ANTIVIRAL ACTION OF ACYCLIC NUCLEOSIDE PHOSPHONATES: BASIC PRINCIPLES

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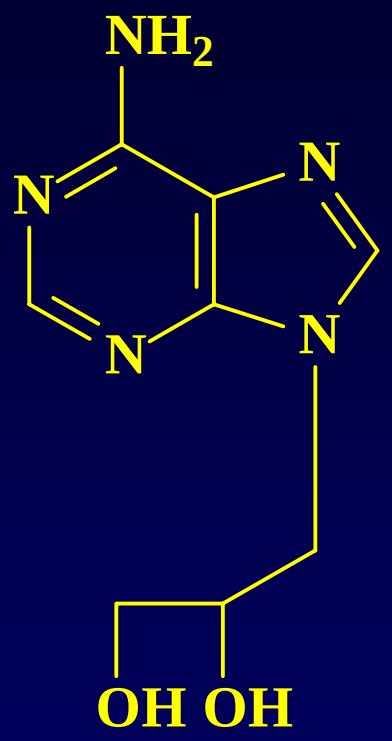
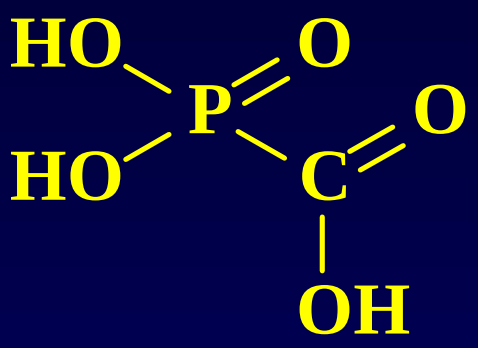
Symposium on Synthetic Nucleosides, Nucleotides and Polynucleotides, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany, 3-5 May 1976

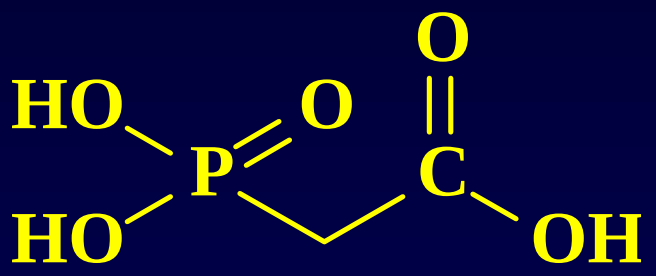


DHPA

De Clercq *et al*., Science 200, 563-565 (1978)

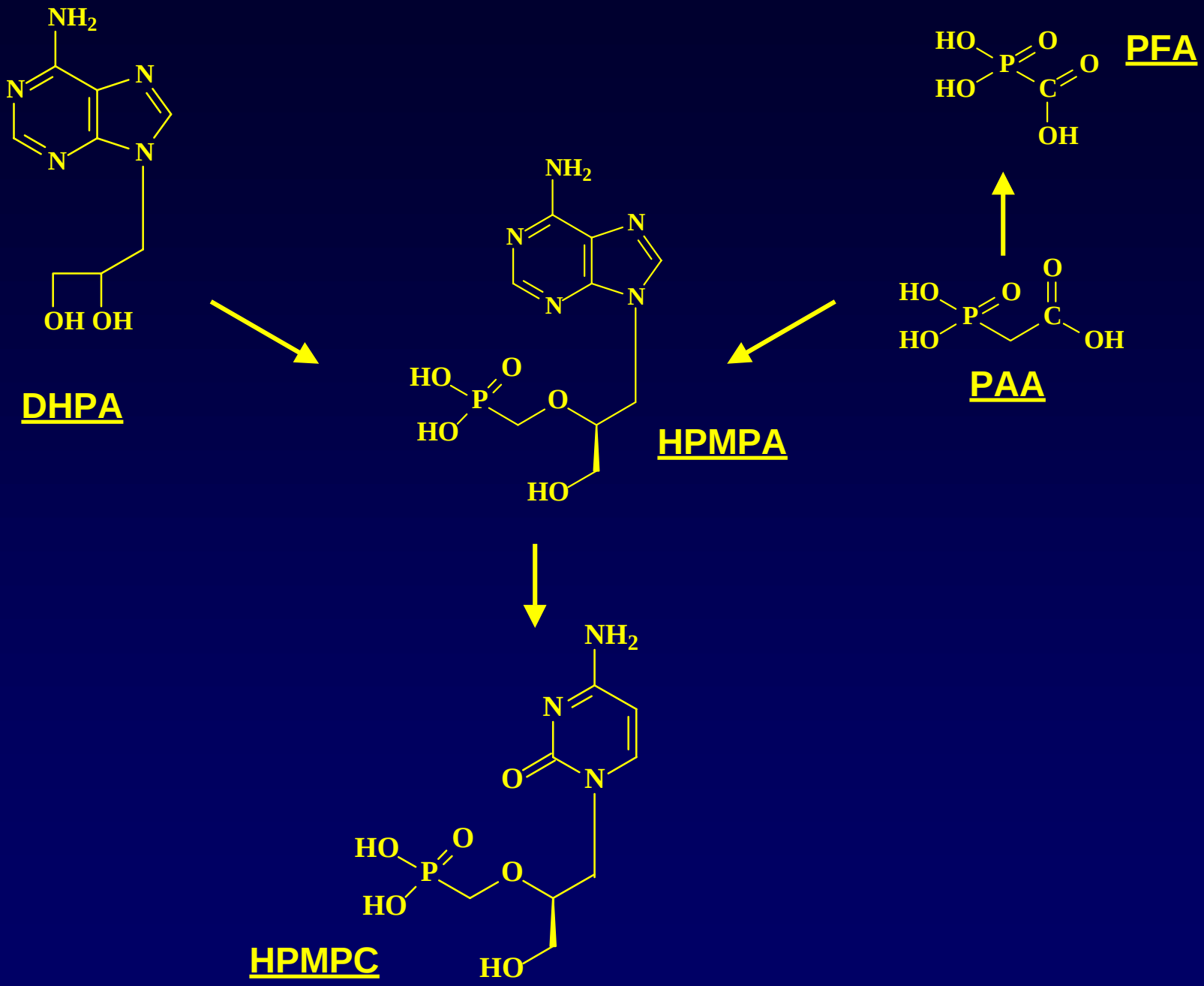


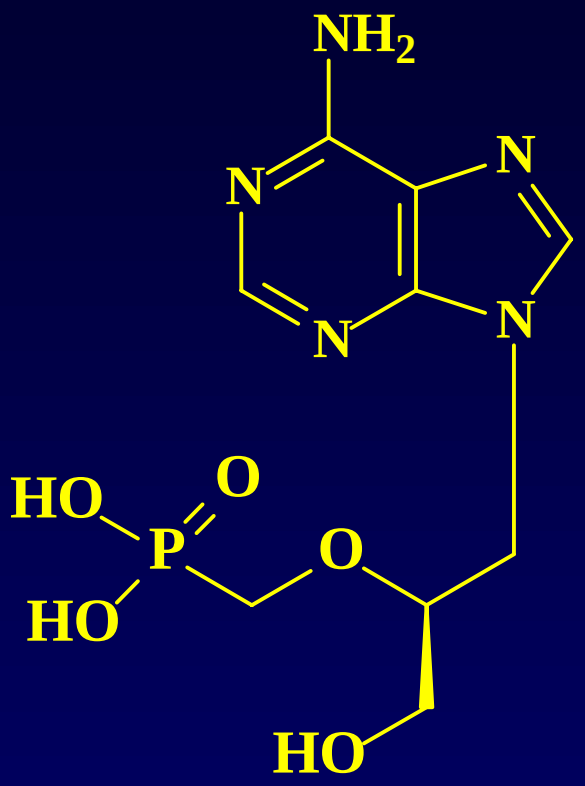
PFA



PAA

Öberg, Pharmacol. Ther. 19, 387-415 (1983)

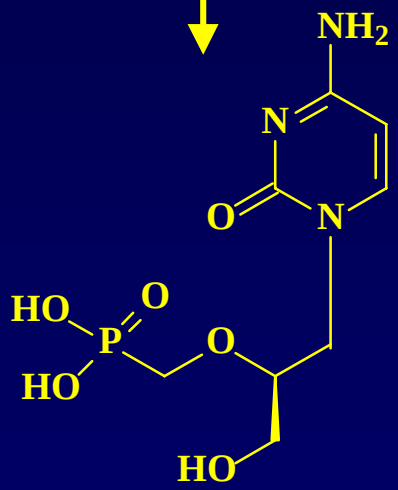

DHPA
HPMPA
PFA
PAA
HPMPC



HPMPA

De Clercq *et al*., Nature 323, 464-467 (1986)

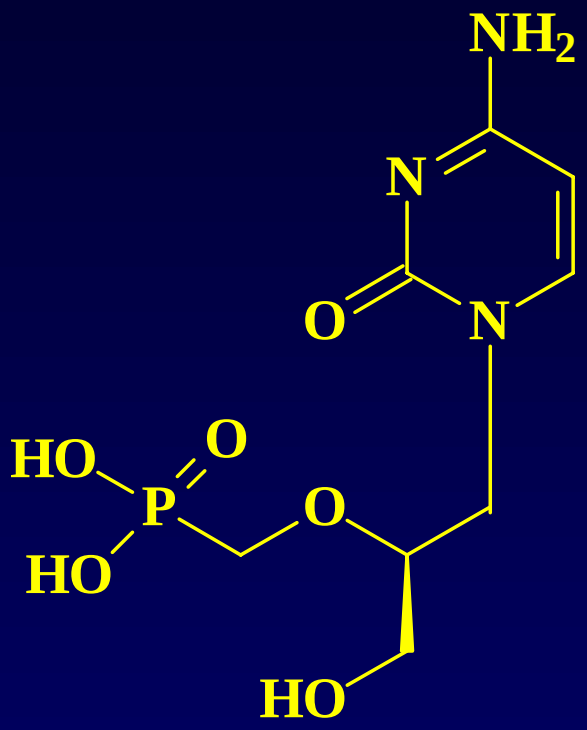
HPMPA



HPMPC

De Clercq *et al.*, Antiviral Res. 8, 261-272 (1987)

(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine



HPMPC
Cidofovir
Vistide®

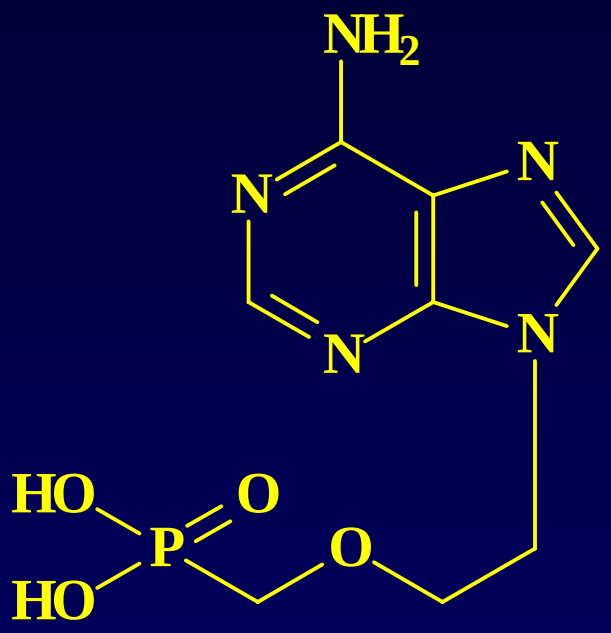
Antiviral activity of HPMPC first described in 1987

E. De Clercq, T. Sakuma, M. Baba, R. Pauwels, J. Balzarini, I. Rosenberg & A. Holý.
Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines.
Antiviral Res. 8, 261-272 (1987).

