The Potential of Nucleotide Analogs as Inhibitors of Retroviruses and Tumors

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Abstract: The biologically active form of most purine or pyrimidine analogs is the nucleoside 5′-mono, di- or triphosphate. The nucleoside form is most often administered because of the ease with which it penetrates cells by facilitated transport. However, many nucleoside derivatives fail to exhibit significant antiviral or antitumor activity because they are not phosphorylated by cellular enzymes to the active nucleotide form. In this review, the potential use of suitable nucleotide analogs as selective inhibitors of ribonucleotide reductase and viral reverse transcriptase is considered. Masked nucleotides such as phosphoramidates or methyl phosphates could be employed to allow transport across cellular membranes. Furthermore, phosphonocarboxamide, phosphonoformate or sulfamidophosphoramidate may mimic nucleotide di- and triphosphates. Tumor cells and virally infected cells are often more permeable to nucleotides and their analogs than normal cells, which could provide a therapeutic advantage. There could be considerable therapeutic potential for nucleotide analogs that can penetrate the tumor cell membranes and that are resistant to enzymatic hydrolysis and are non-incorporable into DNA or RNA.

The Role of Retroviruses in Cellular Transformation

RNA viruses are known to cause malignant neoplasms in a wide range of species from amphibia to primates (1,2). Recently, a unifying theory of malignant transformation involving oncogenic viruses and chemical carcinogens has been proposed (3). Striking evidence for the existence of a unique RNA virus associated with malignant proliferation of human T lymphocytes has emerged from two independent sources (4–6). This virus has has been named human T-cell Leukemia Virus (HTLV). Gallo and co-workers (7) have shown that HTLV is acquired by infection; moreover, HTLV proviral DNA is integrated into DNA of peripheral leukemic cells of HTL patients (8, 9). Hinuma and co-workers (9) were able to transmit HTLV into fresh leukocytes from normal humans and to consistently transform these cells by subsequent X-irradiation. Identification of HTLV-like particles in patients with acquired immune deficiency syndrome (AIDS) also implicates this virus in the etiology of AIDS, which is characterized by the development of Kaposi's sarcoma and various infections (10).

Retrovirus-like particles containing RNA-directed DNA polymerase (reverse transcriptase) have also been isolated from human prostatic cells (11). Gallo and Gelman (12) have reviewed the possibility of a Type-C virus in Hodgkins disease. Moreover, Chandra (13) has found RNA-dependent polymerase activity in human granulocytic sarcoma, human primary melanoma and human osteosarcoma tissue. These studies suggest RNA oncogenic viruses as potential causative agents in certain types of human cancers.

Temin (14) has described some of the possible roles of RNA tumor viruses in the etiology of human cancer: 1. Viruses could act as direct transforming agents, so that genetic information in the virus is responsible for initiating and maintaining tumor cell transformation (such as Rous sarcoma virus in chickens and Friend murine leukemia virus in mice); 2. Viruses could effect genetic changes in an infected host that result in tumorigenesis such as the induction of leukemia in AKR mice, where extended replication of the genetically transmitted Gross virus leads to a mutation or recombination in the viral genome (15). 3. Endogenous virus-related products might cause the induction of tumors by the activation of preexisting "cancer genes", or by recombination of viral genes and cellular genes to form such "transforming genes".RNAs related to the genes of certain transforming retroviruses are frequently detectable in human cancer cells (15).

The feature that unites RNA tumor viruses (retroviruses) and distinguishes them from all other animal viruses is the transcription of their single-stranded RNA into double-stranded DNA. Details of this process have been reviewed by Varmus (17). RNA tumor viruses (retroviruses) thus are characterized by the presence of reverse transcriptase, an RNA-dependent DNA polymerase (RNA-dependent DNA nucleotidyl transferase) that is found in *all* RNA oncogenic viruses as part of the virion. Mutant RNA viruses lacking reverse transcriptase lost their ability to initiate infection and cell transformation (18–20). The presence of reverse transcriptase in *all* oncornaviruses strongly suggests its role in the neoplastic transformation by such viruses. The proviral DNA copy of the RNA viral genome is incorporated into the host cell DNA where it carries information for viral replication and for transformation of the normal cell to a neoplastic cell (21). After the incorporation of a DNA-provirus, a virus can replicate by using the DNA and RNA polymerases of the host cell.

