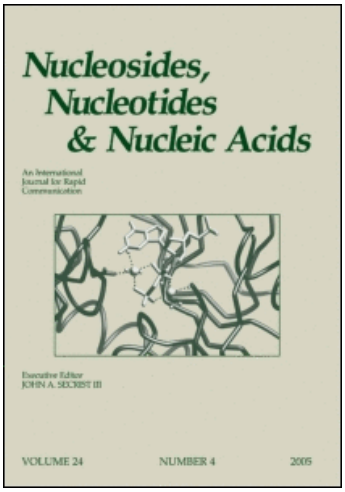

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Antiviral Activity Spectrum and Target of Action of Different Classes of Nucleoside Analogues

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To cite this Article De Clercq, Erik(1994) 'Antiviral Activity Spectrum and Target of Action of Different Classes of Nucleoside Analogues', Nucleosides, Nucleotides and Nucleic Acids, 13: 6, 1271 — 1295
To link to this Article: DOI: 10.1080/15257779408012151
URL: http://dx.doi.org/10.1080/15257779408012151

NUCLEOSIDES & NUCLEOTIDES, 13(6&7), 1271-1295 (1994)

ANTIVIRAL ACTIVITY SPECTRUM AND TARGET OF ACTION OF DIFFERENT CLASSES OF NUCLEOSIDE ANALOGUES

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INTRODUCTION

Depending on the target enzyme with which they interact, nucleoside analogues exhibit marked differences in their antiviral activity spectrum. The activity spectrum of those nucleoside analogues that interact with cellular enzymes involved in the biosynthesis of the RNA and DNA precursor nucleotides encompasses virtually all RNA and DNA viruses. In contrast, the activity spectrum of those nucleoside analogues that are targeted at a specific viral enzyme will be limited to those specific virus types whose enzyme(s) they interact with. For a given compound, the antiviral activity spectrum can often be predicted from identification of the target enzyme, and, *vice versa*, the mode of action can be deduced from the activity spectrum.[1] This concordance between antiviral activity spectrum and mode (target) of antiviral action will be scrutinized for the following categories of nucleoside analogues: AICAR analogues (i.e. ribavirin) that are targeted at IMP dehydrogenase; carbocyclic adenosine analogues (i.e. neplanocin A) that are targeted at AdoHcy hydrolase; OMP decarboxylase inhibitors such as pyrazofurin; CTP synthetase inhibitors such as cyclopentenylcytosine; acyclic guanosine analogues (i.e. acyclovir, ganciclovir) which act as viral DNA chain terminators following initial phosphorylation by the virus-encoded thymidine kinase (TK) or protein kinase; thymidine analogues (i.e. BVDU) whose antiviral action (at the viral DNA polymerase level) also depends on a preferential phosphorylation by the viral TK; acyclic nucleoside phosphonates which can be divided into different subcategories depending on whether

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Dedicated to Prof. Dr. Morio Ikehara at his 70th birthday.

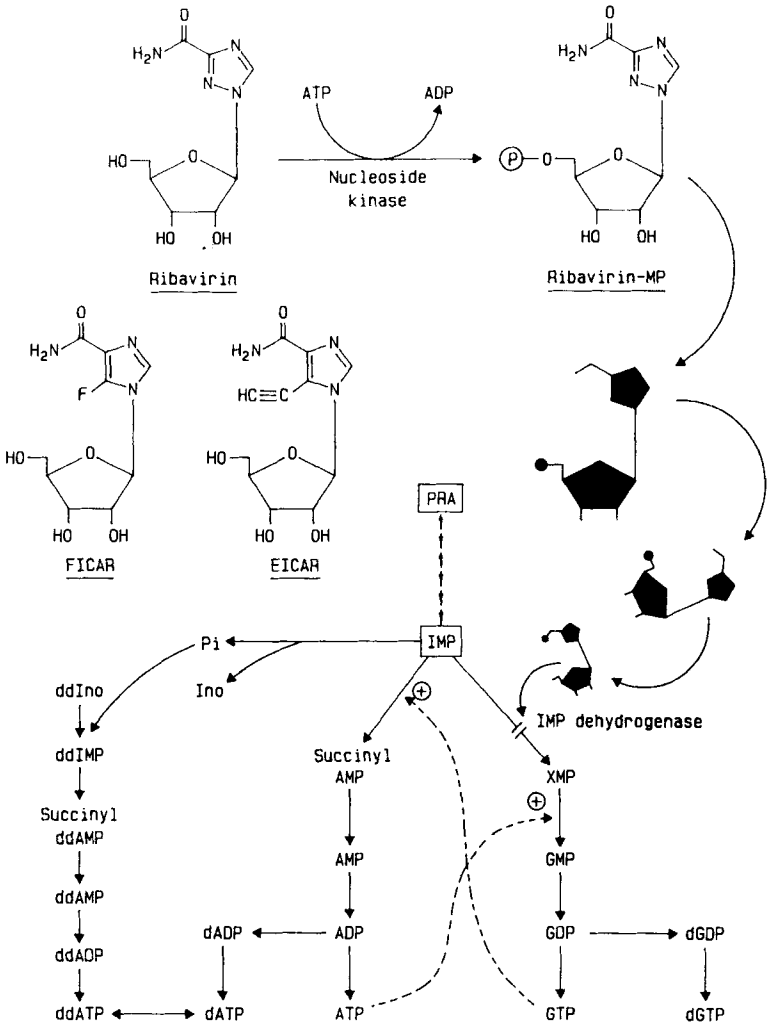
their activity spectrum includes all major DNA viruses (herpes, adeno, pox, papova and hepadna, or herpes-, hepadna-, and retroviruses, or only hepadna- and retroviruses); 2',3'-dideoxynucleoside analogues which, following phosphorylation to the 5'-triphosphate form by cellular enzymes, act as chain terminators of the retro- and hepadnaviral reverse transcriptase; and certain nucleoside derivatives (i.e. HEPT, TSAO) which act as highly specific inhibitors of the reverse transcriptase of human immunodeficiency virus type 1 (HIV-1) (Table 1).

IMP DEHYDROGENASE INHIBITORS (Scheme I)

Ribavirin was the first nucleoside analogue shown to be active against a broad spectrum of RNA and DNA viruses.[2] This broad-spectrum antiviral activity may be primarily ascribed to inhibition of IMP dehydrogenase by ribavirin 5'-monophosphate,[3] although in its 5'-triphosphate form ribavirin may interact with yet other target enzymes, i.e. guanylyl transferase. This may at least partially account for the specific inhibitory effects of ribavirin on those viruses (i.e. influenza) that depend for their mRNA 5'-capping on such enzyme.[4] Several ribavirin derivatives, i.e. FICAR[5] and EICAR[6], exhibit an activity spectrum that is reminiscent of the activity spectrum shown by ribavirin. Thus, FICAR and EICAR, like ribavirin, may be assumed to interact with IMP dehydrogenase, which has, in fact, been recently demonstrated for EICAR.[7] IMP dehydrogenase being a key enzyme in the biosynthesis of purine mononucleotides, IMP dehydrogenase inhibitors may also be expected to interfere with normal cell growth and metabolism. Indeed, EICAR has been found to inhibit the proliferation of tumor cells,[8] and this cytostatic action could be ascribed to inhibition of IMP dehydrogenase.[9] By blocking the conversion of IMP to XMP, IMP dehydrogenase inhibitors lead to a depletion of the guanylate (GMP, GDP, GTP, dGTP) pools, and, since GTP is an obligatory cofactor in the conversion of IMP to AMP (*via* succinyl AMP), ATP and dATP pools are depleted as well.[10] Concomitantly, IMP dehydrogenase inhibitors lead to an accumulation of the intracellular pool levels of IMP, which can serve as phosphate donor for the phosphorylation by 5'-nucleotidase of 2',3'-dideoxyinosine (ddI) to ddIMP. Consequently, IMP dehydrogenase inhibitors are able to potentiate the anti-HIV activity of ddI by, on the one hand, increasing the metabolism of ddI to its active form ddATP, and, on the other hand, reducing the supply of its competing substrate (dATP) for the HIV reverse transcriptase.[10,11] IMP levels can also be increased following addition of AICAR, the normal precursor of IMP, and, thus AICAR has also been found to potentiate the metabolism and anti-HIV activity of ddI.[12]

