3.41 (t, J = 6 Hz, 2H); 1.83–1.97 (m, 2H); 1.50–1.60 (m, 2H); 1.05–1.45 (m, 26H); 0.89 (t, J = 7H). ^{31}P NMR ($CDCl_3$ + MeOD-d_4): δ 16.97. MS-ESI (*m/z*) 571 $(M)H^+$, 569 $(M-H)^-$.

*2-Amino-6-cyclopropylamino-9-(2-hydroxyethyl)purine (**30**)* was synthesized from 2-amino-6-cyclopropylaminopurine (**28**) using the same procedure as **29**, in 73% yield. ^{1}H NMR ($CDCl_3$): δ 7.45 (s, 1H); 4.06 (t, J = 5 Hz, 2H); 3.78 (t, J = 5 Hz, 2H); 2.40 (br s, 1H); 0.76–0.90 (m, 2H); 0.55–0.65 (m, 2H).

*Hexadecyloxypropyl 2-amino-6-cyclopropylamino-9-[2-(phosphonomethoxy)ethyl]purine (**32**)* was synthesized from compound 30 using the same procedure as **20** in 33% yield. ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 7.51 (s, 1H); 3.90–4.08 (m, 2H); 3.48–3.71 (m, 4H); 3.39 (d, J = 9 Hz, 2H); 3.00–3.21 (m, 4H); 2.64 (br s, 1H); 1.45–1.70 (m, 2H); 1.20–1.38 (m, 2H);

0.88–1.1.17 (m, 26H); 0.50–0.78 (m, 5H); 0.35–0.50 (m, 2H). ^{31}P NMR ($CDCl_3$ + MeOD-d_4): δ 13.85. MS-ESI (*m/z*) 611 $(M)^+$, 633 $(M)Na^+$, 609 $(M-H)^-$.

*Oleyloxyethyl 2-amino-6-cyclopropylamino-9-[2-(phosphonomethoxy)ethyl]purine (**33**)* was synthesized from **30** and 2-(oleyl-oxy)ethyl *p*-toluenesulfonyloxymethylphosphonate (Beadle et al., 2006) using the procedure from compound **20**. The yield was 3%. ^{1}H NMR ($CDCl_3$ + MeOD-d_4): δ 7.73 (s, 1H); 5.27–5.42 (m, 2H); 3.92–4.08 (m, 2H); 3.80–3.92 (m, 2H); 3.62–3.92 (m, 2H); 3.50–3.62 (m, 2H); 3.38–3.48 (m, 2H); 3.29–3.38 (m, 2H); 3.00 (br s, 1H); 1.81–2.12 (m, 2H); 1.44–1.65 (m, 2H); 1.11–1.40 (m, 24H); 0.78–0.98 (m, 5H); 0.60–0.73 (m, 2H). ^{31}P NMR ($CDCl_3$ + MeOD-d_4): δ 16.63. MS-ESI (*m/z*) 623 $(M)^+$, 621 $(M-H)^-$.

*2-Amino-6-cyclopropylamino-9-[2-(phosphonomethoxy) ethyl]purine (**35**).* Diethyl *p*-toluenesulfonyl-oxymethylphosphonate (0.18 g, 0.55 mmol) was added to a mixture of 2-amino-6-cyclopropylamino-9-(2-hydroxyethyl)purine (**30**, 0.12 g, 0.50 mmol) and sodium hydride (0.07 g, 3.00 mmol) in dry DMF (5 ml). The mixture was stirred at 50 °C overnight. DMF was evaporated. The residue was dissolved in chloroform (50 ml) and washed with water (2× 5 ml). The chloroform solution was dried over $MgSO_4$ and evaporated. The residue was dissolved in acetonitrile (15 ml), and then bromotrimethylsilane (1.0 ml) was added to the solution that was stirred at room temperature for 20 h. Acetonitrile was evaporated and a new portion of acetonitrile was added and evaporated (2× 10 ml), and then co-evaporated with toluene (10 ml). Water (15 ml) was added to the residue. The mixture was stirred at room temperature for 1 h. The solvents were evaporated, the residue was recrystallized from ethanol–water (1:1) to give product **35** (0.08 g, 48%). ^{1}H NMR (D_2O): δ 7.95 (s, 1H); 4.07–4.20 (m, 2H); 3.64–3.80 (m, 2H); 3.52 (d, J = 9 Hz); 2.61 (br s, 1H); 0.75–0.90 (m, 2H); 0.55–0.70 (m, 2H). ^{31}P NMR (D_2O): δ 18.34. MS-ESI (*m/z*) 329 $(M)^+$, 351 $(M)Na^+$.

