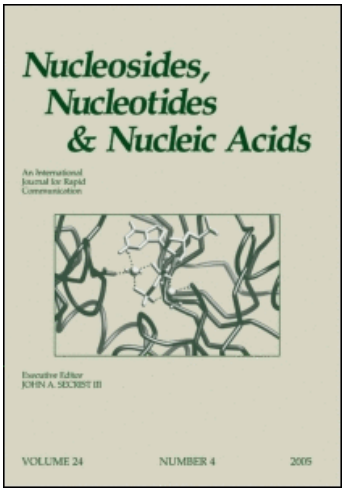


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Nucleoside Analogues as Chemotherapeutic Agents: A Review

C. Périgaud[a]; G. Gosselin[a]; J. L. Imbach[a]
[a] Laboratoire de Chimie Bio-Organique, U.R.A. n° 488 et U.M.R. n° 112 du C.N.R.S., Université de Montpellier II, Sciences et Techniques du Languedoc, Montpellier Cédex, France

To cite this Article Périgaud, C. , Gosselin, G. and Imbach, J. L.(1992) 'Nucleoside Analogues as Chemotherapeutic Agents: A Review', Nucleosides, Nucleotides and Nucleic Acids, 11: 2, 903 — 945
To link to this Article: DOI: 10.1080/07328319208021748
URL: http://dx.doi.org/10.1080/07328319208021748

NUCLEOSIDES AND NUCLEOTIDES, 11(2-4), 903-945 (1992)

NUCLEOSIDE ANALOGUES AS CHEMOTHERAPEUTIC AGENTS: A REVIEW

C. Périgaud, G. Gosselin* and J.-L. Imbach

Laboratoire de Chimie Bio-Organique, U.R.A. n° 488 et U.M.R. n° 112 du C.N.R.S., Université de Montpellier II, Sciences et Techniques du Languedoc, case courrier 008, Place Eugène-Bataillon, 34095 Montpellier Cédex 5, France

Abstract. The importance of nucleoside analogues in chemotherapy and in other potential therapeutic approaches as immunomodulation or regulation of gene expression, is reviewed.

INTRODUCTION

One of the most striking advances in medicine during the twentieth century has been the discovery of antibiotics which inhibit the proliferation of bacteria in an infected organism without detrimental effect against the host. Comparatively, progress in the treatment of cancers and viral infections which are the main causes of morbidity and mortality in industrialized countries, has been tardy and limited. However, cumulative knowledge of viral and cellular replication events has made it possible to identify compounds that might interfere selectively with viral functions or malignant cells. Among these compounds, nucleoside analogues are of great importance.[1,2] For instance, out of the nine antiviral drugs licensed at this time only two (amantadine and interferon, the latter having been approved for therapy of *condyloma acuminatum* which is caused by a papillomavirus) are not nucleosides (Figure 1).

The term "nucleoside(s)" introduced by Levene and Jacobs in 1909, is originally associated with nucleic acids, by hydrolysis of which they were isolated for the first time.[3] Natural nucleosides are constituted by the association of a purine (adenine and guanine) or a pyrimidine (cytosine, uracil and thymine) base, with a pentose residue (β-D-ribofuranose or

* *Dedicated to the memory of Professor Tohru Ueda* *

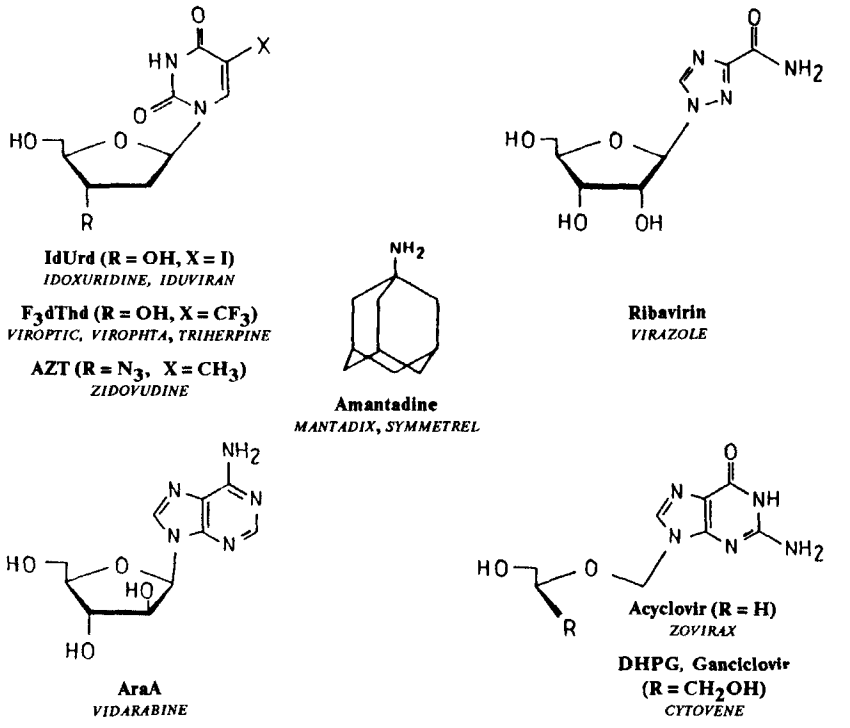


Figure 1. Structurally-defined drugs licensed for clinical antiviral use .

β-D-deoxyribofuranose).[4] Esterification of their 5'-hydroxyl group with phosphoric acid leads to nucleotides. Natural nucleosides and nucleotides play a key role in many biosynthetic and regulatory processes, an extremely important function takes place at the level of the structure of ribo- and deoxyribonucleic acids (RNA and DNA, Figure 2).

As structural units of nucleic acids, nucleosides take part in the molecular mechanisms of conservation, replication and transcription of the genetic information.[5] In each cell, DNA is the support of this information which depends only on the sequence of nucleosides in their chain. The transfer of this information (based on the complementarity guanine-cytosine and adenine-thymine or uracil) is effected owing to ribonucleic acids. Thus, DNA (in the nucleus) is transcripted into messenger RNAs (mRNAs), which are transported into the cytoplasm where they are translated by the ribosomes in proteins. This process involves transfer RNAs (tRNAs) which act as adapter molecules between the nucleotide sequences (codons) on the mRNA and the amino acids they carry. Ribosomes themselves contain a

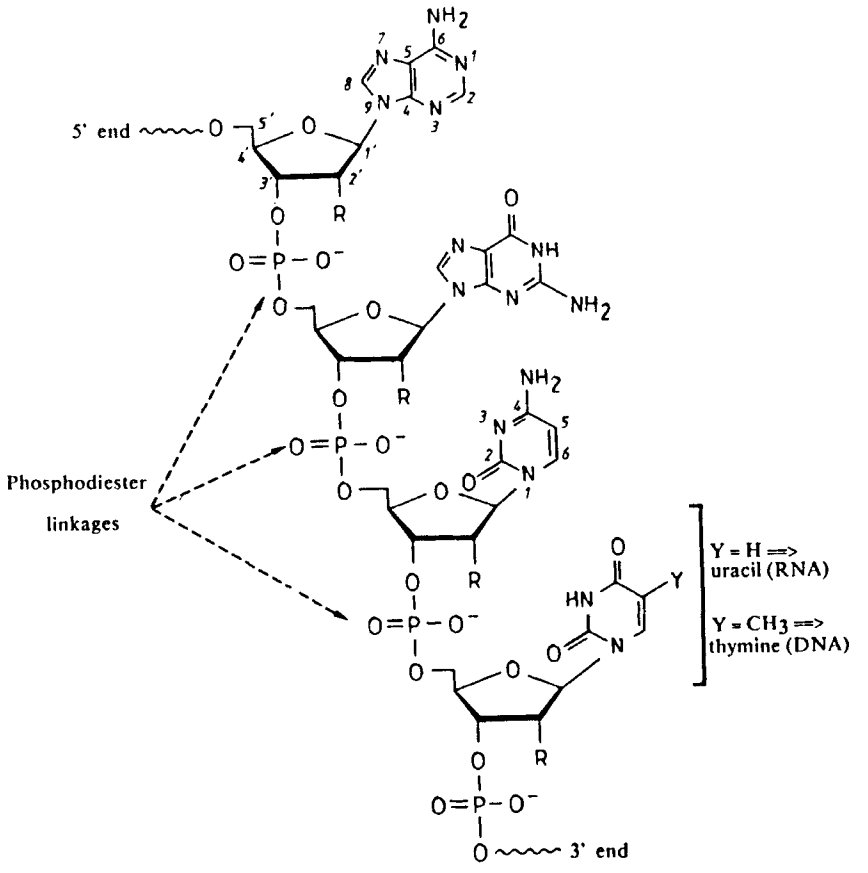
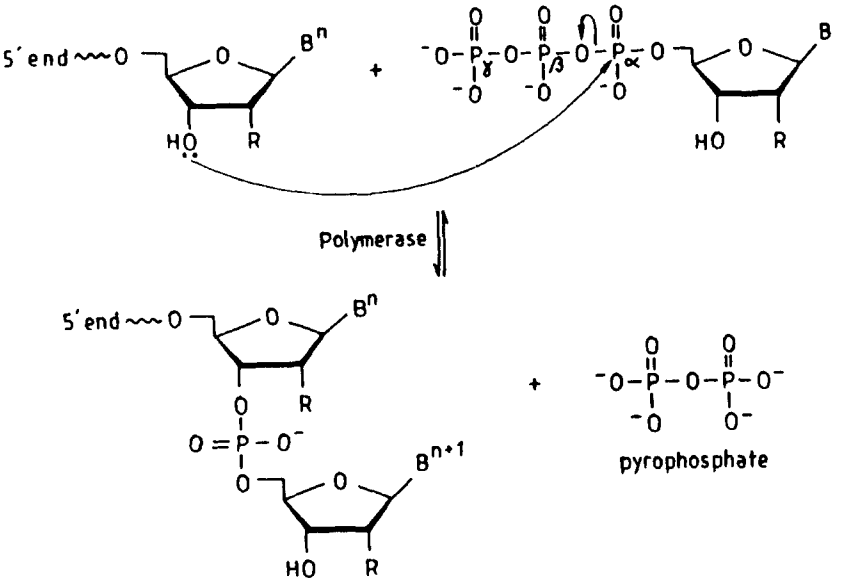


Figure 2. Primary structure of a nucleic acid fragment
(R = OH ==> RNA; R = H ==> DNA)

third class of RNAs called ribosomal RNAs (rRNAs) which constitute for a part their structure. The mechanisms of replication (DNA --> DNA) and of transcription (DNA --> RNA) involve the polymerization of nucleosides (in the form of their triphosphate precursors) (Scheme 1).

Chain elongation is effected by polymerases which catalyse the covalent binding of new triphosphate nucleoside units by their α phosphate at the free 3'-hydroxylated end of the chain with elimination of pyrophosphate. The biosynthesis of nucleoside triphosphates is a vital cellular process which occurs following two different mechanisms: (i) *de novo* pathway



Scheme 1. Mechanism of nucleic acid chain elongation.
(R = OH ⟹ RNA; R = H ⟹ DNA)

from simple precursor metabolites (CO_2, NH_3, formic acid, etc...), (ii) salvage pathway from purine and pyrimidine bases and nucleosides arising from the degradation of nucleic acids or from nutrition. Owing to the fundamental place occupied by nucleotides in cellular life, the conception of their analogues apt to interfere with processes involved in the rapid proliferation of cancer cells or with the multiplication of pathogenic agents, seems to be an attractive approach in chemotherapy. Unfortunately, nucleotides are polyanionic compounds which cannot cross easily through cellular membranes. On the other hand, nucleosides and their analogues are neutral molecules which can enter cells and therefore are able to assume a therapeutic role. However, their biological activity usually depends on their subsequent intracellular phosphorylation by kinases.

The synthesis of nucleoside analogues has been the subject of an extensive development during these last years. The main modifications which, from a natural nucleoside, can lead to analogues are summarized in Figure 3.

In this review, we will not describe the different possible synthetic approaches to nucleoside analogues which have already been the subject of excellent reviews.[6-10] Rather

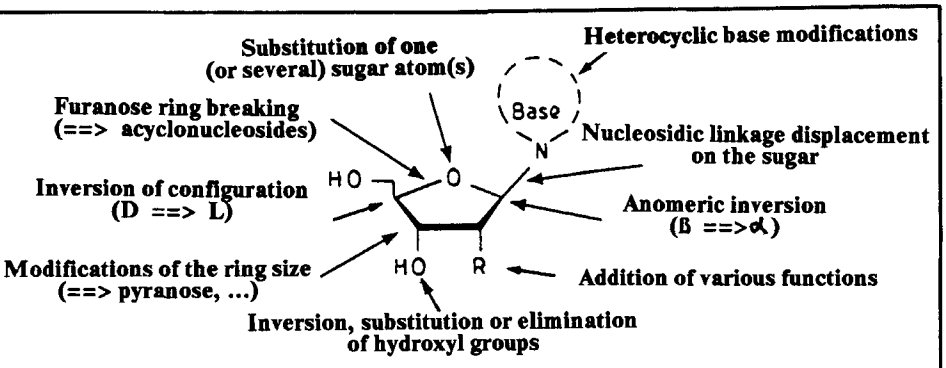


*Figure 3. Main structural modifications susceptible to transform a natural nucleoside (*R = H or OH*) into one of its analogues.*

we will try to evaluate the importance of nucleoside analogues in several established chemotherapies (anticancer, antiviral and antibacterial) and in other attractive fields like immunomodulation or regulation of gene expression which could constitute, in the future, new therapeutic approaches.

NUCLEOSIDE ANALOGUES AND ANTICANCER CHEMOTHERAPY

Malignant cells anarchically grow in spite of the restraints that regulate normal tissue growth (e.g., differentiation, organ size limitation, hormonal regulation). Thus, in these cells the genes coding for differentiation appear to be shut off or inadequately expressed while the genes coding for cell proliferation are expressed when they should not be. Effective anticancer drugs must present some selective toxicity towards malignant cells. In the case of nucleoside analogues, an explanation for their selectivity could be that most of the cancer cells are in cycle, that is they continuously undergo mitosis. Consequently, these cells in cycle are more sensitive to nucleoside analogues (and to their nucleotide metabolites) than resting cells. Nucleoside analogues and modified bases commonly used in anticancer chemotherapy are represented in Figure 4.

Aglycone-modified nucleoside analogues

5-Fluoro-2'-deoxyuridine

Fluorinated pyrimidines and their nucleosides constitute a very important class of antitumour agents,[11] and among them 5-fluorouracil (FUra, Figure 4) and

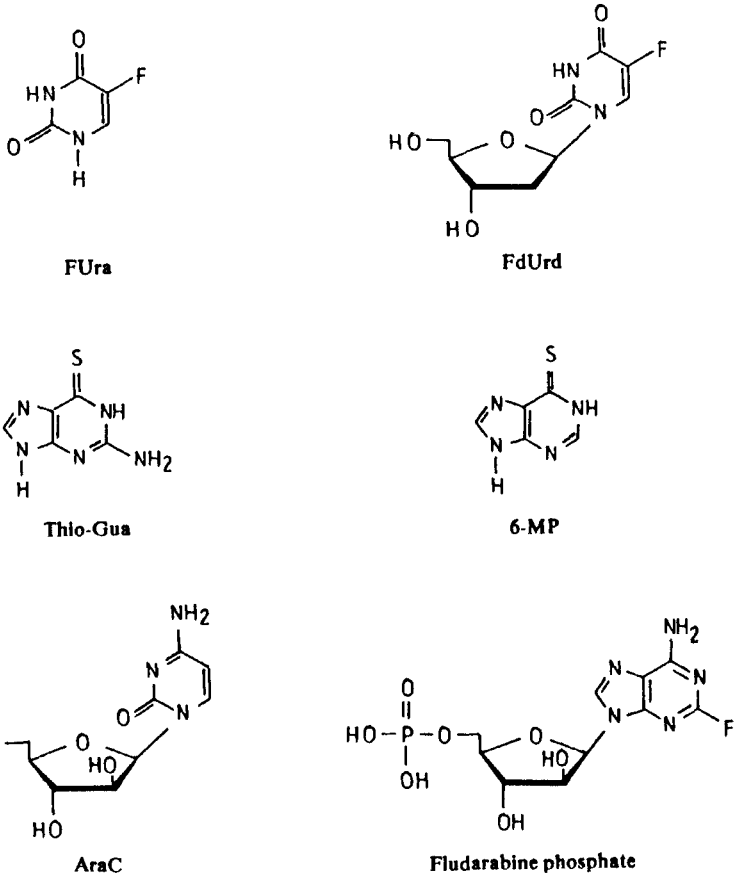
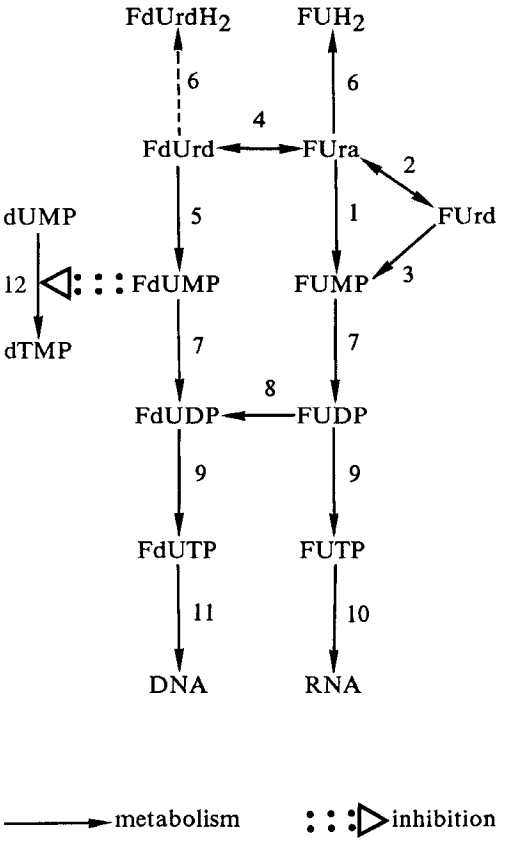


Figure 4. Modified pyrimidine and purine bases and nucleoside or nucleotide analogues licensed for clinical anticancer use.