TABLE 1
Antiviral activity spectrum of different classes of nucleoside analogues

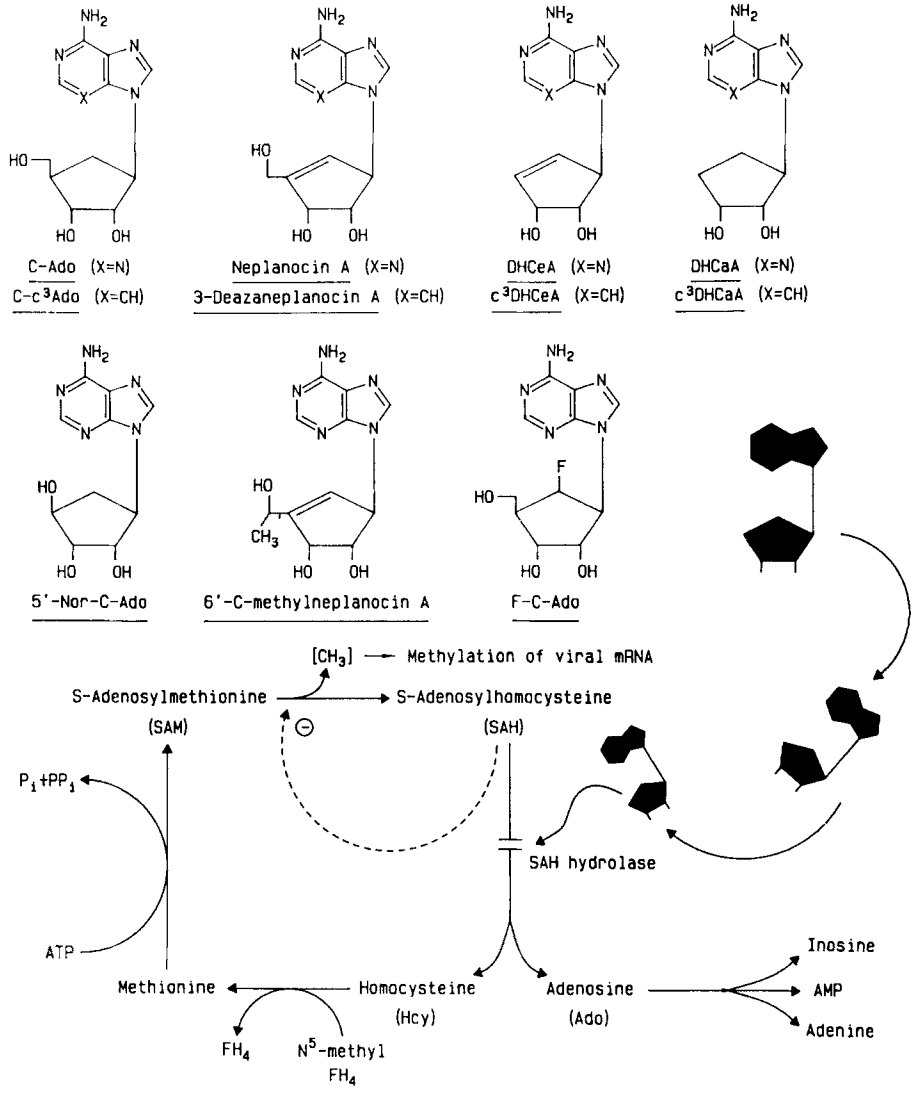
Virus family	Virus type	IMP dehydrogenase inhibitors (i.e. ribavirin)	SAH hydrolase inhibitors (i.e. neplanocin A)	OMP decarboxylase inhibitors (i.e. pyrazofurin)	CTP synthetase inhibitors (i.e. Ce-Cyd)	Acyclic and carbocyclic guanosine analogues (i.e. acyclovir, ganciclovir)	Thymidine analogues (i.e. BVDU, BVaraU)	Acyclic nucleoside phosphonates			Dideoxynucleoside analogues (i.e. AZT)	HIV-1-specific RT inhibitors (HEPT, TSAO)
								HPMPA HPMPC	PMEA	FPMPA PMPA		
Papova	Papilloma							▬	▬			
Adeno								▬				
Herpes	Herpes simplex (HSV-1)			?	?	▬	▬	▬	▬			
	Herpes simplex (HSV-2)			?	?	▬		▬	▬			
	TK⁻ HSV			▬	▬			▬	▬			
	Varicella-zoster (VZV)			?	?	▬	▬	▬	▬			
	TK⁻ VZV			▬	▬			▬	▬			
	Epstein-Barr (EBV)					▬	▬	▬	▬		?	
	Cytomegalo (CMV)	▬	▬	▬	▬	▬		▬	▬			
Hepadna	Hepatitis B (HBV)					?		▬	▬	▬	▬	
Pox	Vaccinia	▬	▬	▬	▬			▬				
Picorna		▬		▬	▬							
Toga		▬		▬	▬							
Flavi		▬		▬	▬							
Bunya		▬	▬	▬	▬							
Arena		▬	▬	▬	▬							
Rhabdo	Vesicular stomatitis (VSV)	▬	▬	▬	▬							
Orthomyxo	*Influenza*	▬	—	▬	▬							
Paramyxo	Parainfluenza	▬	▬	▬	▬							
	Measles	▬	▬	▬	▬							
	Respiratory syncytial (RSV)	▬	▬	▬	▬							
Reo		▬	▬	▬	▬							
Retro	Murine leukemia/sarcoma	▬	▬	▬	▬			▬	▬	▬	▬	
	Human immunodeficiency (HIV-1)								▬	▬	▬	▬
	Human immunodeficiency (HIV-2)								▬	▬	▬	
	Simian immunodeficiency (SIV)								▬	▬	▬	
	Feline immunodeficiency (FIV)								▬	▬	▬	



SCHEME I

SAH HYDROLASE INHIBITORS (Scheme II)

S-adenosylhomocysteine (AdoHcy, SAH) hydrolase has been recognized as a target for antiviral chemotherapy,[13] and various acyclic and carbocyclic adenosine analogues that block the enzyme have proved to be active against a characteristic spectrum of viruses, encompassing, in particular, poxviruses (vaccinia), (-)RNA viruses [bunya, arena (i.e. Junin, Tacaribe), rhabdo (vesicular stomatitis), paramyxo (parainfluenza, measles, respiratory syncytial)] and (±)RNA viruses (reo).[14] As shown