*2,4-Diamino-6-hydroxyethylamino-pyrimidine (**37**).* A mixture of 2,4-diamino-6-chloro-pyrimidine (36, 0.73 g, 5.0 mmol) and tetrahydropyranyloxyethylamine (1.1 g, 7.5 mmol) in ethanol (30 ml) was refluxed for 2 days. Solvents were evaporated and then co-evaporated with ethanol (20 ml) and acetone (20 ml). The residue was dried in vacuum and triturated with boiling ether. The precipitate was filtered and con. HCl (0.15 ml) in mixture of water:dioxane (1:1) (10 ml) was added to the precipitate. The mixture was refluxed for 1 h. The solvent was evaporated, and the residue was purified by column chromatography (eluent: chloroform–methanol–water–ammonium hydroxide 80:20:1:1) to give 37 (0.16 g, 19%). ^{1}H NMR (D_2O): δ 5.21 (s, 1H); 3.73 (t, J = 6 Hz, 2H); 3.39 (t, J = 6 Hz, 2H). MS-ESI (*m/z*) 170 $(M)^+$.

*Hexadecyloxypropyl 2,4-diamino-6-[2-(phosphonomethoxy) ethyl]pyrimidine (**38**)* was synthesized from compound **37** using the procedure for compound **20**. The yield was 15%. ^{1}H NMR ($CDCl_3$): δ 3.90–4.09 (m, 2H); 3.61–3.78 (m, 2H); 3.45–3.60 (m, 4H); 3.30–3.45 (m, 4H); 1.80–1.98 (m, 2H); 1.45–1.61 (m, 2H); 0.95–1.39 (m, 26H); 0.88 (t, J = 6 Hz, 3H). ^{31}P NMR ($CDCl_3$): δ 16.88. MS-ESI (*m/z*) 546 $(M)^+$, 568 $(M)Na^+$.

2.2. Cells and viruses

2.2.1. HIV assays

MT-2 cells (AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health) were maintained in RPMI 1640 supplemented with 10% FBS (JRH Biosciences, Lenexa, Kans.), 10 mM HEPES buffer, 50 IU of penicillin/ml, and 50 μg of streptomycin/ml. HIV-1_{LAI} was obtained from the AIDS Research and Reference Reagent Program. The antiviral activity of each compound was determined by inoculating MT-2 cells with HIV-1_{LAI} at a multiplicity of infection (MOI) of 0.001 $TCID_{50}$/cell, followed by incubation in the presence of three-fold serial drug dilutions (three wells per dilution) as previously described (Hammond et al., 2001). Four days after infection, culture supernatants were harvested, lysed with 0.5% Triton X-100, and assayed for p24 antigen concentration using a commercial enzyme-linked immunosorbent assay (ELISA) (Perkin Elmer Life Sciences, Boston, MA). The antiviral activity of each compound is expressed as the EC_{50}, which is the concentration required to inhibit p24 antigen production by 50%.

2.2.2. Cytotoxicity

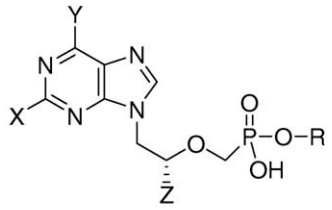
To assess cytotoxicity, MT-2 cells were incubated with drug for 72 h and harvested. Flow count beads (Beckman Coulter, Miami, FL) were added to the cell suspension followed by propidium iodide staining and analysis using an Epics Elite flow cytometer (Beckman Coulter). The 50% cytotoxic concentration (CC_{50}) was calculated from the cell counts and viability.