Summers and Mason (22) have recently shown that hepatitis B virus resembles retroviruses in that it has a reverse transcription step in the viral life cycle. Since hepatitis B has been linked with an increased incidence of cancer of the liver both in man and animals (23), this suggests that hepatitis B may be carcinogenic in the same way as retroviruses. Chronic hepatitis B infection afflicts about 200 million people (24) and is associated with the most common fatal cancer of man, primary hepatic carcinoma (25). Some of the unique aspects of the interaction of retroviruses with vertebrate cells have been reviewed by Aaronson (26).

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Viral Reverse Transcriptase as a Target for Chemotherapy

Reverse transcriptase from a purified avian myoblatosis virus (AMV) is a zinc metalloenzyme (27). The purified enzyme also catalyzes a pyrophosphate exchange reaction between deoxyribonucleotide triphosphates. Thus, reverse transcriptase is different from cellular DNA polymerases in that it fails to degrade polydeoxyribonucleotides or hydrolyze deoxynucleoside triphosphates (28). The hydrolytic activity of the enzyme is directed *only* against the ribo strand of the ribodeoxyribopolynucleotide complex (29). These properties appear to be general for all classes of the reverse transcriptases present in various RNA tumor viruses (30). The cellular DNA polymerases α, β and γ are distinguished from reverse transcriptase by their inability to copy natural RNA (31).

A transformed murine cell line actively producing a murine sarcoma virus (MSV) and a murine leukemia virus (MLV) was found to contain reverse transcriptase activity indistinguishable from the same enzyme of extracellular virions of MLV (32). Uninfected control cells did not exhibit any reverse transcriptase activity. Thus the potent selective binding of a nucleotide analog substrate to the newly synthesized reverse transcriptase should prevent formation of viable new oncogenic virions even in the transformed cell. There is a direct correlation between inhibition of reverse transcriptase and the loss of the ability of the retrovirus to cause transformation of cells in culture (33) and to induce leukemia in experimental animals (34).

F. M. Schabel, Jr. (35) was one of the first to suggest strongly antiviral agents as an adjunct to cancer chemotherapy. Following effective chemotherapy of spontaneous leukemia-lymphoma in AKR mice with a very high probability of drug cure of the cellular phase of the disease, all animals ultimately die of the pathologically classical disease, presumably virally reinduced, often as much as several months after the apparent drug cure. This is an interval well beyond the anticipated maximum time to death from one viable tumor cell that may have survived drug treatment. A chemical compound which would selectively interfere with RNA tumor virus replication of virus-induced cellular transformation should be highly effective in preventing "reinduction" of viral neoplasia in animals and in man (36).

Nucleotide Inhibition of Reverse Transcriptase

The importance of finding specific inhibitors of reverse transcriptase was recognized soon after the discovery (37, 38) of the enzyme. The anthracycline antibiotics, doxorubicin (adriamycin) and daunorubicin are potent inhibitors of the RNA-directed DNA polymerase of Rauscher leukemia virus and avian myeloblastosis virus (17). However, these compounds are also strong inhibitors of cellular DNA polymerases (21). Since the expression of integrated viral genes is responsible for initiation and maintenance of the transformed cell (39, 40), a selective inhibition of retrovirus reverse transcriptase could prevent further virus mediated spread of a tumor (41). A summary of the Russian attempt to find such inhibitors has recently been published (42). Kit (43) and Verma (44) have published similar reviews. Varmus (45) has recently stated that despite enormous synthetic efforts of various investigators, no highly potent specific inhibitor of reverse transcriptase is as yet available.