Licensed for clinical use for the treatment of CMV retinitis in AIDS patients in June 1996.

Launching of VISTIDE®
by Gilead Sciences on 27 June 1996

9-(2-phosphonylmethoxyethyl)adenine



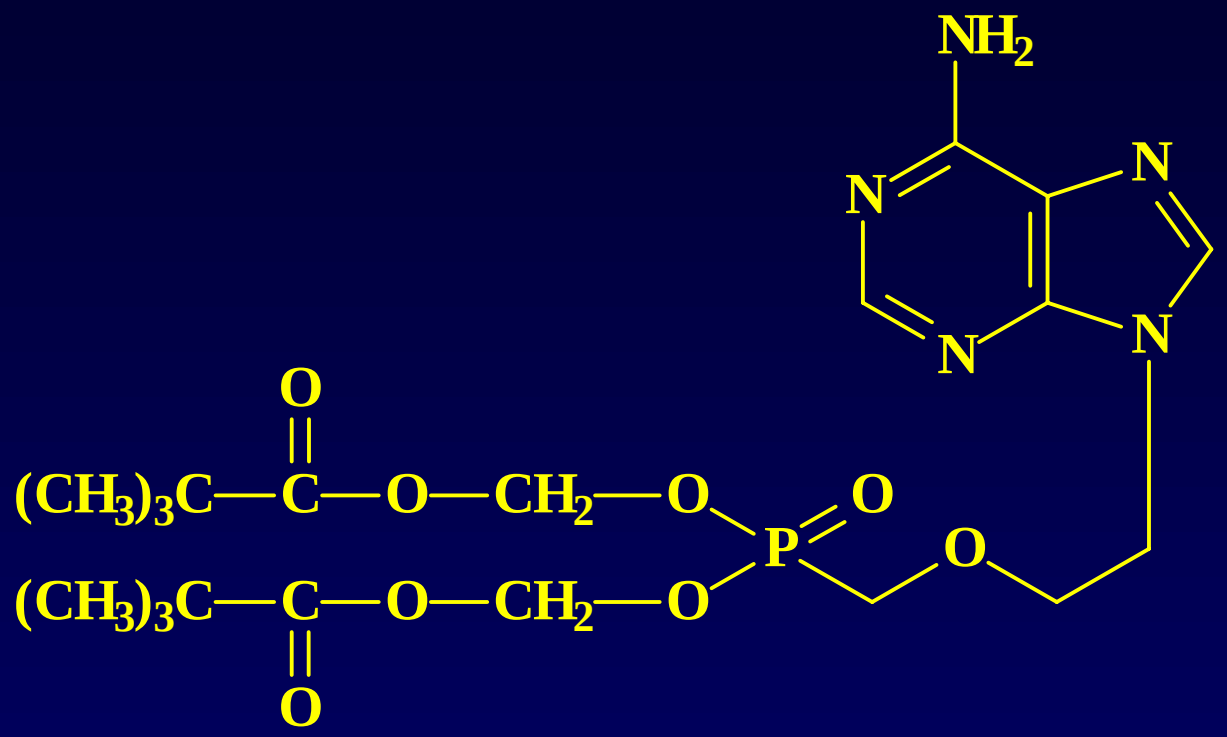
PMEA
Adefovir

De Clercq *et al*., Nature 323, 464-467 (1986)

Antiviral activity of PMEA first mentioned in 1986

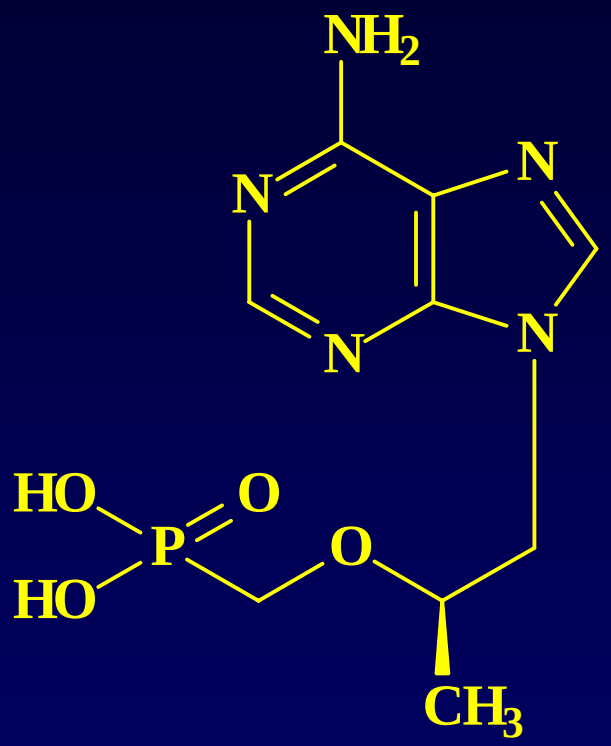
E. De Clercq, A. Holý, I. Rosenberg, T. Sakuma, J. Balzarini & P.C. Maudgal.
A novel selective broad-spectrum anti-DNA virus agent.
Nature 323, 464-467 (1986).

Licensed for clinical use for the treatment of HBV infections (chronic hepatitis B) in September 2002.



Bis(POM)PMEA
Adefovir dipivoxil
Hepsera®

(*R*)-9-(2-phosphonylmethoxypropyl)adenine



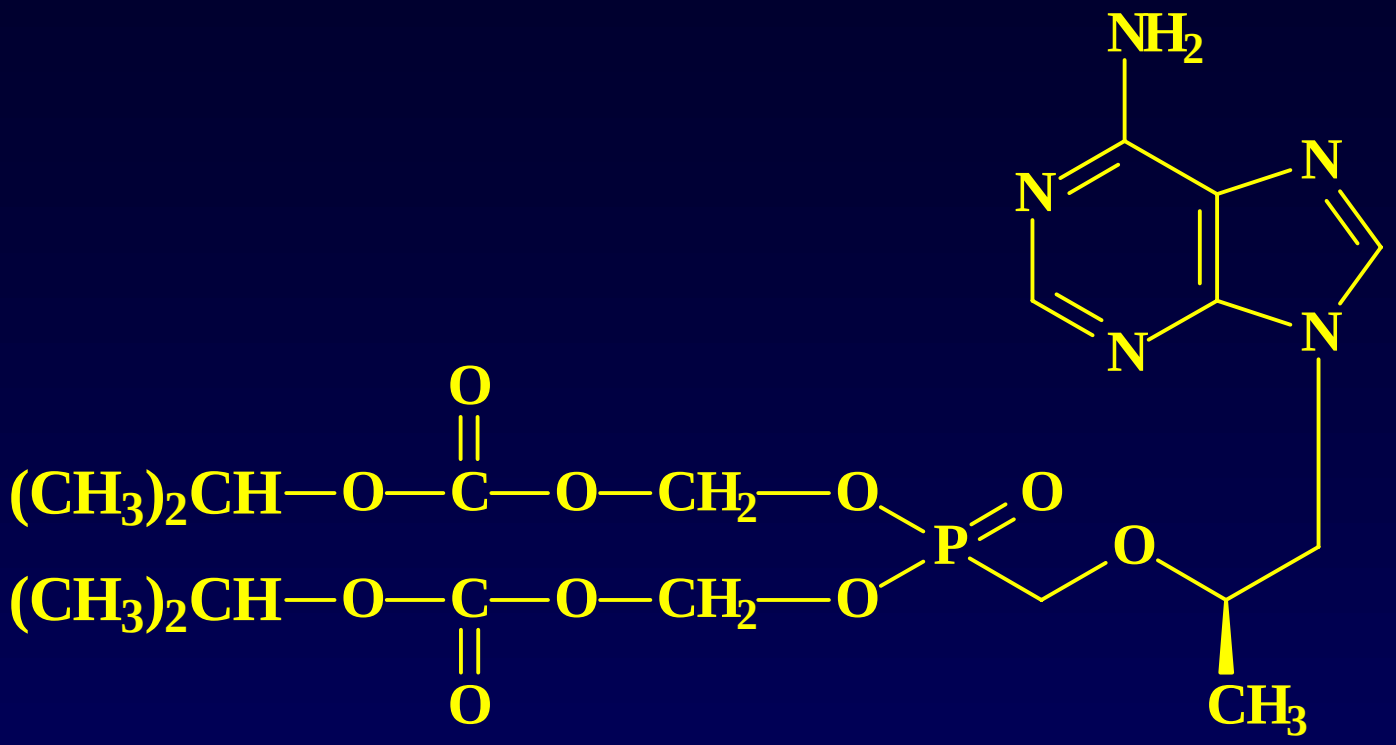
PMPA
Tenofovir

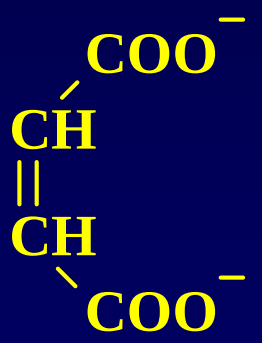
Balzarini *et al*., Antimicrob. Agents Chemother. 37, 332-338 (1993)

Antiviral activity of PMPA first described in 1993

J. Balzarini, A. Holý, J. Jindrich, L. Naesens, R. Snoeck, D. Schols & E. De Clercq.
Differential antiherpesvirus and antiretrovirus effects of the (*S*) and (*R*) enantiomers of acyclic nucleoside phosphonates: potent and selective *in vitro* and *in vivo* antiretrovirus activities of (*R*)-9-(2-phosphono-methoxypropyl)-2,6-diaminopurine.
Antimicrob. Agents Chemother. 37, 332-338 (1993).