3. Results

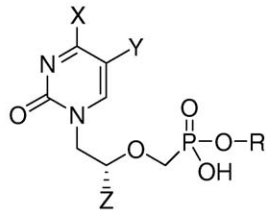
3.1. Chemistry

Fig. 1 summarizes the structures and abbreviations of the acyclic purine and pyrimidine nucleosides that were synthesized for this study. Many of the phosphonomethoxyethyl derivatives were prepared by reaction of diisopropyl 2-chloroethoxymethylphosphonate (**1**) with the appropriate nucleobases as shown in Scheme 1, using methodology described by Holy et al. (1999). The diisopropyl esters were, in turn, converted to the free phosphonic acids, and then esterified with 3-hexadecyloxy-1-propanol (HDP), 2-octadecyloxy-1-ethanol (ODE) or 2-oleyloxy-1-ethanol (OLE) using the coupling reagent 1,3-dicyclohexylcarbodiimide (DCC). To obtain the HDP and ODE esters of PMEG (**15a** and **15b**), the corresponding esters of PME-2-amino-6-chloropurine (**14a** and **14b**) were prepared and hydrolyzed to PMEG esters by heating in 1N HCl/ethanol. PME-5-fluorouracil (**10**) was obtained from the cytosine derivative **9** by deaminolysis in the presence of $NaNO_2$ since alkylation of 5-fluorouracil by diisopropyl 2-chloroethoxymethylphosphonate led to a mixture of the N^1-isomer and O^4-substituted regioisomers that was difficult to resolve. Esterification of **10** with 3-hexadecyloxy-1-propanol afforded HDP-PME-5FU (**10a**).

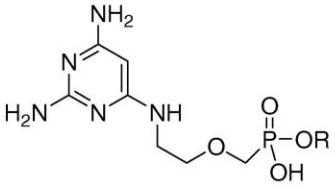
The synthesis of HPMP-5FC and HPMP-5FU analogs is detailed in Scheme 2. The key intermediate, N^4-



X	Y	Z	R[a]	abbreviation	cmpd number
NH_2	OH	H	H	PMEG	**15**
NH_2	OH	H	HDP	HDP-PMEG	**15a**
NH_2	OH	H	ODE	ODE-PMEG	**15b**
H	NH_2	H	H	PMEA	**12**
H	NH_2	H	HDP	HDP-PMEA	**12a**
H	NH_2	H	ODE	ODE-PMEA	**12b**
NH_2	NH_2	H	H	PME-DAP	**13**
NH_2	NH_2	H	HDP	HDP-PME-DAP	**31**
NH_2	cyclopropyl-NH	H	H	PME-cPr-DAP	**35**
NH_2	cyclopropyl-NH	H	HDP	HDP-PME-cPr-DAP	**32**
NH_2	cyclopropyl-NH	H	OLE	OLE-PME-cPr-DAP	**33**
NH_2	NH_2	CH_2OH	HDP	HDP-HPMP-DAP	**27**



NH_2	H	H	H	PMEC	**8**
NH_2	H	H	H	HDP-PMEC	**8a**
NH_2	F	H	H	PME-5FC	**9**
NH_2	F	H	HDP	HDP-PME-5FC	**9a**
OH	F	H	H	PME-5FU	**10**
OH	F	H	HDP	HDP-PME-5FU	**10a**
NH_2	F	CH_2OH	H	HPMP-5FC	**23**
NH_2	F	CH_2OH	HDP	HDP-HPMP-5FC	**22**
OH	F	CH_2OH	H	HPMP-5FU	**19**
OH	F	CH_2OH	HDP	HDP-HPMP-5FU	**21**
NH_2	Br	H	H	PME-5BrC	**11**
NH_2	Br	H	HDP	HDP-PME-5BrC	**11a**