5-fluorodeoxyuridine (FdUrd, Floxuridine, Figure 4) are the most representative. These compounds are converted to 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP, Scheme 2).[12-15] FdUMP which is the active cytotoxic form of these drugs is a potent inhibitor of thymidylate synthetase.[16] The fraudulent incorporation of the fluorinated aglycone FUra, into DNA[17] and/or RNA[16-18] could be another mode of action.

5-Fluoro-2'-deoxyuridine and 5-fluorouracil are used regularly for the treatment of breast cancer, tumors of the gastrointestinal tract and other solid tumors.[6,12] *In vitro*, the effective



1 Pyrimidine phosphoribosyl transferase.

2 Uridine phosphoribosyl transferase

3 Uridine kinase

4 Deoxyuridine phosphorylase

5 Thymidine kinase

6 Dihydrouracil dehydrogenase

7 Nucleoside monophosphate kinase

8 Ribonucleotide reductase

9 Nucleoside diphosphate kinase

10 RNA polymerases

11 DNA polymerases

12 Thymidylate synthetase

Scheme 2. Metabolism and mechanism of action of 5-fluorouracil and of its nucleosides.

dose of FdUrd is much lower than of FUra, possibly because of its lack of activity as substrate for dihydrouracil dehydrogenase.[13] *In vivo*, FdUrd is commonly given i.v. at 0.5-1.0 mg/kg/day for 6-15 days, whereas FUra is given as a weekly i.v. bolus for 15 mg/kg, which is comparable probably because of the rapid cleavage of FdUrd to FUra after administration.

6-Thioguanine and 6-mercaptopurine nucleosides

6-Thioguanine (Thio-Gua, 6-TG) and 6-mercaptopurine (6-MP) (Figure 4) are 6-thio analogues of the naturally occurring 6-keto purine bases guanine and hypoxanthine,

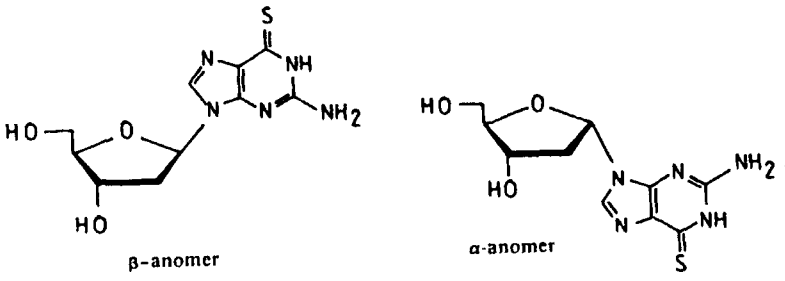


Figure 5. α and β 2'-deoxythioguanosine.

respectively. Their ribosides are metabolized into the corresponding nucleoside 5'-monophosphates which exert several inhibitory actions on *de novo* purine biosynthesis.[13,14,19] These ribonucleotides act as "pseudo"-feedback inhibitors of phosphoribosylpyrophosphate amidotransferase and, in addition, prevent the conversion of inosine 5'-monophosphate (IMP) to the purine nucleotides GMP and AMP. The cytotoxicity of thioguanine and 6-mercaptopurine ribosides is as well correlated with their incorporation into nucleic acids.[13,14,19,20]

Moreover, the anomeric β- and α-2'-deoxythioguanosines (Figure 5) have also been widely studied.[20-26]

It was found that these anomers have carcinostatic effects on murine tumors. The α-anomer is less potent that the β-anomer but it is less toxic for normal cells, apparently because this deoxynucleoside was not phosphorylated to a significant extent in bone marrow cells. In vivo, the low toxicity and the significant activity of α-2'-deoxythioguanosine against an appreciable fraction of human tumors supported the conclusion that this nucleoside analogue is a potential candidate for clinical trials in the treatment of cancer. Surprisingly, no clinical development is known for this compound. Only, 6-thioguanine and 6-mercaptopurine are used in the treatment of acute leukaemias.[12]

Azapyrimidine nucleosides

Azapyrimidine analogues are derived from pyrimidine compounds through the replacement of one of the methine groups with an atome of nitrogen.

5-Azacytidine: the first synthesis of 4-amino-1-(β-D-ribofuranosyl)-1,3,5,-triazin-2-one (5-azaCyd, Figure 6) was reported in 1964. Clinical activity of 5-azaCyd against leukaemias is well documented.[6,14,26,27] 5-Azacytidine acts on a number of important cellular processes

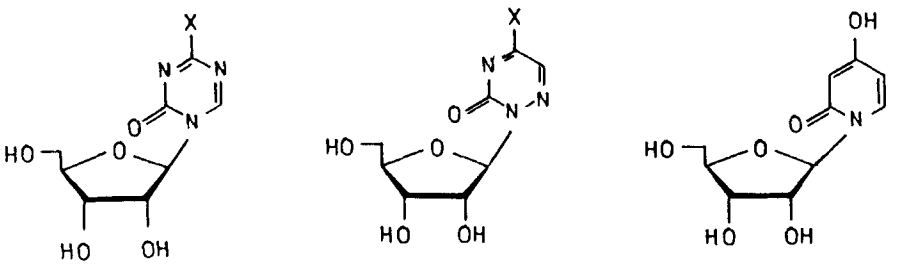


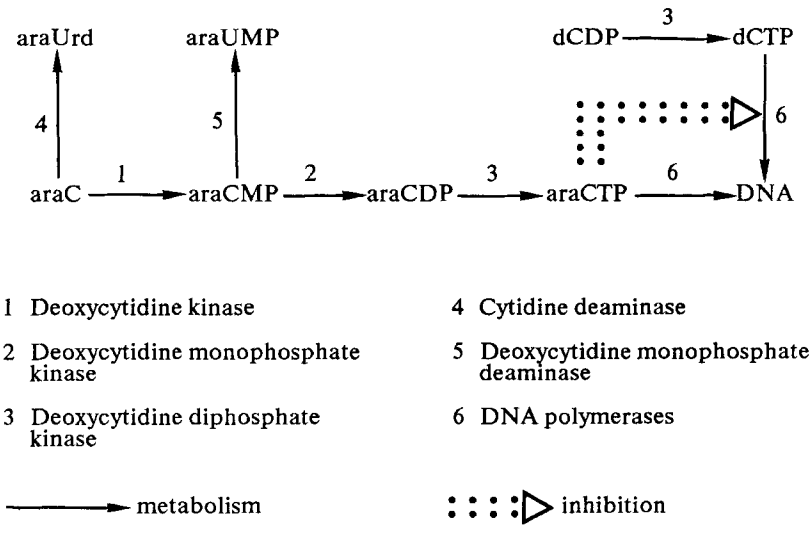
Figure 6. Azapyrimidine nucleosides and 3-deazauridine endowed with antineoplastic properties.

but the degree to which each of its effects contributes to the cytotoxicity is not known. Interference with the *de novo* synthesis of pyrimidine nucleotides[14,27] on the one hand and the formation of fraudulent nucleic acids due to incorporation of 5-azaCyd[14,15,18,26,27] on the other represent the main molecular consequences of exposure to this drug. 5-AzaCyd is deaminated to 5-azauridine (5-azaUrd, Figure 6), but the possible contribution of 5-azaUrd to the observed cytotoxicity is not known.[14,27] The use in anticancer chemotherapy of 5-azacytidine seems to be restricted by serious toxicity problems[6,14] and by the instability of triazine ring system in aqueous solution.[6,14,27]

Other azapyrimidine nucleosides: 6-azacytidine (6-azaCyd, Figure 6) interferes with the *de novo* biosynthesis of pyrimidine nucleotides by inhibiting oritidine 5'-monophosphate decarboxylase. This nucleoside analogue also inhibits the incorporation of cytidine into the RNA of fowl leukemic cells and normal myeloblasts.[27] 6-AzaCyd is deaminated to 6-azauridine (6-azaUrd, Figure 6) which after phosphorylation by uridine kinase, is also an inhibitor of orotidine 5'-monophosphate decarboxylase showing antineoplastic activity.[15,27,28]

3-Deazauridine

3-Deazapyrimidine nucleosides have a methine group in place of the nitrogen atom at the 3 position of the pyrimidine ring. The synthesis of 4-hydroxy-1-(β-D-ribofuranosyl)pyridin-2-one (3-deazaUrd, Figure 6) was reported 20 years ago. 3-DeazaUrd exhibits significant anticancer activity, more particularly against araC-resistant tumors.[13,29] The primary action of this nucleoside analogue, which is not incorporated into nucleic acids, involves its activation to a triphosphate nucleoside which is a potent inhibitor



Scheme 3. Metabolism and mechanism of action of araC.

of CTP synthetase (the unique enzyme for the *de novo* biosynthesis of cytosine metabolites). Other actions of 3-deazaUrd and its nucleotides involve inhibition of several enzymes including cytidine deaminase, deoxycytidylate deaminase and ribonucleotide reductase.[13,29] Clinical trials have shown limited response to 3-deazaUrd as a single agent, but its toxicity is manageable and its combination with other drugs seems to be attractive.[6,13]

Sugar-modified nucleoside analogues

AraC

AraC (1-β-D-arabinofuranosylcytosine, cytosine arabinoside, cytarabine, cytosar, Figure 4) was synthesized in 1959. Acute leukaemias and lymphomas, especially acute non-lymphocytic leukaemia, often respond successfully to araC.[12] The cytotoxic activity of araC is due to its triphosphorylated metabolite araCTP (Scheme 3), and its activation pathway involves the same enzymes required by the physiologic nucleoside deoxycytidine. The cytotoxic effects of araC are believed to be inhibition of DNA polymerase by araCTP,[13-15,28,30,31] and its incorporation into nucleic acids.[6,14,15,28]

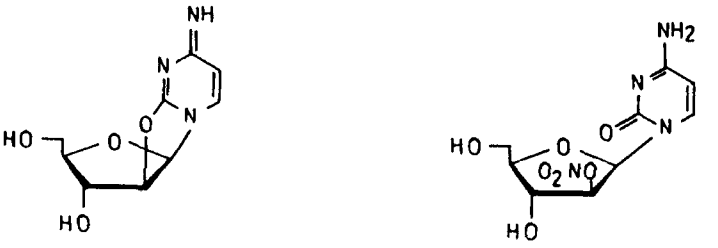


Figure 7. Illustrative prodrugs of araC.

AraC and its 5'-monophosphate derivative are susceptible to degradation by cytidine deaminases, enzymes found in liver, gastrointestinal tract, plasma, and some malignant cells. The products of deamination are inactive in terms of cytotoxicity and one mechanism of araC resistance in human leukaemias is associated with high levels of deaminase activity.[13-15,30] As a consequence, araC has a very short plasma half-life which necessitates continuous intravenous infusion in man to maintain plasma levels and to provide maximum therapeutic efficacy. In an effort to circumvent the scheduling problems of araC therapy and also to minimize the effects of deaminases a variety of prodrugs of araC have been prepared and evaluated.[6,32] Among them, illustrative examples are 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine (cyclocytidine[6,26,30,32,33], Figure 7) and 2'-O-nitro-1-(β-D-arabinofuranosyl)cytosine (2'-O-nitroaraC[34], Figure 7) which are resistant to the action of deoxycytidine deaminase and undergo spontaneous hydrolysis in aqueous systems to give araC. However, on the one hand initial trials have not supported the need for further investigation with cyclocytidine, on the other hand 2'-O-nitroaraC has never, to our knowledge, been tried clinically.

Other nucleotide and nucleoside analogues (For a very recent review, see : Plunkett, W.; Saunders, P.P. *Pharmac. Ther.*, 1991, **49**, 239-268).

Notably, fludarabine phosphate (Figure 4) has been approved by the Food and Drug Administration (FDA) for sale for the treatment of human leukaemias. Other nucleoside analogues with demonstrated anticancer activity are the 2-haloadenine 2'-deoxy-ribonucleosides and 2-chloroadenine arabinonucleoside. Of these, 2-chloro-2'-deoxyadenosine has shown significant activity in humans, as has 2'-deoxycoformycin. Potentially useful nucleoside analogues for cancer treatment include also the 2-halo-2'-fluoroarabinonucleosides (Parker, W.B.; Shaddix, S.C.; Chang, C.H.; White, E.L.; Rose, L.M.; Brockman, R.W.; Shortnacy, A.T.; Montgomery, J.A.; Secrist, J.A. III; Bennett, L.L. Jr *Cancer Res.*, 1991, **51**, 2386-2394).

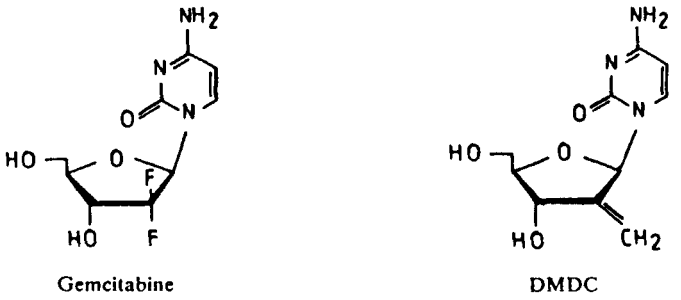
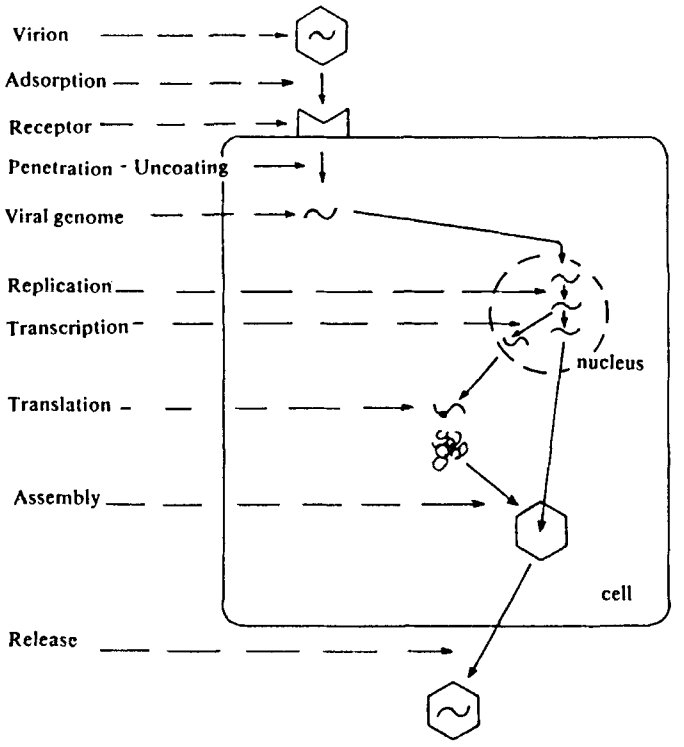


Figure 8. New antineoplastic sugar-modified cytidine analogues.