Holland and co-workers (47) have shown that AKR mice with spontaneously induced lymphoma, despite an apparent cell cure, relapse nonetheless due to probable viral reinduction of the lymphatic leukemia. These investigators showed that ribavirin exhibited 80% inhibition against the Gross (RNA) murine leukemia virus *in vitro*. Treatment of AKR mice with vincristine plus prednisone followed by ribavirin resulted in a significant delay of the reappearance of viable lymphoma cells and a moderate increase in the life-span of the animals compared with treatment with vincristine plus predisone alone (46). These authors therefore suggested the application of this combined therapy to certain viral suspected human neoplastic diseases (46). Ribavirin has been reported independently (47) to increase significantly survivors of spontaneous leukemia in AKR/J female mice. Shannon (48) points out that it is quite possible to have selective antiviral agents that work against RNA tumor viruses that also may posses independent significant antitumor activity. Ribavirin shows activity against L-1210 leukemia (49, 50) and adenocarcinoma 755 in mice (50). Ribavirin also has been shown to suppress the development of adenovirus (49) induced tumors in CBA mice (51) and to inhibit Rauscher murine leukemia virus splenomegaly in mice (48). Furthermore, ribavirin inhibits cellular transformation in rat kidney cells by a temperature sensitive mutant of Rous sarcoma virus (50) and the replication of the Rous sarcoma virus in chicken embryo fibroblasts (52). Ribavirin, which was first prepared in our laboratory (53) in 1972 as an antiviral agent (54), is readily converted by adenosine kinase (55, 56) to ribavirin-5'-phosphate and then to the corresponding 5'-di- and triphosphates (57) (Fig. 1).

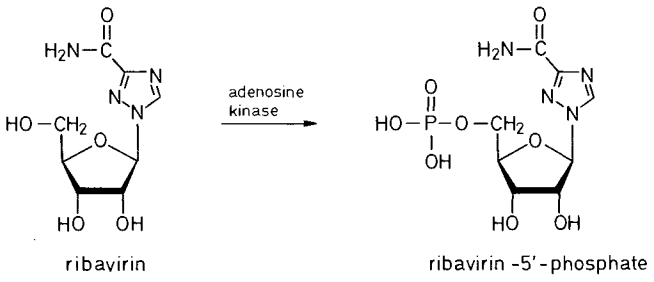


Fig. 1

One of the most promising approaches to the design of a selective inhibitor of reverse transcriptase is the synthesis of specific substrate analogs. Although RNA is utilized as a template, the substrates should be analogous to the natural substrate; a 5'-triphosphate of a 2'-deoxynucleoside. AraCTP is highly inhibitory against the RNA-directed DNA polymerase of Rauscher leukemia virus *in vitro* (58), and it inhibits the DNA polymerase from oncogenic RNA viruses to a greater extent than the DNA polymerase from mammalian cells (59, 60). Similarly, 2',3'-dideoxy-1-β-ribofuranosylthymine (2',3'-dideoxythymidine) inhibits reverse transcriptase from Rous sarcoma virus and Moloney mouse leukemia virus (61, 62). Since phosphorylation of 2',3'-dideoxythymidine has been demonstrated in mammalian cells (63), it is assumed that its 5'-triphosphate (ddTTP) is the active form of the drug. ddTTP was found to inhibit the viral reverse transcriptase of AMV 100-fold more than the cellular α polymerase (64). Furthermore, DNA polymerase γ from adenovirus is

sensitive to inhibition of ddTTP (65). It would appear that a 2',3'-dideoxynucleotide can exert selective inhibition against reverse transcriptase.

Nucleotide Analog Inhibitors of Ribonucleotide Reductase as Potential Antitumor Agents

Ribonucleotide reductase represents the rate limiting enzyme in DNA biosynthesis (66). This enzyme is present only at very low levels in normal mammalian tissues, but its activity rapidly increases with cellular proliferation. In Ehrlich ascites tumor cells Cory and Whitford (67) have shown a good correlation between cell growth and ribonucleotide reductase activity. The relationship between ribonucleotide reductase and tumor growth rate has also been measured in rat hepatomas where an excellent correlation exists between tumor growth rate and specific activity or ribonucleotide reductase (68).

George Weber (69) has identified ribonucleotide reductase as such a *key enzyme* in tumor metabolism. He has shown that ribonucleotide reductase increased 20,800 % in the rapidly growing hepatoma 3683-F over that in normal rat liver. This is a greater increase in enzyme activity in tumor relative to normal tissue over that of any other known enzyme (69). The commitment to neoplasia is accompanied by a profound rise in concentrations of deoxynucleotide triphosphates in neoplastic cells (70). This malignant-transformed enzyme imbalance detailed by Weber and colleagues (71) provides an ideal target enzyme for the design of chemotherapeutic agents (66, 69). Certain known antitumor chemotherapeutic agents such as hydroxyurea (72), guanazole (73) and heterocyclic thiosemicarbazones (74) inhibit ribonucelotide reductase in mammalian systems.