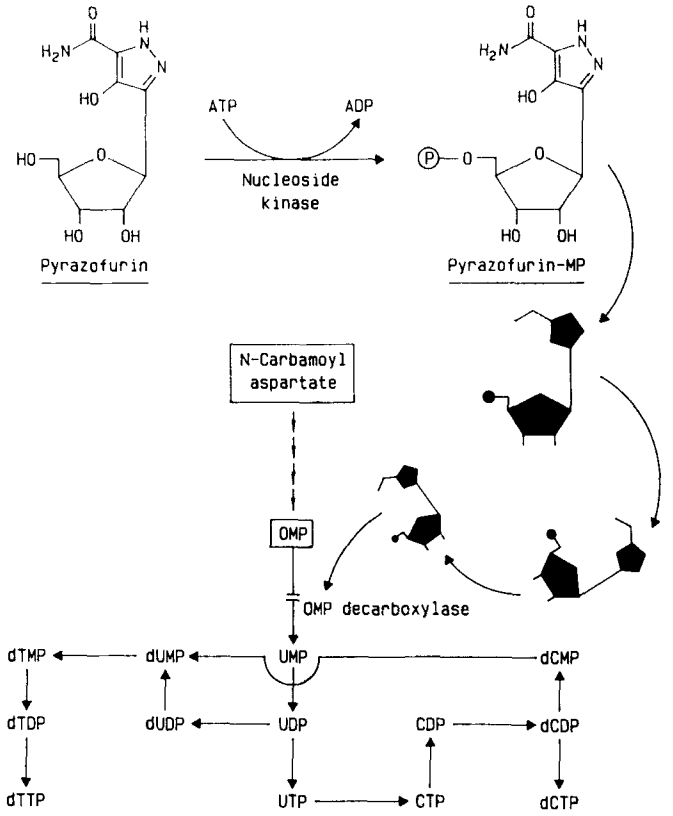
SCHEME II

recently,[15] this activity spectrum also extends to cytomegalovirus (CMV). Among the carbocyclic adenosine analogues, the following have been demonstrated to be potent inhibitors of SAH hydrolase, on the one hand, and to exhibit the antiviral activity spectrum typical of SAH hydrolase inhibitors, on the other hand: C-Ado, C-c^{3}Ado, neplanocin A, 3-deazaneplanocin A,[16,17] 9-(*trans*-2',*trans*-3'-dihydroxycyclopent-4'-enyl)adenine (DHCeA) and its 3-deazaadenine counterpart, c^{3}DHCeA,[17,18] and the

saturated dervatives thereof (DHCaA, c^3DHCaA),[19] (±)-5'-noraristeromycin (5'-nor-C-Ado),[20] 6'-C-methylneplanocin A[21] and (±)-6'ß-fluoroaristeromycin (F-C-Ado)[22] . A close correlation has been found between the antiviral activity of the acyclic and carbocyclic adenosine analogues and their inhibitory effect on SAH hydrolase.[23] In intact cells, carbocyclic adenosine analogues (i.e. C-c^3Ado, neplanocin A, DHCeA, c^3DHCeA, DHCaA, and c^3DHCaA) cause a significant increase in the intracellular AdoHcy levels, which apparently results from their inhibitory effect on SAH hydrolase.[24-26] The characteristic antiviral activity spectrum shown by the SAH hydrolase inhibitors permits the prediction whether a given adenosine analogue would function as a SAH hydrolase inhibitor or not. Thus, 3'-fluoro-3'-deoxyadenosine, which shows an "aberrant" activity spectrum in that it is active against (+)RNA viruses rather than (-)RNA viruses, may be assumed not to act *via* the SAH hydrolase pathway. The viruses that fall within the activity spectrum of the SAH hydrolase inhibitors may correspond to those that for the maturation of their viral mRNA most heavily depend on methylations (i.e. 5'-capping) requiring S-adenosylmethionine (SAM) as the methyl donor. SAH is a product/inhibitor of these reactions and should thus be removed by the SAH hydrolase for the methylations to go unabated. SAH hydrolase inhibitors interfere with this course of events. By suppressing the hydrolysis of SAH into its two components adenosine and homocysteine, they cause an accumulation of SAH. This then leads to an inhibition of the methylation reactions including those that are involved in the maturation of viral mRNA, and an inhibition of the replication of those viruses [(-)RNA viruses, (±)RNA viruses and certain (±)DNA viruses (i.e. CMV)] that are most depending on such methylations.

OMP DECARBOXYLASE INHIBITORS (Scheme III)

OMP decarboxylase has received little attention as a potential target enzyme for antiviral agents, plausibly because compounds that interfere at this level may be expected to shutt off the pyrimidine biosynthetic pathway and, hence, impair normal cell growth and metabolism. Yet, the prototype inhibitor of OMP decarboxylase, pyrazofurin, has long been known to inhibit the replication of a large variety of viruses at concentrations that are apparently not toxic to the host cells.[28] In its activity against poxviruses (i.e. vaccinia), rhabdoviruses (i.e. vesicular stomatitis),[28] retroviruses (i.e. murine leukemia),[29] orthomyxoviruses (influenza A, B and C),[30] and paramyxoviruses (measles, respiratory syncytial),[31,32] pyrazofurin exceeds other antiviral compounds (including ribavirin) both in potency and selectivity. While pyrazofurin has been considered too toxic for systemic use *in vivo*, few attempts have been made to overcome this toxicity. Some pyrazofurin derivatives appear to be equally active but less toxic in cell culture.[33]

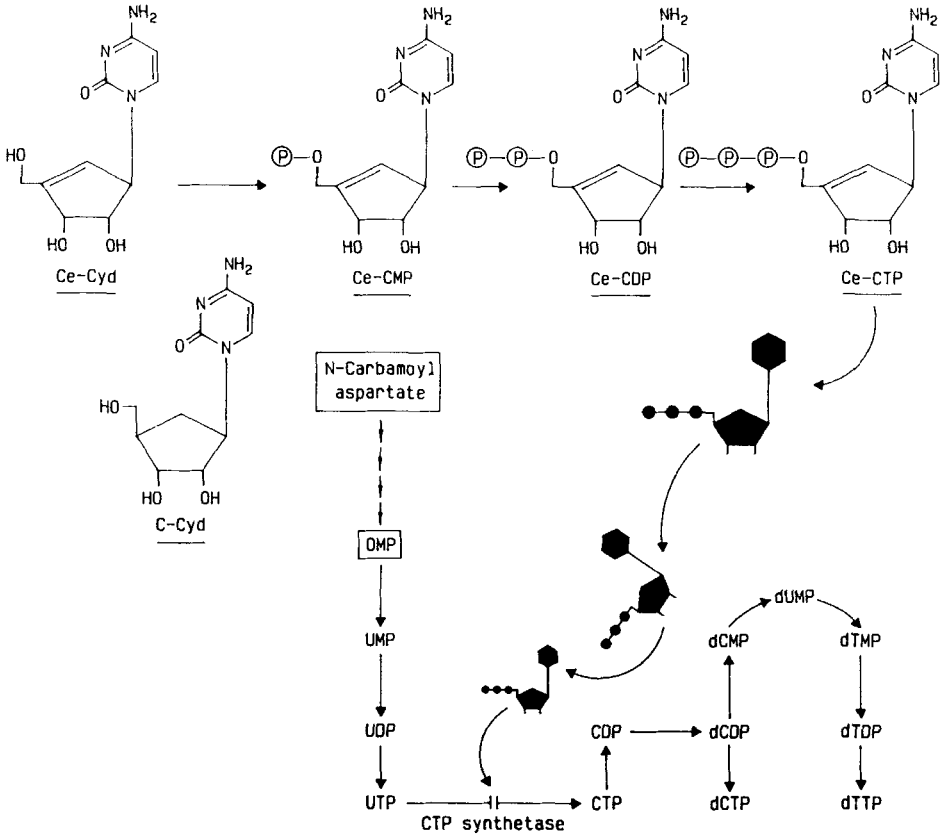


SCHEME III

Given the wealth of *in vitro* virus systems in which pyrazofurin has shown effectiveness, it would seem justified to further explore pyrazofurin and derivatives thereof in the appropriate animal models, particularly following topical drug application as a higher therapeutic ratio may be achieved by this route.[34]

CTP SYNTHETASE INHIBITORS (Scheme IV)

The CTP synthetase inhibitors share a number of common properties with the OMP decarboxylase inhibitors: they are inhibitory to a large variety of viruses, including virtually the whole viral spectrum; they are targeted at a crucial step in the biosynthesis of pyrimidine mononucleotides; and they are fairly toxic to the host cell metabolism and cytostatic to rapidly growing cells. Cyclopentylcytosine (C-Cyd) and cyclopentenylcytosine (Ce-Cyd) can be considered as the prototype inhibitors of CTP synthetase.