Today, chemotherapy remains one of the hopes to control the mechanisms involved in the growth of cancer cells. Cytotoxic drugs have reduced mortality and restored many cancer patients to normal, and the emergence of new antineoplastic nucleoside analogues like successful clinically tried 2',2'-difluorodeoxycytidine (dFdCyd, gemcitabine, Figure 8)[35-37] and the more recently synthesized 2'-deoxy-2'-methylidenecytidine (DMDC, Figure 8)[37-39] appears as a promising prospect in the treatment of cancers.

NUCLEOSIDE ANALOGUES AND ANTIVIRAL CHEMOTHERAPY

Until the beginning of the 1960's the problem of distinguishing viral functions from cellular ones was thought to be insurmontable, and the main strategy for controlling viral infections was (and to a large extent it still is) the development of vaccines, which do not attack a virus directly but forestall infection by stimulating the immune system in advance. Over the past two decades, however, accumulating knowledge of viral replication[40] has made it possible to define specific targets which could be affected by antiviral agents.[41] The virus replicative cycle (Scheme 4) consists in: (i) adsorption of the virion to the cell membrane (generally on specific host cell-receptors), (ii) penetration and uncoating, (iii) replication of the viral genome and protein synthesis, (iv) assembly of macromolecules into a virion, and finally (v), release of virions from the cell. Any drug interfering selectively with one of these events is a candidate for clinical use. In fact, the main targets of antiviral nucleoside analogues are intracellular biosynthetic events, and their selectivity is generally due to the inhibition of virus-associated or induced enzymes involved in nucleoside and nucleotide metabolism.



Scheme 4. Oversimplified virus replicative cycle.

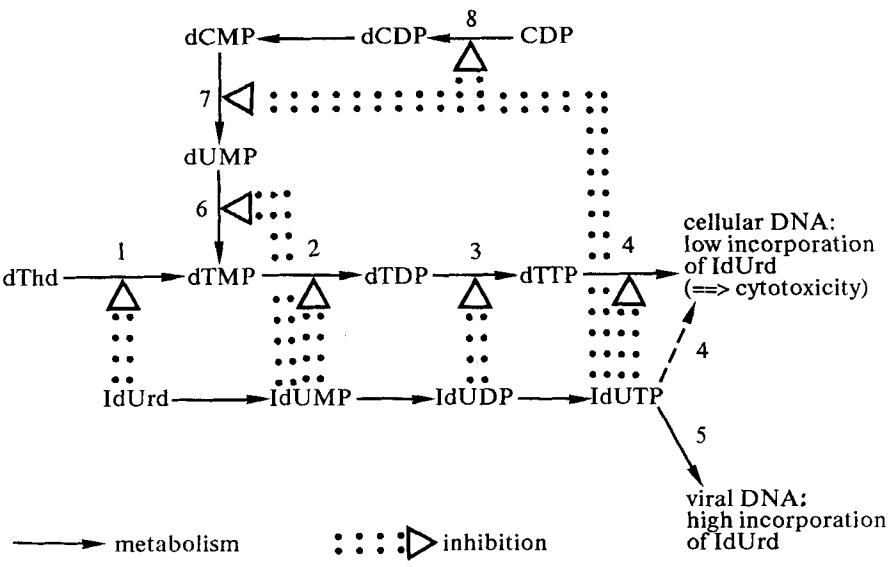
NUCLEOSIDE ANALOGUES ACTIVE AGAINST DNA VIRUSES

The main human pathogenic DNA virus families include herpesvirus, poxvirus (e.g., vaccinia, smallpox) and adenovirus. Antiviral nucleoside analogues exhibit principally activity against herpesvirus including the herpes simplex virus (HSV) types 1 and 2 (causing "cold sores", encephalitis, eye and genital infections), the varicella zoster virus (VZV, causing chickenpox and shingles), the cytomegalovirus (CMV, causing pneumonia and central nervous system diseases), and the Epstein-Barr virus (EBV, which causes mononucleosis *i.e.* glandular fever).

Aglycone-modified nucleoside analogues

5-Iododeoxyuridine

Synthesized in 1959, 5-iododeoxyuridine (IdUrd, IDU, idoxuridine, iduviran, Figure 1) was the first clinically effective antiviral nucleoside analogue. IdUrd is used in the topical



1,2,3: cellular kinases; 4: cellular DNA polymerase; 5: viral DNA polymerase; 6: thymidylate synthetase; 7: dCMP deaminase; 8: CDP reductase.

Scheme 5. Metabolism and mechanism of action of 5-iododeoxyuridine.

treatment of herpetic keratitis and ocular herpes virus infections.[42,43] The mechanism of action of IdUrd involves its activation to its triphosphate derivative (IdUTP) and its incorporation in newly synthesized viral DNA (Scheme 5). Possible explanations for the direct effects of IdUrd incorporation into viral DNA on virus replication have been suggested:[42] (i) high mutation rate for the viral genome during DNA synthesis, (ii) high susceptibility to deoxyribonucleases, (iii) imbalance of gene expression, (iv) distortion of the double-stranded viral DNA, (v) sensitization to ultraviolet light.

In addition to the inhibitory effects related to its incorporation into viral DNA, IdUrd and its phosphorylated metabolites can also affect a number of enzyme systems including thymidine kinase, thymidine monophosphate kinase, deoxycytidine monophosphate deaminase, cytidine diphosphate deaminase, cytidine diphosphate reductase and DNA polymerase.[42,44,45] Since the use of IdUrd for treatment of systemic herpes infections is limited by its toxicity (due to its concomitant incorporation in cellular DNA) and also by rapid development of drug resistance, safer and more effective antiviral agents that possess greater selectivity have been developed for clinical use.

Trifluoromethylthymidine

The first synthesis of trifluoromethylthymidine (5-trifluoromethyl-2'-deoxyuridine, F_3dThd, CF_3dUrd, TFT, viroptic, virophta, triherpine, trifluridine, Figure 1) was reported in 1964. F_3dThd has been approved for topical use in herpetic eye infections. It is considered to be superior to IdUrd in the treatment of herpetic ocular diseases and it is effective against IdUrd-resistant herpesvirus.[42] The main basis for F_3dThd antiviral effects appears to be its preferential incorporation into viral DNA and a subsequent inhibition of the late transcription. Other actions of F_3dThd and its nucleotides include inhibition of several enzymes, like deoxythymidine kinase, deoxythymidylate synthetase and DNA polymerase.[42,45] As for IdUrd, cytotoxic effects produced by F_3dThd are related to its concomitant incorporation into uninfected cell DNA. Potent and irreversible inhibition of thymidylate synthetase is also probably involved in its toxicity.[42,45]

Other 5-substituted pyrimidine nucleoside analogues

5-Ethyldeoxyuridine (EDU, Figure 9): this compound has been marketed (as Acedurid) for the topical treatment of herpetic keratitis, and clinical trials have been initiated in the topical treatment of genital herpes.[4,46-48] In contrast with 5-iododeoxyuridine and trifluoromethylthymidine, which have been in clinical use for almost 25 years despite their well-established mutagenicity, EDU is non-mutagenic (although it has been reported to be incorporated into the DNA of uninfected mouse and human cells). This compound has a much higher affinity for the herpesvirus-induced deoxythymidine kinase (dTK) than for the cellular dTK[42] and it has been shown that, within the HSV-1-infected cell, EDU is incorporated to a larger extent into viral DNA.[46,49]

(E)-5-(2-Bromo- and 2-iodovinyl)deoxyuridine (BVDU and IVDU, Figure 9): BVDU and its iodovinyl analogue IVDU emerged as the most potent and most selective inhibitors of HSV-1 and VZV.[42,43,46,49-53] BVDU offers attractive perspectives for the peroral treatment of HSV-1 and VZV infections in immunosuppressed patients and for topical treatment of herpetic eye infections. The mechanism of action of this compound involves its preferential phosphorylation by HSV-1 and VZV-encoded kinases which convert BVDU successively to its 5'-monophosphate and 5'-diphosphate derivatives. Once it has reached the 5'-triphosphate stage by a cellular kinase, BVDU may either inhibit viral DNA polymerases or serve as substrate and be incorporated into viral DNA.[42,44,46,51,53,54]

5-(2-Chloroethyl)deoxyuridine (CEDU, Figure 9): although this compound appears to be effective at a 5 to 15-fold lower dose than BVDU in the treatment of systemic HSV-1 infection in mice,[43,46] its clinical potential seem to be limited by its mutagenic properties.[46]

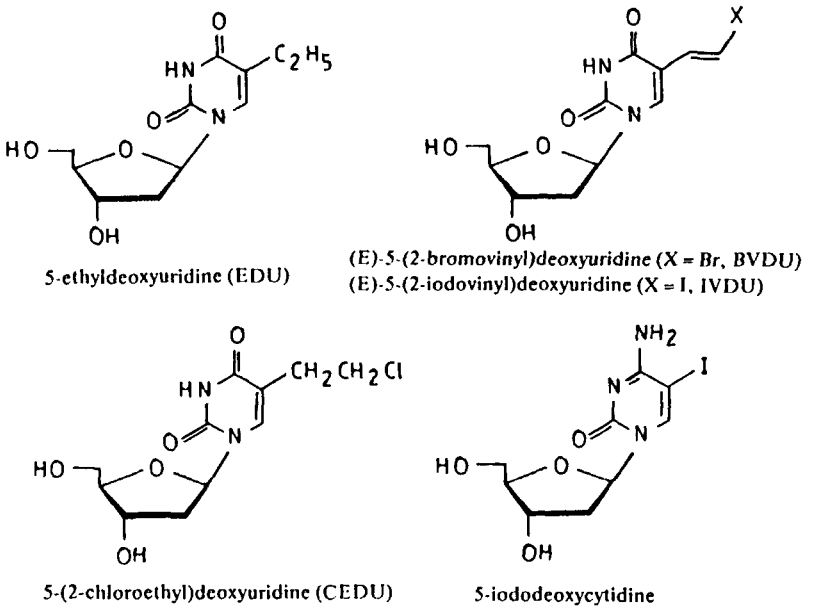
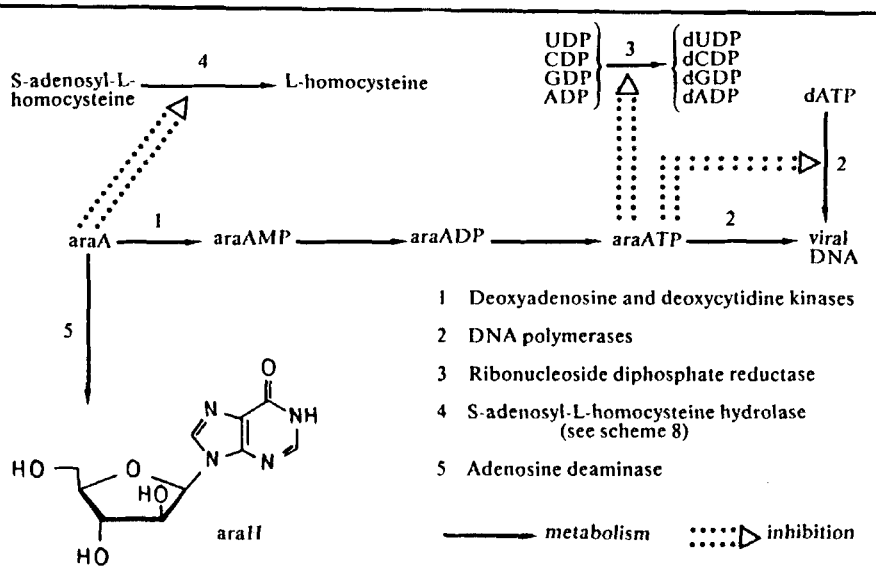


Figure 9. 5-Substituted pyrimidine nucleoside analogues endowed with significant antiviral activities.



Scheme 6. Metabolism and mechanism of action of araA.

5-Substituted deoxycytidines: the antiviral spectrum of 5-substituted deoxycytidine derivatives is similar to their 5-substituted dUrd derivatives to which they are converted by enzymatic deamination.[42,52] Some of them, like 5-iododeoxycytidine (Figure 9, marketed in France as Cebeviran or Cuterherpes), have emerged as potent and selective inhibitors of HSV-1 replication. As a rule, 5-substituted deoxycytidine analogues are equally or slightly less potent, but markedly more selective in their antiherpes activity than the corresponding 5-substituted deoxyuridine analogues,[42,51,52] probably because of their preferential use as substrates by the virus-induced enzymes.[42]

Sugar-modified nucleoside analogues

AraA

9-β-D-Arabinofuranosyladenine (araA, vidarabine, vira-A, Figure 1) was first synthesized in 1960 and is used in the treatment of herpetic keratitis and encephalitis.[55,56] In herpesvirus-infected cells, araA treatment results in the selective inhibition of virus replication. Thus araA is metabolized to its 5'-triphosphate (araATP), which can:[42,57,58] (i) inhibits the virus-specific DNA polymerase, (ii) inhibits the virus-specific ribonucleoside diphosphate reductase, (iii) allows incorporation of araA into viral DNA (Scheme 6).

Although this nucleoside analogue inhibits S-adenosyl-L-homocysteine hydrolase (a key enzyme in transmethylation reactions), such effects may contribute primarily to the cytotoxicity of the drug.[59] The main disadvantages to the clinical use of araA include its extremely low water solubility and its *in vivo* rapid deamination to 9-β-D-arabinofuranosylhypoxanthine (araH, Scheme 6) by adenosine deaminase.

Since araH is considerably less potent than araA, attempts have been made to develop araA derivatives that are deaminase-resistant. Thus, its 5'-phosphate and its carbocyclic analogue (cyclaradine, Figure 10, prepared in 1977) are adenosine deaminase-resistant, hydrolytically stable compounds that have demonstrated significant activity against certain DNA viruses *in vitro* and herpes simplex viruses types 1 and 2 *in vivo*.[42,43]

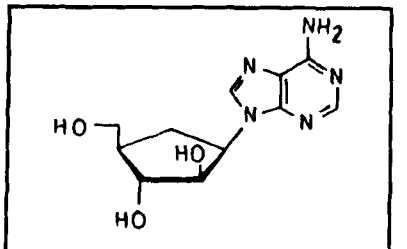
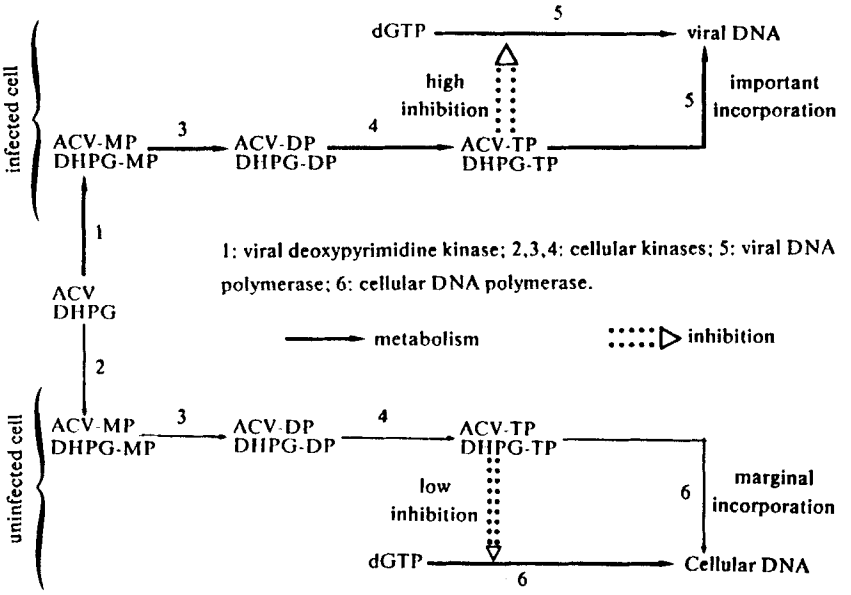


Figure 10. Structure of cyclaradine.

Acyclonucleosides

Significant progress has been made in the development of antiviral chemotherapy owing to the discovery of several acyclonucleosides endowed with potent activities and two of them (acyclovir and DHPG, Figure 1) have been marketed as antiherpetic drugs.