Licensed for clinical use for the treatment of HIV infections (AIDS) in October 2001





bis(POC)-PMPA
Tenofovir disoproxil
Tenofovir disoproxil fumarate: Viread™

fumarate

Milestones (keynote papers) in the development of cidofovir

R. Snoeck, T. Sakuma, E. De Clercq, I. Rosenberg & A. Holý.
(*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine [(*S*)-HPMPC]: a potent and selective inhibitor of human cytomegalovirus replication.
Antimicrob. Agents Chemother. 32, 1839-1844 (1988).

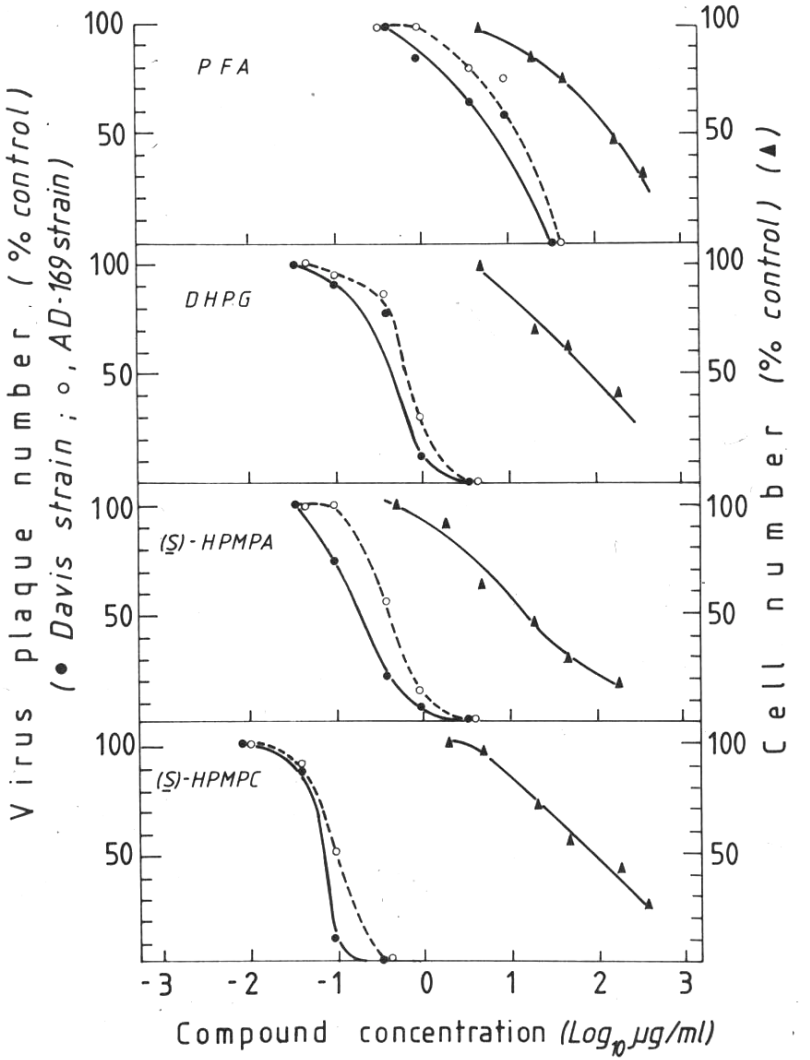
J. Neyts, R. Snoeck, D. Schols, J. Balzarini & E. De Clercq.
Selective inhibition of human cytomegalovirus DNA synthesis by (*S*)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine [(*S*)-HPMPC] and 9-(1,3-dihydroxy-2-propoxymethyl)-guanine (DHPG).
Virology 179, 41-50 (1990).

E. De Clercq & A. Holý.
Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine in various models of herpes simplex virus infection in mice.
Antimicrob. Agents Chemother. 35, 701-706 (1991).

J. Neyts, J. Balzarini, L. Naesens & E. De Clercq.
Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine and 9-(1,3-dihydroxy-2-propoxy-methyl)guanine for the treatment of murine cytomegalovirus infection in severe combined immunodeficiency mice.
J. Med. Virol. 37, 67-71 (1992).

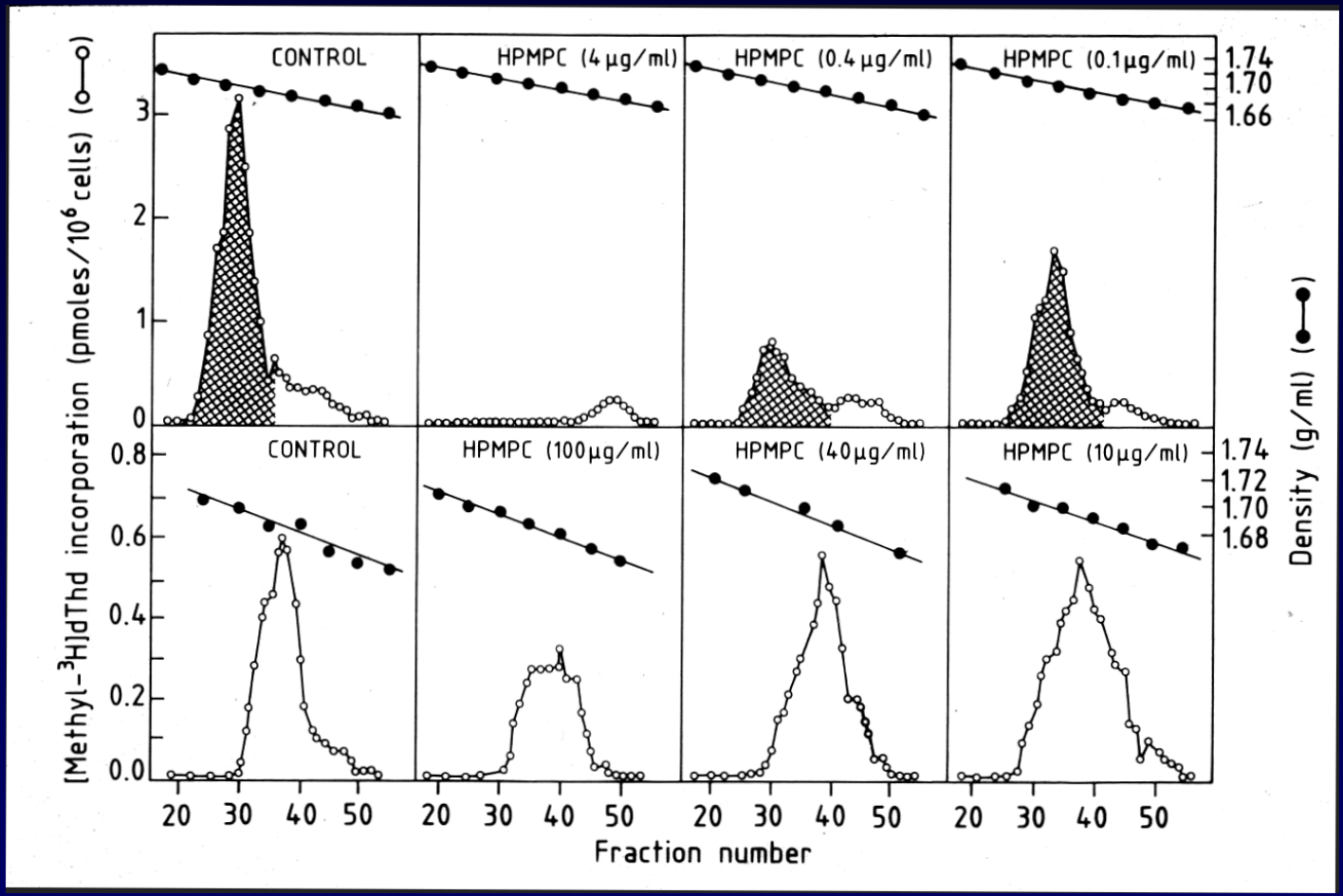
J. Neyts & E. De Clercq.
Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine for the treatment of lethal Vaccinia virus infections in severe combined immune deficiency (SCID) mice.
J. Med. Virol. 41, 242-246 (1993).

Dose-response curves for anti-CMV activity and cytotoxicity of PFA, DHPG, (*S*)-HPMPA and (*S*)-HPMPC in HEL cells



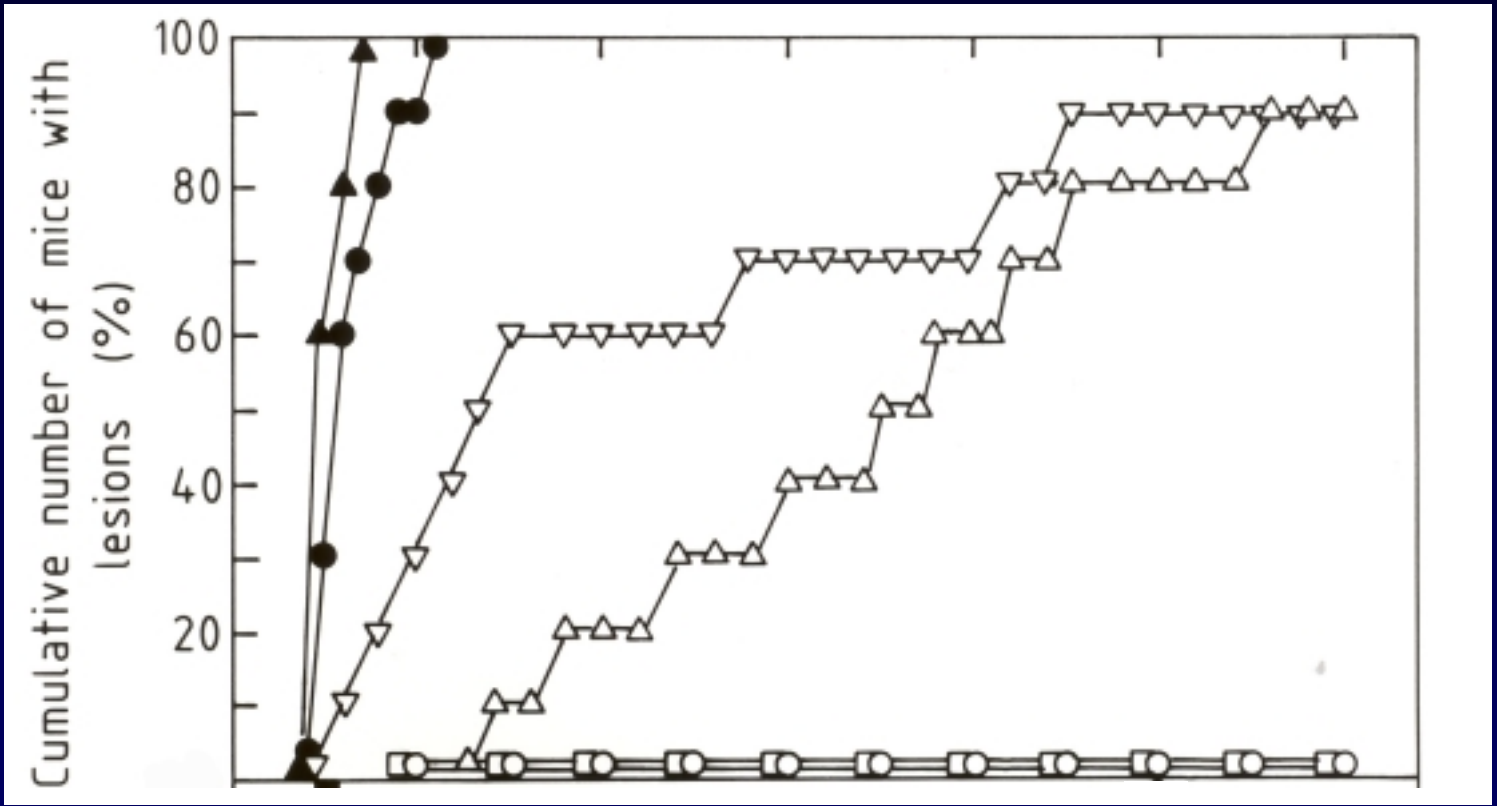
Snoeck *et al*., Antimicrob. Agents Chemother. 32, 1839-1844 (1988)