Mammalian ribonucleotide reductase uses nucleoside diphosphates as substrates and requires a nucleoside triphosphate as an allosteric effector (75). Indeed, a natural inhibitor of ribonucleotide reductase in Chinese hamster ovary cells (76) has recently been shown (77) to consist of an adenosine diphosphate derivative whose structure is yet to be established. Moreover, deoxyribonucleotide triphosphate, such as 2'-deoxy-ATP, 2'-deoxy-GTP and 2'-deoxy-TTP, are powerful inhibitors of ribonucleotide reductase isolated from several mammalian sources (78–83). Chang and Cheng (84) have shown that 2'-deoxyadenosine triphosphate acts as a noncompetitive inhibitor with respect to the specific nucleoside triphosphate activator for the reduction of all four common ribonucleoside diphosphates in human T-type lymphoblast cells. Nucleoside di- and triphosphates are, therefore, attractive as potential inhibitors of ribonucleotide reductase. The reductases from *E. coli* and *L. leishmannii* are inhibited by Ara-ATP and Ara-GTP *in vitro* (85). This inhibitory activity may significantly contribute to the known antitumor activity of Ara-A and Ara-C (84). For example, inhibition of ADP reduction by Ara-ATP, observed in human lymphoblast cells (84), reduces the endogenous dATP pool, which in turn potentiates the inhibitory action of Ara-ATP at the DNA level (85) (Fig. 2). As an alternative mechanism, appropriate nucleoside diphosphate analogs could bind instead of the natural substrate to the catalytic site of ribonucleotide reductase and thereby interfere with this important process of 2'-deoxyribonucleotide synthesis in a specific manner.

Reviews of mammalian ribonucleotide reductase (86, 87) and its importance to cancer cells (70) have recently appeared.

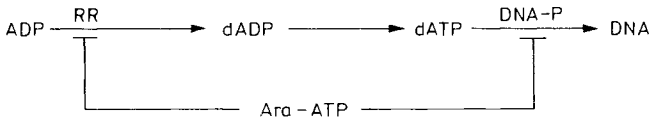


Fig. 2 Proposed mechanism of Ara-ATP cell toxicity involving inhibition of ribonucleotide reductase (RR) and DNA polymerase (DNA-P). Note that Ara-ATP and dATP interact competitively at the DNA site, while Ara-ATP and ADP are noncompetitive at the RR site.

Nucleosides versus Nucleotides as Chemotherapeutic Agents

Most nucleosides that possess significant antitumor activity in animals and in man are phosphorylated *in vivo* to an active nucleotide and exert their biological effect as the corresponding mono-, di- or triphosphate or are incorporated into nucleic acid after conversion to the triphosphate form (88–91). The few samples of nucleosides that exert an inhibitory effect at the nucleoside level in general have not exhibited sufficient potency or specificity to be of practical utility as antitumor agents (92). One of the major problems of the clinical use of nucleosides is the resistance that often develops. Summarizing these problems, Brockman (93) has noted that resistance toward nucleosides is usually due to one of the following:

1. Decreased activity of purine and pyrimidine nucleotide forming enzymes.
2. Failure of resistant cells to convert mononucleotides to a higher form (di- or triphosphate).
3. Increased degradation of the nucleotide form.
4. Failure of the proper form (nucleotide) of the nucleoside to be transported to the site of action.
5. Increased production of a natural metabolite (nucleotide) capable of reversing the inhibitory effects of the analog.

All of these resistance mechanisms are concerned with the production and metabolism of the active nucleotide form within the cell. Therefore, a novel approach would be to design di- or triphosphate analogs that transport satisfactorily into the cell and are not readily susceptible to enzymatic cleavage. These may prove to be superior chemotherapeutic agents, especially if they bound tighter to an enzyme such as reverse transcriptase or ribonucleotide reductase than the natural substrate. The design of active nucleotide analogs capable of penetrating cellular membranes is, however, difficult since the polar character necessary for enzyme binding is also responsible for the poor transport of nucleotides across cellular lipid membranes (94). However, certain nucleotides may enter cells more readily than had previously been supposed. LePage and Naik (95) have shown that 9-β-*D*-xylofuranosyl-6-purinethiol-5'-phosphate (Fig. 3 A) is active against sarcome 180 ascites tumors when given i.p. to mice. The nucleotide penetrates tissues and is excreted unchanged in the urine. It is not converted to the nucleoside or other nucleotides (95). Cohen and Plunkett (96) have considered this problem in their work with ara-AMP (Fig. 3 B) which