Scheme 7. Mechanism of action of acyclovir and ganciclovir against herpes simplex viruses-1 and -2.

Acyclovir: the synthesis and antiviral activity of 9-(2-hydroxyethoxymethyl)guanine (acyclovir, acycloguanosine, ACV, Figure 1) was first reported by Elion et al in 1977.[60] Major indications for the clinical use of acyclovir are the treatments of HSV and VZV infections. Acyclovir is also given prophylactically and increases the time between symptomatic recurrences of genital herpes. The high antiviral potency and selectivity of acyclovir result from:[42,43,47] (i) its better substrate specificity for the herpes virus-induced deoxypyrimidine kinase rather than for the cellular deoxythymidine and purine kinases, (ii) its much greater inhibition of the virus-induced DNA polymerase rather than of cellular DNA polymerase, (iii) its selective incorporation into viral DNA, where it acts as a chain terminator (Scheme 7).

However, acyclovir suffers from a number of drawbacks such as (i) a poor solubility in water, (ii) a low oral adsorption, (iii) a narrow activity spectrum, essentially confined to HSV and VZV and excluding such important pathogens as CMV, (iv) emergence of acyclovir-resistant mutants.

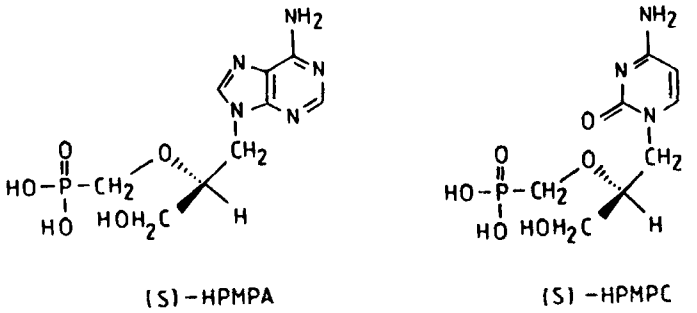


Figure 11. Structures of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)-adenine and cytosine.

DHPG: the synthesis and antiviral activity of 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, 2'-Nor-2'-deoxyguanosine, 2'NDG, BIOLF-62, BW B759U, ganciclovir, cytovene, Figure 1) was reported originally by Martin et al.,.[62,63] Field et al.[64] and Ogilvie et al.[65,66] This compound has been introduced in the clinic for the treatment of severe CMV infections, though it is also active against other herpes viruses for which its mode of action is quite similar to that of acyclovir (Scheme 7). The antiviral activity of DHPG against CMV, a virus which does not induce thymidine kinase activity, seems to involve its phosphorylation in virus-infected cells by cellular kinases and a potent and selective inhibition of the viral DNA polymerase.[67] The clinical development of DHPG suffers from poor absorption[68] and seems to be confined to severe CMV infections due to toxic effects of the drug.

Phosphonylmethoxyalkyl purines and pyrimidines: these acyclonucleoside analogues exhibit a potent and selective activity against adenovirus, herpesvirus and poxvirus.[71] The prototypes of this novel class of antiviral agents are (S)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)adenine [(S)-HPMPA] and -cytosine [(S)-HPMPC] (Figure 11).

HPMPA was found to be active *in vitro* against a variety of DNA viruses including HSV types 1 and 2, CMV, VZV, EBV, adenovirus, and against a retrovirus (Maloney sarcoma virus).[72-74] *In vivo*, its activity against HSV type 1 was demonstrated following i.p. and topical administrations.[72-74] The mechanism of action of HPMPA involves two steps of phosphorylation giving a triphosphate derivative which then inhibits viral DNA polymerase.[75] Contrary to acyclovir, the metabolization of HMPA is not dependent on virus-specified enzymes. That explains why HPMPA is active against CMV and thymidine kinase deficient mutants of herpesvirus.[71]

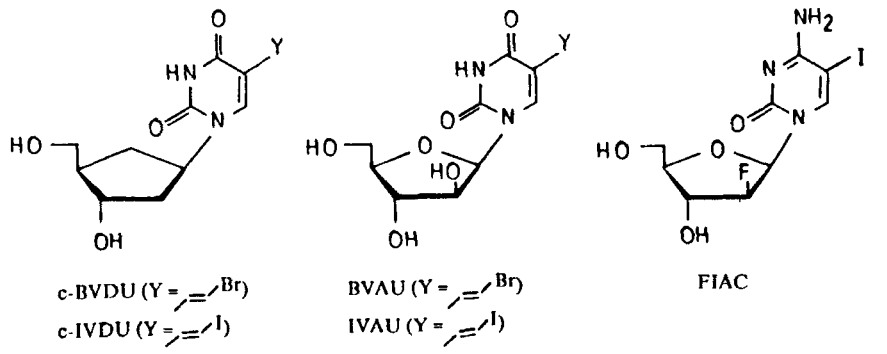


Figure 12. Sugar-modified 5- substituted pyrimidine nucleoside analogues endowed with significant antiviral activities.

On the other hand, (S)-HPMPC is *in vitro* a more potent and selective inhibitor of CMV replication than DHPG.[76,77] *In vivo*, HPMPC is also more effective than acyclovir in the topical treatment of HSV type 1.[77]

Sugar-modified 5-substituted pyrimidine nucleoside analogues

A general characteristic of most, if not all, 5-substituted 2'-deoxyuridines is their propensity to serve as substrate for pyrimidine nucleoside phosphorylases. As a consequence, 5-substituted 2'-deoxyuridines such as EDU, BVDU, CEDU (Figure 9) are converted to their aglycone following cleavage of the N-glycosidic linkage. Resistance to phosphorolytic degradation can be introduced by replacement of the ring oxygen atom with a methylene group (thus leading to carbocyclic analogues[46,49,78]; see also: Shealy, Y.F.; O'Dell, C.A.; Shannon, W.M.; Arnett; G. *J. Med. Chem.*, 1984, **27**, 1416-1421, and Marquez, V.E.; Lim, M.-I. *Med. Res. Rev.*, 1986, **6**, 1-40) or by inversion of the furanose 2'-hydroxylic group (thus leading to the arabinofuranose configuration)[46,48,49] (Figure 12).

The potency, selectivity and activity spectra of these derivatives are quite similar to those of their parent compounds and usually involve inhibition of viral DNA polymerase by their 5'-triphosphate active forms.[42,46]

Other sugar-modified 5-substituted pyrimidine nucleoside analogues have been synthesized. Among them, several 2'-fluoro-5-substituted arabinofuranosylpyrimidines (as 2'-fluoro-5-iodo-β-D-arabinofuranosylcytosine or FIAC, Figure 12) have been found to be potent and selective inhibitors of herpesvirus replication both *in vitro* and *in vivo*.[42,46,48,52,54] However, toxicity problems encountered with these compounds has so far precluded their general use in humans.

NUCLEOSIDE ANALOGUES ACTIVE AGAINST RNA VIRUSES

The major respiratory diseases in humans are caused by RNA viruses like orthomyxovirus (influenza virus), paramyxovirus (respiratory syncytial virus, parainfluenza virus), and picornavirus (rhinovirus). RNA viruses are implicated in a variety of other diseases. It is the case of rotaviruses which have been recognized as the single most important causative agents of acute diarrhoea, of haemorrhagic fever viruses (*i.e.* Lassa, Junin, Machupo) which rank among the most deadly pathogens and are difficult to control by vaccination, and of hepatitis B virus which counts 200 million carriers in the world, of which 40 million may die from cirrhosis and another 10 million from hepatocarcinoma.

Ribavirin

Ribavirin or 1-β-ribofuranosyl-1,2,4-triazole-3 carboxamide (virazole, Figure 1) is a broad-spectrum antiviral agent active against both DNA and RNA viruses. Its major clinical potentials lie in the treatment of respiratory syncytial virus (RSV) and influenza A or B virus infections, where it can be administered as a small-particle aerosol. Ribavirin is also effective in the therapy of Lassa fever and other haemorrhagic fever virus infections. This compound is rapidly monophosphorylated by cellular adenosine kinase and then further phosphorylated to its 5'-triphosphate presumably by cellular enzymes. The antiviral action of ribavirin[42,46,52,54,57] is correlated with the inhibition by its 5'-monophosphate derivative of inosine monophosphate dehydrogenase, resulting in a depletion of the cellular guanosine triphosphate (GTP) pool. This lowering of cellular GTP is a "self-potentiating" effect since GTP competes with ribavirin 5'-triphosphate in the inhibition of viral-specific RNA polymerases. With certain RNA and DNA viruses, the 5'-triphosphate of ribavirin also inhibits the viral-specific mRNA capping enzymes, including guanylyl transferase and N^7-methyltransferase, and that explains why ribavirin has some selectivity towards influenza.

Adenosine analogues[78]

Several adenosine analogues (Figure 13) exhibit a broad-spectrum antiviral activity including RNA viruses. The remarkable similarity in the antiviral spectrum of these compounds points to a common mechanism of action, and the target has been identified as S-adenosyl-L-homocysteine hydrolase (Wolfe, M.S.; Borchardt, R.T. *J. Med. Chem.*, 1991, **34**, 1521-1530), a key enzyme involved in the methylation of many biomolecules using S-adenosyl-L-methionine as a methyl donor (Scheme 8). The inhibition of S-adenosyl-L-homocysteine hydrolase leads to an accumulation of S-adenosyl-L-homocysteine which inhibits transmethylation reactions by a feedback mechanism (Scheme 8). Viral mRNAs that depend on such methylations for their maturation are unfunctional.

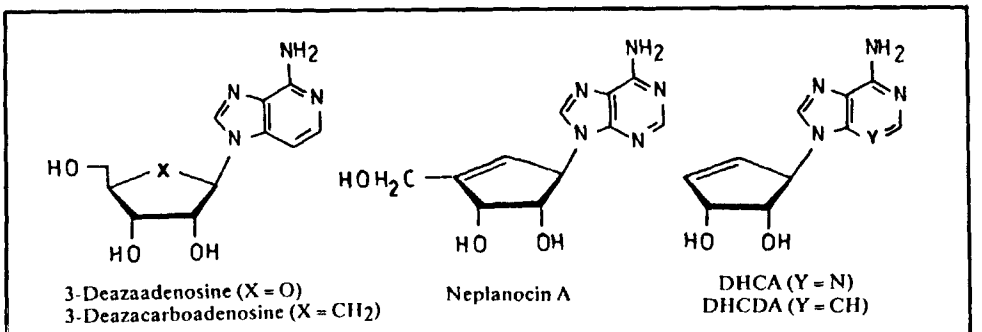
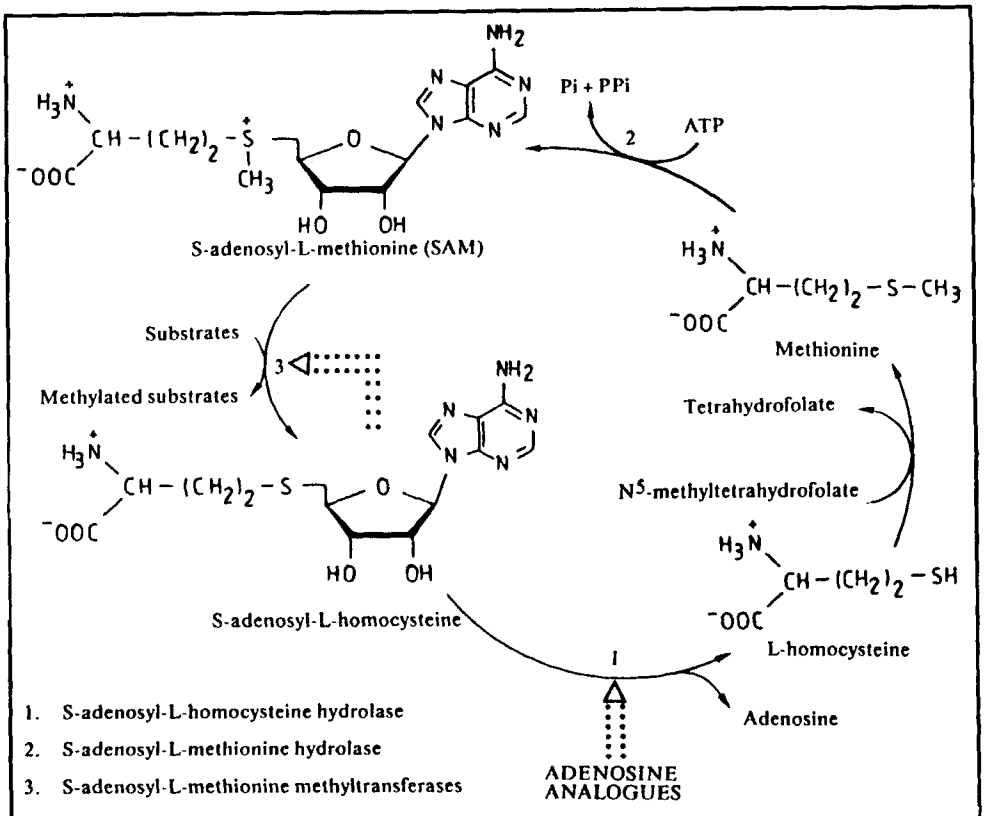
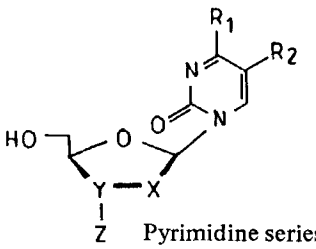


Figure 13. Adenosine analogues active against RNA viruses.

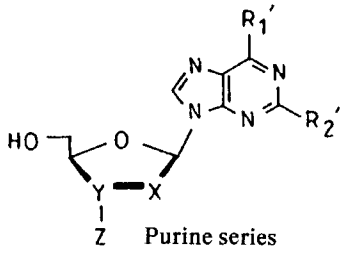


Scheme 8. Mechanism of action of adenosine analogues and consequent inhibition of biological methylations (⟵ metabolism, ◁::: inhibition).

Table 1. *Comparative anti-HIV-1 activity of 2',3'-dideoxynucleosides in MT-4 cell cultures.*[83]



Pyrimidine series



Purine series

	Compound	R_1 ou R_1'	R_2 ou R_2'	Y-X / Z	ED_{50} [a] (μM)	CD_{50} [b] (μM)	SI [c]
Pyrimidine series	**AZT**	OH	CH_3	CH-CH_2 / N_3	> 0,004 < 0,013	> 5,2 < 8,0	> 150 <2000
	AzddUrd	OH	H	CH-CH_2 / N_3	0,36	244	677
	AzddMeCyd	NH_2	CH_3	CH-CH_2 / N_3	1,8	> 1000	> 555
	ddThd	OH	CH_3	CH_2-CH_2	0,2	> 125	> 625
	ddCyd	NH_2	H	CH_2-CH_2	0,06	37	616
	ddeThd	OH	CH_3	CH=CH	0,05	19	380
	FddThd	OH	CH_3	CH-CH_2 / F	0,001	0,197	197
Purine series	**AzddGuo**	OH	NH_2	CH-CH_2 / N_3	1,4	190	136
	AzddDAP	NH_2	NH_2	CH-CH_2 / N_3	0,3	44	147
	ddIno	OH	H	CH_2-CH_2	10	> 500	> 50
	ddAdo	NH_2	H	CH_2-CH_2	2,5	> 250	> 100

a Fifty percent effective dose, based on the inhibition of HIV-1-induced cytopathogenicity in MT-4 cells.

b Fifty percent cytotoxic dose, based on the reduction of the viability of mock-infected MT-4 cells.

c Ratio of CD_{50} to ED_{50}.

However, whatever the potency of their antiviral activity is none of these adenosine analogues has been introduced in medical practice at this time.

NUCLEOSIDE ANALOGUES ACTIVE AGAINST RETROVIRUSES

To date, two important pathogenic human retrovirus have been discovered, namely human T-cell leukaemia virus (HTLV)[80] and human immunodeficiency virus (HIV) which is the etiologic agent of acquired immune deficiency syndrome (AIDS).[81,82]

AZT

3'-Azido-2',3'-dideoxythymidine (azidothymidine, AZT, AzddThd, BW A509U, retrovir, zidovudine, Figure 1) is the only drug approved for the treatment of patients with AIDS and ARC (AIDS-related complex). Its mechanism of action depends on its phosphorylation by cellular enzymes to its 5'-triphosphate derivative. The latter then has the dual ability to act as a competitive inhibitor or alternate substrate of HIV reverse transcriptase, the incorporation of AZT in viral DNA leading to chain termination.[83-86] Despite its activity, AZT has to be administered with caution because severe side effects, particularly bone marrow suppression (involving anaemia and leukopenia) may develop in a subset of patients.[83,87-89] Moreover, AZT-resistant HIV strains have been recently isolated from AIDS patients.[90,91] In view of the shortcomings of AZT an extensive search for new anti-AIDS drugs has been initiated.