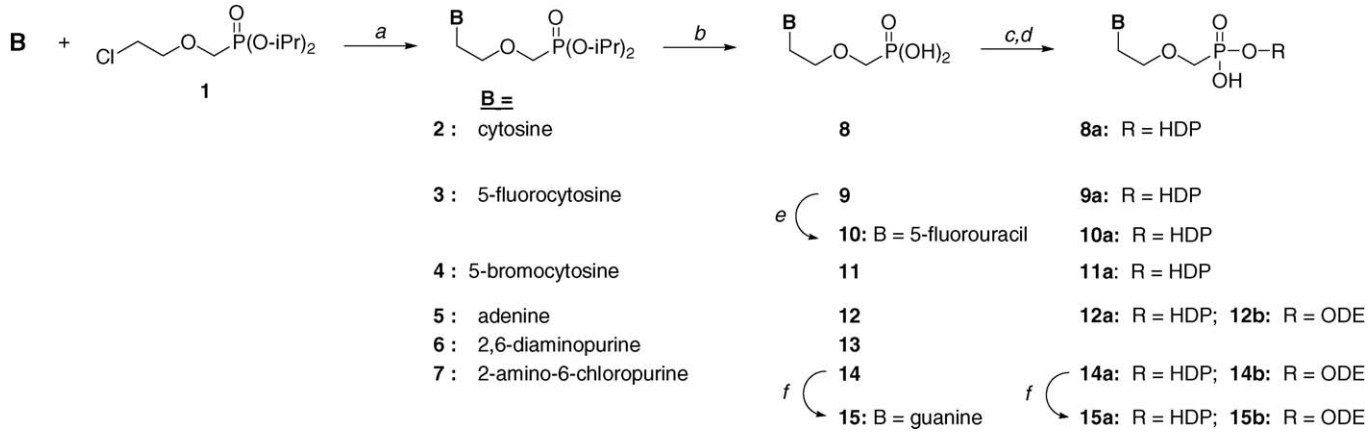
na	na	na	HDP	HDP-TAPym	**38**

[a]HDP = $(CH_2)_3O(CH_2)_{15}CH_3$; ODE = $(CH_2)_2O(CH_2)_{17}CH_3$; OLE = $(CH_2)_2O(CH_2)_8CH{=}CH(CH_2)_7CH_3$

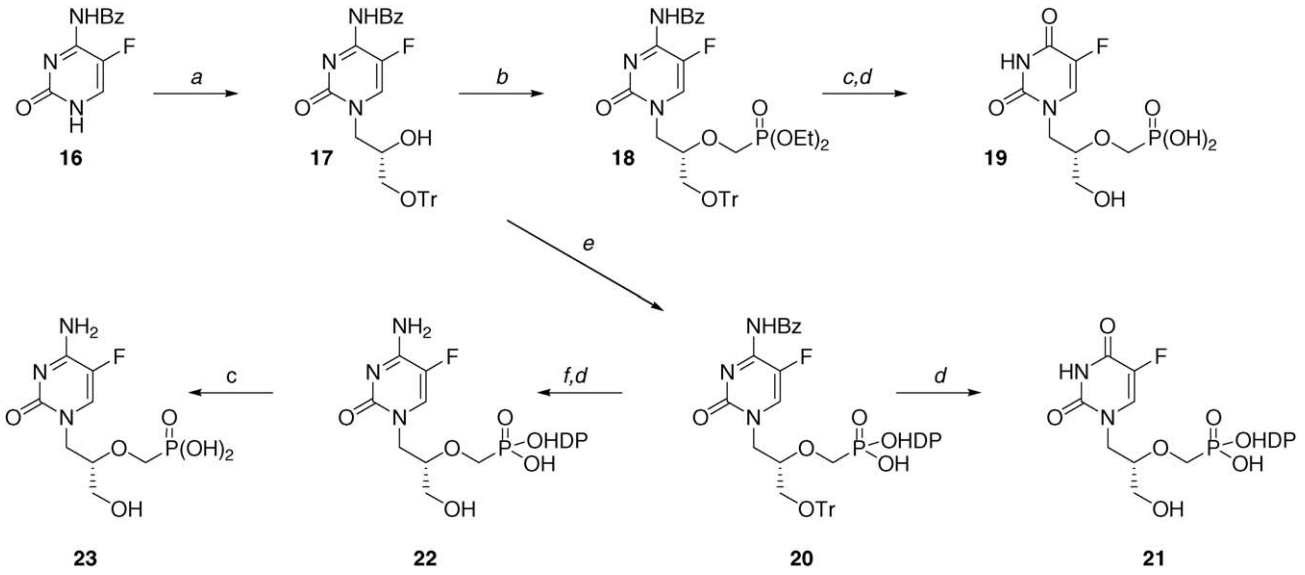
Fig. 1. Structures of compounds.