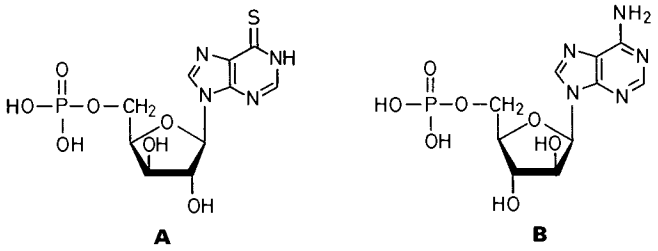


Fig. 3

had been doubly labeled in the base as well as the phosphate moiety. Ara-AMP slowly entered the cells and accumulated in both acid soluble and acid-insoluble materials. A small amount of ara-AMP was phosphorylated intracellularly to the triphosphate and was subsequently incorporated into DNA (97). LePage and co-workers (98) have also shown that ara-AMP (Fig. 3 B) given intravenously to cancer patients, may serve as a depot form of ara-A.

It has been shown (99) that L-cells treated with 2',5'-dideoxyadenosine-5'-phosphate (supplied from our laboratory) are extensively killed after 48 h, whereas 2',3'-dideoxyadenosine is essentially nontoxic to L-cells. This was interpreted as the intact 5'-phosphate entering the L-cells, while the 2',3'-dideoxyadenosine, although transported across the cellular membrane, was not phosphorylated to the corresponding 5'-phosphate (99). Since normal cellular membranes are not readily penetrated by nucleotides (100), perhaps the more leaky membranes of the malignant cell may be more permeable to nucleotides. This would permit nucleotides to enter tumor cells preferentially by passive diffusion.

How do Nucleotides Enter the Cell?

Although purine and pyrimidine nucleosides are transported across the plasma membrane by facilitated active transport (100, 101), nucleotides do not readily enter into cells (101–103). Sirotnak and associates (104) have suggested that the role of membrane transport might be successfully applied to enhance the drug distribution to the target tumor cells. Although maintenance of a selective permeability barrier is an essential feature of the cellular membrane, little is known about the regulation of such membrane permeability in mammalian cells. Heppel and co-workers (105) have found that treatment of spontaneously or viral transformed mouse cells with ATP causes a striking increase in membrane permeability, so that p-nitrophenyl phosphate readily penetrates the cell and is hydrolyzed by an internal phosphatase (106). ATP greatly increased the passive permeability of cultured, transformed cells for nucleotides (107). In contrast, there was little or no effect of ATP on untransformed 3T3 cells or mouse embryo fibroblasts (107). With Ehrlich ascites cells treatment of ATP provided increased entry of ATP itself into the cancer cell (108). The alteration of membrane permeability by ATP in transformed 3T6 cells is reversible (109). Makan (109) suggests that an ATP requiring protein kinase on the transformed cell surface is involved in controlling membrane permeability. Although molecules as large as NADPH are able to cross the leaky membrane, no cytoplasmic enzymes leave the cell. Kitagawa (110) has shown that β, γ-methylene guanosine-5'-triphosphate inhibited protein synthesis in ATP-treated transformed cells but not in untreated normal cells. This indicates that the methylene analog of GTP successfully penetrated the ATP permeabilized cell. Makan (111) has presented evidence that ATP catalyzes the phosphorylation of the inner plasma membrane surface which opens channels in the cellular membrane in the transformed 3T3 cell. Stillwell and Winter (112) have studied the diffusion rates of adenine nucleotides across bimolecular lipid membranes. The diffusion rates were surprisingly ATP > ADP > AMP. Of considerable interest is the fact that ADP is specifically transported across inner mitochondrial membranes by a special carrier mechanism which involves specific binding of adenosine-5'-diphosphate (113) to an inner membrane lipoprotein. Therefore, ADP analogs could specifically inhibit oxidative phosphorylation. Nucleotide diffusion has been shown to be stimulated by divalent metal cations (112). Pardee (114) has found that the greater polarity induced by the presence of salt, made baby hamster cells permeable to nucleotides. Ara CTP readily penetrated these cells and prevented cellular proliferation (114). Once nucleotides have gained cellular entry, nucleotides may transfer to a similar adjacent cell via intercellular (gap) junctions that are freely permeable to small ions and molecules but not to macromolecules (115), resulting in equilibration of purine nucleotide pools between cells (115, 116). The size of such channels may be regulated by a transmembrane protein whose regulatory function in turn is determined by the degree of phosphorylation on the membrane surface (94) by a *c*AMP-dependent protein kinase (117, 118).