Other 2',3'-dideoxynucleosides

Various 2',3'-dideoxynucleoside analogues (Table 1) have been described which show an anti-HIV potency and selectivity,[83,92,93] and among them 2',3'-dideoxyinosine (ddIno, Table 1) has recently been approved by the FDA for therapy of AIDS. The mechanism of action of these 2',3'-dideoxynucleosides is quite similar to that of AZT, *i.e.* inhibition of viral reverse transcriptase by their 5'-triphosphate.

Carbovir (C-2',3'-didehydro-2',3'-dideoxyguanosine, C-D4G, NSC 614846, Figure 14), another 2',3'-dideoxynucleoside but a carbocyclic one, is also a potent and selective inhibitor of HIV-1 in different cell lines.[92,94]

Its marked antiviral synergistic effect when used in combination with AZT demonstrated *in vitro* against HIV-1 seems to justify further developments.[94]

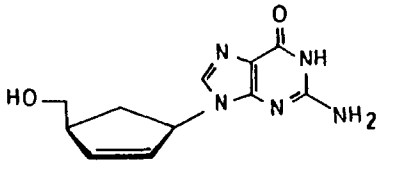


Figure 14. Structure of carbovir.

Other nucleoside analogues

In addition to the 2',3'-dideoxynucleosides, several members of new structural classes of nucleoside analogues (Figure 15) are endowed with a potent and selective anti-HIV activity. Representative compounds are iso-ddA, 9-(2-phosphonylmethoxyethyl)adenine (PMEA), carbocyclic oxetanocin analogues (cyclobut-A, cyclobut-G), allenic (adenallene and cytallene) and oxolane (NGPB-21, DDIII-30A) derivatives of purines and pyrimidines.[94]

It is important to point out that the difficulty with the development of new drugs for AIDS is there is no animal test system for their evaluation; and hence there is no good way to eliminate compounds that are only active at toxic levels. Lack of cytotoxicity to the cells in culture is not a reliable criterion. On the other hand, most of the new compounds found active in the cell culture test systems are likely to be too toxic at effective doses in humans, or for one reason or another to show little activity in humans.

Recently, a series of novel acyclouridine derivatives substituted at both the C-5 and C-6 positions have been recently reported as selective anti-HIV-1 agents.[71,95-99] The prototype of this class of compounds is HEPT,[95] but 1-(2-ethyloxymethyl)-5-ethyl-6-(3,5-dimethylphenylthio)uracil (Figure 16) is at the present time the most potent and selective derivative.[96] The basis for the unique specificity of these analogues as HIV-1 inhibitors resides in a specific interaction with HIV-1 reverse transcriptase.[71,98] However, the HEPT derivatives do not need to be phosphorylated by cellular kinases to exhibit this activity.[100]

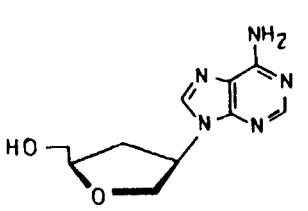
Nucleoside analogues have improved the treatment of several viral infections like herpesvirus and RSV infections. The discovery of AZT as an anti-HIV drug has increased their importance in antiviral chemotherapy, and extensive search for novel nucleoside analogues remains one of the most promising approaches in the curative treatment of viral infections and improvement of AIDS (or ARC) patient conditions.

NUCLEOSIDE ANALOGUES AND ANTIBIOTHERAPY

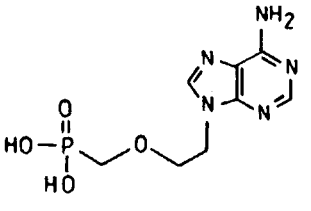
Nucleoside antibiotics[101-105] are by definition produced by microorganism fermentation, and most of them have many biologic activities including anticancer, antiviral and antiparasitic activity. However, in this section we will discuss mainly on nucleoside antibiotics which are more or less selectively toxic for bacteria and fungi.

SIMPLE NUCLEOSIDE ANTIBIOTICS

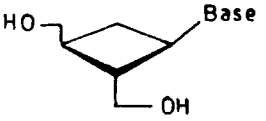
Simple nucleoside antibiotics (Figure 17) can be subdivided into different groups depending on whether the structural modifications concern the sugar (psicofuranine), the



Iso-ddA

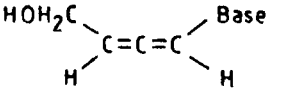


PMEA



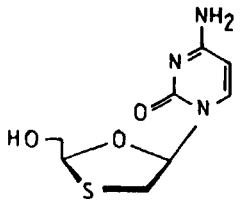
Cyclobut-A (Base = Adenine)

Cyclobut-G (Base = Guanine)

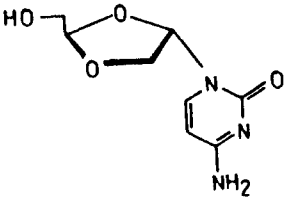


Adenallene (Base = Adenine)

Cytallene (Base = Cytosine)

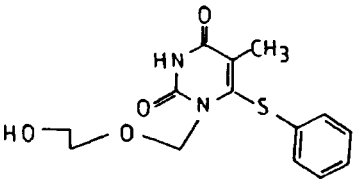


NGPB-21



DD III 30 A

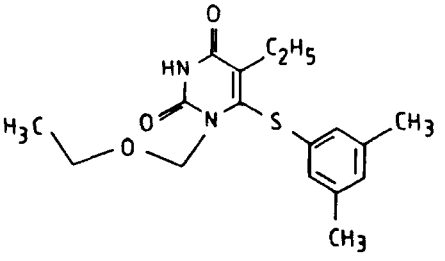
Figure 15. New reported anti-HIV nucleoside analogues.



HEPT

ED_{50} (μM) 7.0; CD_{50} (μM) 740

S.I. 106



ED_{50}(μM) 0.0044; CD_{50} (μM) > 100

S.I. > 22 700

Figure 16. Inhibition of HIV-1 replication in MT-4 cells by HEPT and one of its analogues.[96]

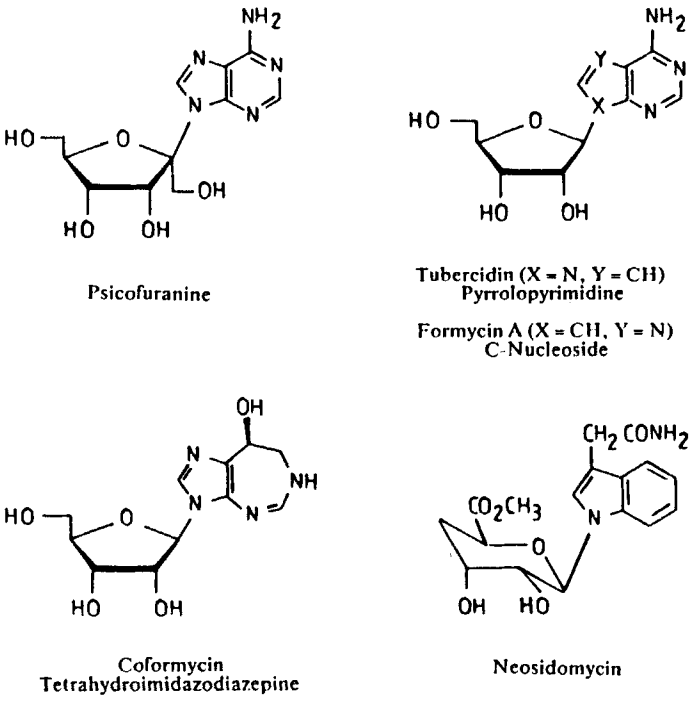


Figure 17. Illustrative simple nucleoside analogues endowed with antibacterial and antifungal activities.

aglycone (tubercin, coformycin and formycin A), or the sugar and the aglycone (neosidomycin).

Simple nucleoside antibiotics often act as inhibitors of nucleic acid synthesis. Psicofuranine (9-β-D-psicofuranosyladenine, angustmycin C) is a naturally occurring adenine-ketose nucleoside isolated from the culture filtrates of *Streptomyces hygroscopicus var. decoyicas*.[102] Pharmacologically, this compound is an important inhibitor of GMP synthetase and hence causes significant reduction in guanylic acid biosynthesis.[106,107] Unlike most bioactive nucleosides, activity of psicofuranine does not depend on a metabolic conversion to its corresponding nucleotides, and experimental evidence suggests that it binds to the enzyme at a regulatory site as a free nucleoside.[106,107] Tubercidin (7-deazaadenosine, a constituent of the culture filtrates of *S. tubercidicus*) is an analogue of adenosine which can be phosphorylated in microorganisms and red blood cells. Tubercidin and its nucleotides inhibit purine synthesis *de novo*, rRNA processing, methylation of tRNA, protein and nucleic acid synthesis, causing visible nuclear damage.[101,102] The inhibition of bacterial cells by tubercidin can be attributed to faulty regulation of phosphofructokinase

(blocking glucose utilization) by its 5'-triphosphate derivative.[102] Formycin A (8-aza-9-deazaadenosine) is also phosphorylated enzymically to its 5'-mono-, di- and triphosphates, the latter is a substrate for amino acyl-tRNA synthetase.[101,102] Psicofuranine, tubercidin and formycin A all have also shown anticancer activity in experimental animal systems, and tubercidin has been used topically effectively in humans. Coformycin, or (R)-3-(β-D-*erythro*-pentofuranosyl)-3,4,7,8-tetrahydroimidazo-[4,5-d][1,3]diazepin-8-ol, is a potent inhibitor of adenosine deaminase.[101,102] Coformycin alone does not exhibit antibacterial activity but shows a strong synergistic effect with formycin for the inhibition of the growth of bacteria.[102,105] Finally, neosidomycin, an indole nucleoside produced by *S. hygroscopicus* is an inhibitor of Gram-negative bacteria.[105]

ACYL AND GLYCOSYL NUCLEOSIDE ANTIBIOTICS

Acyl and glycosyl nucleoside antibiotics (Figure 18) have more complex structures and their mode of action generally implies several mechanisms. They can act as inhibitors of nucleic acid synthesis but also of protein or glycan synthesis.[105] Thus, puromycin, bamicetin, blasticidin S and nucleocidin inhibit protein synthesis. Puromycin or 6-dimethylamino-9-[3-(p-methoxy-L-β-phenylalanylamino)-3-deoxy-β-D-ribofuranosyl]-purine, a broad-spectrum antibiotic elaborated by *S. alboniger*, acts as a codon-independent functional analogue of aminoacyl-tRNA. It blocks protein synthesis by reacting with the nascent polypeptide on the peptidyl-tRNA site of the ribosome and by catalyzing the release of incomplete peptide chains from the peptidyl-tRNA-messenger-ribosome complex. The reaction terminates with the formation of peptidyl-puromycin.[101] Bamicetin (amicetin C) and blasticidin S have similar structural features and similar inhibitory patterns. They inhibit peptidyl-transferase and block the transfer of aminoacids from aminoacyl-tRNA to polypeptide.[102] Blasticidin S inhibits both gram-positive and gram-negative bacteria and several fungi.[101] Nucleocidin (antibiotic T-3018 or 4'-fluoro-5'-O-sulfamoyladenosine) is a more potent inhibitor of protein synthesis than puromycin.[102] It does not affect the binding of tRNA to ribosomes, nor does it inhibit RNA synthesis. Finally, nikkomycins (neopolyoxins) were the first nucleoside antibiotics found to inhibit fungal cell wall chitin biosynthesis. Their structures mimic UDP-N-acetylglucosamine, a substrate for chitin synthetase.[105] This class of compounds has been shown to possess antifungal, insecticidal and acaricidal activity.[105,108,109]

Although none of these compounds is used for clinical treatment as antibacterial or antifungal agent, some of them have shown *in vivo* activity in fungal systemic infections,[109] and the search of new nucleoside analogue antibiotics active against opportunistic fungal pathogens (as *Candida albicans*) remains of fundamental importance.

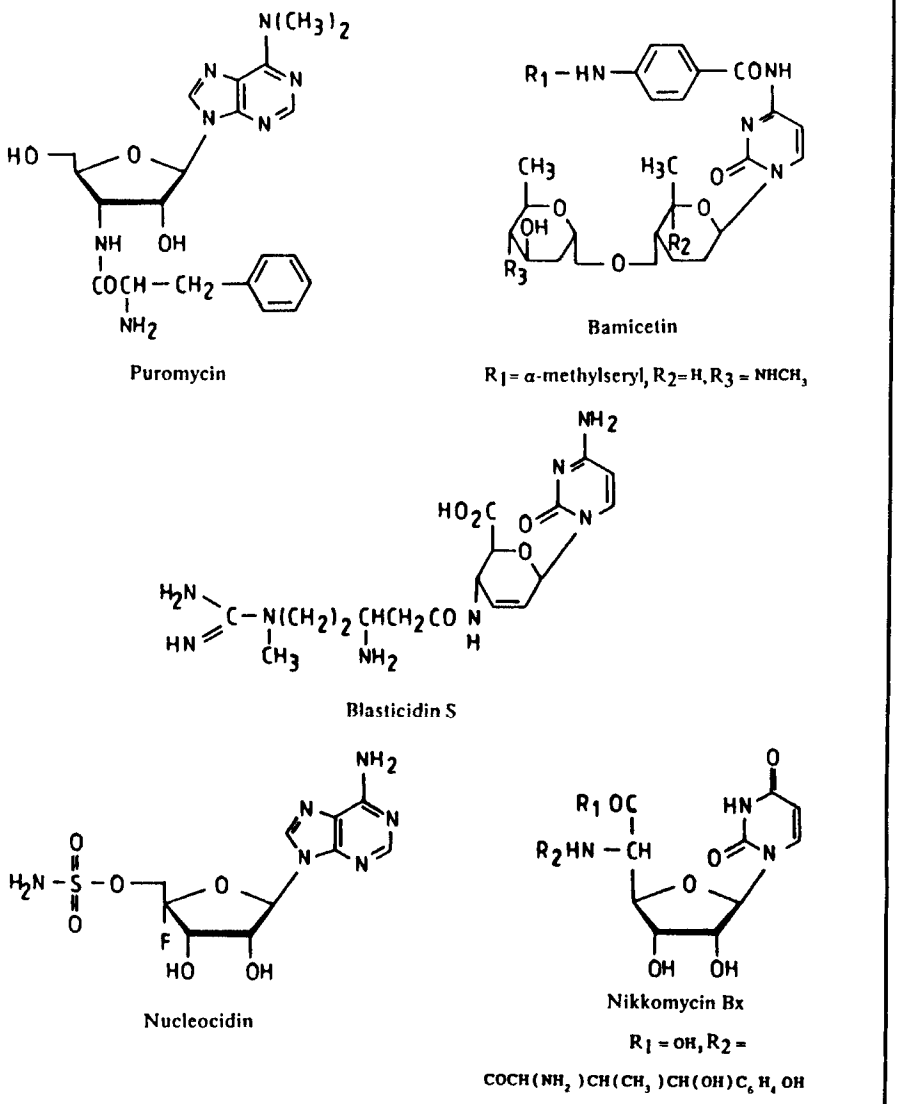


Figure 18. Illustrative acyl and glycosyl nucleoside analogues endowed with antibacterial and antifungal activities.

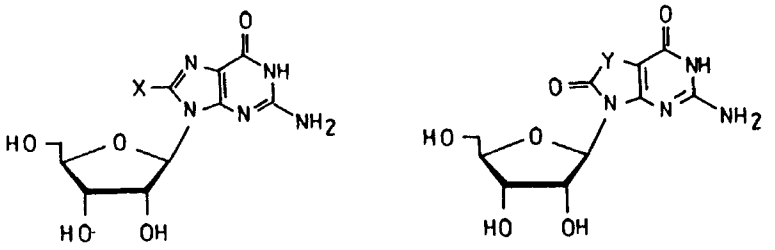


Figure 19. Immunostimulating nucleoside analogues.