SCHEME IV

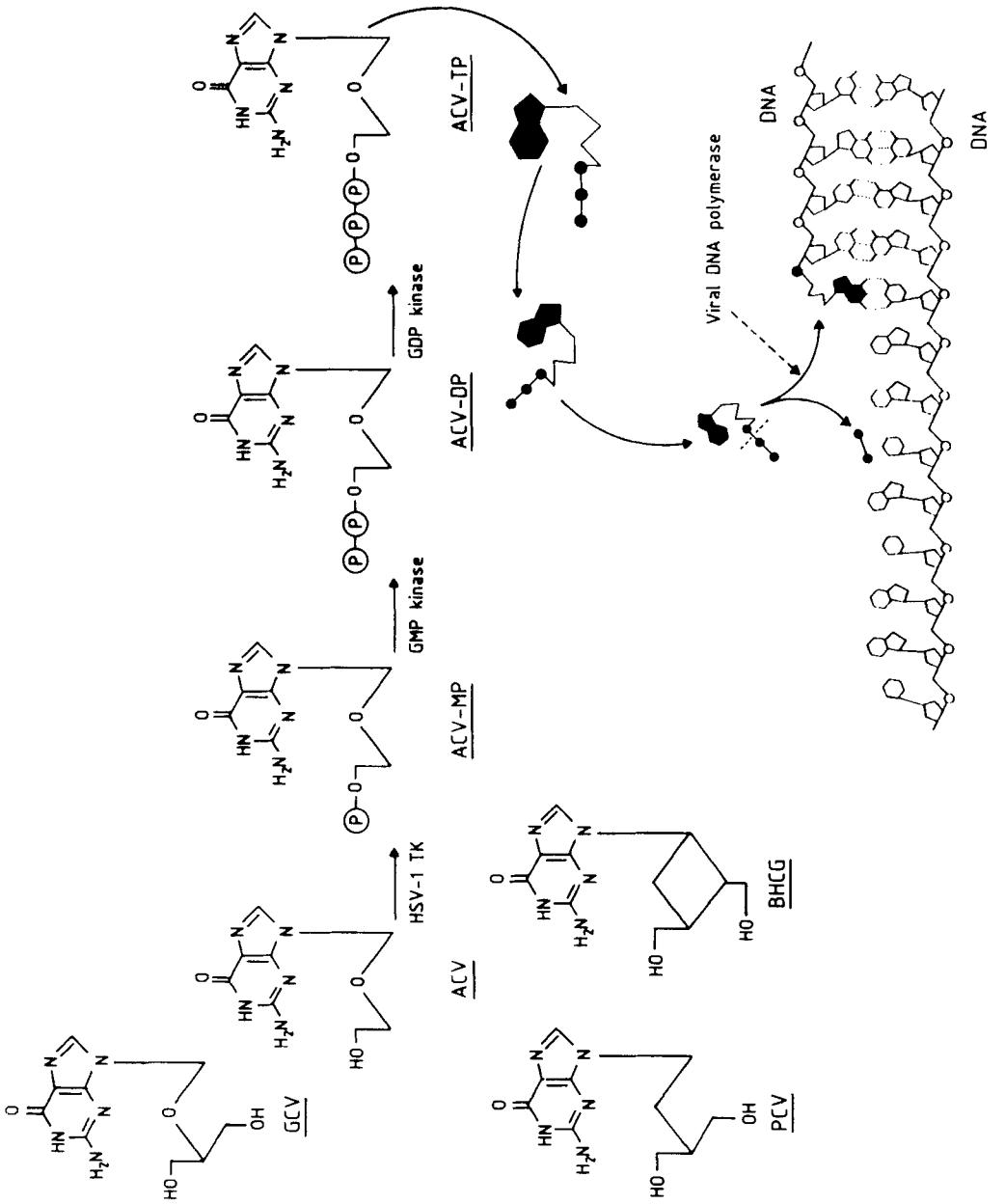
While originally pursued for its inhibitory activity against influenza virus,[35] C-Cyd has proved effective against a wide array of RNA and DNA viruses.[36] Ce-Cyd is even more potent an antiviral agent than C-Cyd, and encompasses an activity spectrum that is even wider than that of C-Cyd.[37,38] Among the viruses that are sensitive to C-Cyd and Ce-Cyd are the thymidine kinase-deficient (TK^-) herpes simplex virus (HSV) mutants which are resistant to the HSV TK-dependent drugs such as BVDU and acyclovir.[39] To exert their antiviral, antimetabolic and cytostatic effects, Ce-Cyd must be phosphorylated intracellularly to its triphosphate,[40] which then acts as a potent inhibitor of CTP synthetase,[41] and thus produces a profound depletion of CTP as well as CDP, dCDP and dCTP pools.[42] As noted above for pyrazofurin, the potential of Ce-Cyd (or derivatives thereof) in the systemic or topical treatment of virus infections *in vivo* has not been sufficiently explored.

ACYCLIC AND CARBOCYCLIC GUANOSINE ANALOGUES (Scheme V)

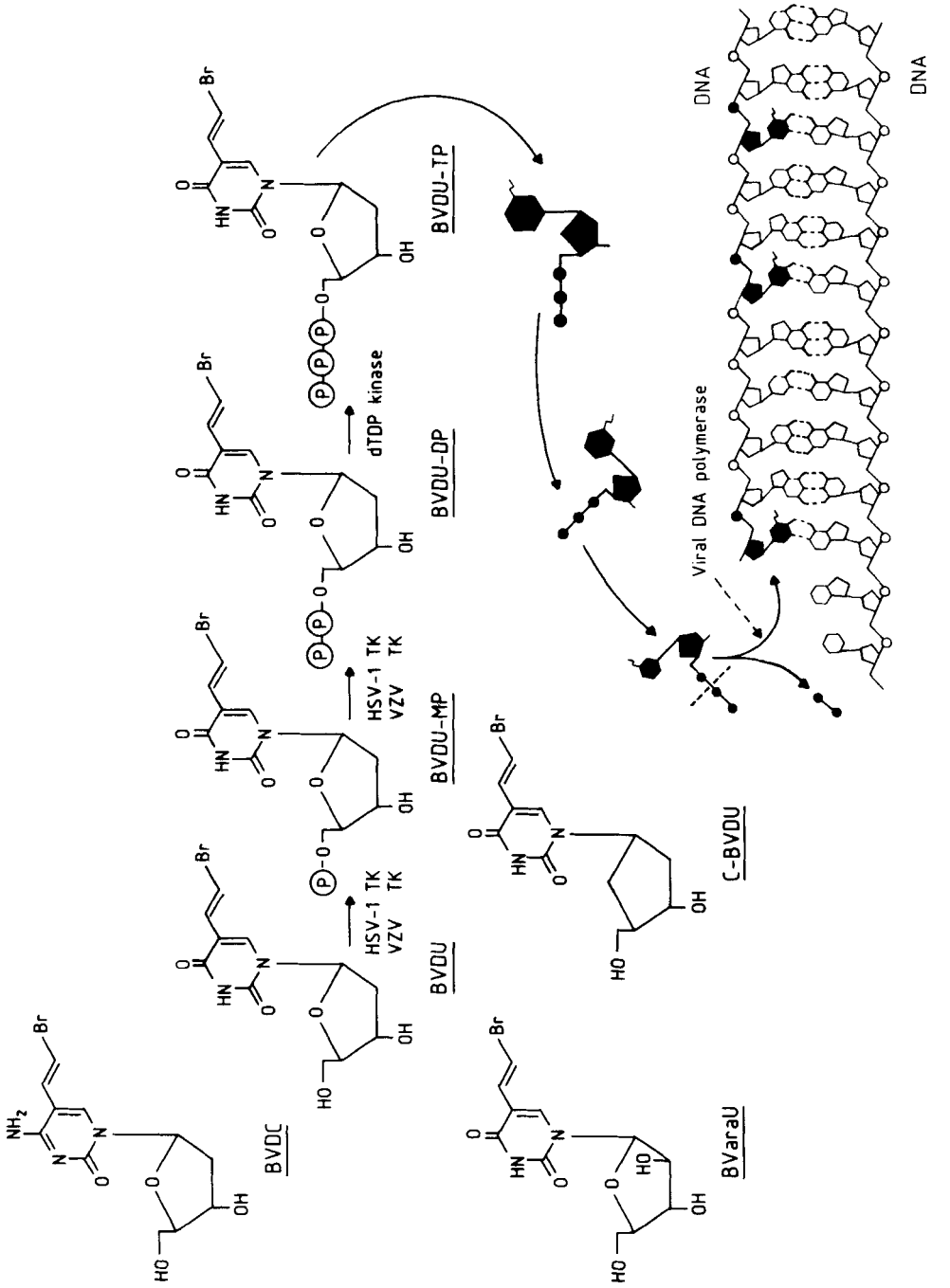
Following acyclovir (ACV), the first acyclic nucleoside analogue shown to be a selective inhibitor of HSV replication,[43,44] several other acyclic/carbocyclic guanosine analogues have been found to selectively inhibit the replication of HSV-1, HSV-2, VZV and other herpesviruses (i.e. CMV and EBV): ganciclovir (GCV), buciclovir (BCV), penciclovir (PCV) and BHCG, the latter being 9-[2,3-bis(hydroxymethyl)cyclobutyl]-guanine. All these compounds share a number of common features. They are recognized as substrate by the HSV- and VZV-encoded TK (acting as a deoxycytidine kinase, rather than thymidine kinase), which explains why the acyclic/carbocyclic guanosine derivatives are active against TK^+, and not TK^-, strains of HSV and VZV. The viral TK converts ACV and its congeners to their monophosphate, and cellular enzymes then take care of the further phosphorylation to the di- and triphosphate. In their triphosphate form, the compounds act as inhibitor/substrate of the viral DNA polymerase.[45,46] If incorporated into DNA, ACV triphosphate must function as a chain terminator, whereas the others could, at least theoretically, be incorporated internally *via* an internucleotide linkage. GCV (ganciclovir) can be distiguished from the other guanosine analogues in that it demonstrates a pronounced activity against CMV. This virus is not known to encode a viral specific TK, and if GCV is specifically inhibitory to CMV, this must stem from the compound being recognized as substrate by a virus-specified protein kinase.[47,48] The acyclic/carbocyclic guanosine analogues are only poorly bioavailable by the oral route. In attempts to increase the oral bioavailability, prodrugs have been designed [i.e. the valyl ester of acyclovir (valacyclovir) and the diacetyl ester of the 6-deoxy derivative of penciclovir (famciclovir)] that are indeed better taken up orally than the parent compounds. Famciclovir[49] and penciclovir have been accredited with higher *in vivo* efficacy against herpesvirus infections than ACV[50] because of the accumulation (long half-life) of PCV triphosphate in the herpesvirus-infected cells.[51]