benzoyl-5-fluoro-1-(*S*)-[3-trityloxy-2-hydroxypropyl]cytosine (**17**), was synthesized by alkylation of N^4-benzoyl-5-fluorocytosine with *S*-trityl glycidyl ether (Vemishetti et al., 1997). Reaction of **17** with 3-(hexadecyloxy)propyl *p*-toluenesulfonyloxymethylphosphonate (Beadle et al., 2006) gave the fully protected compound **20**, which could be deprotected in two steps to give HDP-HPMP-5FC (**22**). Alternatively, treatment of **20** with 80% AcOH (reflux) yielded HDP-HPMP-5FU (**21**). Reaction of **17** with diethyl *p*-toluenesulfonyloxymethylphosphonate (Holy and Rosenberg, 1987), followed by hydrolysis gave HPMP-5FU (**19**).

HPMP derivatives of 2,6-diaminopurine were prepared using a similar strategy, which is shown in Scheme 3. Reaction of 2,6-diaminopurine with (*S*)-trityl glycidyl ether gave interme-



Scheme 1. Synthesis of phosonomethoxyethyl nucleoside phosphonates. Reagents: (a) DBU, *N*,*N*-DMF; (b) bromotrimethylsilane, acetonitrile; (c) 3-hexadecyloxy-1-propanol or 2-octadecyloxy-1-ethanol, DCC, pyridine; (d) 1N HCl, EtOH (1:1).