NUCLEOSIDE ANALOGUES AND IMMUNOMODULATION

The knowledge of the intricate relations and multiple levels of interactions between various components of the immune system[110-113] has been of paramount importance in the understanding of how the immune system provides protection against foreign intruders and against a number of pathological conditions. This understanding has led to a major effort to develop drugs that might modulate the host's natural defense mechanisms.[114-117]

Among these drugs, C7- and C8-substituted guanine ribonucleosides constitute a class of immunostimulatory agents. C8-Substituted guanine ribonucleosides, exemplified by 8-bromoguanosine and 8-mercaptoguanosine (Figure 19), have be shown to be potent B-cell activators,[118] inducing polyclonal proliferation and differentiation of B cells *in vitro* and acting as adjuvants for antigen-specific B-cell responses *in vitro* and *in vivo*.[119] In addition, 8-mercaptoguanosine stimulates the differentiation of cytotoxic T cells without causing proliferation.[120] 8-Bromoguanosine also acts as an interferon inducer by activating natural killer cells and macrophages *in vitro*.[121]

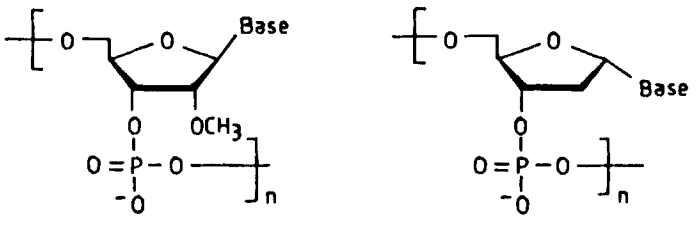
7-Methyl-8-oxoguanosine (7,8-dihydro-7-methyl-8-oxoguanosine, Figure 19) selectively stimulates proliferation of B lymphocytes.[122] In addition, this mitogenic agent is a potent adjuvant for hummoral immune responses.[123] Several other guanosine analogues substituted at the 7- and/or 8-positions have been studied.[124] Among them, 7-thia-8-oxoguanosine [5-amino-3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine-2,7(3H,6H)-dione, Figure 19], a novel thiazolopyrimidine nucleoside, exhibits great immunological activity. This compound induces interferon,[125] activates natural killer cells *in vivo*[125] and has broad-spectrum antiviral activity *in vivo* against many DNA and RNA viruses.[126-130]

The mode of action by which these nucleoside analogues activate the immune system is still unclear. It has been postulated that these compounds act intracellularly[131] according to a biochemical pathway common to several cell types.[119] C7- and/or C8-substituted guanine ribonucleosides are not phosphorylated by the major cellular enzyme systems that commonly phosphorylate nucleosides, and the capacity for phosphorylation is not relevant to the ability of these compounds to exert their biological activity.[132] Several studies suggest the involvement of guanine nucleotide (G) binding proteins of the phosphatidylinositol-protein kinase C pathway as the site of action of these low molecular weight compounds.[124,133]

A therapeutic approach based on immunomodulation could represent in the future an additional curative response to cancer or viral infections.

A NEW APPROACH IN CHEMOTHERAPY: POTENTIAL USE OF SYNTHETIC ANTISENSE OLIGONUCLEOTIDES

Gene expression in living organisms is generally controlled by proteins.[134-136] However, it has been also shown that small RNAs could play a similar role in bacteria[137,138] and in eukaryotes.[139] Each of these regulatory RNAs (or antisenses or antimessengers) has a sequence which is complementary to the mRNA coding for one of the proteins under control and binds by base pairing, thus inhibiting the functions of the target mRNA. These results corroborate the idea put forward by several groups[140-149] that nucleic acids could be used as antimessengers for artificial control of gene expression. A first approach (which will not be developed here) consists in the use of recombinant DNA techniques, and in this perspective antisense RNAs can be introduced into cells by microinjection or they can be expressed after transfection of the cells with plasmids carrying an antisense gene.[150-153] The preparation, introduction and expression of antisense RNAs require rather sophisticated molecular biology techniques. Another simpler approach consists in using natural or synthetic oligonucleotides. From statistical considerations, a sequence of 17 bases should be unique within the human genome. As only a small fraction of the genome is transcribed into messenger RNA, shorter sequences (about 12 nucleotides) can be used. It would be desirable, in order to keep an homology with endogenous mRNAs, that the synthetic oligonucleotide must be constituted by ribonucleotide units. However, because of the difficulties in the chemical synthesis of oligoribonucleotides and owing to the *in vivo* poor stability of natural RNA, most of the to date studies concern others types of oligomers. In fact, the ability of any oligonucleotide to bind selectively to a mRNA lies on Watson-Crick (or Hoogsteen) complementary base pairing, and it could seem difficult to



2'-O-Methyloligoribonucleotide α-Oligodeoxyribonucleotide

Figure 20. Illustrative synthetic oligonucleotides built with sugar-modified nucleoside analogues.

modify the aglycones without compromising the selectivity of hybridization. On the contrary, a β-D-ribofuranose does not appear to be essential, and according to this assumption synthetic β-D-oligodeoxynucleotides, whose synthesis could be automated, have been successfully used as antimessengers.[154] Moreover, hybridization of the latters with their target mRNA sequence induces the degradation of RNA component by ribonuclease H, resulting in an amplification of the antisense effect. Whatever the activity of these antisense agents is, the main factors limiting their use were perceived early on to be cellular uptake and the rate of their degradation. Owing to their polyanionic structure, oligonucleotides cannot cross membranes efficiently despite the possible participation of a cell-surface receptor protein.[155] In order to prevent this drawback, several techniques as electroporation or microinjection have been used, but none of them seems useful *in vivo,* and the problem of uptake of exogenous oligonucleotides in an organism still remains. The second obstacle is their degradation by numerous nucleases which can be found in all living organisms.[156]

In order to circumvent these problems, several chemical modifications have been introduced in oligodeoxyribonucleotides.[157] From Miller and Ts'O studies in the early 70's[158] until 1985, these transformations only concerned the phosphate backbone and have led for instance to phosphotriester, methylphosphonate, phosphoramidate and phosphorothioate derivatives.[154]

Sugar moiety may also be modified in an antisense approach, and to date two kinds of oligonucleotides built with nucleoside analogues, namely oligo-2'-O-methyl-ribonucleotides[159] and α-oligodeoxyribonucleotides[160-172] (Figure 20) have been intensively studied.

Moreover, the synthesis and preliminary results on stability and base pairing of chimeric nuclease resistant homooligomers built with α and/or β-L-deoxynucleosides,[173-177] acyclonucleosides,[178-180] carbocyclic nucleosides[181,182] and α-D-ribonucleosides[183] have been recently reported.

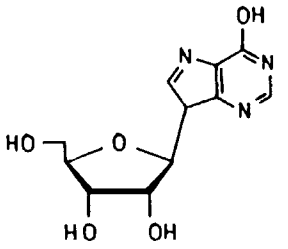
The potential therapeutic applications of synthetic antisense oligonucleotides explain the great deal of interest for this type of compounds.[184-186] mRNAs are not the unique targets of synthetic oligomers and the demonstration that homopyrimidine oligonucleotides could bind to the major groove of duplex DNA at homopurine-homopyrimidine sequences to form a local triple helix gives new insight to the development of potent transcriptional regulators (antigene oligonucleotides).[187,188] Finally, whatever their target and structure are, oligonucleotides can be associated to various conjugates, improving some of their already existing features (like their strength of hybridization or their uptake by cells) or endowing them with some new properties (like cleaving, crosslinking or alkylating abilities).[189,190]

The use of nucleoside analogues as monomeric units of modified oligomers allows one to believe in a quick development of a new and more selective therapeutic strategy. All diseases caused by infectious agents and the most part of the affections linked to a mutated gene (genetic diseases, tumors) are potentially accessible to this antisense approach. In many other pathologies (high blood pressure, inflammatory processes ...) the modulation of gene expression could lead to a lowering of the observed effects.

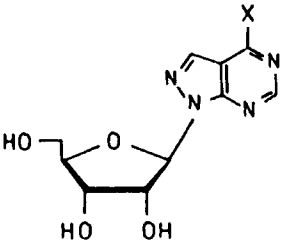
MISCELLANEOUS OTHER ACTIVITIES AND PROPERTIES OF NUCLEOSIDE ANALOGUES

The field of applications for nucleoside analogues is very large. As discussed above some of them are able to interfere selectively with the uncontrolled growth of a cell cancer, others show antiproliferative activity against various pathogens. A crucial feature in their activity is the effectiveness by which they are transported across the plasma cell membrane and today several different types of mammalian nucleoside transport systems of broad permeant specificity have been identified.[191]

The potential use of nucleoside analogues in antiparasite chemotherapy is also of great importance and about three billion people in the world are affected by parasitic diseases.[192] Protozoan and helminthic parasites have both a deficiency in *de novo* synthesis of purine nucleotides, and the purine salvage pathways are essential for their survival and growth.[193,194] Thus, formycin B,[195] allopurinol riboside[196] and thiopurinol riboside[197] (Figure 21) are converted by a nucleoside phosphotransferase (the unique salvage enzyme of



Formycin B



Allopurinol riboside (X = OH)
Thiopurinol riboside (X = SH)

Figure 21. Illustrative nucleoside analogues endowed with antiparasitic activities.

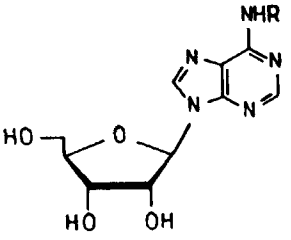


Figure 22. Illustrative cytokinin N^6-substituted adenosines.

Leishmania) to their corresponding nucleotides which can either act as inhibitors of other essential enzymes in purine metabolism[194,197] or be incorporated into the nucleic acids of these organisms.[196,198]

The potentiality of nucleoside analogues in chemotherapy justifies current intense research in several areas and may provide new drugs with nucleoside structure in the future. For instance it has been suggested that modified adenosines migh provide therapeutically useful antihypertensive agents owing to their agonist activity with the natural parent nucleoside.[199]

Apart from their importance in chemotherapy, nucleoside analogues are able to play other essential roles in various fields.

Thus, some 6-substituted adenosines, like N^6-isopentenyl- [R = $CH{=}C(CH_3)_2$] or N^6-benzyl- [R = $CH_2C_6H_5$] adenosine (Figure 22) have shown cytokinin activities.[200-202]

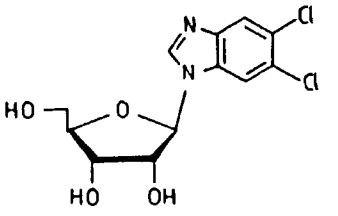


Figure 23. Structure of DRB.

Nucleoside analogues can also be used as biochemical tools, and a method for determining nucleotide sequences in nucleic acids relies upon the use of 2',3'-dideoxynucleoside-5'-triphosphates as base-specific chain terminators of enzymatic DNA synthesis.[203,204]

Finally, some nucleoside analogues are able to interfere specifically with cellular enzymes involved in the metabolism, the transport and functions of natural nucleosides. For example 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole[218] (DRB, Figure 23) is a powerful inhibitor of casein kinase-2,[205,206] enzyme which plays a central role in numerous physiologic processes,[207-213] more particularly in the regulation of transcription processes.[214-217]

In conclusion, by their multiple potential applications, nucleoside analogues represent a very important class of compounds which are still intensively studied.

ACKNOWLEDGMENTS

We thank Synthelabo Recherche, France, for a partial support of this research. We would also like to thank both referees for helpful comments. The assistance of C. Duguet in typing this manuscript is also greatly appreciated.

REFERENCES

1. *Antineoplastic and Immunosuppressive Agents* ; Sartorelli, A.C.; David, G.J., Eds; Springer-Verlag: Berlin, 1974.
2. Hirsch, M.S.; Kaplan, J.C. *Sci. Amer.*, 1987, **256**(4), 76-85.
3. Levene, P.A.; Jacobs, W.A. *Chem. Ber.*, 1909, **42**, 2474-2478.
4. For a recent and exhaustive book on nucleosides, nucleotides and nucleic acids, see: *Nucleic Acids in Chemistry and Biology*; Blackburn, G.M.; Gait, M.J., Eds ; IRL Press: Oxford, 1990.

5. *The Biochemistry of the Nucleic Acids*; Adams, R.L.P.; Knowler, J.T.; Leader, D.P., Eds; Chapman and Hall: London, 1986.
6. McCoss, M.; Robins, M.J. in *The Chemistry of Antitumor Agents*; Wilman, D.E.V., Eds; Blackie: Glasgow, 1990, pp. 261-298.
7. Robins, R.K.; Kini, G.D. in *The Chemistry of Antitumor Agents*; Wilman, D.E.V., Ed.; Blackie: Glasgow, 1990, pp. 299-321.
8. Ueda, T. in *Chemistry of Nucleosides and Nucleotides*; Townsend, L.B., Ed.; Plenum Press: New York, 1988, Vol. 1, Chapter 1, pp. 1-112.
9. Srivastava, C.; Robins, R.K.; Meyer, R.B. in *Chemistry of Nucleosides and Nucleotides*; Towsend, L.B., Ed.; Plenum Press: New York, 1988, Vol. 1, Chapter 2, pp. 113-281.
10. *Nucleoside Analogues, Chemistry, Biology and Medical Applications*; Walker, R.T.; De Clercq, E.; Eckstein, F., Eds; Nato Advanced Study Institutes Series, Series A: Life Sciences; Plenum Press: New York, 1979, Vol. 26.
11. Heidelberger, C. in reference 1, Part II, Chapter 41, pp. 193-231.
12. Black, D.J.; Livingston, R.B. *Drugs*, 1990, **39**, 489-501.
13. Chabner, B.A. in *Cancer and Chemotherapy*; Crooke, S.T.; Prestayko, A.W., Eds; Academic Press: New York, 1981, Vol. 3, pp. 3-24.
14. *The Anticancer Drugs*; Pratt, W.B.; Ruddon, R.W., Eds; Oxford University Press: New York, 1979.
15. Prusoff, W.H.; Fischer, P.M. in reference 10, pp. 281-318.
16. Heidelberger, C.; Danenberg, P.V.; Moran, R.G. in *Advances in Enzym and Related Areas of Molecular Biology*; Meister, A., Ed.; Wiley, J.: New York, 1983, Vol. 54, pp. 57-119.
17. Broom, A.D. *J. Med. Chem.*, 1989, **32**, 2-7.
18. Randerath, K.; Tseng, W.C.; Harris, J.S.; Lu, L.J.W. in *Recent Results in Cancer Research, Modified Nucleosides and Cancer;* Nass, G., Ed.; Springer-Verlag: Berlin, 1983, Vol. 84, 283-297.
19. Paterson, A.R.P.; Tidd, D.M. in reference 1, Part II, Chapter 47, pp. 384-403.
20. Tidd, D.M.; Paterson, A.R.P. *Cancer Res*, 1974, **34**, 738-746.
21. Le Page, G.A.; Junga, I.G.; Bowman, B. *Cancer Res.*, 1964, **24**, 835-840.
22. Le Page, G.A.; Junga, I.G. *Mol. Pharmacol.*, 1967, **3**, 37-43.
23. Le Page, G.A.; *Can. J. Biochem.*, 1968, **46**, 655-661.
24. Peery, A.; Le Page, G.A. *Cancer Res.*, 1969, **29**, 617-623.
25. Le Page, G.A.; Loo, T.L. in *Cancer Medicine*; Holland, J.F.; Frei, E., Eds; Lea and Febiger: Philadelphie, 1973, pp. 754-768.
26. Rossi, A. in reference 10, pp. 409-436.
27. Skoda, J. in reference 1, Part II, Chapter 45, pp. 348-372.
28. Renis, H.E. *Antibiotics Chemother.*, 1980, **27**, 164-207.
29. Brockman, R.W.; Shaddix, S.C.; Williams, M.; Nelson, J.A.; Rose, L.M.; Schabel, F.M. in *Chemistry, Biology and Clinical Uses of Nucleoside Analogues*; Bloch, A., Ed.; *Ann. N.Y. Acad. Sci.*, 1977, Vol.255, Part III, pp. 501-522.
30. Creasey, W.A. in reference 1, Part II, Chapter 42, pp. 232-256.
31. North, T.W.; Cohen, S.S. in *Viral Chemotherapy [International Encyclopedia of Pharmacology and Therapeutics,* Vol. 111*]*; Shugar, D., Ed.; Pergamon Press LTD: Oxford, 1984, Vol. 1, Chapter 8, pp. 303-341.
32. Hadfield, A.F.; Sartorelli, A.C. *Adv. Pharmacol. Chemother.*, 1984, **20**, 21-67.
33. Burchenal, J.H.; Currie, V.E.; Dowling, M.D.; Fox, J.J.; Krakoff, I.H. in *Chemistry and Clinical Uses of Nucleoside Analogs* ; Bloch, A., Ed.; *Ann. N.Y. Acad. Sci.*, 1977, Vol. 255, Part II, pp. 202-212.
34. Chwang, T.L.; Fridland, A.; Avery, T.L. *J. Med. Chem.*, 1983, **26**, 280-283.
35. Braakhuis, B.J.M.; Van Dongen, G.A.M.S.; Vermorken, J.B.; Snow, G.B. *Cancer Res.*, 1991, **51**, 211-214.
36. Grunewald, R.; Abbruzzese, J.L.; Tarassoff, P.; Plunkett, W. *Cancer Chemother. Pharmacol.*, 1991, **27**, 258-262.