THYMIDINE ANALOGUES (Scheme VI)

Shortly after acyclovir, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) was discovered as a potent and selective anti-herpesvirus agent,[52] with, as compared to acyclovir, an approximately 5-fold higher potency against HSV-1, 50-fold lower potency against HSV-2, and 1000-fold higher potency against VZV. BVDU and its congeners (i.e. BVDC, BVaraU and C-BVDU) are even better substrates of HSV TK and VZV TK than the acyclic/carbocyclic guanosine analogues, and this explains their highly selective activity against HSV-1 and VZV. In fact, the HSV-1 TK and VZV TK are able to convert BVDU *via* BVDU monophosphate to BVDU diphosphate, whereas HSV-2 TK converts BVDU to the mono- but not further onto the diphosphate, and this explains



SCHEME V



SCHEME VI

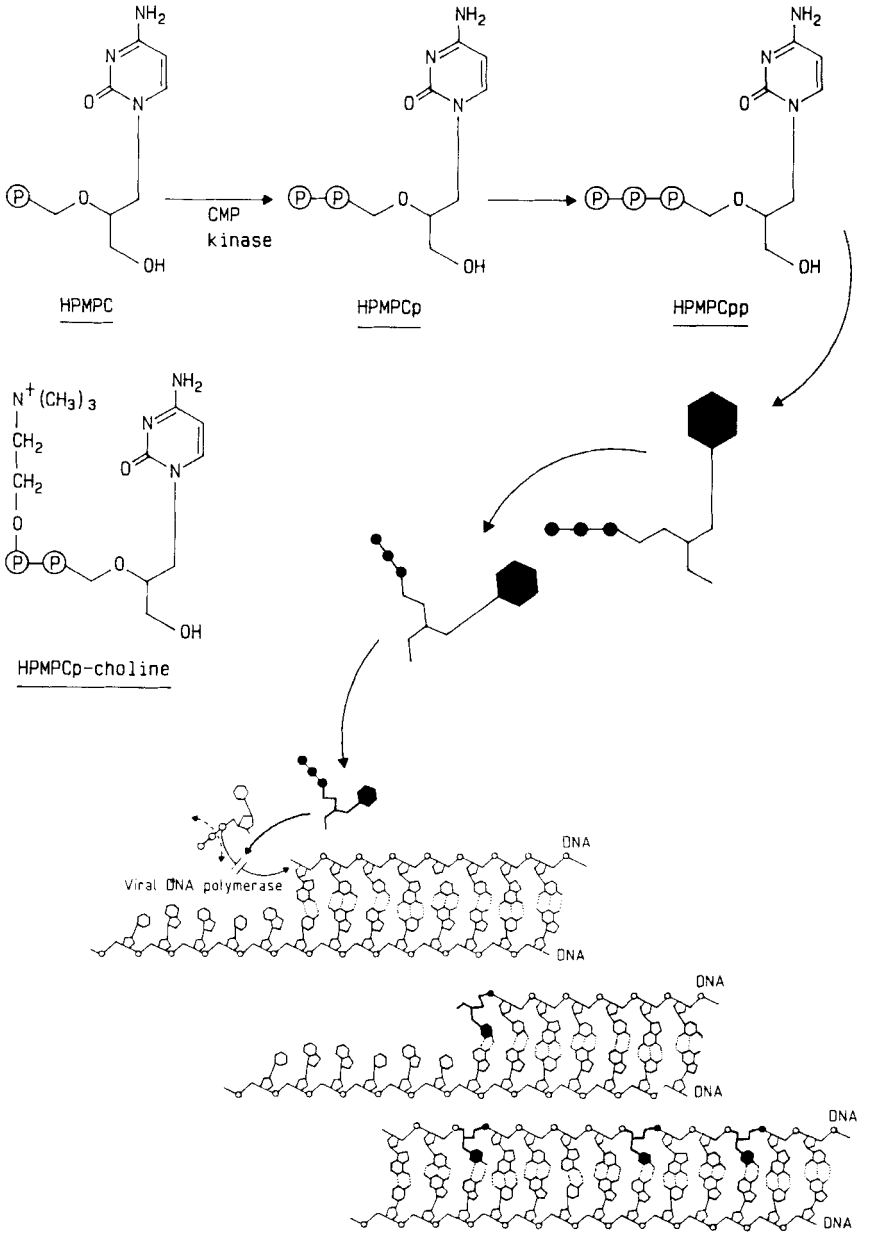
why HSV-2 is significantly less sensitive than HSV-1 to the inhibitory effects of BVDU and its congeners (i.e. BVaraU).[53] After BVDU diphosphate has been converted to the triphosphate (by cellular enzymes), the latter may act as competitive inhibitor/substrate of the viral DNA polymerase. If acting as substrate it can be incorporated into the interior of the DNA chain, and this may lead to breakage of the DNA strands.[54] The remarkable specificity of BVDU as an anti-HSV-1 and anti-VZV agent is determined by the presence of the (*E*)-5-(2-bromovinyl) substituent [with the hydrogens at C-1 and C-2 of the vinyl group in the trans (*Entgegen*) position]. Hence, it is not surprising that the various analogues that have been synthesized after BVDU, *viz.* BVaraU, BVDC and C-BVDU, demonstrate an antiviral activity similar to that of BVDU. Recently, 5-heteroaryl-substituted 2'-deoxyuridines, i.e. 5-(3-bromoisoxazol-5-yl)-2'-deoxyuridine, 5-(bromothien-2-yl)-2'-deoxyuridine and 5-(5-chlorothien-2-yl)-2'-deoxyuridine, have been synthesized that share with BVDU a common activity spectrum: activity against different strains of HSV-1 and VZV, but not HSV-2, CMV or TK^- HSV-1.[55-57] From their activity spectrum, it can be readily inferred that these compounds, like BVDU, are dependent on a specific phosphorylation by the HSV-1- and VZV-encoded thymidine kinase.