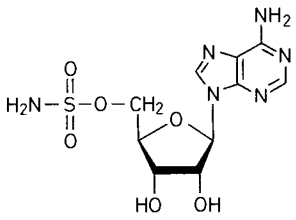
Nucleotides seem to penetrate virally infected and virally transformed cells more readily than normal cells because of a virally induced increase in the membrane permeability (119–121). Carrasco (120) has shown that β,γ-methylene guanosine-5'-triphosphate penetrates EMC infected 3T6 cells and exerts a direct inhibitory effect on protein synthesis in these cells. This could provide an excellent basis for selective action since the normal uninfected 3T6 cells are impermeable to this GTP analog (120). Furthermore, virus-infected cells are more permeable than normal cells to high molecular weight polar nucleoside antibiotics such as anthelmycin and gougerotin (122, 123).

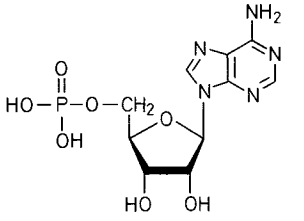
The treatment of mouse L cells with sodium dextran sulfate 500 has been shown to render the cell permeable to ribonucleoside diphosphates (124). This is an example of permeable cells which allow one to study the properties of ribonucleotide reductase (125). The use of ribonucleoside diphosphates as substrates for deoxynucleotide formation was found to parallel the degree of permeabilization (124).

Nucleotides Modified at the Phosphate Moiety

The design of nucleotide analogs with modified phosphate groups may yield therapeutic agents that readily enter the cell, do not require intracellular activation and resist enzymatic degradation. Such agents would overcome many of the shortcomings of nucleoside analogs which are dependent on enzymatic conversion to an active form. For example, the ionic phosphate moiety can be converted to an amide or ester function to obtain less ionic character and better cellular penetrability. Ara-A-5'-*O*-methyl phosphate, reported first from our laboratory (126) exhibited *in vitro* antiviral activity equivalent to Ara-A.

It has been shown by Cass (127) that ara-A-5'-*O*-methyl phosphate in culture against L-1210/C2 cells exhibited significant antiproliferative activity equivalent to Ara-A. These points were recognized quite early by the late B. R. Baker who suggested that the ionized phosphate moiety was probably necessary for substrate binding to a cationic enzyme site. He suggested that other anionic groups such as sulfate, phosphonate or carboxylate should be able to replace the phosphate in the binding process. Baker, in a series of papers (128–134) entitled "Simulation of 5'-Phosphoribosyl Binding" demonstrated that with selected isolated enzymes *in vitro*, certain 9-adenylcarboxylic acids (128) could simulate the binding of 5'-adenylic acid. However, 5'-*O*-carbamoyl-2'-deoxy-5-fluorouridine failed to simulate the binding of 2'-deoxy-5-fluoro-5'-uridylic acid (131) to thymidylate synthetase. In an early attempt to prepare nucleotide analogs in which the 5'- phosphate group might be mimicked, Shuman, Robins and Robins (135) prepared a series of nucleoside 3'- and 5'-sulfamates. Of these derivatives, adenosine-5'-sulfamate (Fig. 4) inhibited the growth of *Trypanosoma rhodesiense* (136) and protein synthesis in *E. coli* (137).