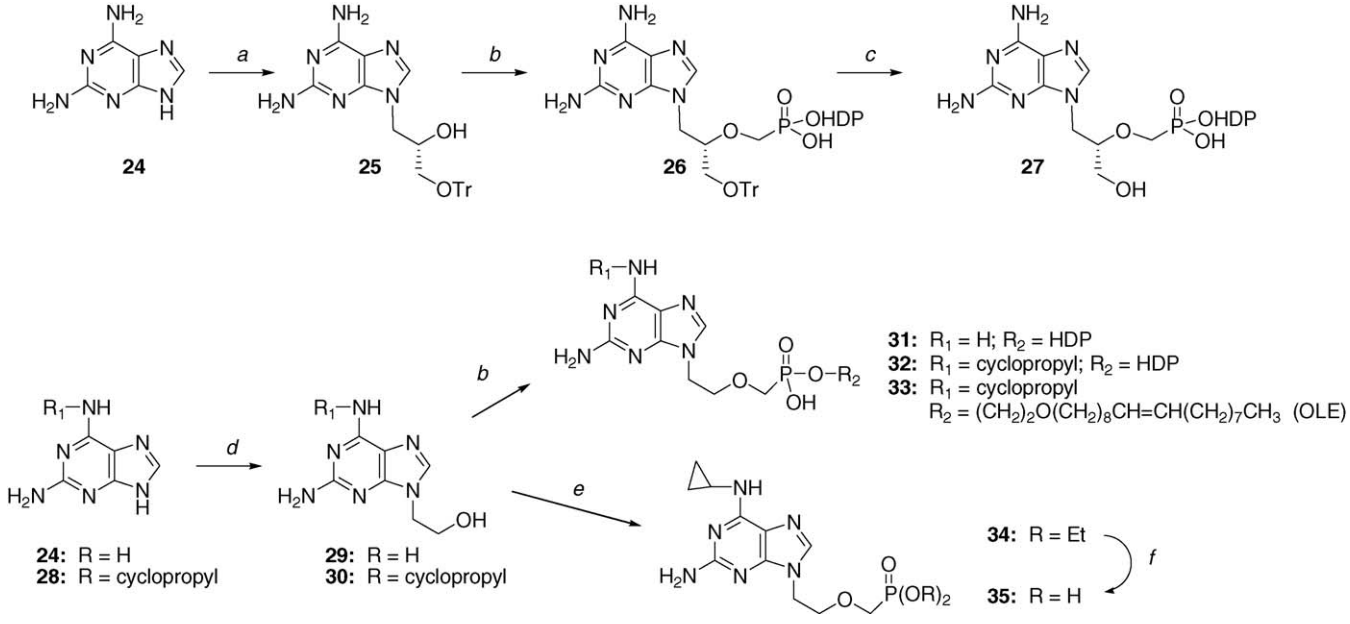


Scheme 2. Synthesis of 5-fluoropyrimidine nucleoside phosphonates. Reagents: (a) (*S*)-trityl glycidyl ether, NaH, *N*,*N*-DMF; (b) diethyl *p*-toluenesulfonyloxymethylphosphonate, NaH, *N*,*N*-DMF; (c) (i) bromotrimethylsilane, acetonitrile; (ii) water; (d) 80% acetic acid; (e) hexadecyloxypropyl *p*-toluenesulfonyloxymethylphosphonate, NaH, *N*,*N*-DMF; (f) NH_3, MeOH.

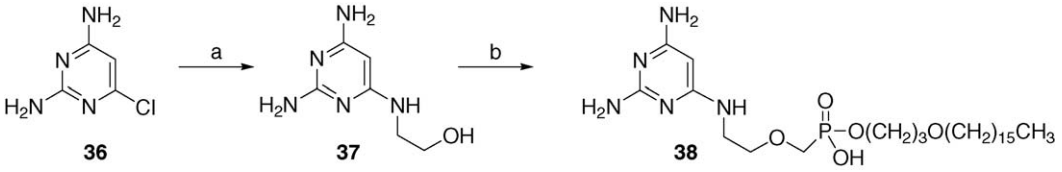
diate **25** which was alkylated with 3-(hexadecyloxy)propyl *p*-toluenesulfonyloxymethylphosphonate to give **26**. Deprotection of **26** gave HDP-HPMP-DAP (**27**).

Since 2,6-diaminopurine (**24**) and 2-amino-6-cyclopropylaminopurine (**28**) do not react with diisopropyl 2-chloroethoxymethyl-phosphonate under the conditions of Scheme 1, an alternative method was needed to obtain the PME derivatives of these bases. As shown in Scheme 3, **24** and **28** reacted with ethylene carbonate (Bischofberger and Kent, 1996) to give 2,6-diamino-9-(2-hydroxyethyl)purine (**29**) or 2-amino-6-cyclopropylamino-9-(2-hydroxyethyl)purine (**30**) and these intermediates could be alkylated with alkoxyalkyl or diethyl *p*-toluenesulfonyloxymethylphonates to provide HDP-PME-DAP (**31**), PME-cyclopropyl-DAP (**35**) or PME-cyclopropyl-DAP alkoxyalkyl esters (**32** and **33**).

We also prepared an open ring analog of HDP-PME-DAP, hexadecyloxypropyl 2,4-diamino-6-[2-(phosphonomethoxy)ethylamino]pyrimidine (HDP-PME-TAPym, **38**). Our synthesis is shown in Scheme 4. Reaction of tetrahydropyranyloxyethylamine (Bernady et al., 1979) with



Scheme 3. Synthesis of 2,6-diaminopurine nucleoside phosphonates. Reagents: (a) (*S*)-trityl glycidyl ether, NaH, *N*,*N*-DMF; (b) hexadecyloxypropyl *p*-toluenesulfonyloxymethylphosphonate, NaH, *N*,*N*-DMF; (c) 80% acetic acid; (d) ethylene carbonate, NaH, *N*,*N*-DMF; (e) diethyl *p*-toluenesulfonyloxymethylphosphonate; NaH, *N*,*N*-DMF; (f) (i) bromotrimethylsilane, acetonitrile; (ii) water.