CsCl equilibrium gradient analysis of DNA from CMV- and mock-infected HEL cells exposed for 96 hours to varying concentrations of cidofovir (HPMPC)



Neyts *et al.*, Virology 179, 41-50 (1990)

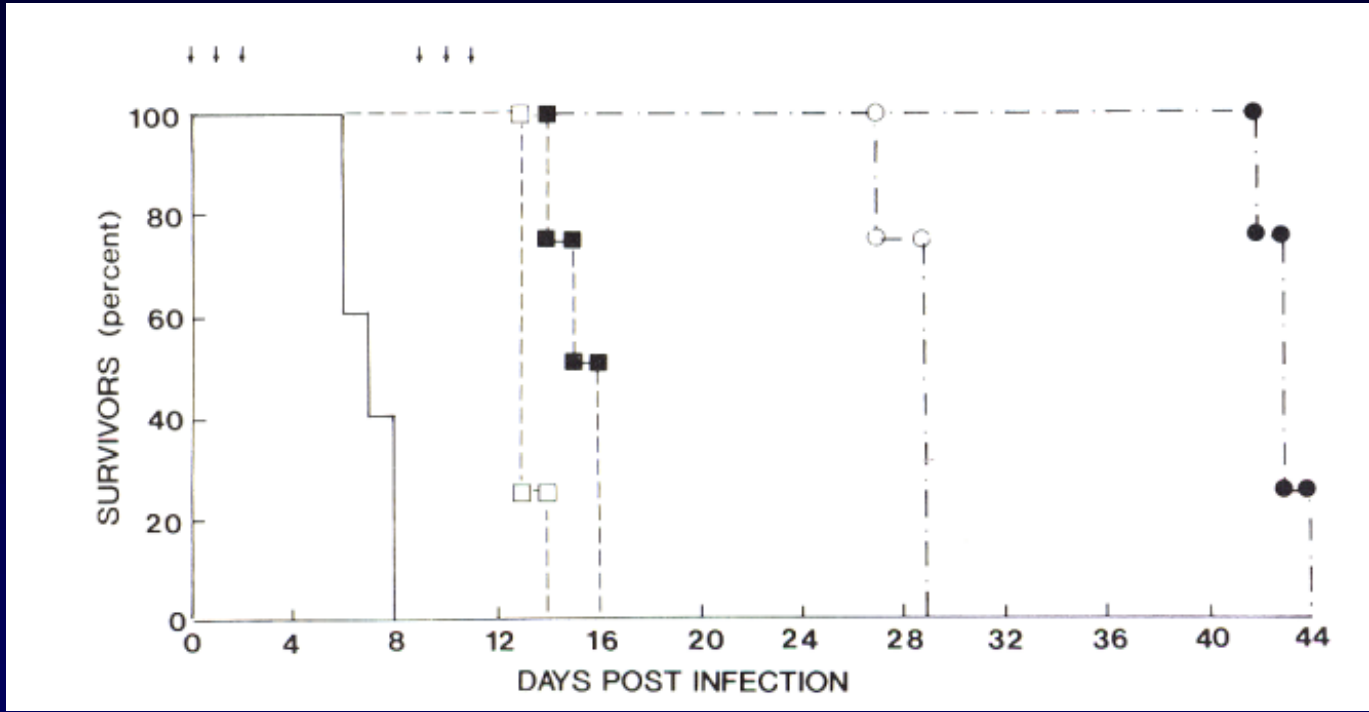
Inhibitory effects of HPMPC or ACV administration on the development of skin lesions and/or paralysis of the hind legs



The compounds were administered i.p. twice a day for 5 days starting 1 h after virus infection at the following doses: 0 mg/kg/day (control (●), HPMPC at 20 mg/kg/day (σ), HPMPC at 50 mg/kg/day (ρ), HPMPC at 100 mg/kg/day (O), HPMPC at 250 mg/kg/day (≤), and ACV at 100 mg/kg/day (π). There were 10 mice per group.

De Clercq & Holý, Antimicrob. Agents Chemother. 35, 701-706 (1991)

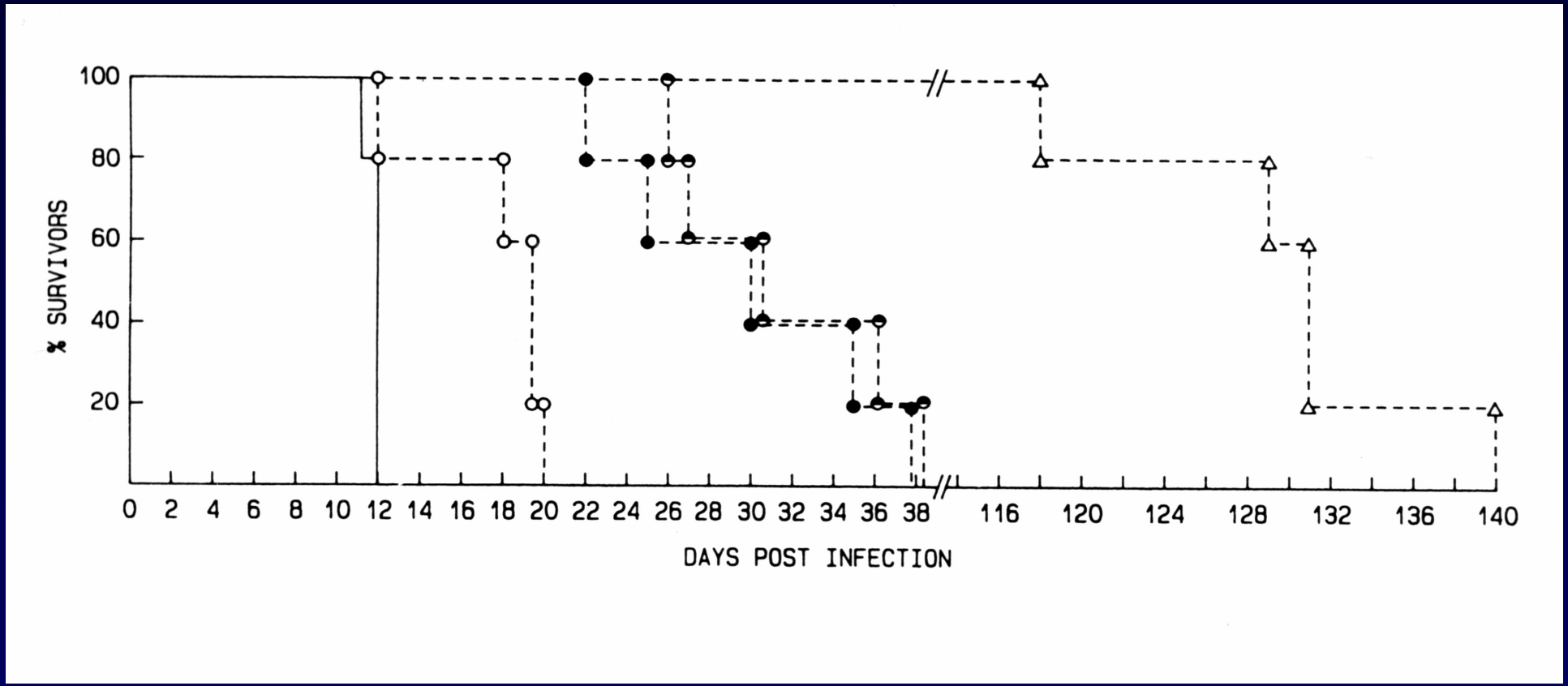
Survival of SCID mice infected i.p. with MCMV and treated s.c. with DHPG or HPMPC



For first set of mice, treatment was initiated 2 hours after infection and was continued for the next 2 days; the second set of mice was treated again during a second period (days 9, 10, and 11 after infection, as indicated by the arrows). Symbols: (—) untreated controls (n = 5); (---) DHPG-treated mice; (– –) HPMPC-treated mice; (□) DHPG at 20 mg/kg/day at days 0, 1 and 2 (n = 4); (■) DHPG at 20 mg/kg/day at days 0, 1, 2, 9, 10, and 11 (n = 4); (O) HPMPC at 20 mg/kg/day at days 0, 1, and 2 (n = 4); ( ) HPMPC at 20 mg/kg/day at days 0, 1, 2, 9, 10 and 11 (n = 4).

Neyts *et al*., J. Med. Virol. 37, 67-71 (1992)

Survival of SCID mice infected i.v. with VV and treated s.c. with cidofovir



Treatment was initiated at 2 h after infection and was either continued for the next 4 days or repeated on day 4 p.i. and then twice every week (on day 1 and 4 of each week). Symbols: untreated controls (-) (n=5); treated at 1 mg/kg/day for 5 days (O) (n=5); at 5 mg/kg/day for 5 days (●) (n=5); at 20 mg/kg/day for 5 days (⦿) (n=5); or at 20 mg/kg/twice a week for up to 20 weeks (Δ) (n=5).

Neyts & De Clercq, J. Med. Virol. 41, 242-246 (1993)

Milestones (keynote papers) in the development of adefovir

R. Pauwels, J. Balzarini, D. Schols, M. Baba, J. Desmyter, I. Rosenberg, A. Holý & E. De Clercq.
Phosphonylmethoxyethyl purine derivatives: a new class of anti-human immunodeficiency virus agents.
Antimicrob. Agents Chemother. 32, 1025-1030 (1988).