The stringent dependence of the anti-herpetic drugs, belonging to the acyclic/carbocyclic guanosine (i.e. ganciclovir) or thymidine (i.e. BVDU) class, on phosphorylation by the HSV- or VZV-encoded thymidine kinase, offers the potential for therapeutic use of these agents not only for the treatment of HSV and VZV infections but also for the treatment of various cancers. The latter premise is based on the fact that tumor cells that have been transfected with the HSV-1 TK gene or HSV-2 TK gene become exquisitely sensitive to the cytostatic action of some of the anti-herpetic agents (i.e. BVDU).[58-60] Also, ganciclovir acquires an increased cytostatic activity if the tumor cells have been transfected by the HSV TK gene. Whereas the cytostatic activity of ganciclovir resides in the inhibition of DNA synthesis by the compound's triphosphate, that of BVDU is based on the inhibition of thymidylate synthase by BVDU's monophosphate.[61] Viral TK-dependent drugs such as BVDU could be advocated for a combined gene therapy/chemotherapy approach, pending, of course, the availability of the necessary technology to introduce the viral TK gene into the tumor cells. This has recently proved to be feasible, and thus *in vivo* HSV TK gene-transfected brain tumors as well as malignant melanomas were found to regress following antiviral drug treatment.[62-64] The combined viral TK gene therapy/antiviral drug chemotherapy approach may herald a new and effective strategy towards the eradication of such tumors (i.e. brain and liver tumors, and malignant melanomas) that are not readily amenable to the usual cytotoxic chemotherapy.

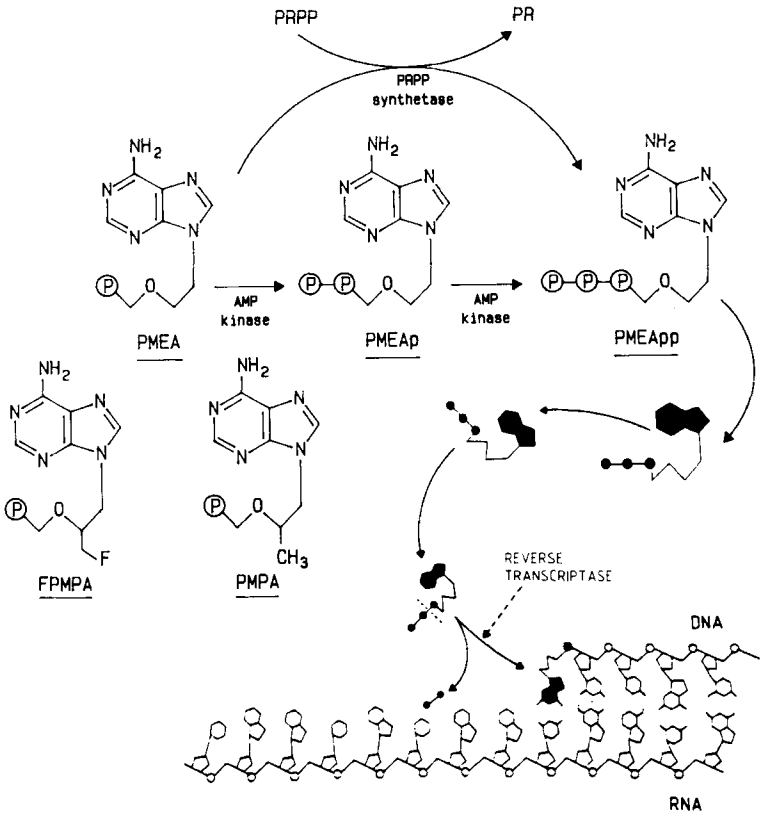
ACYCLIC NUCLEOSIDE PHOSPHONATES (Schemes VII and VIII)

(*S*)-9-(3-Hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA), the prototype of the acyclic nucleoside phosphonates can be regarded as a hybrid molecule between (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA) and phosphonoacetic acid, two agents that have since long been recognized as broad-spectrum antivirals.[65] HPMPA was found to exhibit a broad-spectrum activity against a wide variety of DNA viruses, including adeno-, herpes-, hepadna-, irido-, and poxviruses.[66] Among the herpesviruses, TK^- strains of HSV and VZV (that are resistant to the acyclic/carbocyclic guanosine and thymidine analogues) are equally, if not more sensitive, to HPMPA than the wild-type TK^+ strains. In fact, the compound is phosphorylated by cellular enzymes [i.e. AMP kinase, PRPP (5-phosphoribosyl-1-pyrophosphate) synthetase] to its active form, HPMPA diphosphate (HPMPApp), which then inhibits viral DNA synthesis.[67] The cytosine counterpart of HPMPA, HPMPC, has an activity spectrum similar to that of HPMPA,[68] and, as it has proved less toxic than HPMPA *in vitro* and *in vivo* (i.e. mice), it has been chosen as an antiviral drug candidate for further development.[69] HPMPC is particularly promising as a potential anti-CMV drug[70]: it inhibits CMV DNA synthesis at a concentration that is 1000-fold lower than the concentration required to affect cellular DNA synthesis.[71] After it has been taken up by the cells, HPMPC is metabolized to HPMPC monophosphate (HPMPCp), HPMPC diphosphate (HPMPCpp) and HPMPCp-choline (which, because of its long half-life, could be considered as an intracellular depot form of HPMPC).[72,73] In its activity (diphosphate) form, HPMPC would be targeted at the viral DNA polymerase. In principle, HPMPCpp may act as a competitive inhibitor/substrate of the viral DNA polymerization, and, as substrate, it could be incorporated either terminally (and thus prevent chain elongation) or internally (and thus allow DNA chain growth). Which of these mechanisms prevails and how HPMPC achieves a preferential inhibition of viral DNA synthesis (as compared to cellular DNA synthesis) remain issues worth further pursuing. As a point mutation in the CMV DNA polymerase gene can confer resistance to HPMPC (as well as ganciclovir),[74] it is obvious that the CMV DNA polymerase must serve as target for these drugs.