37. Baker, C.H.; Banzon, J.; Bollinger, J.M.; Stubbe, J. *J. Med. Chem.*, 1991, **34**, 1879-1884.
38. Matsuda, A.; Takenuki, K.; Tanaka, M.; Sasaki, T.; Ueda, T. *J. Med. Chem.*, 1991, **34**, 812-819.
39. Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T. *Cancer Res.*, 1991, **51**, 2319-2323.
40. *Comprehensive Virology;* Fraenkel-Conrat, H.; Wagner, R.R., Eds; Plenum Publishing Corp.: New York, 1979.
41. Prusoff, W.H.; Lin, T.S.; Zucker, M. *Antiviral Res.*, 1986, **6**, 311-328.
42. Shannon, W.M. in *Antiviral Agents and Viral Diseases of Man*; Galasso, G.J., Ed.; Raven Press: New York, 1984, Chapter 3, pp. 55-121.
43. Robins, R.K.; Revenkar, G.R. in *Antiviral Drug Development. A Multidisciplinary Approach*; De Clercq, E.; Walker, R.T., Eds; *Nato Advanced Institutes Series,* Series A: Life Sciences; Plenum Press: New York, 1987, Vol. 143, pp. 11-36.
44. Oberg, B. in *Problems of Antiviral Therapy*; Stuart-Harris, C.H.; Oxford, J., Eds; Academic Press: London, 1982, pp. 35-69.
45. Fischer, P.H.; Prusoff, W.H. in *Chemotherapy of Viral Infections, Handbook of Experimental Pharmacology*; Came, P.E.; Caliguiri, L.A., Eds; Springer-Verlag: Berlin, 1982, Vol. 61, Part A, Chapter 2, pp. 95-116.
46. De Clercq, E. in *Advances in Drug Research*; Testa, B., Ed.; Academic Press LTD: London, 1988, Vol. 17 , pp. 1-59.
47. Mansuri, M.M.; Martin, J.C. in *Annual Reports in Medicinal Chemistry*; White, R.J., Ed.; Academic Press: New York, 1987, Vol. 22, Section III, Chapter 15, pp. 147-157.
48. Mansuri, M.M.; Martin, J.C. in *Annual Reports in Medicinal Chemistry*; Allen, R.C., Ed.; Academic Press: New York, 1988, Vol. 23, Section III, Chapter 13, pp. 161-170.
49. De Clercq, E. in *Applied Virology Research*; Kurstak, E.; Marusyk, R.G.; Murphy, F.A.; Van Regenmortel, M.H.V., Eds; Plenum Publishing Corporation, 1988, Vol. 1 , pp. 219-232.
50. De Clercq, E. *Chem. Scripta*, 1986, **26** , 41-47.
51. De Clercq, E.; Walker, R.T. *Pharmac. Ther.*, 1984, **26**, 1-44.
52. De Clercq, E. in *Targets for the Design of Antiviral Agents*; De Clercq, E.; Walker, R.T., Eds; Nato Advanced Institutes Series, Series A: Life Sciences; Plenum Press: New York; 1983, Vol. 73, pp. 203-230.
53. De Clercq, E. in *Problems of Antiviral Therapy*; Stuart-Harris, C.H.; Oxford, J., Eds; Academic Press: London, 1982, pp. 295-314.
54. Robins, R.K. *Chem. Eng. News*, 1986, **64**, 28-40.
55. *Adenine Arabinoside: An Antiviral Agent*; Pavan-Langston, D.; Buchanan, R.A.; Alford, C.A., Eds; Raven-Press: New York, 1975.
56. Whitley, R.; Alford, C.A.; Hess, F.; Buchanan, R.A. *Drugs*, 1980, **20** , 267-282.
57. Drach, J.C. in *Target for the Design of Antiviral Agents*; De Clercq, E.; Walker, R.T., Eds; Nato Advanced Institutes Series, Series A: Life Sciences; Plenum Press: New York, 1983, Vol. 33, pp. 231-257.
58. Park, N.H.; Pavan-Langston, D. in *Chemotherapy of Viral Infections, Handbook of Experimental Pharmacology*; Came, P.E.; Caliguiri, L.A., Eds; Springer-Verlag: Berlin, 1982, Vol. 61, Part A, Chapter 3, pp. 117-136.
59. Sacks, S.L.; Merigan, T.C.; Kaminska, J.; Fox, I.H. *J. Clin. Invest.*, 1982, **69**, 226-230.
60. Elion, G.B.; Furman, P.A.; Fyfe, J.A.; De Miranda, P.; Beauchamp, L.; Schaeffer, H.J. *Proc. Natl Acad. Sci. USA*, 1977, **74**, 5716-5720.
61. Schaeffer, H.J., Beauchamp, L.; De Miranda, P.; Elion, G.B.; Bauer, D.J.; Collins, P. *Nature* , 1978, **272**, 583-585.
62. Martin, J.C.; Dvorak, C.A.; Smee, D.F.; Matthews, T.R.; Verheyden, J.P.H. *J. Med. Chem.*, 1983, **26**, 759-761.

63. Smee, D.F.; Martin, J.C.; Verheyden, J.P.H.; Matthews, T.R. *Antimicrob. Agents Chemother.*, 1983, **23**, 676-682.
64. Field, A.K.; Davies, M.E.; Dewitt, C.; Perry, H.C.; Liou, R.; Germershausen, J.; Karkas, J.D.; Ashton, W.T.; Johnston, D.B.R.; Tolman, R.L. *Proc. Natl Acad. Sci. USA*, 1983, **80**, 4139-4143.
65. Ogilvie, K.K.; Cheriyan, U.O.; Radatus, B.K.; Smith, K.O.; Galloway, K.S.; Kennell, W.L. *Can. J. Chem.*, 1982, **60**, 3005-3010.
66. Smith, K.O.; Galloway, K.S.; Ogilvie, K.K.; Cheriyan, U.O. *Antimicrob. Agents Chemother.*, 1982, **22**, 1026-1030.
67. Freitas, V.R.; Smee, D.F.; Chernow, M.; Boehme, R.; Matthews, T.R. *Antimicrob. Agents Chemother.*, 1985, **28**, 240-245.
68. De Miranda, P.; Burnette, T.; Cederberg, D.; Blum, M.R.; Brodie, H.R.; Mills, J. *Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother.*, 1986, abst. n° 566.
69. Bowden, R.A.; Digel, J.; Reed, E.C.; Meyers, J.D. *J. Infect. Dis.*, 1987, **156**, 899-903.
70. Shepp, D.H.; Dandliker, P.S.; De Miranda, P. *Ann. Intern. Med.*, 1985, **103**, 368-373.
71. De Clercq, E. *Nucleosides Nucleotides*, 1991, **10**, 167-180.
72. Holy, A. *Chem. Scripta*, 1986, **26**, 83-89.
73. Bronson, J.J.; Ghazzouli, I.; Hitchcock, M.J.M.; Webb II, R.R.; Kern, E.R.; Martin, J.C. in *Nucleotide Analogues as Antiviral Agents*; Martin, J.C., Ed.; Am. Chem. Soc.: Washington DC, 1989, Chapter 6, pp. 88-102.
74. De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P.C. *Nature*, 1986, **323**, 464-467.
75. Votruba, I.; Bernaerts, R.; Sakuma, T.; De Clercq, E.; Merta, A.; Rosenberg, I.; Holy, A. *Mol. Pharmacol.*, 1987, **32**, 524-529.
76. Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. *Antimicrob. Agents Chemother.*, 1988, **32**, 1839-1844.
77. Bronson, J.J.; Ghazzouli, I.; Hitchcock, M.J.M.; Webb II, R.R.; Martin, J.C. *J. Med. Chem.*, 1989, **32**, 1457-1463.
78. Montgomery, J.A. *Antiviral Res.*, 1989, **12**, 113-132.
79. Wolfe, M.S.; Borchardt, R.T. *J. Med. Chem.*, 1991, **34**, 1521-1530.
80. Poiez, B.J.; Ruscetti, F.W.; Reitz, M.S.; Kalyanamaran, V.S.; Gallo, R.C. *Nature*, 1981, **294**, 268-271.
81. Barre-Sinoussi, F., Chermann, J.C.; Rey, R.; Nugeyre, M.T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science*, 1983, **220**, 868-871.
82. Gallo, R.C.; Salahuddin, S.Z.; Popovic, M.; Shearer, G.M.; Kaplan, M.; Haynes, B.F.; Palker, T.J.; Redfield, R.; Olesky, J.; Safai, B.; White, G.; Foster, P.; Markham, P.D. *Science*, 1984, **224**, 500-503.
83. Herdewijn, P.; De Clercq, E. in *Design of Anti-AIDS Drugs*; De Clercq, E., Ed.; Elsevier: Amsterdam, 1990, Vol. 14, pp. 141-174.
84. Sarin, P.S. *Ann. Rev. Pharmacol.*, 1988, **28**, 411-428.
85. Broder, S. in *Human Retrovirus, Cancer, and AIDS: Approaches to Prevention and Therapy*; Bolognes, D., Ed.; Liss, A.R.: New York, 1988, Vol. 71, pp. 365-380.
86. De Clercq, E. *Anticancer Res.*, 1987, **7**, 1023-1038.
87. Cretton, E.M.; Xie, M.Y.; Bevan, R.J.; Goudgaon, N.M.; Schinazi, R.F.; Sommadossi, J.P. *Mol. Pharmacol.*, 1990, **39**, 258-266.
88. Kolata, G. *Science*, 1987, **235**, 1464-1465.
89. Richman, D.D.; Fischl, M.A.; Grieco, M.H.; Gottlieb, M.S.; Volberding, P.A.; Laskin, O.L.; Leedom, J.M.; Groopman, J.E.; Mildvan, D.; Hischl, M.S.; Jackson, G.G.; Durack, D.T.; Phil, D.; Nusinoff-Lehrman, S. *New Engl. J. Med.*, 1987, **317**, 192-197.
90. Richman, D.D. *Am. J. Med.*, 1990, **88**, 8-10.

91. Larder, B.A.; Darby, G.; Richman, D.D. *Science*, 1989, **243**, 1731-1734.
92. Nasr, M.; Litterst, C.; Mc Gowan, J. *Antiviral Res.*, 1990, **14**, 125-148.
93. De Clercq, E. *Antiviral Res.*, 1989, **12**, 1-20.
94. Balzarini, J.; De Clercq, E. in *Design of Anti-AIDS Drugs*; De Clercq, E. Ed.; Elsevier: Amsterdam, 1990, Vol. 14, pp. 141-174.
95. Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R.T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.*, 1989, **32**, 2507-2509.
96. Tanaka, H.; Baba, M.; Hayakawa, H.; Haraguchi, K.; Miyasaka, T.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Walker, R.T.; De Clercq, E. *Nucleosides Nucleotides*, 1991, **10**, 397-400.
97. Tanaka, H.; Baba, M.; Hayakawa, H.; Sakamaki, T.; Miyasaka, T.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R.T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.*, 1991, **34**, 349-357.
98. Tanaka, H.; Baba, M.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R.T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.*, 1991, **34**, 1394-1399.
99. Tanaka, H.; Baba, M.; Saito, S.; Miyasaka, T.; Takashima, H.; Sekiya, K.; Ubasawa, M.; Nitta, I.; Walker, R.T.; Nakashima, H.; De Clercq, E. *J. Med.Chem.*, 1991, **34**, 1511-1514.
100. Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J.; Schols, D.; Nakashima, H.; Perno, C.-F.; Walker, R.T.; Miyasaka, T. *Biochem. Biophys. Res. Commun.*, 1989, **165**, 1375-1381.
101. *Nucleoside Antibiotics*; Suhadolnick, R.J., Ed.; Wiley-Interscience: New York, 1970.
102. Suhadolnick, R.J. in *Progress in Nucleic Acid Research and Molecular Biology*; Cohn, W.E., Ed.; Academic Press: New York, 1979, Vol. 22, pp. 193-291.
103. Goodchild, J. *Topics in Antibiotic Chemistry*, 1982, **6**, 99-228.
104. Buchanan, J.G.; Wightman, R.H. *Topics in Antibiotic Chemistry*, 1982, **6**, 229-239.
105. Isono, K. *J. Antibiotics*, 1988, **41**, 1711-1739.
106. Yagisawa, N.; Takita, T.; Ishizuka, M.; Takeuchi, T.; Umezawa, H. *J. Antibiotics*, 1982, **35**, 435-759.
107. Nichol, C.A. in *Handbook of Experimental Pharmacology*; Springer-Verlag: Berlin, 1975, Vol. 38, pp. 434-457.
108. Barett, A.G.M.; Lebold, S.A. *J. Org. Chem.*, 1990, **55**, 5818-5820.
109. Saksena, A.K.; Girijavallabhan, V.M.; Cooper, A.B.; Loebenberg, D. in *Annual Reports in Medicinal Chemistry*; Allen, R.C., Ed.; Academic Press: San Diego, 1990, Vol. 24, Section III, Chapter 12, pp. 111-120.
110. *The Cellular Basis of the Immune Response*; Golub, E.S., Ed.; Sinauer Associates: Massachusetts, 1981.
111. *Essential Immunology*; Roitt, I.M., Ed.; Blackwell Scientific Publications: Oxford, 1984.
112. Samuel, C.E. in *Progress in Nucleic Acid Research and Molecular Biology*; Cohn, W.E.; Moldave, K., Eds; Academic Press: New York, 1988, Vol. 35, pp. 27-72.
113. Sehgal, P.B.; Pfeffer, L.M.; Tamm, I. in *Chemotherapy of Viral Infections, Handbook of Experimental Pharmacology*; Came, P.E.; Caliguiri, L.A., Eds; Springer-Verlag: Berlin, 1982, Vol. 61, Part A, Chapter 6, pp. 205-311.
114. *Biological response modifiers*; Torrence, P.F., Ed.; Academic Press: New York, 1985.
115. *Synthetic Adjuvants*; Adam, A., Ed.; Wiley-Interscience: New York, 1985.
116. Hadden, J.W. *J. Am. Med. Assoc.*, 1987, **258**, 3005-3010.
117. St Georgiev, V. *Trends Pharmacol. Sci.*, 1988, **9**, 446-451.
118. Weigle, W.O. *C.R.C. Crit. Rev. Immunol.*, 1987, **7**, 285-324.
119. Wicker, L.S.; Ashton, W.T.; Boltz, R.C.; Meurer, L.C.; Miller, B.J.; Nichols, E.A.; Sigal, N.H.; Tolman, R.L.; Peterson, L.B. *Cell. Immunol.*, 1988, **112**, 156-165.