Scheme 4. Synthesis of 2,4,6-triaminopyrimidine phosphonate. Reagents: (a) (i) 2-tetrahydropyranyloxyethylamine, EtOH; (ii) 0.15N HCl, water, *p*-dioxane;(b) hexadecyloxypropyl *p*-toluenesulfonyloxymethylphosphonate, NaH, *N*,*N*-DMF.

2,4-diamino-6-chloropyrimidine followed by acidic hydrolysis gave 2,4-diamino-6-(hydroxyethylamino)pyrimidine (**37**) which was then alkylated with hexadecyloxypropyl *p*-toluenesulfonyloxymethylphosphonate (Beadle et al., 2006) to give the compound **38**.

3.2. Biology

Esterification of the acyclic purine nucleoside phosphonates, PMEA, PME-DAP, PME-cyclopropyl-DAP and PMEG resulted in dramatic increases in antiviral activity (Table 1). Increases in antiviral activity of 3–5 orders of magnitude were observed versus the unmodified acyclic nucleoside phosphonate. HDP- and ODE-PMEA had EC_{50}s of 15 and 20 pM, respectively, versus 1.1 nM for PMEA; cytotoxicity was increased but selectivity indexes of 4000 and 900 were noted. PME-DAP had an average EC_{50} of 0.08 μM compared with 16 pM for HDP-PME-DAP; the selective index of the latter was 1250. Similar results were noted for HDP- and OLE-PME-cPr-DAP which had EC_{50} values of <10 pM and selective indexes of >12,000 and >400, respectively. HDP- and ODE-PMEG had EC_{50} values of 7.4–11 pM but their cytotoxicity was <0.1 to <100 pM, indicating a lack of antiviral selectivity. The HDP esters of HPMP-DAP and TAPym had EC_{50} values of 0.04 and 0.14 nM and selectivity indexes of 1700 and 143, respectively (Table 1).

We also prepared HDP esters of selected pyrimidines and tested them against HIV-1 in vitro (Table 2). Most HDP esters of the pyrimidines studied were not highly active against HIV-1 and did not show antiviral selectivity. The only exception was HDP-PME-5FC which had an EC_{50} of 0.14 μM and a selectivity index of 174.

Table 2
Antiviral activity and selectivity of acyclic pyrimidine phosphonates and their alkoxyalkyl esters in MT-2 cells infected with HIV-1_{LAI}

Compound	EC_{50} (μM)	CC_{50} (μM)	SI
PMEC	>100 (1)	>100 (2)	–
HDP-PMEC	>10 (1)	39 (2)	<3.9
PME-5FC	>100 (2)	>100 (3)	–
HDP-PME-5FC	0.14 ± 0.02 (3)	24 ± 14 (3)	174
PME-5BrC	>100 (2)	100 (2)	<1
HDP-PME-5BrC	31 (2)	38 ± 28 (3)	1.2
PME-5FU	>100 (2)	>100 (2)	–
HDP-PME-5FU	24 (2)	36.5 (2)	1.5
HPMP-5FC	>100 (2)	>100 (2)	–
HDP-HPMP-5FC	39 (2)	41 (2)	1.1
HPMP-5FU	>100 (2)	>100 (2)	–
HDP-HPMP-5FU	>100 (2)	35 (2)	<0.35

4. Discussion

Alkoxyalkyl esterification of acyclic purines and some pyrimidine phosphonates having activity against HIV-1 dramatically increases their antiviral activity in vitro. PMEA, PME-DAP and PME-cPr-DAP all show at least 10,000-fold increases in anti-HIV activity as their HDP- or ODE-esters (Table 1). PME-5FC, which has no discernable activity against HIV-1 (EC_{50} > 100 μM), becomes active at a submicromolar EC_{50} of 0.14 μM as HDP-PME-5FC (Table 2). While this strategy also increases the cytotoxicity of the compounds, the increases in antiviral activity are several orders of magnitude greater than the increase in cytotoxicity, resulting in increased antiviral selectivity. This extends to HIV-1 our previous work with alkoxyalkyl esters of CDV and HPMPA showing >100-fold increases in