While conceived at the same time as HPMPA,[66] 9-(2-phosphonylmethoxyethyl)-adenine (PMEA) was later found to exhibit an antiviral activity spectrum that only partially overlaps with that of HPMPA. While active against herpes- and hepadnaviruses, PMEA is not active against pox- and adenoviruses.[68] Yet, it has marked activity against HIV-1 and HIV-2[75] and various other retroviruses, including murine sarcoma virus (MSV),[76] murine AIDS (MAIDS) virus,[77] feline leukemia virus (FeLV),[78] feline immunodeficiency virus (FIV)[79] and simian immunodeficiency virus (SIV)[80], both *in vitro* and *in vivo*. HPMPA has little, if any, activity against retroviruses.[75] The differences



SCHEME VII



SCHEME VIII

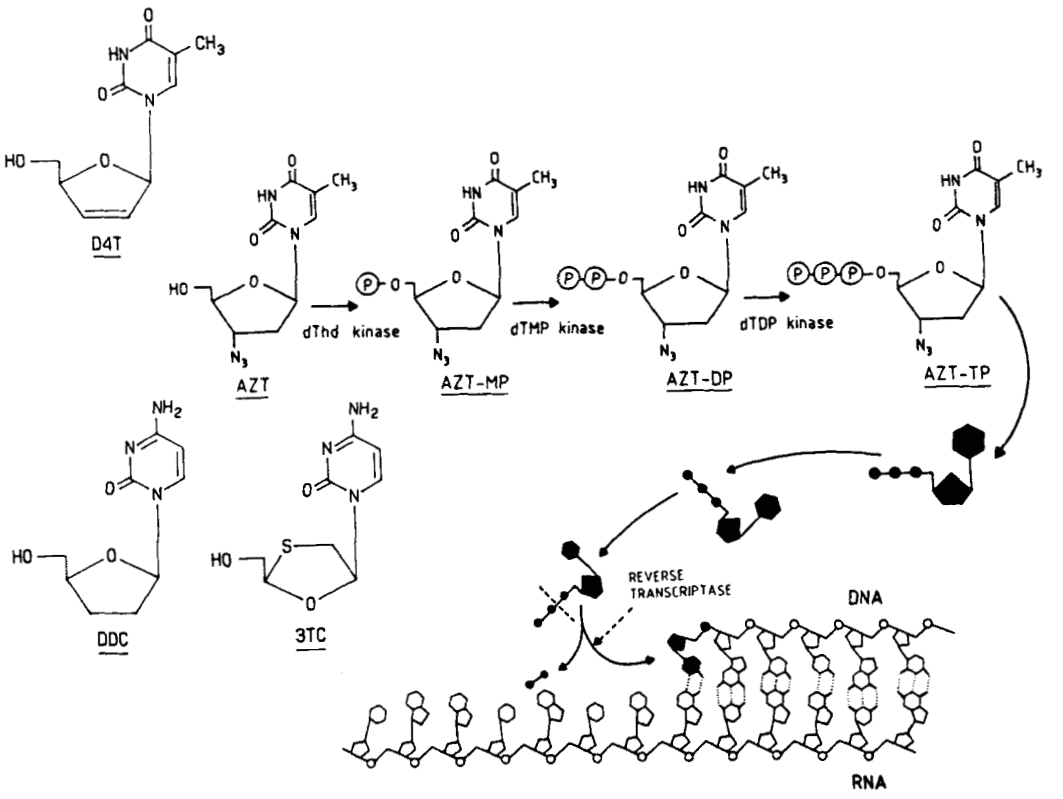
in activity spectrum between PMEA and HPMPA may be related to differences in the mode of action of their diphosphates (PMEApp, HPMPApp) at the viral DNA polymerase level. While HPMPApp, if acting as substrate, could be incorporated internally (*via* an internucleotide linkage) into the DNA chain,[81] PMEApp must obligatorily act as a chain terminator if incorporated, because it does not contain the 3'-hydroxyl group needed for further elongation. PMEApp has indeed been shown to act as a chain terminator in both the HIV-1 reverse transcriptase reaction[82] and HSV-1 DNA polymerase reaction.[83]

If the acyclic side chain of HPMPA is modified to 3-fluoro-2-phosphonylmethoxypropyl (as in FPMPA) or 2-phosphonylmethoxypropyl (as in PMPA), the antiviral activity spectrum is further shifted away from the DNA viruses to the retroviruses. FPMPA and PMPA are specifically active against retroviruses and no

longer active against herpesviruses or other DNA viruses (except for hepadnaviruses).[84,85] Following phosphorylation of FPMPA and PMPA (i.e. by AMP kinase or PRPP synthetase) to their diphosphate form (FPMPApp, PMPApp), the latter function as chain terminators of the reverse transcriptase, and this may explain their inhibitory effects on the replication of retro- and hepadnaviruses.

DIDEOXYNUCLEOSIDE ANALOGUES (Scheme IX)

The three anti-HIV drugs that have now been formally approved for therapeutic use, namely 3'-azido-2',3'-dideoxythymidine (AZT), 2',3'-dideoxyinosine (DDI) and 2',3'-dideoxycytidine (DDC), were among the first dideoxynucleoside analogues shown to specifically inhibit HIV infectivity *in vitro*.[86,87] Subsequently, 2',3'-didehydro-2',3'-dideoxycytidine (D4C),[88] 2',3'-didehydro-2',3'-dideoxythymidine (D4T),[89] 5-chloro-3'-fluoro-2',3'-dideoxyuridine[90] and various other 3'-azido- or 3'-fluoro-substituted analogues were reported to inhibit the replication of HIV in cell culture.[91,92] Dideoxynucleoside analogues, in which the 3'-carbon has been replaced by a sulfur atom, *viz.* 2',3'-dideoxy-3'-thiocytidine (3TC) and its 5-fluoro derivative (FTC) have also been shown to inhibit the replication of HIV,[93-95] and that of hepatitis B virus (HBV) as well;[96,97] and the anti-HIV and anti-HBV effects of 3TC and FTC primarily reside with their (-)enantiomers. Yet, other 2',3'-dideoxynucleoside analogues, i.e. 2',3'-dideoxyuridine (ddUrd), 5-ethyl-ddUrd and numerous 3'-substituted (other than the 3'-azido- or 3'-fluoro-substituted) dideoxynucleosides fail to inhibit HIV replication.[92] How could the marked anti-HIV activity of some dideoxynucleoside analogues (i.e. AZT, DDC, D4T) and the inactivity of others (i.e. ddUrd) be accounted for ? As first demonstrated with AZT,[98] the 2',3'-dideoxynucleoside analogues must first be phosphorylated (by cellular enzymes) to their 5'-triphosphate before the latter can act, as either competitive inhibitor or alternative substrate (chain terminator) at the reverse transcriptase level.[99] Akin to AZT 5'-triphosphate,[100] all 2',3'-dideoxynucleoside 5'-triphosphates may be assumed to block the reverse transcriptase action by premature chain termination. The fact that some 2',3'-dideoxynucleoside analogues are effective in suppressing HIV, whereas others are not, is dependent of their intracellular metabolism to the 5'-triphosphate form.[101] Marked differences have been noted in the pattern of intracellular metabolism of different 2',3'-dideoxynucleosides: for example, AZT is efficiently phosphorylated to the 5'-monophosphate but then hampered in its further phosphorylation, apparently because AZT-MP inhibits dTMP kinase that catalyzes the conversion of AZT-MP to AZT-DP; for D4T, there is no hindrance in the phosphorylation from the mono- to the diphosphate form.[102,103] The intracellular metabolism of ddUrd is blocked at the first phosphorylation step, and this explains why the compound is inactive against HIV. If ddUrd would be converted intracellularly to the

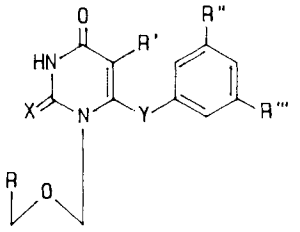


SCHEME IX

5'-triphosphate form (ddUTP), it should be effective in inhibiting HIV replication, since ddUTP has indeed proved to be a potent chain terminator in the HIV reverse transcriptase reaction.[104] As the anabolic pathway of ddUrd is blocked at the first phosphorylation step, it would thus suffice to deliver ddUrd intracellularly in its monophosphate form to re-gain anti-HIV activity. This can be achieved by a masked monophosphate prodrug approach, i.e. based on the use of the bis[*S*-(2-hydroxyethylsulfidyl)-2-thioethyl]ester of ddUMP which is as such taken up by the cells, and, once inside the cell, releases the parent monophosphate ddUMP.[105]