Adenosine 5'-phosphate

Fig. 4

In another instance of substitution of sulfamate for phosphate, Mungall and co-workers (138) have prepared 5'-sulfamino-5'-deoxyadenosine (Fig. 5). It would be interesting to carry this concept over to nucleoside di- and triphosphate analogs with the substitution of more than one phosphate group. Phelps, Woodman and Danenberg (139) have recently prepared 5-fluoro-2'-deoxyuridine-5'-phosphorodiamidate (Fig. 6) which, on a molar basis, produced a cytostatic effect in tumor bearing mice comparable to 5-fluoro-2'-deoxyuridine. These authors suggest that phosphorodiamidates of nucleoside-5'-phosphates are neutral compounds that should be able to cross cell membranes by facilitated transport similar to nucleosides (139). The phosphorodiamidate could then be hydrolyzed enzymatically within the cell to give the corresponding nucleoside-5'-phosphate. A 5'-simulated nucleotide with other than a phosphate or phosphonate group has little chance of being active as a di- or triphosphate analog, since it may *not* be readily phosphorylated to the di- or triphosphate level by a nucleotide kinase. It should be noted, however, that 6'-cyano-6'-deoxyhomoadenosine-6'-phosphonic acid (Fig. 7) and 5'-deoxy-5'-(C-dihydroxyphosphinyl) hydroxymethyladenosine (Fig. 8) prepared by Hampton and co-workers (140, 141) are both substrates for rabbit and pig AMP kinases. Moreover, the substances Figure 7 and 8 are deaminated by rabbit AMP aminohydrolyase (140, 141).

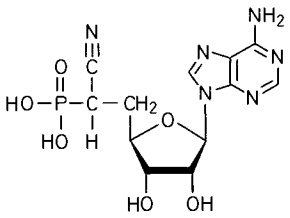


Fig. 7

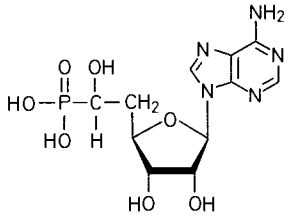


Fig. 8

Since the natural substrate for purine and pyrimidine ribonucleotide reductase is the corresponding nucleoside 5'-diphosphate, a logical approach to the discovery of new and potent ribonucleotide reductase inhibitors might involve a study of various nucleoside diphosphate substrate analogs where the diphosphate moiety is changed to include a phosphonate, sulfamate, phosphocarboxamidate, phosphonoformate, hypophosphate or similar groups to provide the required polar binding characteristics and yet retain the ability to penetrate tumor cells.

Also of considerable interest is the finding that phosphonoformate (Fig. 9) can inhibit reverse transcriptase of Maloney murine leukemia virus and other sources, whereas phosphonoacetate (Fig. 10) is not an inhibitor (142, 143). These compounds are pyrophosphate analogs which are in general active as antiviral agents by binding in the pyrophosphate "well" of the viral DNA and RNA polymerases (144, 145). Phosphonoformate (Fig. 9) is unique in that it inhibits both RNA and DNA-dependent polymerase activities (142, 145). Perhaps the attachment of phosphonoformate at the 5'-position of a 2'-deoxynucleoside would provide the required specificity of an inhibitor of reverse transcriptase which would *not* inhibit normal cellular RNA or DNA polymerases. We have recently succeeded in the synthesis of the adenosine 5'-phosphonoformate derivative (Fig. 11) which has been iso-

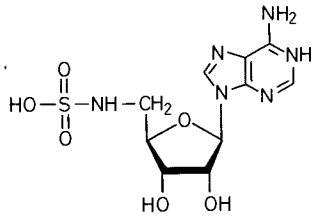


Fig. 5

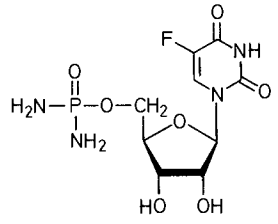


Fig. 6

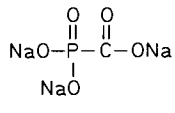
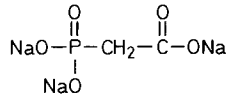


Fig. 9



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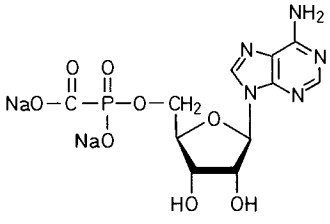

Fig. 11

lated as the disodium salt (146). The chemical and biochemical properties remain to be studied.