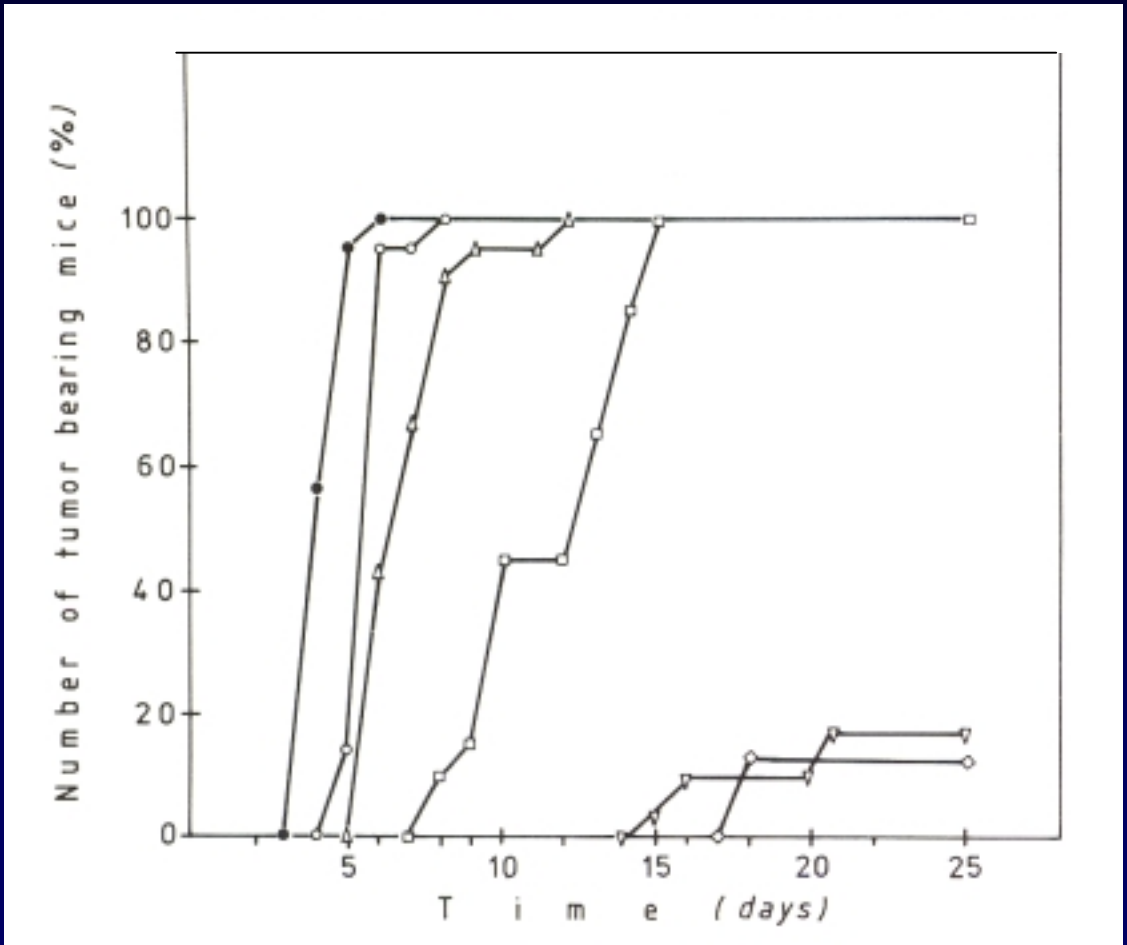
J. Balzarini, L. Naesens, P. Herdewijn, I. Rosenberg, A. Holý, R. Pauwels, M. Baba, D.G. Johns & E. De Clercq.
Marked *in vivo* antiretrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine, a selective anti-human immunodeficiency virus agents.
Proc. Natl. Acad. Sci. USA 86, 332-336 (1989).

J. Balzarini, Z. Hao, P. Herdewijn, D.G. Johns & E. De Clercq.
Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)-adenine, a potent anti-human immunodeficiency virus compound.
Proc. Natl. Acad. Sci. USA 88, 1499-1503 (1991).

T. Yokota, S. Mochizuki, K. Konno, S. Mori, S. Shigeta and E. De Clercq.
Inhibitory effects of selected antiviral compounds on human hepatitis B virus DNA synthesis.
Antimicrob. Agents Chemother. 35, 394-397 (1991).

L. Naesens, J. Balzarini, N. Bischofberger & E. De Clercq.
Antiretroviral activity and pharmacokinetics in mice of oral bis(pivaloylmethyl)-9-(2-phosphonylmethoxy-ethyl)adenine, the bis(pivaloyloxymethyl) ester prodrug of 9-(2-phosphonylmethoxyethyl)adenine.
Antimicrob. Agents Chemother. 40, 22-28 (1996).

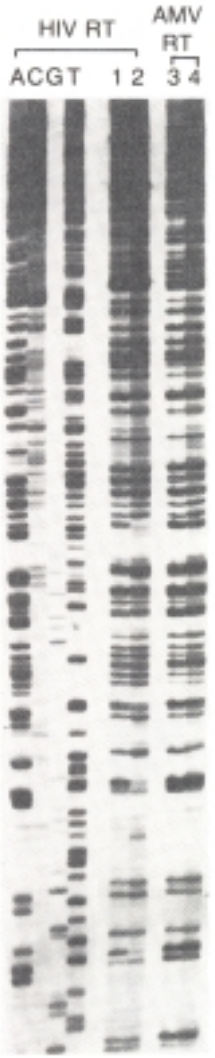
Tumor formation of NMRI mice inoculated with Mo-MSV after treatment with indicated doses of PMEA



PMEA (in a 25-µl vol) was injected i.p. at doses of either 1 (O), 2.5 (ρ), 5 (□), 20 (σ) or 50 (↓) mg/kg per day starting on the day of infection and continuing for an additional 9 days. Control mice received vehicle only ( ).

Balzarini *et al*., Proc. Natl. Acad. Sci. USA 86, 332-336 (1989)

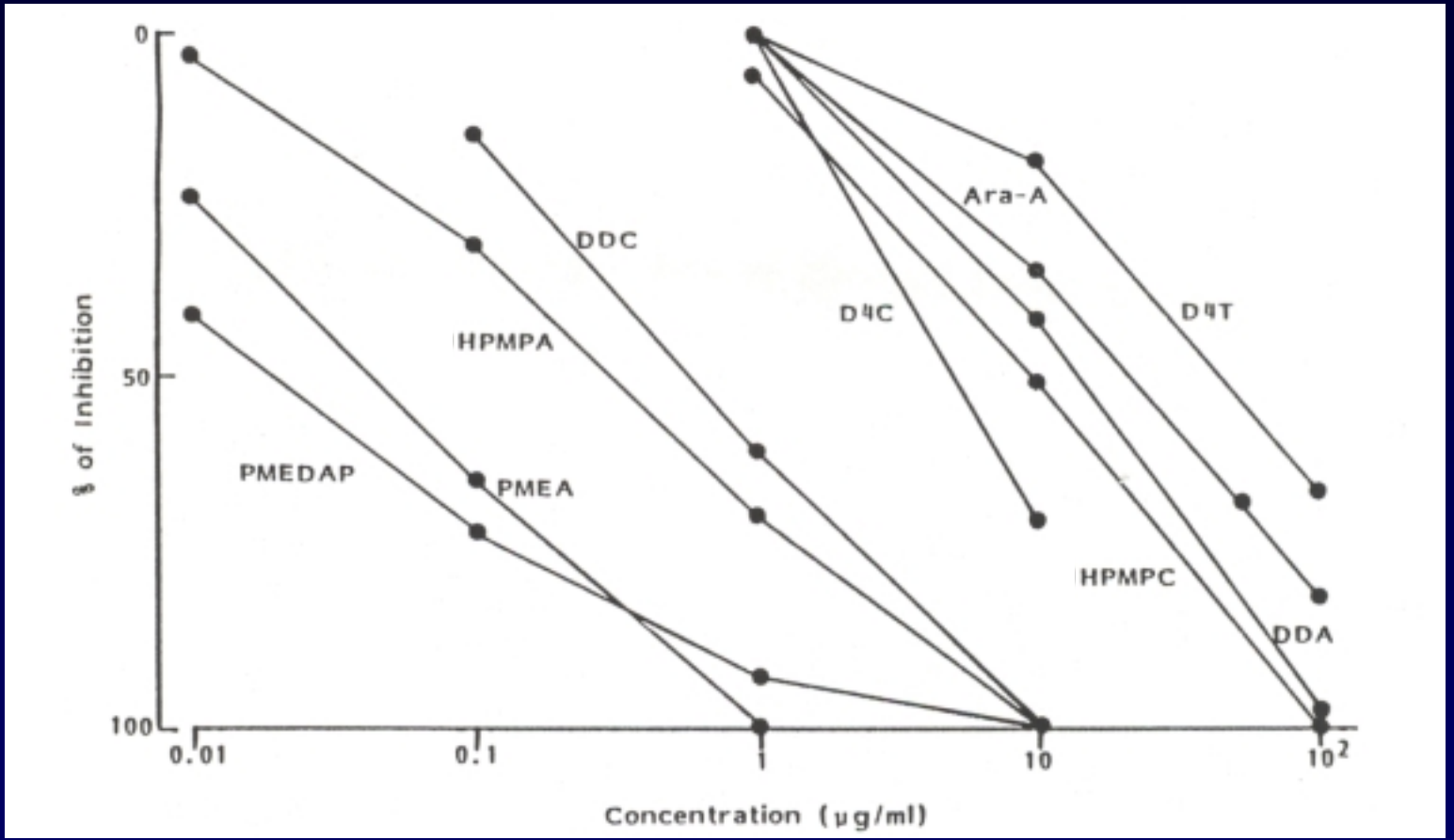
Sanger sequencing reaction with HIV-1 or AMV RT



Lanes A, C, G and T: DNA chain termination by ddATP, ddCTP, ddGTP and ddTTP, respectively.
Lanes 1 and 3: DNA chain termination by ddATP for HIV-1 RT and AMV RT, respectively.
Lanes 2 and 4: DNA chain termination by PMEApp for HIV-1 RT and AMV RT, respectively.