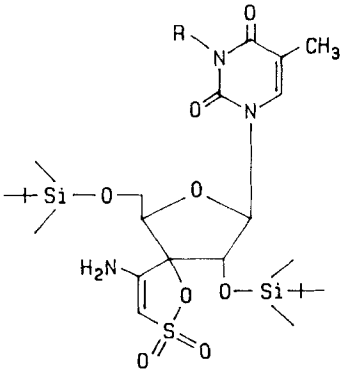
HIV-1-SPECIFIC REVERSE TRANSCRIPTASE INHIBITORS (Scheme X)

The first nucleoside analogue shown to be a specific HIV-1 inhibitor was 1-(2-hydroxyethoxymethyl)-6-phenylthiothymine (HEPT)[106,107]: the compound was found to



HEPT DERIVATIVES

	X	Y	R	R'	R''	R'''
HEPT	O	S	CH_2OH	Me	H	H
E-EPU	O	S	Me	Et	H	H
E-EBU	O	CH_2	Me	Et	H	H
E-EPU-S	S	S	Me	Et	H	H
E-EPU-dM	O	S	Me	Et	Me	Me
E-EBU-dM	O	CH_2	Me	Et	Me	Me
I-EBU	O	CH_2	Me	iPr	H	H
I-EBU-dM	O	CH_2	Me	iPr	Me	Me



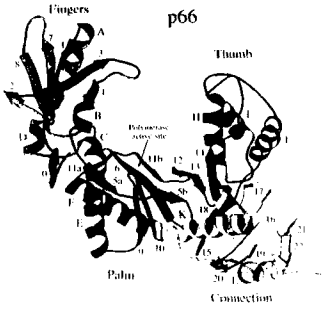
TSAO derivatives

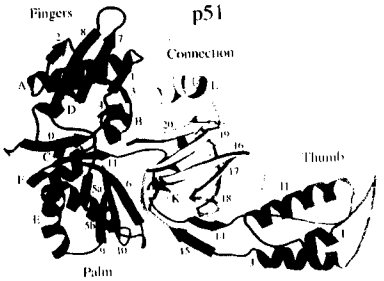
	R
TSAO-T	H
TSAO-m^3T	Me
TSAO-e^3T	Et

HIV-1 Reverse transcriptase

E-EBU-dM 106
HEPT 188
E-EBU 181

TSAO 138





SCHEME X

inhibit the replication of several strains of HIV-1 in several cell culture systems but did not affect the replication of other retroviruses including HIV-2. Several HEPT derivatives, i.e. 5-ethyl-1-ethoxymethyl-6-phenylthiouracil (E-EPU), 5-ethyl-1-ethoxymethyl-6-benzyluracil (E-EBU), 5-ethyl-1-ethoxymethyl-6-phenylthio-2-thiouracil (E-EPU-S), 5-ethyl-1-ethoxymethyl-6-(3,5-dimethylphenylthio)uracil (E-EPU-dM), 5-ethyl-1-ethoxymethyl-6-(3,5-dimethylbenzyl)uracil (E-EBU-dM), 5-isopropyl-1-ethoxymethyl-6-benzyluracil (I-EBU) and 5-isopropyl-1-ethoxymethyl-6-(3,5-dimethylbenzyl)-uracil (I-EBU-dM), were later found to exceed by far the parent HEPT compound in both potency and selectivity.[108-112] The HEPT congeners owe their selective inhibitory effect on HIV-1 replication to a specific interaction with the HIV-1 reverse transcriptase (RT).[108,109] Akin to the tetrahydro-imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one (TIBO) derivatives,[113,114] the HEPT derivatives interact with an allosteric site at the viral RT, which, while different from the substrate (dNTP) binding site, may be functionally and even spatially associated with the substrate binding site.[115,116] Another class of nucleoside analogues that show a behavior similar to that of the HEPT derivatives are the 2',5'-bis-O-(*tert*-butyldimethylsilyl)-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide)pyrimidine (TSAO) nucleosides, i.e. with thymine (TSAO-T), 3-methylthymine (TSAO-m^3T) or 3-ethylthymine (TSAO-e^3T) as the pyrimidine base.[117-120] Like HEPT, TSAO derivatives effect a selective inhibition of HIV-1 replication, due to a specific interaction with a non-substrate binding site of the HIV-1 RT.[121] In addition to TIBO, HEPT and TSAO, various other, non-nucleoside, analogues have been identified as specific HIV-1 RT inhibitors,[122] and all these compounds seem to interact with a common target site ("pocket") within the HIV-1 RT p66 subunit.[123,124] The crucial amino acids involved in the binding of the HIV-1-specific RT inhibitors have been delineated by tracing the amino acid substitutions that confer resistance to the drugs, followed by site-directed mutagenesis of the HIV-1 RT to confirm that the observed mutations indeed made the HIV-1 RT resistant. These studies revealed that the amino acids at position 100 (Leu), 103 (Lys), 106 (Val), 108 (Val), 179 (Val), 181 (Tyr), 188 (Tyr), 190 (Gly) and 236 (Pro) of the p66 subunit, and position 138 (Glu) of the p51 subunit, must play a critical role in the binding of the HIV-1-specific RT inhibitors and/or the conformation of their binding site ("pocket").[125] Mutations at any of these positions led to a reduced sensitivity of the HIV-1 RT to one or more of the compounds. Whereas the mutation at position 138 (Glu → Lys) of the p51 subunit is responsible for resistance to TSAO,[126-128] the HEPT derivatives E-EBU-dM, E-EBU and HEPT itself select for resistance at the p66 subunit positions 106 (Val → Ala), 181 (Tyr → Cys) and 188 (Tyr → His), respectively.[129] In addition to the mutation at the p51 position 138, mutations at the p66 positions 181, 188 and 106, also lead to reduced

sensitivity to TSAO. These amino acids may either serve as direct attachment points for TSAO or at least help securing the desirable conformation of the TSAO binding site. While all the HIV-1-specific RT inhibitors may be postulated to interact with the same non-substrate binding site at the HIV-1 RT, significant differences must exist in the exact way by which the different compounds bind, as suggested by the differences in drug sensitivity/resistance patterns generated by different mutations.[125]

CONCLUSION

Depending on their antiviral activity spectrum, nucleoside analogues can be divided into different classes: IMP dehydrogenase inhibitors, SAH hydrolase inhibitors, OMP decarboxylase inhibitors, CTP synthetase inhibitors, acyclic and carbocyclic guanosine analogues, thymidine analogues, acyclic nucleoside phosphonates, dideoxynucleoside analogues, and HIV-1-specific RT inhibitors (Table 1). From inspection of the activity spectrum shown by a given nucleoside it is often clear to which class it belongs, how it must act and at which enzyme it must be targeted. The antiviral activity spectrum can thus be considered as predictive of the mode of action of the compound. When reviewing the antiviral activity spectrum of the different classes of nucleoside analogues, it is evident that, as we proceed from those nucleoside analogues that interfere with cellular enzymes (i.e. OMP decarboxylase, CTP synthetase, SAH hydrolase, IMP dehydrogenase) to those that are targeted at a virus-specified enzyme (i.e. reverse transcriptase), the antiviral activity spectrum narrows, and thus the antiviral specificity increases. At the extremes are the OMP dicarboxylase and CTP synthetase inhibitors (i.e. pyrazofurin and Ce-Cyd), which are active against virtually all viruses, and the HIV-1-specific reverse transcriptase inhibitors (i.e. HEPT and TSAO), which are only active against HIV-1. Whereas those nucleoside analogues that exhibit the narrowest activity spectrum (or highest antiviral specificity) may also be the least toxic, they should be most prone to lead to the emergence of virus-drug resistance. Conversely, those compounds that have the widest activity spectrum may also be the most toxic but should be the least inclined to give rise to resistance development.

ACKNOWLEDGMENTS

The original investigations of the Author are supported by the AIDS Basic Research Programme of the European Community, and grants from the Belgian Geconcerteerde Onderzoeksacties, the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek, the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek, and the Janssen Research Foundation. I thank Christiane Callebaut for her dedicated editorial assistance.

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Received 12/7/93
Accepted 12/15/93