120. Feldbush, T.L.; Ballas, Z.K. *J. Immunol.*, 1985, **134**, 3204-3211.
121. Koo, G.C.; Jewell, M.E.; Manyak, C.L.; Sigal, N.H.; Wicker, L.S. *J. Immunol.*, 1988, **140**, 3249-3252.
122. Henry, E.M.; Kini, G.D.; Larson, J.B.; Robins, R.K.; Alaghamandan, H.A.; Smee, D.F. *J. Med. Chem.*, 1990, **33**, 2127-2130.
123. Goodman, M.G.; Hennen, W.J. *Cell. Immunol.*, 1986, **102**, 395-402.
124. Bontems, R.J.; Anderson, J.D.; Smee, D.F.; Jin, A.; Alaghamandan, H.A.; Sharma, B.S.; Jolley, W.B.; Robins, R.K.; Cottam, H.B. *J. Med. Chem.*, 1990, **33**, 2174-2178.
125. Nagahara, K.; Anderson, J.D.; Kini, G.D.; Dalley, K.; Larson, S.B.; Smee, D.F.; Jin, A.; Jolley, W.B.; Robins, R.K.; Cottam, H.B. *J. Med. Chem.*, 1990, **33**, 407-415.
126. Smee, D.F.; Alaghamandan, H.A.; Cottam, H.B.; Sharma, B.S.; Jolley, W.B.; Robins, R.K. *Antimicrob. Agents Chemother.*, 1989, **33**, 1487-1492.
127. Smee, D.F.; Alaghamandan, H.A.; Cottam, H.B.; Jolley, W.B.; Robins, R.K. *J. Biol. Response Mod.*, 1990, **9**, 24-32.
128. Smee, D.F.; Alaghamandan, H.A.; Jin, A.; Sharma, B.S.; Jolley, W.B. *Antiviral Res.*, 1990, **13**, 91-102.
129. Smee, D.F., Alaghamandan, H.A.; Bartlett, M.L.; Robins, R.K. *Antiviral Chem. Chemother.*, 1990, **1**, 47-52.
130. Smee, D.F.; Huffman, J.H.; Gessaman, A.C.; Huggins, J.W.; Sidwell, R.W. *Antiviral Res.*, 1991, **15**, 229-239.
131. Goodman, M.G.; Weigle, W.O. *Proc. Natl Acad. Sci. USA*, 1984, **81**, 862-866.
132. Goodman, M.G. *J. Immunol.*, 1988, **141**, 2394-2399.
133. Rollins-Smith, L.A.; Lawton, A.R. *J. Mol. Cell. Immunol.*, 1988, **4**, 9-19.
134. Watson, J.D.; Hopkins, N.H.; Roberts, J.W.; Argetsinger-Steitz, J.; Weiner, A.M. in *Molecular Biology of the Gene*; Gillen, J.R., Ed.; The Benjamin/Cummings, 1987, Vol. 1, Chapter 3, pp.65-94.
135. Schleif, R. *Science*, 1988, **241**, 1182-1187.
136. Hélène, C.; Lancelot, G. *Prog. Biophys. Mol. Biol.*, 1982, **39**, 1-168.
137. Green, P.J.; Pines, O.; Inouye, M. *Annu. Rev. Biochem.*, 1986, **55**, 569-597.
138. Simons, R.W.; Kleckner, N. *Annu. Rev. Genetics*, 1988, **22**, 567-600.
139. Heywood, S.M. *Nucl. Acids Res.*, **14**, 6771-6772.
140. Paterson, B.M.; Roberts, B.E.; Kuff, E.L. *Proc. Natl Acad. Sci. USA*, 1977, **74**, 4370-4374.
141. Stephenson, M.L.; Samecnik, P.C. *Proc. Natl Acad. Sci. USA,*, 1978, **75**, 285-288.
142. Summerton, J. *J. Theor. Biol.*, 1979, **78**, 77-99.
143. Jayaraman, K.; McParland, K.; Miller, P.; Ts'O P.O.P. *Proc. Natl Acad. Sci. USA*, 1981, **78**, 1537-1541.
144. Trudel, M.; Dondon, J.; Grunberg-Manago, M.; Finelli, J.; Buckingham, R.H. *Biochimie*, 1981, **63**, 235-240.
145. Asseline, U.; Thuong, N.T.; Hélène, C. *C.R. Acad. Sci. Paris, 1983, 297* (III), 369-372.
146. Asseline, U.; Delarue, M.; Lancelot, G.; Toulmé, F.; Thuong, N.T.; Montenay-Garestier, T.; Hélène, C. *Proc. Natl Acad. Sci. USA* , 1984, **81**, 3297-3301.
147. Asseline, U.; Toulmé, F.; Thuong, N.T.; Delarue, M.; Montenay-Garestier, T.; Hélène, C.; *EMBO. J.*, 1984, **3**, 795-800.
148. Knorre, D.G.; Vlassov, V.V. *Prog. Nucl. Acid Res. Mol. Biol.*, 1985, **32**, 291-320.
149. Hélène, C.; Montenay-Garestier, T.; Saison, T.; Takasugi, M.; Toulmé, J.J.; Asseline, U.; Lancelot, G.; Maurizot, J.C.; Toulmé, F.; Thuong, N.T. *Biochimie*, 1985, **67**, 777-783.
150. Takayama, K.M.; Inouye, M. *Crit. Rev. Biochem. Mol. Biol.*, 1990, **25**, 155-184.
151. Weintraub, H.; Izant, J.G.; Harland, R.M. *Trends Genet.*, 1985, **1**, 22-25.
152. Izant, J.G.; Weintraub, H. *Science*, 1985, **229**, 345-352.
153. Mizuno, T.; Chou, M.; Inouye, M. *Proc. Natl Acad. Sci. USA*, 1984, **81**, 1966-1970.

154. *Oligodeoxyribonucleotides, Antisense Inhibitors of Gene Expression*, Topics in Molecular and Structural Biology; Cohen, J.S., Ed.; McMillan Press: London, 1989, Vol. 12.
155. Locke, S.L.; Stein, C.A.; Zhang, X.H.; Mori, K., Nakanishi, M.; Subasinghe, C.; Cohen, J.S.; Neckers, L.M.; *Proc. Natl Acad. Sci. USA*, 1989, **86**, 3474-3478.
156. Wickstrom, E. *J. Biochem. Biophys. Methods*, 1986, **13**, 97-102.
157. Uhlmann, E.; Peyman, A. *Chem. Rev.*, 1990, **90**, 543-584.
158. Miller, P.S.; Fang, K.N.; Kondo, N.S.; Ts'O, P.O.P. *J. Am. Chem. Soc.*, 1971, **93**, 6657-6665.
159. Sproat, B.S.; Beijer, B.; Iribarren, A. *Nucleic Acids Res.*, 1990, **18**, 41-49, and references cited therein.
160. Rayner, B.; Malvy, C.; Paoletti, J.; Lebleu, B.; Paoletti, C.; Imbach, J.L. in reference 154, pp. 119-136.
161. Morvan, F.; Rayner, B.; Imbach J.L. in *Genetic Engineering*; Setlow, J.K., Ed.; Plenum Press: New York, 1990, **12**, pp. 37-52.
162. Gagnor, C.; Bertrand, J.R.; Thenet, S.; Lemaitre, M.; Morvan, F.; Rayner, B.; Malvy, C.; Lebleu, B.; Imbach, J.L.; Paoletti, C.; *Nucl. Acids Res.*, 1987, **15**, 10419-10436.
163. Cazenave, C.; Stein, C.A.; Loreau, N.; Thuong, N.T.; Neckers, L.M.; Subasinghe, C.; Hélène, C.; Cohen, J.S.; Toulmé, J.J. *Nucl. Acids Res.*, 1989, **17**, 4255-4273.
164. Gagnor, C.; Rayner, B.; Leonetti, J.P.; Imbach, J.L.; Lebleu, B. *Nucl. Acids Res.*, 1989, **17**, 5107-5114.
165. Paoletti, J.; Bazile, D.; Morvan, F.; Imbach, J.L.; Paoletti, C. *Nucl. Acids Res.*, 1989, **17**, 2693-2704.
166. Ts'O, P.O.P.; Miller, P.S.; Greene, J.J. in *Development of Target-Oriented Anticancer Drugs*; Cheng, Y.C.; Goz, B.; Minkoff, M., Eds; Raven Press: New Yord, 1983, pp. 189-206.
167. Morvan, F.; Rayner, B.; Imbach, J.L.; Lee, M.; Hartley, J.A.; Chang, D.K.; Lown, J.W. *Nucl. Acids Res.*, 1987, **15**, 7027-7044.
168. Morvan, F.; Rayner, B.; Imbach, J.L.; Thenet, S.; Bertrand, J.R.; Paoletti, J.; Malvy, C.; Paoletti, C. *Nucleic Acids Res.*, 1987, **15**, 3421-3437.
169. Thenet, S.; Morvan, F.; Bertrand, J.R.; Gautier, C.; Malvy, C. *Biochimie*, 1988, **70**, 1729-1732.
170. Bacon, T.A.; Morvan, F.; Rayner, B.; Imbach, J.L.; Wickstrom, E. *J. Biochem. Biophys. Methods*, 1988, **16**, 311-312.
171. Pauwels, R.; Debyser, Z.; Baba, M.; Rayner, B.; Morvan, F.; Imbach, J.L.; De Clercq, E. *Arch. Int. Physiol. Biochim.*, 1988, **96**, B-49.
172. Bloch, E.; Lavignon, M.; Bertrand, J.R.; Pognon, F.; Morvan, F.; Malvy, C.; Imbach, J.L.; Paoletti, C. *Gene*, 1988, **72**, 349-360.
173. Damba, M.J.; Giannaris, P.A.; Marfey, P.; Reid, L.S. *Tetrahedron Lett.*, 1991, **32**, 2573-2576.
174. Morvan, F.; Genu, C.; Rayner, B.; Gosselin, G.; Imbach, J.L. *Biochem. Biophys. Res. Commun.*, 1990, **172**, 537-543.
175. Fujimori, S.; Shudo, K. *J. Am. Chem. Soc.*, 1990, **112**, 7436-7438.
176. Van Boeckel, C.C.A.; Visser, G.M.; Hegstrom, R.A.; Van Boom, J.H. *J. Mol. Evol.*, 1987, **25**, 100-105.
177. Anderson, D.J.; Reischer, D.J.; Taylor, A.J.; Wechter, W.J. *Nucleosides Nucleotides*, 1984, **3**, 499-512.
178. Ogilvie, K.K.; Gillen, M.F. *Tetrahedron Lett.*, 1980, **21**, 327-330.
179. Ogilvie, K.K.; Nguyen-Ba, N.; Gillen, M.F.; Radatus, B.K.; Cheriyan, U.O.; Hanna, H.R.; Smith, K.O.; Galloway, K.S. *Can. J. Chem.*, 1984, **62**, 241-252.
180. Schneider, K.C.; Benner, S.A. *J. Am. Chem. Soc.*, 1990, **112**, 453-455.
181. Perbost, M.; Lucas, M.; Chavis, C.; Pompon, A.; Baugartner, H.; Rayner, G.; Griengl, H.; Imbach, J.L. *Biochem. Biophys. Res. Commun.*, 1989, **165**, 742-747.

182. Sagi, J.; Szemzo, A.; Szecsi, J.; Otvos, L. *Nucleic Acids Res.*, 1990, **18**, 2133-2140.
183. Debart, F.; Rayner, B.; Imbach, J.L. *Tetrahedron Lett.*, 1990, **31**, 3537-3540.
184. Morvan, F.; Rayner, B.; Malvy, C.; Paoletti, J.; Paoletti, C.; Imbach, J.L. in *Advances in Chemotherapy of AIDS*; Diasio, R.B.; Sommadossi, J.P., Eds; Pergamon Press: New York, 1990, Chapter 2, pp. 11-22.
185. Miller, P.S. in *Herpes Viruses, The Immune System and AIDS: Development in Medicinal Virology*; Aurelian, L., Ed.; Klumer Academic: Norwell, 1990, Vol. 6, pp. 343-363.
186. Koga, M.; Moore, M.F.; Beaucage, S.L. *J. Org. Chem.*, 1991, **56**, 3757-3759.
187. Helene, C.; Toulmé, J.J. *Biochem. Biophys. Acta*, 1990, **1049**, 99-125.
188. Dervan, P.B. in reference 154, pp. 197-210.
189. Hélène, C.; Toulmé, J.J. in reference 154, pp. 137-172.
190. Goodchild, J. *Bioconjugate Chem.*, 1990, **2**, 165-187.
191. Zimmerman, T.P.; Domin, B.A.; Mahony, W.B.; Prus, K.L. *Nucleosides Nucleotides*, 1989, **8**, 765-774.
192. Wang, C.C. *Trends Biochem. Sci.*, 1982, **7**, 354-356.
193. Wang, C.C. *J. Med. Chem.*, 1984, **27**, 1-9.
194. Jaffe, J.J. *Ann. N.Y Acad. Sci.*, 1975, **255**, 306-316.
195. Carson, D.A.; Chang, K.P. *Biochem. Biophys. Res. Commun.*, 1981, **100**, 1377-1383.
196. Nelson, D.J.; La Fon, S.W.; Tuttle, J.V.; Miller, W.H.; Miller, R.L.; Krenitsky, T.A.; Elion, G.B.; Berens, R.L.; Marr, J.J. *J. Biol. Chem.*, 1979, **254**, 11544-11549.
197. Marr, J.J.; Berens, R.L.; Nelson, D.J.; Krenitsky, T.A.; Spector, T.; La Fon, S.W.; Elion, G.B. *Biochem. Pharmacol.*, 1982, **31**, 143-148.
198. Rainey, P.; Santi, D.V. *Proc. Natl Acad. Sci. USA*, 1983, **80**, 288-292.
199. Trivedi, B.K.; Blankley, C.J.; Bristol, J.A.; Hamilton, H.W.; Patt, W.C.; Kramer, W.J.; Johnson, S.A.; Bruns, R.F.; Cohen, D.M.; Ryan, M.J. *J. Med. Chem.*, 1991, **34**, 1043-1049.
200. Mittelman, A.; Evans, J.T.; Chheda, G.B. *Ann. N.Y. Acad. Sci.*, 1975, **255**, 225-234.
201. Hashizuma, T.; Yoshida, K. *Agr. Biol. Chem.*, 1976, **40**, 2001-2004.
202. Shibata, H.; Ohnishi, N.; Takeda, K.; Fukunaga, H.; Shimamura, K.; Yasunobu, E.; Tani, I. *Can. J. Microbiol.*, 1986, **32**, 186-189.
203. Sanger, F.; Nicklen, S.; Coulson, A.R. *Proc. Natl Acad. Sci. USA*, 1977, **74**, 5463-5467.
204. Prober, J.M.; Trainor, G.L.; Dam, R.J.; Hobbs, F.W.; Robertson, C.W.; Zagursky, R.J.; Cocuzza, A.J.; Jensen, M.A.; Baumeister, K. *Science*, 1987, **238**, 336-341.
205. Meggio, F.; Shugar, D.; Pinna, L.A. *Eur. J. Biochem.*, 1990, **187**, 89-94.
206. Stevens, A.; Maupin, M.K. *Biochem. Biophys. Res. Commun.*, 1989, **159**, 508-515.
207. Schneider, H.R.; Reichert, G.H.; Issinger, O.G. *Eur. J. Biochem.*, 1986, **6**, 733-738.
208. Perez, M.; Grande, J.; Itarte, E. *FEBS Lett.*, 1988, **238**, 273-276.
209. Brunati, A.M.; Saggioro, D.; Chieco-Bianchi, L.; Pinna, L.A. *FEBS Lett.*, 1986, **206**, 59-63.
210. Issinger O.G.; Martin, T.; Richter, W.W.; Olson, M.; Fujiki, H. *EMBO J.*, 1988, **7**, 1621-1627.
211. Sommercorn, J.; Krebs, E.G. *J. Biol. Chem.*, 1987, **262**, 3839-3843.
212. Haystead, T.A.; Campbell, D.G.; Hardie, D.G. *Eur. J. Biochem.*, 1988, **175**, 347-354.
213. Klarlund, J.E.; Czech, M.P. *J. Biol. Chem.*, 1988, **263**, 15872-15875.
214. Cadena, D.L.; Dahmus, M.E. *J. Biol. Chem.*, 1987, **262**, 12468-12474.
215. Horikoshi, M.; Nakanishi, Y.; Hiroshima, S.; Ohtsuki, M.; Kobayashi, T.; Natori, S. *Chem. Pharm. Bull.*, 1987, **35**, 4181-4187.

216. Zandomeni, R.D.; Carrera-Zandomeni, M.; Shugar, D.; Weinman, R. *J. Biol. Chem.*, 1986, **261**, 3414-3420.
217. Dahmus, M.E. *J. Biol. Chem.*, 1981, **256**, 3322-3339.
218. Recently, blocking of Tat-dependent HIV-1 RNA modification by DRB have been reported: Braddock, M.; Thorburn, A.M.; Kingsman, A.J.; Kingsman, S.M. *Nature*, 1991, **350**, 439-441.

Received 9/9/91
Accepted 12/4/91