Balzarini *et al*., Proc. Natl. Acad. Sci. USA 88, 1499-1503 (1991)

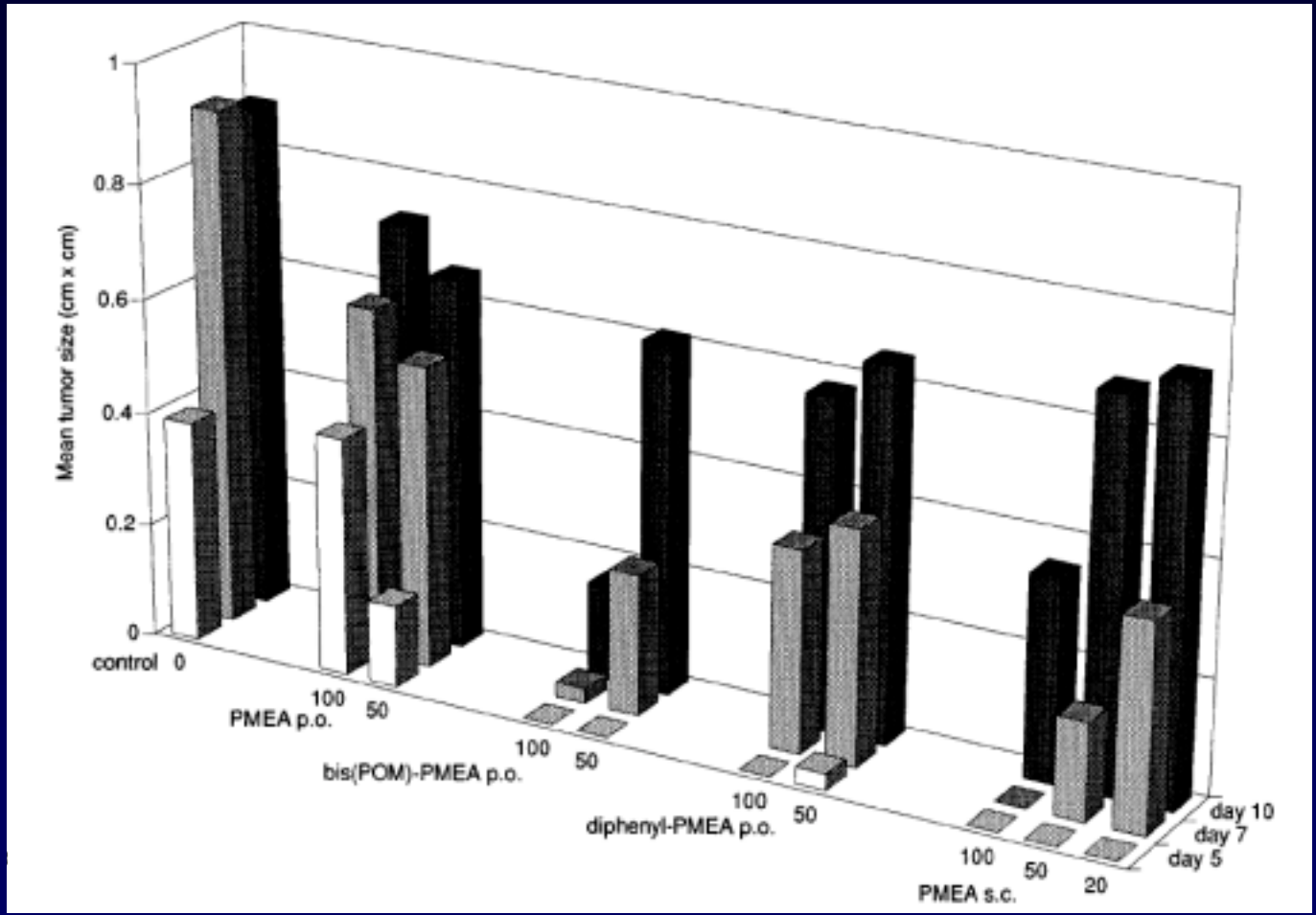
Inhibitory effect of antiviral compounds on HBV DNA synthesis in HB611 cells



^{32}P-labeled HBV DNA determined by Southern blot analysis.

Yokota *et al*., Antimicrob. Agents Chemother. 35, 394-397 (1991)

Inhibitory effects of bis(POM)-PMEA, diphenyl-PMEA, and PMEA on MSV-induced tumor development in SCID mice



Compounds were administered twice daily on days 0 through 4 at the indicated doses [p.o. (oral administration) or s.c. (subcutaneous administration)]. On days 5, 7, and 10 post infection, the tumors were measured with calipers.

Naesens *et al*., Antimicrob. Agents Chemother. 40, 22-28 (1996)

Milestones (keynote papers) in the development of tenofovir

C.C. Tsai, K.E. Follis, A. Sabo, T.W. Beck, R.F. Grant, N. Bischofberger, R.E. Benveniste & R. Black.
Prevention of SIV infection in macaques by (*R*)-9-(2-phosphonylmethoxypropyl)adenine.
Science 270, 1197-1199 (1995).

R.A. Otten, D.K. Smith, D.R. Adams, J.K. Pullium, E. Jackson, C.N. Kim, H. Jaffe, R. Janssen, S. Butera & T.M. Folks.
Efficacy of postexposure prophylaxis after intravaginal exposure of pig-tailed macaques to a human-derived retrovirus (human immunodeficiency virus type 2).
J. Virol. 74, 9771-9775 (2000).

K.K. Van Rompay, M.B. McChesney, N.L. Aguirre, K.A. Schmidt, N. Bischofberger & M.L. Marthas.
Two low doses of tenofovir protect newborn macaques against oral simian immuno-deficiency virus infection.
J. Infect. Dis. 184, 429-438 (2001).

Prophylaxis of parenteral HIV infection

Tenofovir protects macaques against intravenous SIV infection.

Juvenile, long-tailed macaques were inoculated intravenously with SIV_{mne}.

Tenofovir was administered subcutaneously once daily beginning either 48 hours before, 4 hours after or 24 hours after virus inoculation. Treatment continued for 4 weeks.

Tenofovir prevented SIV infection in all macaques without toxicity, whereas all control macaques became infected.

Tsai *et al*., Science 270, 1197-1199 (1995)

Prophylaxis of intravaginal HIV infection

Tenofovir was investigated in the postexposure prophylaxis (PEP) of intravaginal infection of pig-tailed macaques with HIV-2.

Tenofovir was administered subcutaneously at 30 mg/kg for 28 days starting 12, 36 or 72 h after viral inoculation.

Early intervention (i.e. treatment started at either 12 or 36 hours after viral inoculation) prevented HIV infection via vaginal exposure.

Otten *et al*., J. Virol. 74, 9771-9775 (2000)

Prophylaxis of perinatal HIV infection

Tenofovir protects newborn macaques against oral SIV infection.

By the age of 3 days, newborn macaques were inoculated orally with virulent SIVmac251.

Tenofovir was administered subcutaneously at 2 doses of 4 mg/kg either 4 h before or 20 h after, or 1 and 25 h after, or as a single dose of 30 mg/kg at 1 h after SIV inoculation.

The animals remained SIV negative and seronegative.

Van Rompay *et al*., J. Infect. Dis. 184, 429-438 (2001)

Antiviral activity spectrum of cidofovir

- Papovaviridae
  - Polyomaviridae
  - Papillomaviridae
- Adenoviridae
- Herpesviridae
- Poxviridae
- Iridoviridae

Antiviral activity spectrum of cidofovir

- Papovaviridae
  - Polyomaviridae
    - Murine polyomavirus
    - Human polyomavirus
  - Papillomaviridae
    - Rabbit papillomavirus
    - Human papillomaviruses (several types)
- Adenoviridae
  - Human adenoviruses (several types)

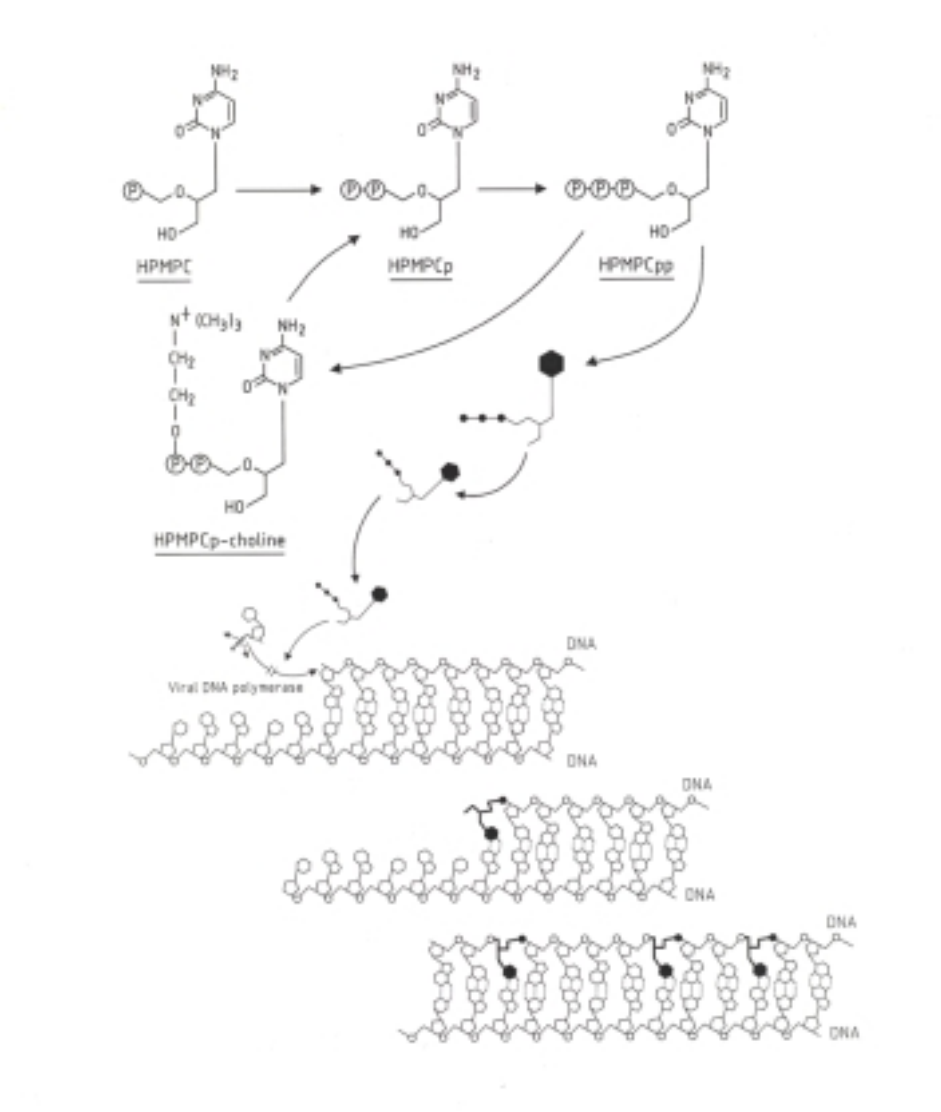
Antiviral activity spectrum of cidofovir (continued)