Richardson (147) has recently summarized a possible scheme for the action of RNA polymerase in its addition of a nucleotide to the 3'-end of a nascent RNA chain. The second and third phosphate of the nucleoside triphosphate and the 2'- and 3'-hydroxyl groups bind to the enzyme while the internucleotide bond is being formed from a nucleophilic attack of the 3'-hydroxyl of the terminal nucleotide of the RNA chain. From this model one would conclude that anionic or polar binding of at least two phosphates or similar polar groups is important for good substrate binding to RNA polymerase.

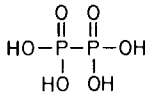

Fig. 12

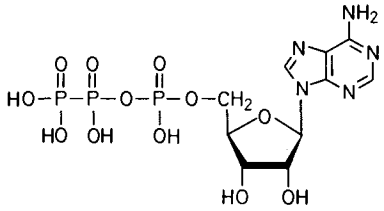

Fig. 13

Hypophosphate (Fig. 12) is a good inhibitor of AMV reverse transcriptase with a ten times greater potency in inhibiting the viral enzyme than DNA polymerase α (40). The chemical synthesis of adenosine-5'-phosphohypophosphate (148) (Fig. 13) and guanosine-5'-phosphohypophosphate (149) (Fig. 14) were reported over ten years ago. The nucleotides shown in Figures 13 and 14 are not cleaved enzymatically at the P-P bond (149), and adenosine-5'-phosphohypophosphate is a good inhibitor of phenylalanine polymerization (149). The formation of the phosphoramidate linkage as an internucleotide linkage has been reported (150). Simoncsits and Tomasz (151) report an ATP analog with a P_1-amino function. The first phosphate-modified ATP analog to be prepared was adenylylmethylenediphosphonate (AMP-PCP) (Fig. 15) in which a methylene group replaced the β,γ-bridge oxygen of ATP (152). The advantage of the methylene linkage is the added stability toward enzymatic hydrolysis. Similarly, Yount and co-workers (153) have synthesized adenylylimidodiphosphate (AMP-PNP) (Fig. 16) which is stable at neutral or alkaline pH for long periods of time (153, 154). The terminal N-P bond was shown to resist cleavage by

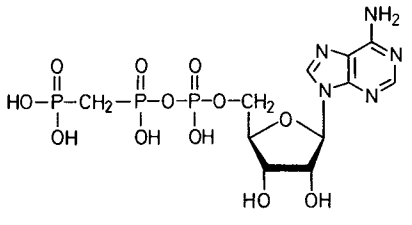

Fig. 14

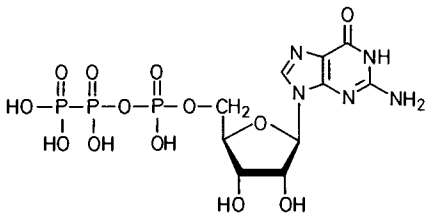

Fig. 15

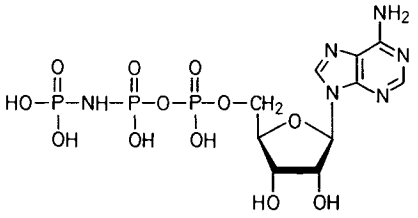

Fig. 16

a large variety of enzymes (155), which offers a distinct advantage over the readily hydrolyzed triphosphate derivatives of various adenosine analogs.

Divalent metal ions such as manganese or magnesium might be employed to form a chelated derivative of a suitable nucleoside 5'-triphosphate analog which could greatly assist its transport across cellular membranes. X-ray data on the magnesium salt of ATP have shown the involvement of the β,γ-phosphate anions and coordination of nitrogen 7 (156). It is known that divalent metal ions increase the diffusion of ATP across bimolecular lipid membranes (112), probably by reducing the ionic nature of the molecule.

A decided advantage to the design of nucleotide analogs with specific groups mimicking the natural phosphate moiety is the reduced likelihood that such nucleotide derivatives would be incorporated into DNA or RNA; therefore, the long term toxicity of these nonincorporable nucleotide analogs may be minimal (157).

As has been pointed out in this review, the need is great and the stakes are high for the medicinal chemist who can meet the challenge to prepare just the right nucleoside diphosphate or nucleoside triphosphate analog that will selectively penetrate the virally infected cell or tumor cell, inhibit the target enzyme specifically and simultaneously resist rapid enzymatic degradation and that in addition will not be subject to incorporation into host RNA or DNA. The challenge does indeed seem to be within our reach.

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