Table 1
Antiviral activity and selectivity of acyclic purine phosphonates and their alkoxyalkyl esters in MT-2 cells infected with HIV-1_{LAI}

Compound	EC_{50} (μM)	CC_{50} (μM)	SI
PMEA	1.1 ± 0.6 (6)	157 ± 54 (3)	143
HDP-PMEA	$1.5 \times 10^{-5} \pm 3 \times 10^{-5}$ (4)	0.06 ± 0.09 (3)	4000
ODE-PMEA	$2.0 \times 10^{-5} \pm 3 \times 10^{-5}$ (3)	0.018 ± 0.013 (3)	900
PME-DAP	0.15; 0.01	5.3 ± 4.0 (5)	66.3
HDP-PME-DAP	$1.6 \times 10^{-5} \pm 1.2 \times 10^{-5}$ (3)	0.02 ± 0.03 (4)	1250
PME-cPr-DAP	0.07 ± 0.06 (3)	4.2 ± 3.3 (5)	60
HDP-PME cPr-DAP	$<1 \times 10^{-5}$ (3)	0.12 ± 0.13 (4)	>12000
OLE-PME cPr-DAP	$<1 \times 10^{-5}$ (3)	0.004 ± 0.005 (3)	>400
PMEG	0.13 ± 0.16 (7)	2.5 ± 1.8 (7)	19.2
HDP-PMEG	$7.4 \times 10^{-6} \pm 1.9 \times 10^{-5}$ (5)	$<1 \times 10^{-4}$ (4)	<13.5
ODE-PMEG	$1.1 \times 10^{-5} \pm 9 \times 10^{-6}$ (3)	$<1 \times 10^{-7}$ (4)	<0.01
HDP-HPMP-DAP	0.04 ± 0.02 (3)	68 ± 7.0 (3)	1700
HDP-TAPym	0.14 ± 0.16 (3)	20 ± 4.6 (3)	143

antiviral activity against poxviruses (Kern et al., 2002; Keith et al., 2004; Beadle et al., 2006), herpes group viruses (Beadle et al., 2002; Williams-Aziz et al., 2005; Wan et al., 2005). Thus, esterification of antiviral nucleoside phosphonates with alkoxyalkyl groups appears to be a generally applicable procedure for increasing antiviral activity and selectivity of this class of antiviral nucleosides.

The phosphonomethoxyethyl nucleosides are phosphorylated to their diphosphates in the cell and the diphosphates cause chain termination after incorporation into viral DNA. For HIV replication, the reasons for the increase in antiviral activity will require further study to establish the exact mechanisms involved. However, in general, acyclic nucleoside phosphonates contain an intrinsic phosphonate which carries a double negative charge. Based on what is known about CDV (HPMPC), compounds of this type do not readily enter cells because of their charge and molecular weight. They are taken up by fluid phase endocytosis, a slow process with limited capacity (Connelly et al., 1993). Their antiviral activity is probably underestimated in vitro because of limited cell penetration and slow anabolic phosphorylation.

When the cell penetration of HDP-[2-^{14}C]CDV and [2-^{14}C]CDV was compared, we found that uptake of HDP-CDV in MRC-5 cells was 11- to 23-fold greater than CDV and conversion to CDV-diphosphate was 100 times greater with HDP-CDV relative to CDV (Aldern et al., 2003). We hypothesize that the same general mechanism of action may be the explanation for the multiple log increases in anti-HIV activity observed with the alkoxyalkyl esters of acyclic nucleoside phosphonates in MT-2 cells infected with HIV-1. Further study of the cellular uptake and metabolism of these compounds and the effects of their diphosphates on the activity of HIV reverse transcriptase will be required to establish the mechanisms of increased activity in HIV infected cells.

HDP- and ODE-esters of CDV are orally bioavailable (Ciesla et al., 2003) and orally active as we have shown previously in lethal poxvirus infections (Quenelle et al., 2004; Buller et al., 2004) and in HCMV and MCMV infection in mice (Bidanset et al., 2004; Kern et al., 2004). We anticipate that the active alkoxyalkyl esters of this report will also be orally active and are worthy of further study as possible anti-HIV agents.

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