- **Herpesviridae**
  - HSV-1 (TK^+ and TK^-)
  - HSV-2 (TK^+ and TK^-)
  - VZV (TK^+ and TK^-)
  - EBV
  - HCMV (PK^+ and PK^-)
  - HHV-6
  - HHV-7
  - HHV-8

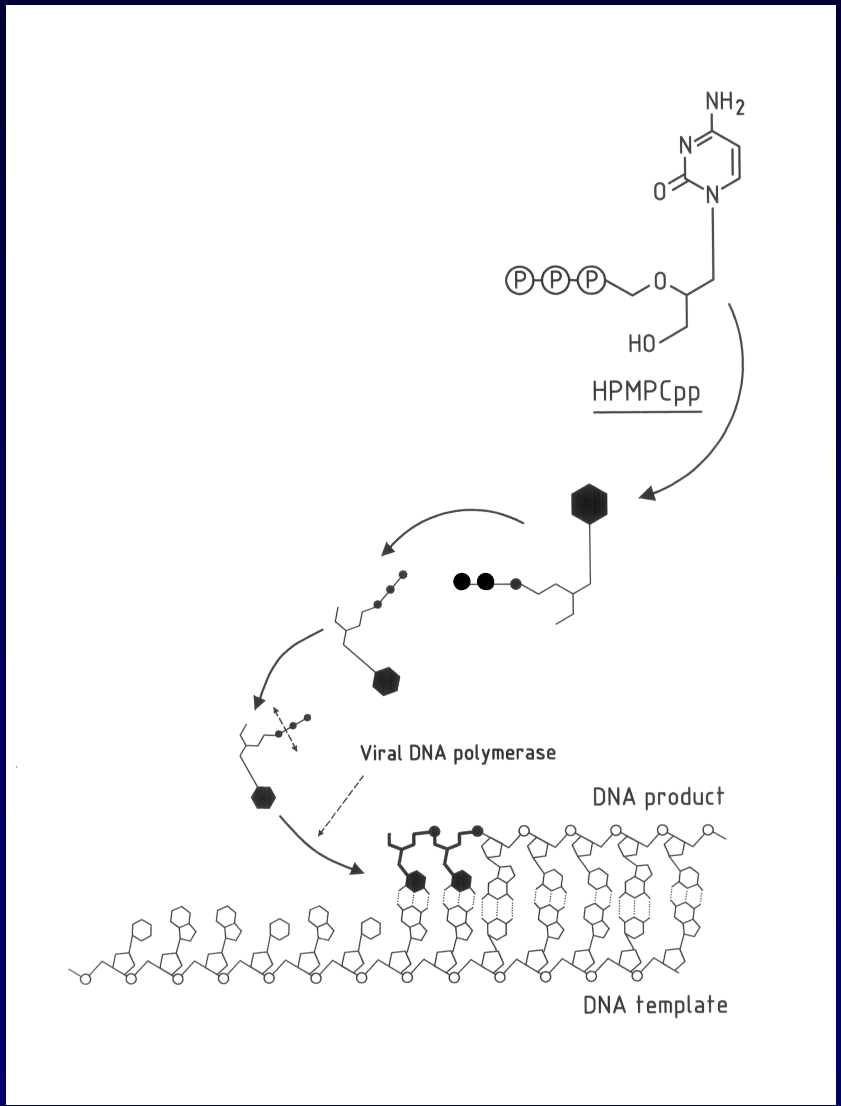
Antiviral activity spectrum of cidofovir (continued)

- **Poxviridae**
  - Vaccinia virus
  - Variola virus
  - Cowpox virus
  - Monkeypox virus
  - Camelpox virus
  - Molluscum contagiosum virus
  - Orf virus
- **Iridoviridae**
  - African swine fever virus

Cidofovir: mechanism of antiviral action (HCMV)



Cidofovir: mechanism of action (HCMV)



HPMPC HDP
(HDP: 1-*O*-hexadecyloxypropyl)

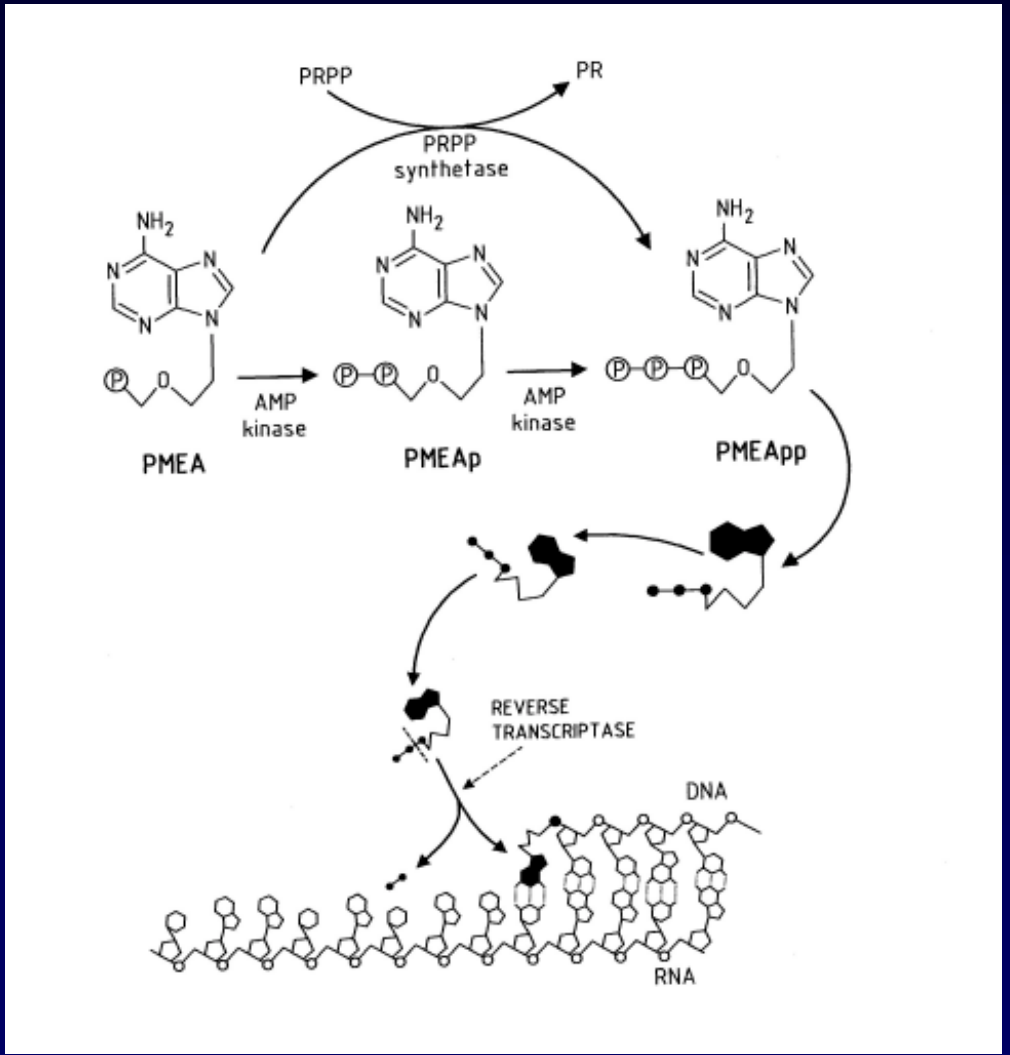
Antiviral activity spectrum of PMEA (Adefovir)

Herpesviridae	
Herpes simplex virus type 1 (HSV-1)	●
Herpes simplex virus type 2 (HSV-2)	●
Varicella-zoster virus (VZV)	●
Epstein-Barr virus (EBV)	●
Human cytomegalovirus (HCMV)	●
Thymidine kinase-deficient HSV (TK^- HSV)	●
Thymidine kinase-deficient VZV (TK^- VZV)	●
Hepadnaviridae	
Human hepatitis B virus (HHBV)	●
Duck hepatitis B virus (DHBV)	●

Antiviral activity spectrum of PMEA (Adefovir)
(continued)

Retroviridae	
Human immunodeficiency virus type 1 (HIV-1)	●
Human immunodeficiency virus type 2 (HIV-2)	●
Simian immunodeficiency virus (SIV)	●
Feline immunodeficiency virus (FIV)	●
Visna/maedi virus	●
Feline leukemia virus	●
LP-BM5 (murine AIDS) virus	●
Moloney (murine) sarcoma virus	●

Mechanism of action of adefovir (PMEA)



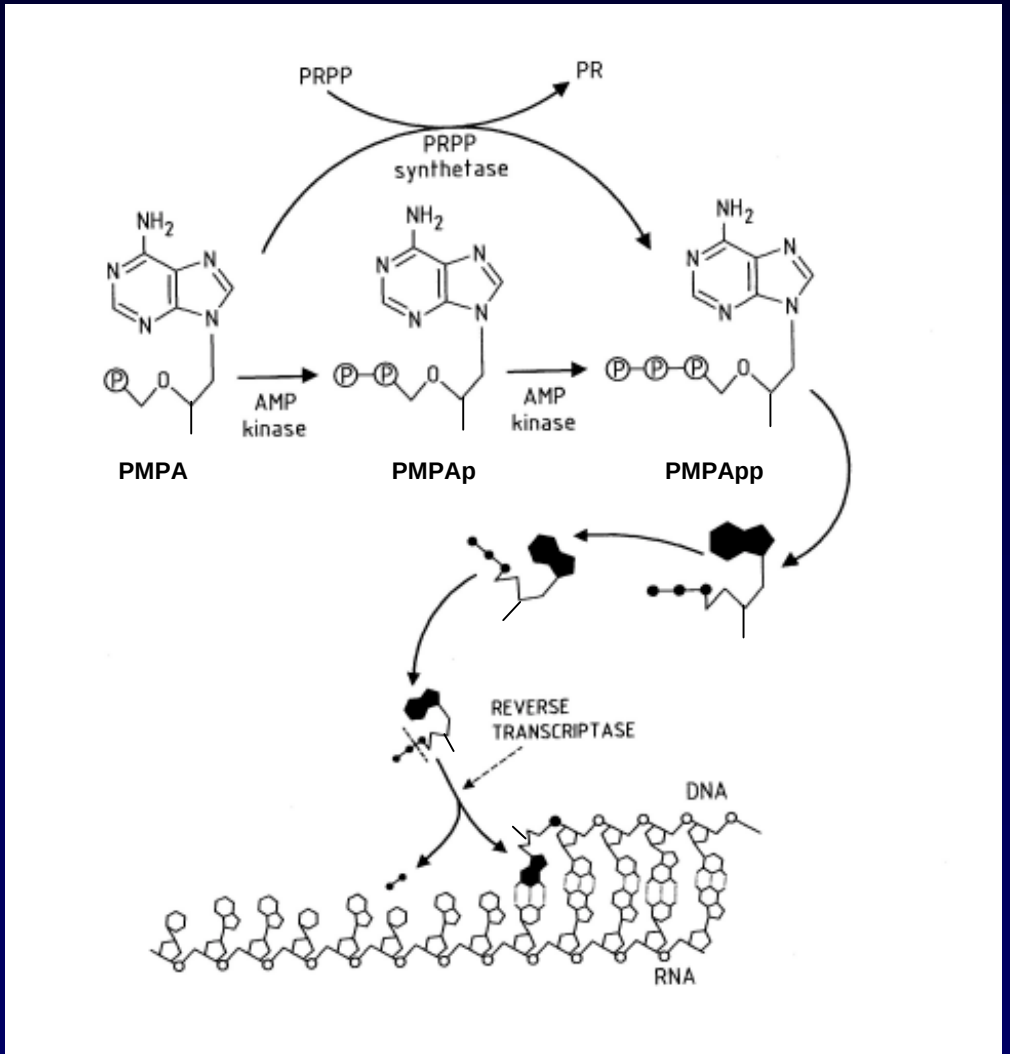
Antiviral activity spectrum of PMPA (Tenofovir)

Herpesviridae	
Herpes simplex virus type 1 (HSV-1)	o
Herpes simplex virus type 2 (HSV-2)	o
Varicella-zoster virus (VZV)	o
Epstein-Barr virus (EBV)	
Human cytomegalovirus (HCMV)	o
Thymidine kinase-deficient HSV (TK^- HSV)	o
Thymidine kinase-deficient VZV (TK^- VZV)	o
Hepadnaviridae	
Human hepatitis B virus (HHBV)	●
Duck hepatitis B virus (DHBV)	●

Antiviral activity spectrum of PMPA (Tenofovir)
(continued)

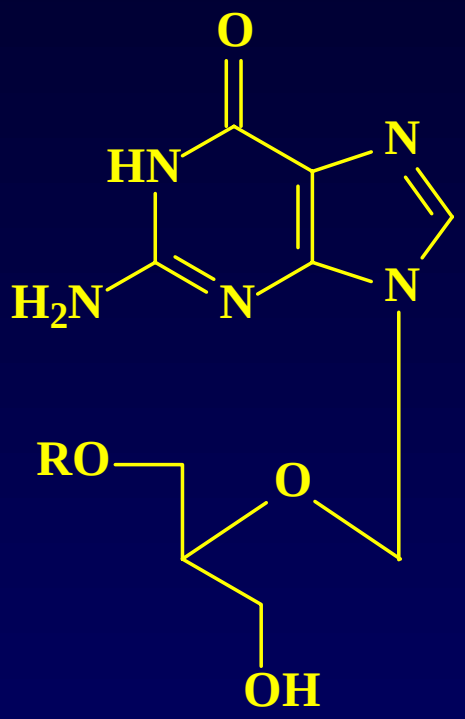
Retroviridae	
Human immunodeficiency virus type 1 (HIV-1)	●
Human immunodeficiency virus type 2 (HIV-2)	●
Simian immunodeficiency virus (SIV)	●
Feline immunodeficiency virus (FIV)	●
Visna/maedi virus	●
Feline leukemia virus	●
LP-BM5 (murine AIDS) virus	●
Moloney (murine) sarcoma virus	●

Mechanism of action of tenofovir (PMPA)

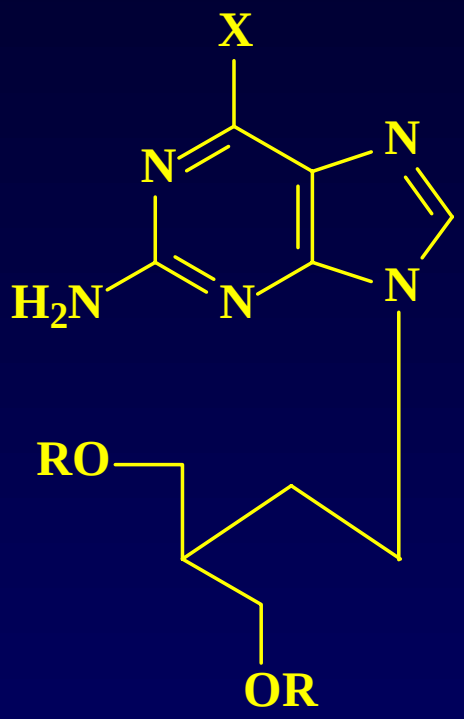


Acyclic nucleoside analogues

R = H: Acyclovir
R = H_2N-CH-CO-: Valaciclovir
$CH(CH_3)_2$

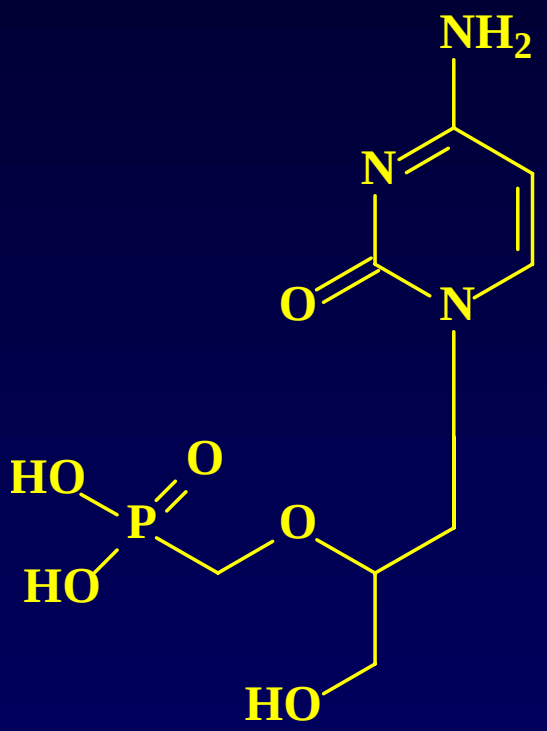


R = H: Ganciclovir
R = H_2N-CH-CO-: Valganciclovir
$CH(CH_3)_2$

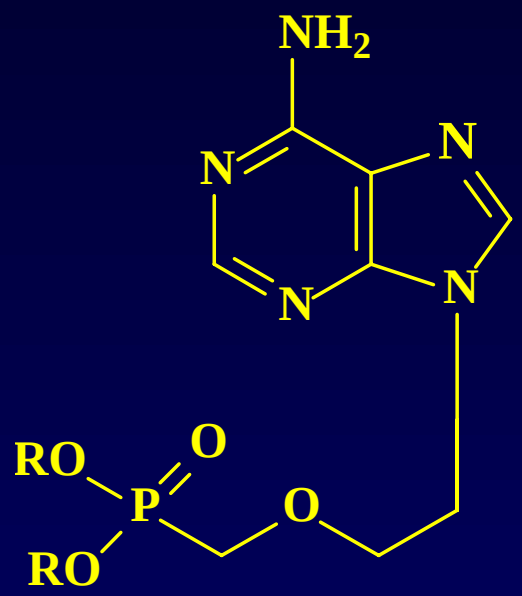


R = H, X = OH: Penciclovir
R = CH_3CO, X = H: Famciclovir

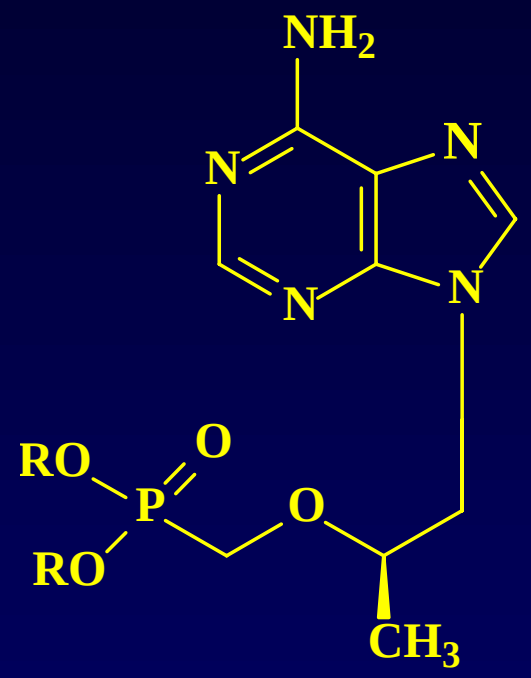
Acyclic nucleotide analogues
Acyclic nucleoside phosphonates



Cidofovir
(HPMPC)

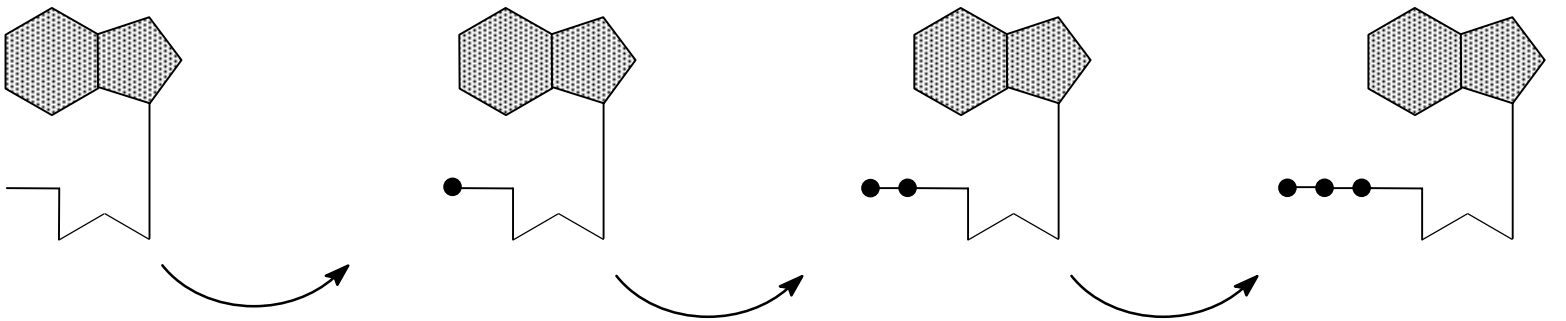


R = H: Adefovir (PMEA)
R = $(CH_3)_3C$-CO-O-CH_2-:
Adefovir dipivoxil
Bis(POM)-PMEA



R = H: Tenofovir (PMPA)
R = $(CH_3)_2CH$-O-CO-O-CH_2-:
Tenofovir disoproxil
Bis(POC)-PMPA

Acyclic nucleoside analogues:



Acyclic nucleotide analogues: Acyclic nucleoside phosphonates: