PRODRUGS OF ANALOGS OF NUCLEIC ACID COMPONENTS

Petr ALEXANDER and Antonin HOLY

Institute of Organic Chemistry and Biochemistry,
Academy of Sciences of the Czech Republic, 166 10 Prague 6, The Czech Republic

Received August 3, 1993
Accepted February 26, 1994

This review regards various attitudes to the rational design of prodrugs derived of nucleoside and nucleotide analogues with pronounced biological (antiviral, anticancer) activity. Particular attention is focused on the recent development of prodrugs of approved therapeutic agents with the above structural association.

1. INTRODUCTION

The analogs of nucleic acids components represent a substantial part of antivirals and cytostatics which have been introduced into clinic during last years. Often the newly discovered biologically active substance does not possess optimal metabolic and pharmacological properties, i.e., maximal effectiveness without toxic side-effects. Hence the efforts to optimize its availability in target tissues and minimize its interaction with uninfected and non-transformed cells. The specific targeting of biologically active compound should assist in directing and concentrating the drug at required site and exclude its distribution in other localities. The term *prodrug* has been used for the first time by Albert[1] for *chemically modified substance lacking pharmacological activity which must be enzymatically or by other means transformed in biological system to obtain the target metabolite with required effects*. Under optimum conditions the biologically active substance is either formed or transported only into the infected or transformed cells. These problems have been dealt with in different reviews and reports[2–9].

2. TRANSPORT OF NUCLEIC ACIDS COMPONENTS AND THEIR ANALOGS INTO CELLS

2.1. Transport of Low-Molecular Substances

The biological activity of the majority of oncolytic and antiviral substances based on structural similarity with natural nucleic acid components depends on their pharmacokinetics, tissue distribution and in vivo activity. Thus e.g. the sensitivity of different types of human leukemic cells to cytosine arabinoside (araC) correlates with the density of transport channels for nucleosides and with the rate of araC penetration[10].

Certain types of cells are not capable to form nucleosides de novo and therefore depend on purines or pyrimidines from other sources; other cells are destined to transport certain substances – including nucleosides – across barriers between different systems. The cells of CNS and cardiovascular system possess surface receptors for nucleosides, and intracellular transport depends on the effect of these transmitters[11]. The transport is defined as a penetration of molecules across cell membrane in the presence or absence of the transmitter. Three different mechanisms are known[12]: *Simple diffusion, facilitated diffusion, and active transport*. The first two processes are non-concentrative and the intracellular concentration of a transported substance may

maximally reach its extracellular level. Simple diffusion is unsaturable and is not suppressed by transport inhibitors; moreover, its rate is not affected by the transport of chemically related substances and is neither stereospecific nor Na^+ dependent. On the contrary, the facilitated diffusion and active transport possess saturation characteristics, they are sensitive towards specific inhibitors and in some cases are stereospecific.

Both of these processes have specific kinetic and concentration parameters. Both diffusion types proceed at substantially higher rates than the active transport and they are involved at higher concentration of transported substances. Active transport becomes important when extracellular concentrations are low; it enables cells to accumulate substances against concentration gradient; since it depends on energy delivery, mainly from ATP, it is inhibited by metabolic toxins like cyanide or iodoacetate and is usually driven by Na^+ ions[11]. The driving force of diffusion processes is related to the difference between the inner and outer concentration of a given substance. The penetration is therefore substantially influenced by the subsequent intracellular modification of the substance, as phosphorylation, deamination etc. which continuously reduces the intracellular concentration of the transported substance. Subsequent metabolic conversions play an important role since they activate those analogs whose biological effect is conditioned by their conversion to nucleotides[2]. Nucleotides are formed by phosphoribosylation of bases with phosphoribosyl transferases, or by base transformation to nucleosides catalyzed by nucleoside phosphorylase and by subsequent nucleoside kinase phosphorylation. Other enzymes like phosphotransferases, 5′-nucleotidases etc. may also be involved. In cell lines resistant to purine and pyrimidine analogs the activity of these enzymes is often affected. This resistance cannot be circumvented by administering phosphates of respective analogs because these derivatives cross the membrane only in small quantities and easily undergo dephosphorylation: in certain cases, however, neutral prodrugs may be applied, e.g. phosphodiesters which penetrate into cells where they are transformed to nucleotide analogs.

To determine whether the analog enters the cell by simple or facilitated diffusion, it is necessary to analyze the parameters of the process during the initial period of contact with regard to their sensitivity towards the presence of transport inhibitors. The facilitated diffusion is nearly completely inhibited in the presence of nanomolar concentration of 6-(4-nitrobenzylthio)-9-(β-D-ribofuranosyl)purine (NBMPR; nitrobenzylthioinosine, NBTI), dilazep, dipyridamol and other inhibitors[13]. Recently series of transport proteins have been isolated and characteristics of transport mechanisms have been studied using liposome–protein complexes and other models[14–16]. The intracellular uptake via transport channels has been found in a number of nucleoside analogs whose structural modification enables also the differentiation of protein receptors. So for instance Mahony et al.[17] studied the transport of DHPG (9-(1,3-dihydroxy-2-propoxymethyl)guanine) across the membrane of erythrocytes. DHPG is taken up preferentially via the receptor for purine bases and to a lesser degree through the channel specific for

nucleosides; in contrast, its parent compound ACV (9-(2-hydroxyethoxymethyl)-guanine) which lacks one hydroxymethyl grouping is taken up – similarly to 5-fluorouracil – via nucleobase transport system. It is obvious that for the transport the presence of hydroxyl group corresponding to position 3′ of sugar residue is necessary. This finding is further strengthened by the observation that azidothymidine and other 2′,3′-dideoxynucleosides where the 3′-OH group is substituted by hydrogen or azido group are not taken up by facilitated transport mechanism; due to their lipophilic properties these compounds cross the membrane by simple diffusion[18,19].

It should also be mentioned that certain pharmacologically active substances may be artificially transported into cells using ionophoresis. This method is based on the effect of external electric field on the skin which becomes more permeable for substances of ionic character. However, in some cases complications and unwanted effects[20] (e.g. inflammations) may occur.

2.2. Transport of Macromolecules

The mechanisms of transport discussed above regards low-molecular weight compounds. The cell in contact with environment is also able to take up and eliminate macromolecular substances by means of *phagocytosis, pinocytosis or endocytosis*. Phagocytosis can take place only in certain types of cells; it is quite nonspecific (e.g. taking up of bacteria or damaged cells). More frequently occurring pinocytosis is nonspecific uptake of droplets of extracellular liquids containing dissolved substances. The rate of permeation in this case is proportional to the concentration of the substance in the medium. The most specific process is endocytosis mediated by receptors which are situated on membrane surface and which distinguish and bind extracellular macromolecules. The region of the membrane which includes receptor–ligand complexes then undergoes endocytosis by means of membrane inclusion. These vesicles fuse with different cell organelles, most frequently lysozomes wherein the content of the vesicle is enzymatically degraded. Receptor-mediated endocytosis is highly specific[21]. The complexes formed by endocytosis are fixed by specific fibrillar proteins, clathrines. The vesicles also contain ATP-dependent proton pump which ensures substantial lowering of intracellular pH value resulting in the degradation of ligand–receptor bond and in the recycling of receptor. The area of endocytosis structures in certain cell types amounts up to 2% of total cell surface.

Similar fusion occurs following viral infection[22] between virus and cell membranes and the vesicle which is formed undergoes endocytosis. The lowering of pH value inside the complex initiates infection[23]. Hepatocytes for instance possess a surface receptor which binds glycoprotein chains with D-galactose termini[24].

The receptor-mediated endocytosis may be instrumental for targeting different drugs:

a) High affinity of receptors to ligands which enables their binding even at very low concentrations;

b) rapid turnover of receptors permitting to reach relatively high ligand concentration;

c) the presence of certain receptors in diverse cell types which allows targeted transport[25]. The ideal form of transported drug should have following components: Element for specific recognition of target cells (antibodies against the cell surface antigen or against other structures which are recognized by surface receptors), element ensuring pharmacological action only at the target site (by suitably chosen chemical binding or structural modification), element facilitating the uptake of the transported form towards the target cell across anatomical barriers.

2.3. Conjugates with Antibodies

A number of immunoconjugates including toxins[26], cytostatic drugs and radioisotopes[27] have been prepared. Theoretically one may obtain extremely specific antibodies recognizing only antigens on the surface of certain cell type; they are used in experimental oncology and at present some of them undergo preclinical and clinical trials. Their practical importance is limited by a large number of surface antigens, low number of molecules of the drug attached to the antibody and by inadequate intracellular uptake of the whole complex with surface-bound molecules. Another alternative may consist in transport systems capable to destroy the cell using for instance radionuclides or peroxidase-antibody conjugates[28] which kill the cell by generation of hydrogen peroxide. Still another approach is the construction of heterobifunctional antibodies which recognize tumor cell surface antigens on the one hand and on the other specifically bind T-cell antigenic receptor complexes resulting in T-cell activation and leading ultimately to tumor cell lysis[29]. The variation of this approach is targeting with hormones or growth factor required by tumor cells (as has been tested in melanoma[30]). Practical use of this technique may be successful if immunogenity is eliminated and the efficiency of intracellular transport enhanced.

2.4. Application of Receptors

The strategy of targeting with the aid of receptors requires detailed knowledge of ligand properties, of relative receptor distribution in different cell types and of metabolic transformations to which the complex receptor–ligand is subjected. Such information is available for a limited number of receptor systems (receptors for transferrin, epidermal growth factor, insulin and others) which are present in many cell types and consequently do not guarantee necessary selectivity. The only exception are hepatocytes and macrophages whose receptor systems permit selective drug transport.

The receptor system which recognizes terminal galactoside residues of oligosaccharides (glycoproteins) and which is present exclusively in hepatocytes has been used for targeted transport of pharmacologically active substances in the form of their mac-

romolecular conjugates. Since hepatocytes are target cells for different infections it is possible to enhance the chemotherapeutic efficacy in different stages of malaria and hepatitis, to decrease the toxicity of certain drugs (halothan, isoniazid), and to treat some diseases caused by the absence of certain enzymes or receptors. In pathological conditions accounted for by increased iron levels, as in thalassemia, it is feasible to transport chelating agents by targeting.

Better results have been obtained with macrophages[31] which function as host cells for bacterial, fungal and parasitic infective agents (causing e.g. tuberculosis, leprosis, brucellosis, histoplasmosis). Also pathogenesis of numerous viral diseases (AIDS, yellow fever, herpetic encephalitis, hepatitis, pneumonia) is characterized by virus replication in macrophages[32].

At present there can be distinguished three receptor systems in macrophages: (i) Receptors recognizing mannosyl or fucosyl terminal residues of oligosaccharides (glycoproteins), (ii) galactose receptors (different from hepatocyte galactose receptors) – limited to Kupffer cells[33], (iii) scavenger receptors which recognize series of polyanionic macromolecules including acetylated and maleonylated proteins, sulfated polysaccharides of fucoidin type and dextrane sulfate, or polyinosinic acids[34].

3. DESIGN OF PRODRUGS OF NUCLEIC ACIDS COMPONENTS AND THEIR ANALOGS

3.1. Prodrugs of Nucleosides and Other Analogs

The intracellular uptake of antimetabolites mediated by active transport is conditioned by certain structural elements which enable receptors to recognize different molecules. For example, in nucleoside analogs the presence of 3′-OH or of certain bases is obligatory for the transport by a specific system for natural nucleosides[35]. In the absence of these elements the analog cannot be taken up by system specific for natural nucleosides and may permeate either by simple diffusion[36] or by other mechanisms mentioned above. In such cases, the transport is influenced by polarity, lipophilic or hydrophilic properties of the analog and also by its binding to structures which mediate the transport[18,19,37,38]. The hydrophobization of the active substance is frequently used to affect these parameters. Many reports deal with acylation or alkylation of the base or sugar residues of nucleoside (see below). The character of such derivatization influences not only the permeation of drugs across cell membranes but also their overall distribution in organism and binding to other macromolecules (glycoproteins) or lipidic tissues. The essence of these diffusion processes is the dissolution in lipophilic membranes and further uptake of the compound in the direction of concentration gradient.

Recently, there has been a significant progress in the study of more specific processes which are based on hydrogen bonds, complementary bases, mutual interaction of aromatic systems, etc. These facts made possible a formulation of molecular recogni-

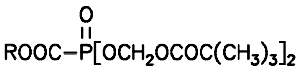
tion principles applicable also to problems connected with recognition and transport of nucleic acid components[38]. The aim of these studies is modeling of certain enzyme processes and development of transport systems suitable for distribution of pharmacologically active substances. Diverse artificial receptor models for the recognition of bases, nucleosides, nucleotides as well as their phosphates[39] have been prepared.

Furuta[40] has described a new approach to the transmembrane transport of nucleosides which is based on the complementary base pairing. He used lipophilic transporter dissolved in the membrane in a ternary system where hydrophobic membrane separates two aqueous phases. This transporter is represented by tris(triisopropylsilyl) nucleoside derivatives complementary to the base of transported analog. In the presence of such transporter the nucleoside transport rate increases by several orders of magnitude.

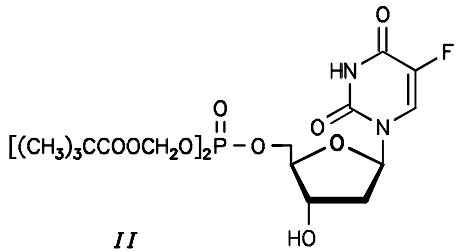
3.2. Prodrugs of Nucleotides and Their Analogs

It is well known that the intracellular transport of nucleotides is minimal[41]. Although the active transport of ATP has been observed in certain cell types[42], a number of experiments with phosphates of natural nucleosides and their analogs indicated that the cleavage of phosphate ester bond occurs on membrane: in those cases where the respective nucleotide is detected inside the cell, this phenomenon is accounted for by subsequent phosphorylation of thus-formed nucleoside. However, a series of biologically active phosphonate nucleotide analogs has been recently synthesized in which such a dephosphorylation cannot occur during their permeation into the cell despite of their outspoken polarity[43–45]. Only a single report on the mechanism of phosphonate uptake by the cell appeared so far[46]. The authors show that substances PMEA [9- (2-phosphonomethoxyethyl)adenine] and HPMPA [9-(3-hydroxy-2-phosphonomethoxypropyl)-adenine] possess very similar characteristics testifying the same mechanism of uptake. The data indicate an active transport which is not affected by classical inhibitors of nucleoside transport into the cell. The involvement of active transport is also confirmed by the fact that at very low extracellular concentrations, the intracellular level of the substances is higher than their concentration in the medium; furthermore, at increasing concentrations the transport shows a saturation characteristics and is temperature dependent. These features are symptomatic for endocytosis. Also certain oligonucleotides which theoretically might not be expected to be taken up by cells enter them by endocytosis[47].

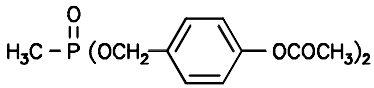
Different ways have been proposed to circumvent the decreased ability of phosphorylated nucleosides to pass the cell membrane. Of some importance is the approach in which polarity of phosphate group is decreased by the esterification with an easily hydrolyzable group. Such triesters bearing two residues of the corresponding nucleoside were proposed as prodrugs of acyclovir and other nucleosides[48a]. Satisfactory results were obtained e.g. with pivaloyloxymethyl esters which afford the neutral precursor of foscarnet *I* or esters of nucleoside phosphate analogs[48b] *II*. Also in the



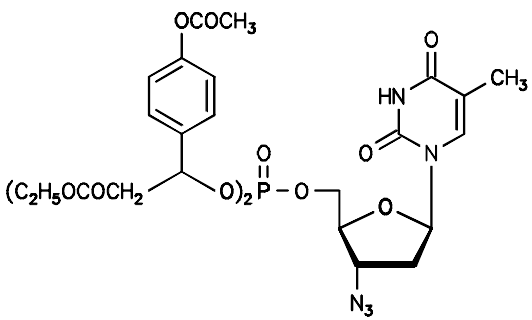
I



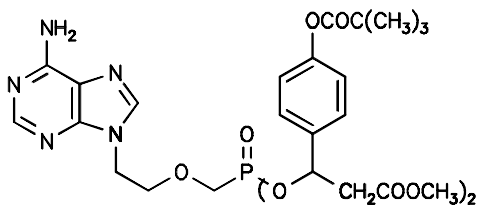
II



III



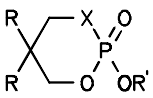
IV

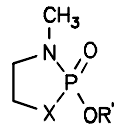


V

series of acyclic phosphonate analogues of nucleotides the bis(pivaloyloxymethyl) esters considerably enhance antiviral activity[49]. Bis(acyloxymethyl) esters undergo rapid enzymatic splitting by carboxyesterase resulting in the formation of monoester and free phosphonate. However, their half-life in plasma is very short. Moreover, during the hydrolysis of this group an equivalent amount of toxic formaldehyde is released. This problem led several authors[50a] to the concept of substituted dibenzyl esters which are more easily degraded enzymatically. Since the site of attack by carboxyesterase in nascent monoester must be sufficiently distant from monoanion of phosphate group 4-acetoxybenzyl derivatives *III* have been proposed. Resulting diester of methanesulfonic acid is rapidly cleaved enzymatically to monoester which is degraded by carboxyesterase at the acetate function of benzene nucleus and nascent 4-hydroxybenzyl ester is cleaved spontaneously with the release of methanesulfonic acid. A neutral lipophilic prodrug of 3′-azidothymidine 5′-monophosphate *IV* which liberates the parent nucleotide by cleavage of the ester groups with esterases and subsequent β-elimination has substantially decreased neurotoxicity in mice. It is equipotent to AZT in vitro in protecting the lymphocytes against HIV induced transformation and displays also activity against HBV in vitro[50b]. Similar mechanism based on spontaneous β-elimination reaction of the enzymatically unmasked *O*-esterified 3-(*p*-hydroxyphenyl)-3-hydroxypropanoate was utilized in the design of the prodrug of acyclic nucleoside phosphonate PMEA (*V*). This compound is reportedly more active against HIV-1, HIV-2 and herpesviruses in vitro compared to the parent compound[50c].

Alkyl esters of nucleotides are chemically and enzymatically stable; neutral cyclic diesters and phosphoramidates of nucleotides[51] *VI – VIII* were also studied; these substances are likely to penetrate membranes by diffusion and subsequently to undergo hydrolysis either spontaneously or by enzymatic oxidation at ester-bound carbon atoms; this process is associated with the degradation of the ring and release of phosphate function.



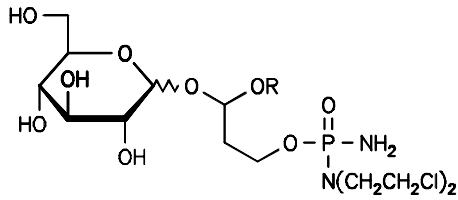


VIa, R = H; X = NH
VIb, R = H; X = O
VIc, R = F; X = O

VII, X = O
VIII, X = NCH_3

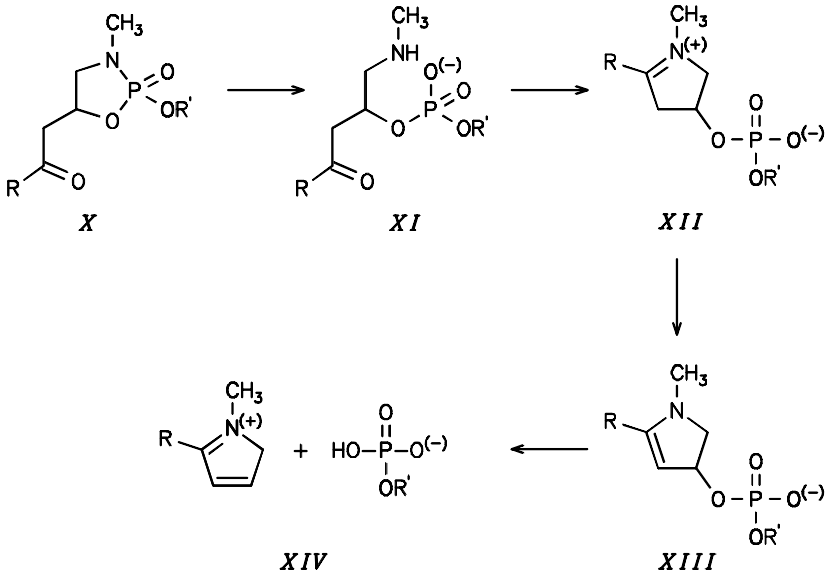
An enhanced glycolysis in tumor cells leads to an increased content of lactic acid resulting in the decreased pH values compared to normal cells. This difference may be utilized for selective degradation of non-toxic prodrug forms of cytostatics. Tietze et

al.[52] made use of the acidolability of acetal glycosides of aldophosphamide *IX*. The non-toxic conjugate *IX* is hydrolyzed to the parent cytostatic agent at lower pH value. Besides aldophosphamide and sugar molecule additional cytostatic alcohol may be bound to the molecule via acetal linkage.



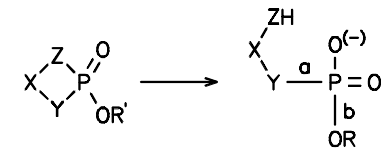
IX

Kumar et al.[53] prepared cyclic phosphoramides *X* which do not require for nucleotide release under physiological conditions any participation of enzymes. Phosphoramidate bonds undergo hydrolysis in plasma under the release of acyclic structure *XI* (Scheme 1) which cyclizes rapidly to compound *XII* owing to the presence of carbonyl group at the side chain. This intermediate is spontaneously converted to compound *XIII* and eliminates nucleotide *XIV*.

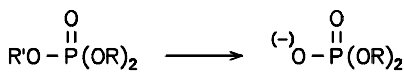


SCHEME 1

Recently an interesting approach has been proposed for the modification of acyclovir[54,55] (Scheme 2): Cyclic phosphotriesters and phosphoramidates of the type *XV* undergo spontaneous hydrolysis at pH 6 – 7 to phosphodiesters *XVI* which preferentially release nucleoside analogue by the action of phosphatase. Phosphotriesters *XVII* with substituted phenols yield by chemical hydrolysis symmetrical diesters *XVIII* which release equimolar quantity of nucleoside and nucleotide by an enzymatic attack at sites a and b. Most suitable esters are derived from 4-(methylthio)phenyl and 4-(methanesulfonyl)phenyl triesters (type *XIX*) whose half-time of hydrolysis (pH 7.7) is 10 – 15 h.

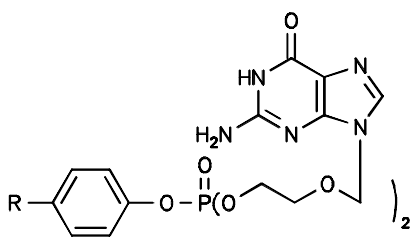


XV *XVI*



XVII *XVIII*

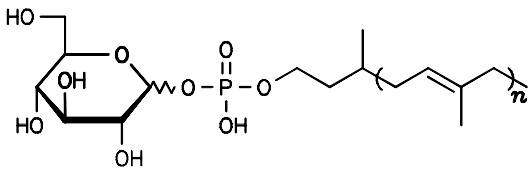
SCHEME 2



XIX

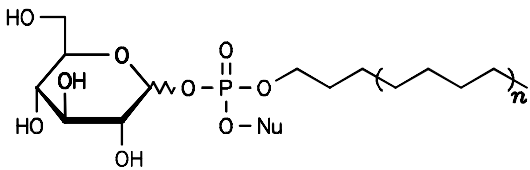
Neumann et al.[56] studied the influence of alkyl chain-length on the transport of mono- and diesters of nucleotides and their analogs across the model membrane. The mechanism of this transport is explained by penetration of the ester by means of its lipophilic terminus into the phospholipid double-layer membrane. The resulting intensive interference at individual membrane layers causes an enhanced exchange of molecules between these layers. This "flip-flop" process is temperature dependent and is influenced by the concentration of Me^{2+}-ions[57]. The esters of nucleotides are then rearranged in the layer and those which are located at the interior side of the membrane are

released inside the cell. This system has been devised in analogy to the structure of 1-glycosyldolichylmonophosphate *XX* which is a lipid carrier and functions as a donor of saccharides for the biosynthesis of oligosaccharides and glycoproteins. In analogy to this structure derivative *XXI* has been prepared in which the phosphate group is esterified by nucleoside. Owing to the presence of the sugar moiety this system is water-soluble and its alkyl chain and overall neutrality of the molecule ensure its hydrophobic character which results in dissolution of the drug in membranes: The sugar moiety may direct the transport of the complex towards cell receptors (see above) and the substance may be transported actively by glucosephosphate and dolichylphosphate transport proteins. This mechanism has been studied with NMR using model membranes[58]. Recently the effect of analogous lipophilic phosphotriesters of AZT (3′-azido-3′-deoxythymidine)[59] was investigated. In accord with results obtained with model membranes, in tissue cultures their antiviral effect was comparable to that of AZT. Oral administration of these compounds to animals resulted in a significant increase of AZTMP (AZT 5′-phosphate) plasma level as well as in the prolongation of its pharmacologically active phase of plasma concentration. Furthermore, an increased AZTMP concentration in the brain was observed which indicates an ability of the triester to cross hematoencephalitic barrier (BBB, blood brain barrier) with subsequent release of AZTMP (ref.[59]).



$n = 16 - 20$

XX



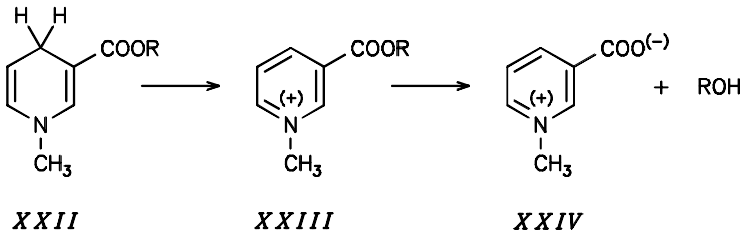
Nu = nucleosid-5′-yl

XXI

3.3. Transport into Central Nervous System

The cells of CNS in mammalian organism are separated from blood circulation system and other body cells by hematoencephalitic barrier which is much less permeable for

polar or even charged substances. Whereas majority of cell types behave rather similarly as erythrocytes as far as nucleoside and nucleotide transport is concerned, neurones are characterized by their increased transport rate, particularly in the case of adenosine[60]. Bodor et al.[61] developed a system based on the oxidation of dihydropyridine (DHP) to pyridinium salt in analogy to NAD+/NADH system. The application of this approach to a series of drugs which do not penetrate in CNS (amines, catecholamins), or are excessively lipophilic and permeate freely in both directions (steroid hormones) led to a selective transport and to a prolonged retention time of these substances in CNS. Lipophilic derivative *XXII* (Scheme 3) easily penetrates into CNS where it is oxidized to hydrophilic pyridinium salt *XXIII* which remains locked by the cell, i.e., it is unable to efflux and slowly releases active substance *XXIV* (ref.[62]). Since initial work on antivirals has been aimed towards the therapy of diseases caused by neurotropic viruses, e.g. herpes encephalitis and cytomegalovirus pneumonia, the reason for application of this attitude to the preparation of 5′-DHP derivatives of ribavirin, trifluorothymidine, ACV, ara-A and other antiviral nucleosides[63] is obvious. Similarly, as the brain tissues and cerebrospinal fluids are HIV sanctuaries, DHP-derivatives of substances active against HIV e.g. AZT have been tested with the purpose of treatment in AIDS patients[64,65].



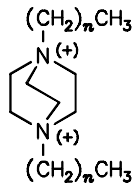
XXII *XXIII* *XXIV*

SCHEME 3

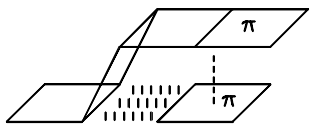
3.4. Artificial Receptors and Transport

In the seventies there was a systematic investigation of the possibilities to affect the transport of nucleotides by means of complexes with various compounds which are able to envelop polar structures thus exposing exclusively the lipophilic parts of their molecules on the surface. Tabushi et al.[66] developed a system composed of lipophilic quaternary amines *XXV*, derived from 1,4-diazabicyclo[2.2.2]octane which was able to transport nucleotides across model membrane. Although this method ensured an efficient transport of adenine nucleotides it failed in case of GMP.

Many authors made use of the analogy of a specific interaction of enzymes with coenzymes (e.g. FAD and NADH) or with substrates as oligonucleotides (in case of restriction enzymes). Weak binding interactions (hydrogen bonds, complementary base pairing, specific aromatic interactions and electrostatic interactions) jointly or individually contribute to recognition processes in biological systems since their strength in non-polar environment of membrane double-layer is substantially increased. Hamilton et al.[67] therefore proposed on the basis of the structure of ribonuclease T1-guanosine complex the general structure of synthetic receptors *XXVI* wherein the base is bound by interaction of heteroaromatic nucleus and by strong hydrogen bonds: Ditopic receptors *XXVI* contain complementary base and aromatic nuclei in a suitable arrangement[68]. The molecule can bind different types of substances including nucleobases and nucleosides by manipulating its structural parameters.

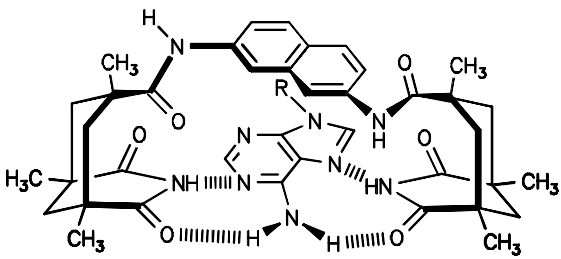


XXV



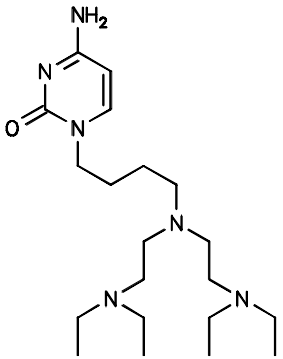
XXVI

Rebek[69] prepared interesting receptors using Kemp's tricarboxylic acid[70]; three carboxyl groups which are fixed in axial conformation thus enable to construct from two molecules cavities which bind very firmly certain substances. In the structure *XXVII*, chelation is so strong that adenosine derivative may be extracted from water and transported across model membranes[71]. Weak intermolecular forces acting only in a near proximity are the key for the high selectivity of artificial receptors, and they simulate very adequately the situation in natural systems.

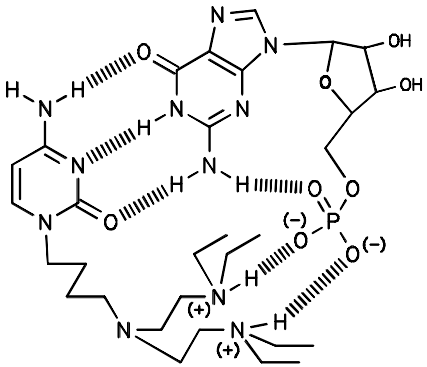


XXVII

Furuta et al.[72] prepared ditopic receptors *XXVIII* containing cytosine and amino groups which form complex *XXIX* with GMP. The strength of binding of these complexes is proportional to number and position of amino groups, and it may be increased by suitable modification of nucleobase or polyamine subunit.



XXVIII



XXIX

For the transport of nucleoside phosphates sapphyrine carriers were used. These homologs of porphyrins possessing cycle with six pyrrole units and with a large and basic inner cavity form complexes with nucleotides and are able to transfer them across model membrane. Conjugate with cytosine acts as a selective GMP carrier at neutral pH in a model membrane system[73]. Rubyrin (macrocyclic molecule with still higher number of pyrrole units) is very efficient for the analogous transfer of nucleoside diphosphates[73]. Lehn's Laboratory[74] which has originated the so-called supramolecular chemistry also studied artificial receptors. They have reported on polyammonium macrocyclic receptors, recognizing and binding ATP, which are able to simulate the enzyme catalysis during hydrolysis of this metabolite[75].

All of the above mentioned systems have the ability to enclose highly polar nucleotides, transport them to lipophilic membrane environment and release them due to concentration gradient. The selectivity of these artificial structures towards respective substrates is in certain cases extraordinary but it does not attain the selectivity of natural systems. In near future one may await further improvement of artificial receptors as well as their use for the construction of transport prodrugs based on nucleotides. The assumption that neutral molecules interact with binding sites only very weakly has not been confirmed[76]. On the contrary, tunicamycin, an inhibitor of dolichylpyrophosphoryl-*N*-acetylglucosamine synthesis – despite of its neutral character – binds at the site of tetraanionic transition state[77]. Also other antibiotics with low charge act as analogs of charged natural metabolites[78].

3.5. Macromolecular Carriers

Much attention has been devoted recently to the use of natural and synthetic polymers as carriers for chemotherapeutics[5]. Ideal carrier should possess sufficient binding capacity for the substance and sufficient solubility of the conjugate in water. Furthermore, its molecular weight must be sufficiently large to avoid renal excretion, but the unmodified carrier should not be absorbed by pinocytosis. The bond between carrier and drug in body fluid compartment must be stable but cleavable in target cells, the rate of its enzymatic degradation must be low and, of course, it must not be toxic or immunogenic. The carrier may be modified by ligation to specific determinants corresponding to receptors in certain tissues and thus it may reach high selectivity of chemotherapeutic effect.

Natural carriers are usually biocompatible. Of natural macromolecules serum albumin (HSA), especially in its glycosylated form, is often used. Molema[79] prepared HSA modified by different saccharides with ligated AZT monophosphate. Lactosamine HSA was used by Fiume for the transport of araC and ACV into hepatocytes[80]. Ouchi et al. tried to bind 5-FU to chitin and chitosan[81]. The synthetic carriers have other advantages as e.g. more exact specification (molecular weight, charge and hydrophobicity) and homogeneity, better stability during manipulation and storing, lower immunogenity and they are less expensive in larger quantities. These advantages cause a constantly increasing demand for their application.

Recent reports on cyclodextrines utilize their complexing properties with different substrates for the transport of peptide drugs[82]. Poly-L-lysine was used in mannosylated form for the transport of antiviral acyclic nucleoside phosphonates into HIV infected macrophages[83]. This polymer has also been used as transporter for antisense oligodeoxynucleotides[84]. Another system with interesting properties is poly(hydroxypropylmethacrylamide), polyHPMA (ref.[5]). In this case the drug is conjugated by means of peptide bond which is stable in blood circulation compartment and enables splitting by lysozomal enzymes in intracellular space. The carrier may be provided with sugar determinants which are able to interact with specific receptors of certain cell types. The use of these polymers as well as their targeting has been dealt with in a monograph[5].

3.6. Use of Liposomes

During the last decade the interest in liposomes as drug transport means has significantly increased[85]. Liposomes are microscopic structures composed of one or more concentric lipid double-layer, separated by aqueous phase. They are able to be taken up by cells by endocytosis. The half-life of liposomes in blood circulation varies from few minutes up to many hours and depends on their size, composition and surface charge. In the organism the liposomes are taken up by macrophages via endocytosis and degraded by lysozomal lipases with subsequent release of transported substances. Again

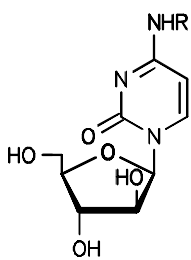
it is possible to exploit the targeting effects of saccharides or of monoclonal antibodies (vide infra). To prolong the half-life of liposomes in blood circulation compartment one may combine them with polystyrene particles which considerably decrease their immunogenicity and affinity towards certain types of scavenger cells[87]. Liposomes may transport either strongly polar substances in their hydrophilic center or lipophilic compounds incorporated in hydrophobic regions of lipid double-layer[86]. Different types of liposomes were developed for these applications: (i) Multilamellar vesicles (MLV) originating by spontaneous hydration of dry phospholipids in the presence of excess water (their size, however, is heterogeneous), (ii) small unilamellar vesicles (SUV) with diameter of 25 nm which may be prepared by ultrasonic disintegration; (iii) large unilamellar vesicles (LUV) prepared by evaporation of a reverse phase[5].

4. PRODRUGS OF SELECTED BIOLOGICALLY ACTIVE NUCLEOSIDES AND NUCLEOTIDES

4.1. Cytostatics

4.1.1. 1-(β-D-Arabinofuranosyl)cytosine

1-(β-D-Arabinofuranosyl)cytosine (araC, *XXX*) is an important cytostatic used for instance in treatment of leukemia[7]. In cells it is phosphorylated to the 5′-triphosphate which competitively inhibits the activity of DNA polymerase. In blood it has a very short half-life and often it is necessary to administer it by continuous intravenous infusion to obtain the therapeutic plasma level[87]. To circumvent these difficulties a series of 3′,5′-esters with saturated and unsaturated acids has been prepared[88–90]. Some of them, especially palmitoyl and stearoyl[91] or 5′-adamantoyl derivatives[92] have a prolonged half-life, increased blood level and can be administered orally.



XXX; R = H

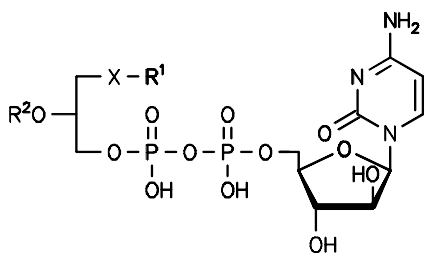
XXXI; R = $CO(CH_2)_nCH_3$

n = 2 – 20

N-Acyl derivatives *XXXI* (C_2 – C_{20}) also possess better pharmacological and cytostatic parameters compared to araC (ref.[93]). *N*-Behenoyl derivative has most advantageous properties since it is taken up by cells rapidly, has substantially longer excretion half-life and lower toxicity as well as longer plasma retention time[94]. Additional 5′-substitution with hydrophilic group, e.g. by phosphate, enhances the solubility in water but also increases cytotoxicity and hemolytic activity.

Interesting transport system for araC was introduced by Schott et al.[95] which not only enhances its cytostatic effect by chemical lipophilization turning it to the N^4-oleyl derivative which was incorporated into liposomes, but further optimizes overall effect by targeting the liposomes with the aid of monoclonal antibodies. The authors used two systems: In the first one they ligated covalently labeled antibodies to liposomes which were functionalized by carboxylate groups, and in the second instance they made use of the affinity of avidin to biotin. Biotinylated isotopically labeled liposomes containing N^4-oleyl-araC were ligated with biotinylated antibodies using avidin. Both systems are substantially more effective than the application of liposomes alone.

Since araC is activated by phosphorylation, transport systems directly releasing araCMP intracellularly have been proposed. AraC-5′-diphosphate-D,L-1,2-dipalmitine[96] *XXXIIa* is an analog of CDP-diglyceride which is a key intermediate in membrane phosphoglyceride biogenesis. As araCDP-diacylglycerols release enzymatically araCMP, these substances are equally active against araC-resistant cell lines which are deficient in deoxycytidine kinase[97]. Hong et al.[98a] prepared araCDP-diglyceride analogs *XXXIIb* with one of the acyl linkages replaced by alkyl ether or thioether bond. This substitution led in certain conjugates to an abundant enhancement of activity. These compounds affect not only the synthesis of nucleic acids but also membrane metabolic processes. Similar delivery system was utilized also for 3′-deoxythymidine[98b] and acyclovir[98c].



XXXIIa, X = O; R^1 = R^2 = $C_{15}H_{31}CO$

XXXIIb, X = S; R^1 = alkyl; R^2 = acyl group

Lier et al.[99] synthesized analogs of nucleosides and of their bases bound to steroid hormones with the aim to enhance specificity and uptake. Esters of prednisone and prednisolone with araCMP was fully active in L1210 murine leukemia model in vivo at doses when araC alone was inactive[100]. A series of similar diphosphates[101] which function as transporters across membranes was also prepared; it is claimed to possess higher antineoplastic activity than araC. This type of prodrugs is easily transported by liposomes on account of their lipophilic properties as documented e.g. by studies of *N*′-[1-(cholesterylcarbonyl)glycyl]araC[102].

Various advantageous properties have been described also for 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine (cyclocytidine, anhydro-araC). It is not deaminated by cytidine deaminase and generates araC spontaneously by hydrolysis. It has a similar effect but higher therapeutic index; its tissue distribution is more specific then in case of araC, due to its association with mucopolysaccharides[103]. Its 3′- and 3′,5′-esters with $C_{11} - C_{12}$ fatty acids have been prepared; some of them have a good cytostatic activity in mouse L1210 leukemia.

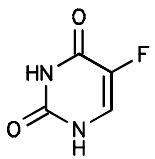
4.1.2. 5-Fluorouracil and Its Nucleosides

5-Fluorouracil (FUra, *XXXIII*) is an established drug often used for postsurgical treatment of different tumors. It is primarily metabolized to 5-fluoro-UMP and -dUMP. The former compound is incorporated into RNA (ref.[104]) following activation to the triphosphate. The cytotoxicity of FUra is mainly due to 5-fluoro-dUMP which strongly inhibits the activity of thymidylate synthase and thus DNA synthesis de novo. FUra is relatively rapidly excreted from organism either directly or following its degradation to α-fluoro-β-alanine so that its therapeutic level rapidly decreases.

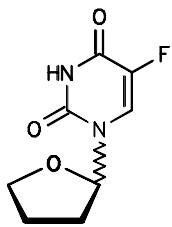
One of the successful prodrugs of FUra is Ftorafur (1-(2-tetrahydrofuranyl)-5-fluorouracil, *XXXIV*, ref.[105]) which has been studied extensively[7] and is in clinical use. Ftorafur is a mixture of two equally active stereoisomers, analogous to α and β anomers of nucleosides. Their activation takes place in a series of metabolic oxidation steps beginning at C-2′ and C-5′(ref.[106]). The end result is the gradual release of FUra with prolonged half-life and notably lowered toxicity. Still more effective than both FUra and Ftorafur is 1,3-bis(2-tetrahydrofuranyl)-5-fluorouracil which is also in clinical use[107].

The finding that Ftorafur functions as a prodrug initiated the intensive search for further analogs with improved therapeutic properties. Ozaki et al.[108] prepared a series of 1-carbamoyl-5-fluorouracil derivatives *XXXV*, nearly all of which are effective against transplanted tumors. Optimal properties has 1-hexylcarbamoyl derivative (HCFU). The same authors have isolated a series of its metabolites formed by enzymatic oxidation of alkyl chain which themselves have antitumor activity but lower therapeutic indices than HCFU(ref.[109]).

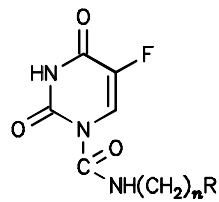
Holy et al.[110–112] synthesized *N*-substituted esters and amides of 5-fluorouracil-1-acetic acid *XXXVI*. However, the degradation of the derivatives and release of the heterocyclic base in cells was not observed. Yamamoto et al.[113] prepared related alkoxycarbonyl derivatives of FUra (ref.[114]). The resorption of these drugs in stomach and intestine is enhanced and their hydrolysis to active base is increased. Benzyl derivative of FUra (ref.[114]) has also been prepared: On account of its lipophilic properties it binds to HSA which functions as its transporter in blood. For the transport across cell membrane Kingsbury[115] synthesized FUra peptide derivative which would be expected to undergo degradation by peptidases to active substance. The intracellular transport may be influenced by a suitable choice of amino acids.



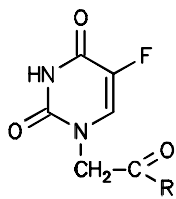
XXXIII



XXXIV



XXXV



XXXVI

R = alkoxy, alkylamino, dialkylamino group

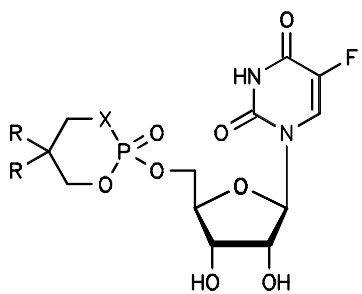
The group of FUra prodrugs also comprizes 5′-deoxy-5-fluorouridine (5′-DFUR, doxifluridin) which makes use of active transport and which is enzymatically degraded to FUra by uridine phosphorylase[116]. 5′-DFUR is substantially less toxic for bone marrow and has higher selectivity towards tumor tissue. Its 3,4,5-trimethoxybenzoyl derivative has a low toxicity and may be administered orally for longer periods[117].

Numerous polymer carriers have been used to increase selectivity and prolong the plasma half-life of FUra. Many of these systems are very easily administered; nevertheless, in some cases there are problems with side-effects caused by the carriers; moreover, their metabolism and excretion have not been fully elucidated.

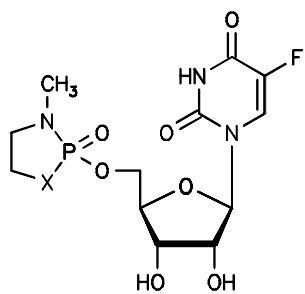
Incidentally, the first member of the later developed "dideoxy-2′,3′-didehydronucleoside" group, 2′,3′-dehydro-2′-deoxy-5-fluorouridine was prepared as FUra prodrug[118]. It is not phosphorylated by cellular kinases and resists to nucleoside phosphorylases which cleave glycosidic bond of nucleosides; however, it is rather cytotoxic. Holy et al.[119] prepared lipophilic 5′-*O*-alkyl ethers of 5-fluorouridine. Only the 5′-*O*-butyl derivative was active to a certain extent but it was still less effective than Ftorafur.

Osawa[120] made use of the important role played by sialic acid as a component of cell membrane and prepared a conjugate of 5′-DFUR with sialic acid which inhibits the transfer of these acids by sialyl transferase to exogenous glycoproteins and glycolipids. In certain types of tumors it suppresses the formation of metastases.

A series of neutral FUMP precursors *XXXVII – XLI* as transport forms[121] has been prepared during the last decade. Compounds *XXXVII* and *XXXVIII* are inactive whereas *XXXIX – XLI* are hydrolyzed under physiological conditions to intermediates with an activity against L1210 cells which is probably due to thymidylate synthase inhibition. Since TK^- strains are resistant towards these drugs, it is likely that the drug is hydrolyzed and cleaved on the outer surface of cells and only then it is taken up intracellularly.



XXXVII, R = H; X = NH
XXXVIII, R = H; X = O
XXXIX, R = F; X = O

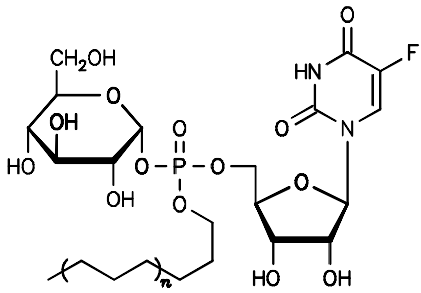


XL, X = O
XLI, X = N–CH_3

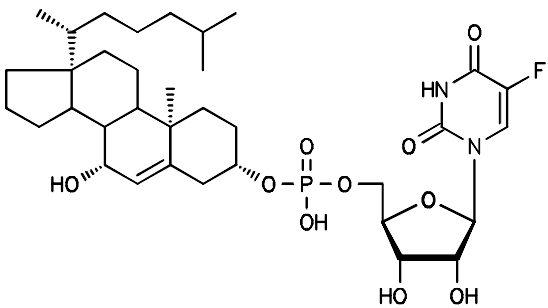
Kumar et al.[53] prepared cyclic phosphoramides which do not require any participation of enzymes for the release of nucleotide; they undergo hydrolysis leading to the formation of acyclic intermediate which rapidly cyclizes – due to the presence of carbonyl group in the side chain – to release nucleotide.

Waalkes et al.[122] prepared 3′,5′-di-*O*-palmitoyl-5-fluoro-2′-deoxyuridine which was encapsulated into liposomes of different composition. Preliminary experiments indicate that the enhanced activity of this substance may be due to higher content of the deoxynucleotide inside cells. Debouzy et al.[58] prepared triester of 5′-FUMP *XLII* with lipophilic alkyl chain and sugar subunit which by itself is able to form liposomes or penetrate membrane double-layer.

Since different oxysterols are more cytotoxic to tumor cells than to untransformed cells and act upon cell membrane, sterol phosphodiester conjugates with hydrophilic molecule of nucleoside have been prepared. The conjugate of FdUMP with 7-α-dehydrocholesterol *XLIII* has high activity and its mechanism of action is under investigation[123].



XLII



XLIII

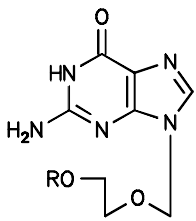
4.2. Antivirals

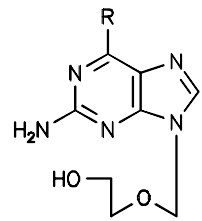
Among analogs of nucleic acids components there is a series of substances with highly selective and broad-spectrum antiviral effect[32,49]. Some of them are currently used in the clinic: 5-Iodo-2′-deoxyuridine (Idoxene, IDU), 5-trifluoromethyl-2′-deoxyuridine (trifluorothymidine, Viroptic, TFT), 9-(β-D-arabinofuranosyl)adenine (Vidarabine, Ara-A), 1-(β-D-arabinofuranosyl)-*E*-(5-bromovinyl)uracil (BV-araU, sorivudine), 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV, Zovirax), 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir, Cytovene, DHPG), 9-(4-hydroxy-3-hydroxymethylbut-1-yl)-

guanine (PCV, Penciclovir), 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (ribavirin, Virazole), 3′-azido-3′-deoxythymidine (azidothymidin, zidovudin, Retrovir), 2′,3′-dideoxyinosine (didanosine, Videx)[9]. Numerous attempts have been performed to influence pharmacological parameters or to target the drugs to specific cells or tissues.

4.2.1. Acyclovir, Ganciclovir and Penciclovir

Following oral administration of ACV, *XLIVa*, 15 – 20% of the substance is absorbed. Higher plasmatic levels necessary for the action against less sensitive viruses result in the exclusion of the substance in renal tubuli with subsequent complications[9]. With the aim to increase the water solubility a series of ACV derivatives *XLIVb – XLIVe* has been prepared. The solubility of these esters in water reaches 6% to compare with 0.2% of ACV, their activity is comparable or slightly lower but their cytotoxicity is substantially decreased compared to the parent drug. However, the oral absorption is not increased[124]. This does not apply to the L-valine ester of ACV, *XLIVf* (VACV, valaciclovir, ref.[125]) which offers a substantial improvement in oral bioavailability. It has been recently approved (Valtrex) for antiviral therapy (herpesviruses). Good et al.[126] prepared 9-(2-hydroxyethoxymethyl)-2,6-diaminopurine (*XLV*) which is transformed to ACV by the action of adenosine deaminase. While antiviral effects of the both compounds are comparable, ED_{50} values for substance *XLV* are higher and the maximum plasma level is twice as large as that of the parent compound.





XLIVa, R = H
XLIVb, R = glycyl ester
XLIVc, R = L-alanyl ester
XLIVd, R = β-alanyl ester
XLIVe, R = 3-carboxypropionyl ester
XLIVf, R = azidoacetyl ester
XLIVg, R = L-valyl ester

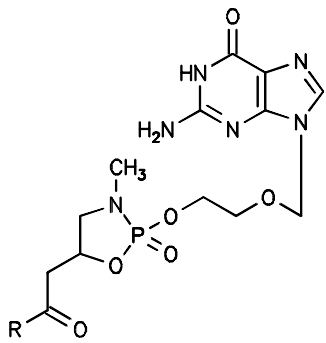
XLV, R = NH_2
XLVI, R = H

6-Deoxyacyclovir *XLVI* is by itself antivirally inactive; however, in vivo it undergoes an oxidation by xanthine oxidase resulting in a mixture of inactive 6-deoxy-8-hydroxyacyclovir and ACV (ref.[124]). On administering 6-deoxyacyclovir to humans, ACV

concentration in plasma is comparable to that reached after the administration of ACV alone at twentyfold dose. Compound *XLVI* has higher water solubility, better absorption, shorter plasma half-life and does not accumulate after repeated dosage. To enhance lipophilic properties of ACV a series of its primary and secondary alkyl ethers of ACV was prepared by Winkler et al.[127].

ACV triphosphate is a substrate-type inhibitor of herpes virus DNA polymerase[128]. The activity depends on the primary transformation of ACV by viral thymidine kinase (TK) to the monophosphate. In TK deficient virus strains the antiviral effect does not occur. On administration of ACV mono-, di- or triphosphates the compounds are dephosphorylated at the membrane. This fact stimulated the preparation of many ACV derivatives which could be taken up inside the cells and hydrolyzed there into monophosphates either chemically or enzymatically.

Farrow et al.[54] prepared triesters of ACV monophosphate with substituted phenols which yield by spontaneous enzymatic hydrolysis simultaneously acyclic nucleoside and its monophosphate in equimolar amounts. Of the different alcohols, 4-(methylthio)phenyl and 4-(methanesulfonyl)phenylesters of the type *XIX* with hydrolysis half-life of 10 – 15 h (pH 7.7) seemed to be the most appropriate candidates for further investigation.



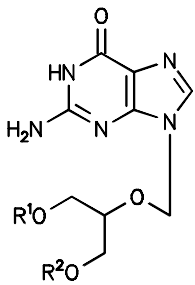
XLVII

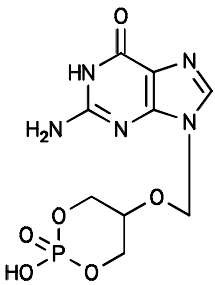
Kumar[53] prepared cyclic ester phosphoramidates whose phosphoramide bond undergoes hydrolysis under physiological conditions without enzyme participation (see above). Different derivatives of this type, e.g. *XLVII*, probably act as nucleotide prodrugs but no direct release of nucleotide has been demonstrated in any of these substances.

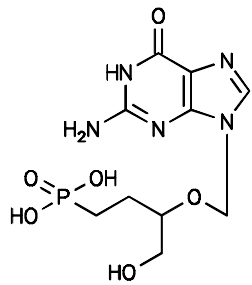
Also the conjugate of ACV monophosphate with lactoseamin–albumin, prepared by Fiume et al.[80], functions as targeted carrier releasing ACVMP in hepatocytes and has good effects against hepatitis B virus.

Another active drug structurally similar to ACV is ganciclovir, *XLVIII* (Cytovene, DHPG, ref.[129]) whose phosphate *IL*, 1,3-diphosphate *L*, cyclic phosphate *LI* and phos-

phonate *LII* are highly efficient agents in vivo against HSV-1, HSV-2, cytomegaloviruses and other DNA viruses. Derivatives *IL – LI* are not activated by kinases, and they have low toxicity and low permeability. Only cyclic phosphate *LI* is taken up by cells where it is hydrolyzed to (*S*)-enantiomer of DHPG monophosphate *IL*. The mechanism of action of this compound has not been completely elucidated since the respective triphosphate[130] is not its target metabolite.







XLVIII, $R^1 = R^2 = H$ ***LI*** ***LII***

IL, $R^1 = P(O)(OH)_2$; $R^2 = H$

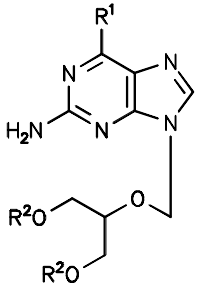
L, $R^1 = R^2 = P(O)(OH)_2$

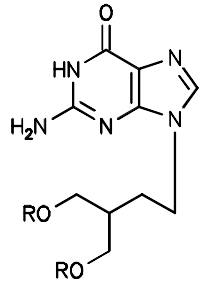
Additional dipropionyl, dioctanoyl DHPG and N^2-butyryl amide have been prepared[131]; they possess certain antiviral activity but no therapeutic advantage.

Similarly to ACV also the 2,6-diaminopurine analog of ganciclovir[132] (*LIII*) is an outstanding substrate of adenosine deaminase, while 6-deoxyDHPG (*LIV*) is a good substrate for xanthine oxidase. Both substances are highly antiviral in vivo; however, in tissue culture they are without effect.

Winkelmann et al.[133] prepared DHPG and ACV alkyl ethers, some of them with important antiviral activity: The best compound is diisopropylether of 6-deoxy-DHPG (*LV*) also known as HOE-602 which in organism is first oxidized by xanthine oxidase and then stepwise releases monoisopropyl derivative and DHPG. Oral administration of HOE-602 leads to higher plasma concentrations of DHPG compared to DHPG alone.

Penciclovir (*LVIa*) can be considered a "carba" analogue of ganciclovir. It has a potent and selective activity against herpes simplex viruses and varicella zoster virus[134]. Its oral prodrug, the di-*O*-acetyl derivative famciclovir (*LVIb*) which has substantially improved pharmacological parameters[135,136] has been recently approved for treatment of shingles and is considered a prospective drug also for treatment of infectious mononucleosis caused by Epstein–Barr virus[137].



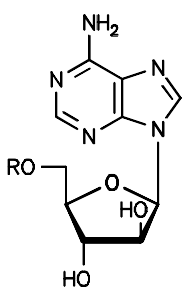


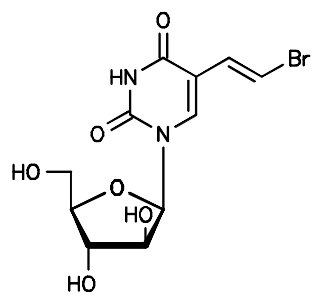
LIII, $R^1 = NH_2$; $R^2 = H$
LIV, $R^1 = R^2 = H$
LV, $R^1 = H$; $R^2 = CH(CH_3)_2$

LVIa, R = H
LVIb, R = CH_3CO

4.2.2. Vidarabin

AraA (*LVII*) which has been widely used in clinic is now often replaced by ACV and used mainly in those cases of infection with TK^-HSV which are resistant to ACV and also in herpes virus encephalitis in newborns. It is scarcely soluble in water and it dissolves only to a limited extent in lipids; thus, its topical and parenteral application is rather difficult. Moreover it undergoes deamination by adenosine deaminase to hypoxanthine derivative which is 30 times less effective. For this reason, different mono-, di- and triacyl derivatives of this nucleoside with aliphatic and aromatic carboxylic acids have been prepared and examined as prodrugs. The esterification of sugar residue by three acyl groups results in an enhanced lipophilicity and in an increased resistance to deamination; however, it is associated in majority of cases with a decrease or loss of





LVII, R = H
LVIII, R = $P(O)(OH)_2$

LIX

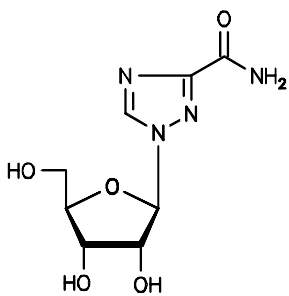
activity[138]. 2′,3′- and 3′,5′-diacyl derivatives with short linear acyl residues retain their activity; however, on branching of the acyl groups their activity is lost[139]. Diacyl derivatives possess suitable water and lipid solubility, resistance to enzyme degradation and good biological availability as well as high activity against HSV-2 and are considered as good prodrug candidates[140].

Ara-A 5′-monophosphate (*LVIII*) is substantially more water-soluble and more resistant to deamination compared to the nucleoside. Nevertheless, during its application an extensive dephosphorylation and subsequent deamination takes place. It was also applied by ionophoresis. Its diesters with aliphatic alcohols (ethyl, propyl, butyl, pentyl) have been suggested as prodrugs of *LVIII*. While in vitro these esters are degraded by esterases and viral DNA synthesis is inhibited, in vivo this effect has not been observed[141].

Another therapeutically important arabinoside, 1-(β-D-arabinofuranosyl)-(*E*)-5-(2-bromovinyl)uracil (*LIX*, BV-araU, sorivudine, Brovavir) is approved in Japan for treatment of varicella zoster infections[142]. 5′-*O*-Alkyl and 5′-*O*-acyl derivatives of this nucleoside were scrutinized as potential oral prodrugs of sorivudine with extended plasma level of the drug. Short 5′-*O*-alkyl (e.g. ethyl) derivatives are excellent oral prodrugs in mice[143].

4.2.3. Ribavirin

Ribavirin *LX* is used predominantly against respiratory syncitial virus in children[32], and at present its combination with AZT has been proposed for the treatment of AIDS patients. Contrary to other nucleoside antivirals, ribavirin is well soluble in water and is rapidly absorbed. It is taken up by cells by active nucleoside transport. To enhance its lipophilicity and thus the transport into CNS its 5′-*O*-acetyl, butyryl, benzoyl and 1-adamantoyl esters have been prepared. Its 2′,3′,5′-tri-*O*-acetyl ester has outstanding effects against a number of virus infections as well as the ability to cross BBB (ref.[144]). On the other hand, 5′-*O*-butyryl, valeryl, caproyl and other acyl derivatives offer no advantage in comparison to free nucleoside *LX*. The increase of lipophilicity of such

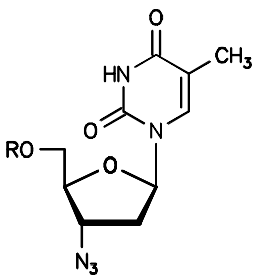


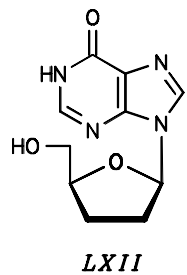
LX

substances enhances not only their penetration into CNS but also decreases their excretion resulting thus ultimately in a decreased retention and short plasma half-life. The end result is also low concentration in CNS which limits the usefulness of such prodrugs. For this reason esters with *N*-methyldihydropyridine-4-carboxylic acid[63] have been prepared (according to Bodor's conception, vide supra); on administering to mice, they exhibit remarkably improved therapeutic effects and decreased toxicity compared to the original compound.

4.2.4. Dideoxynucleosides

Several years ago 3′-azido-2′,3′-dideoxythymidine (azidothymidine, AZT, Retrovir) *LXIa* and 2′,3′-dideoxyinosine (didanosine, d_2I, Videx, *LXII*) have been approved for treatment of patients with AIDS. Few other 2′,3′-dideoxynucleoside analogs which inhibit HIV replication and have a similar selectivity index as AZT (ref.[141]) are also undergoing preclinical and clinical investigation. Their effectiveness depends on three-step enzymatic phosphorylation which gradually converts nucleosides to their 5′-triphosphates that act as substrate inhibitors of HIV reverse transcriptase[145]. The incorporation of these analogs into the growing DNA chain results in the chain termination. The selectivity is explained by different affinity of the modified triphosphate to





LXII

LXIa, R = H
LXIb, R = 4-phenylpiperazin-1-ylacetyl
LXIc, R = morpholin-1-ylacetyl
LXId, R = 1-methyl-1,4-dihydropyridin-3-carbonyl
LXIe, R = retinoyl
LXIf, R = L-phenylalanyl
LXIg, R = L-tyrosinyl
LXIh, R = L-isoleucinyl
LXIi, R = L-lysinyl
LXIj, R = L-glutamyl

HIV reverse transcriptase and to cellular DNA polymerases[146]. The same principle applies to two novel drugs recently approved for AIDS treatment: 2′,3′-dideoxycytidine (ddC, *LXIIIa*) and 2′3′-didehydro-2′,3′-dideoxythymidine (d4T, Lamivudine, *LXIVa*).

Cheung et al.[18] used HPLC to assay the lipophilicity of the series of nucleoside analogs with respect to their adsorption and distribution in target tissue. On account of its lipophilic character the cellular uptake of AZT does not depend on transport mechanism for natural nucleosides and proceeds by diffusion via non-activated transport mechanism[19].

Kawaguchi et al.[147] prepared 5′-esters of AZT and other dideoxynucleosides with diverse carboxylic acids. The rate of enzymatic hydrolysis depends on the chain length and is most rapid for capronic acid ester. With increasing number of carbon atoms the half-life of excretion is prolonged: Higher members of this class bind to lipophilic plasma particles with which they are transported and metabolized.

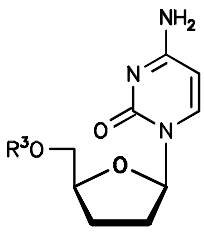
Agarwal[148] and Hanson[149] prepared *N*-piperazinyl and morpholinyl substituted esters *LXIb*, *LXIc* and 1,4-dihydro-1-methyl-3- [(pyridinylcarbonyl)] derivative *LXId* for transport into neuron tissues[150], ester of retinoic acid *LXIe* (ref.[151]) which itself inhibits HIV replication, and finally esters of selected amino acids *LXIf – LXIj* with the intention to use their specific transport properties[152]. Since bone marrow cells lack amino acid transport system[153] it has been presumed that such an AZT derivative may be less toxic for bone marrow cells. These derivatives are generally less toxic and they are taken up by cells more easily. The most active is dihydropyridine derivative of AZT *LXId* which was prepared[63] together with the corresponding uracil analogue (AZU). These derivatives easily penetrate membranes and inside cells they are oxidized to quaternary pyridinium salt and hydrolyzed to AZT (see also *XXII – XXIV*). Biological tests revealed that the transport into CNS of these substances is clearly enhanced.

McGuigan et al.[154] studied neutral AZTMP diesters. Their solubility in membranes is enhanced and their intracellular diffusion increased: Ethyl, butyl and propyl esters, however, are inactive in vivo since their phosphodiester bond is not hydrolyzed. AZT monoester phosphoramides were also prepared; they contain amide-bound amino acid derivative and ester-bound aralkyl alcohols which are split by nonspecific esterases. These neutral nucleotide prodrugs are highly effective also against virus mutants resistant to nucleosides[155a]. Two-step activation mechanism was employed in the design of bis(4-acyloxybenzyl) ester of AZT 5′-monophosphate which are activated by the esterase cleavage of the acyloxy groups[155b].

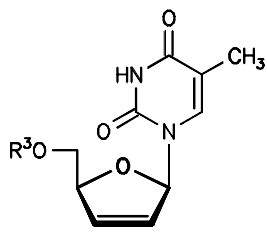
Gouyette et al.[58] made use of the transport system described above and prepared AZT phosphate derivatives esterified by saccharide (D-glucose, D-mannose and ethyl D-mannopyranoside) and by hexadecyl chain. The NMR studies in the presence of model unilamellar vesicles revealed their interaction with external lipid layer and the transport into intravesicular space. The studies in tissue cultures showed antiviral activity comparable with that of AZT and confirmed the presumed mode of transport and of sub-

sequent hydrolysis of these derivatives as well as their decreased toxicity compared to AZT.

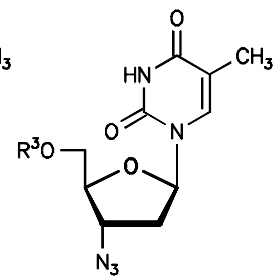
Murata and Achiwa[156] prepared the conjugate consisting of AZT and *N*-acetyl-D-neuraminic acid which is of importance for the cell wall metabolism.



LXIII



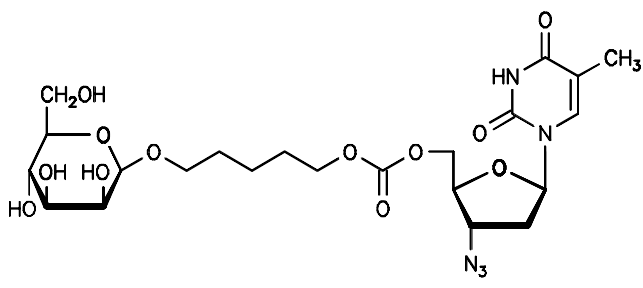
LXIV



LXVI

In formulae *LXIII*, *LXIV*, *LXVI*: *a*, R^3 = H

b, R^3 = $R^1OCH_2CH(OR^2)CH_2OP(O)(OH)$

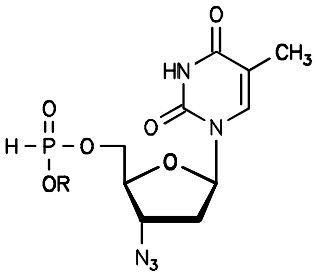


LXV

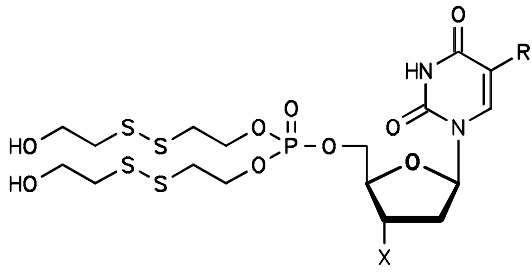
Carbonate derivative *LXV* releases AZT enzymatically as well as by spontaneous hydrolysis. The sugar residue serves as a group directing the molecule to membrane receptors; D-mannose derivative suppresses cytopathic effects due to HIV-1 and inhibits reverse transcriptase. On the other hand, the conjugate of AZT with *N*-acetylglucosamine is not active[157].

Diglycerides of AZT-5′-phosphate *LXVIb* analogous to those mentioned as ara-C prodrugs have been prepared. Following intravenous application these compounds bind specifically to serum lipoproteins with which they are transported and metabolized. They do not undergo glucuronidation and slowly release AZT and its 5′-phosphate[158a]. These lipophilic derivatives of AZT have also been incorporated into mixed liposomes together with glycerophospholipids and cholesterol functioning as their specifically

targeted carrier to macrophages[158b]. Their ammonium salts form unilamellar liposomes spontaneously. Diphosphoglycerol derivatives of antiretroviral dideoxynucleosides AZT, ddC and ddT (*LXIIIb*, *LXIVb*, *LXVIb*) enclosed in liposomes are somewhat less active in vitro, however, better selectivity index and enhanced effect[158c] have been observed in vivo.



LXVII



LXVIIIa, R = CH_3; X = N_3
LXVIIIb, R = X = H

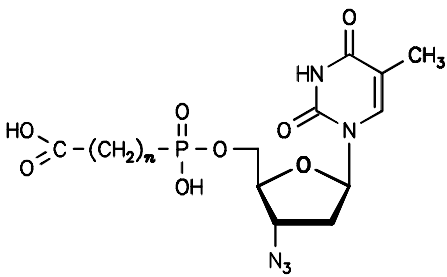
5′-Alkyl hydrogen phosphonates of AZT (*LXVII*) with various length of the alkyl group (C_1 – C_7) retain full antiviral activity of the parent drug whilst exhibiting decreased cellular toxicity. They are also active in a cell line that is poorly responsive to AZT (ref.[159]).

Bis[*S*-(2-hydroxyethylsulfidyl)-2-thioethyl] esters of AZT 5′-monophosphate (*LXVIIIa*) and 2′,3′-dideoxyuridine 5′-monophosphate (*LXVIIIb*) enter the cells and release the corresponding monoester probably by the action of cellular reductases. The remaining ester groups are cleaved by a phosphodiesterase-catalyzed hydrolysis yielding the corresponding nucleotide. An improved antiviral activity was observed particularly in the latter case (*LXVIIIb*). An analogous neutral diester prodrug has been prepared also from 9-(2-phosphonomethoxyethyl)adenine[160].

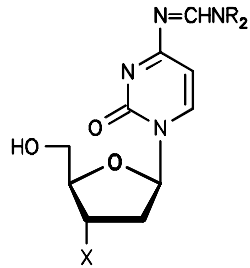
Molema et al.[79] used serum albumin (HSA) for targeted transport of AZTMP to T4 lymphocytes. Following the modification of albumin by D-glucose, D-galactose, D-fucose and D-mannose, nucleotide analogs were bound to the remaining amino groups. The antiviral activity increased depending on the number of sugar residues and it was optimal in D-mannosyl derivative with forty sugar units per HSA molecule.

Lambert[161], Stein[162] and Rosowsky[163] prepared 5′-esters of phosphonoformic and phosphonoacetic acid with antiviral nucleoside analogs including 2′,3′-dideoxynucleosides *LXIX* which by themselves act as inhibitors of viral DNA polymerases. These substances might function as double prodrugs which gradually release by hydrolysis both active substances. However, the effect of these esters in tissue cultures was lower than the activity of individual compounds in vitro.

The amidine derivatives can be easily obtained from nucleoside and nucleotide analogues which contain heterocyclic bases bearing amino functions, by the reaction with *N*,*N*-dialkylformamide dialkyl acetals[164]. They generate the parent compound by chemical hydrolysis in weakly alkaline media, with the rate values dependent on the pH and the character of the *N*-alkyl group. This concept was applied first to the development of araA prodrug[165] and later to the preparation of a series of *N*,*N*-dialkylaminomethylene derivatives of 2′,3′-dideoxycytidine and its 3′-fluoro congener[166]. In the latter series (compounds *LXX*), the optimum properties were encountered with *N*,*N*-di(2-propyl) derivatives which were substantially better water-soluble compared to the parent compounds and showed significant antiretroviral activity.



LXIX, n = 0,1



LXX, X = H,F; R = alkyl

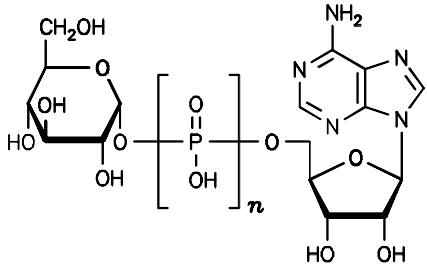
4.2.5. 5-Iodo-2′-deoxyuridine

5-Iodo-2′-deoxyuridine (idoxuridin, Dendrid) is used in ophtalmology for the topical treatment of herpetic keratitis. To enhance its permeability across retina its 5′-esters with aliphatic carboxylic acids[167] have been prepared. This modification enables substantial lowering of dosage necessary to obtain effective concentration.

4.2.6. Oligonucleotides

Recently much work has been devoted to medical application of modified oligonucleotides[168]. In the organism these substances are easily split by nucleases and their ionic character renders the direct intracelullar penetration impossible; apparently their transport takes place predominantly via endocytosis. To enhance their stability and biological accessibility a series of systems has been proposed. The modification by lipophilic groups brings certain advantage[169]: Lipophilization with longer acyl groups results in the increased resistance to diesterases and in an enhanced penetration into cells. In this respect the systems transporting oligonucleotides in liposomes, in particular those

targeted by monoclonal antibodies[170] or bound to polymer carriers[171] exhibit outstanding properties. The targeting effect of D-glucose or D-mannose on the transport of (2′ – 5′)-oligoadenylates is utilized in derivatives *LXXI* which may be transported by endocytosis with the aid of membrane receptors[172].



LXXI

5. CONCLUSION

Transformation of antimetabolites to their prodrugs may result in considerable changes of biological activity, increase of selectivity index, enhancement of biological accessibility and stimulation of the overall effect. The drug transport targeted with the aid of endocytosis and receptors, application of macromolecular carriers and other possibilities described above will considerably contribute to therapeutic success. One may also expect the outcome of the investigation of homing receptors of lymphocytes which will determine their localization in diverse tissues. More profound molecular understanding of viral tropism could assist in using certain viruses as important drug carriers. Moreover, various migrating cell populations (monocytes, macrophages, granulocytes and neutrofiles) could be applied as targeting drug carriers for different purposes[25].

REFERENCES

1. Albert A.: Nature (London) *182*, 421 (1958).
2. Bennett L. L., Brockman R. W., Montgomery J. A.: Nucleosides Nucleotides *5*, 117 (1986).
3. Ayre S. G.: Med. Hypotheses *29*, 283 (1989).
4. Tidd D.: *Antitumor Drug Resistance*, p. 445. Fox Springer-Verlag, Berlin 1984.
5. Johnson P., Lloyd-Jones J. G.: *Drug Delivery System.* VSH Publishers 1987.
6. Bundgaard H.: *Bioreversible Carriers in Drug Delivery*, p. 1. Pergamon Press, New York 1987.
7. Hadfield A. F., Sartoreli A. C.: Adv. Pharmacol. Chemother. *20*, 21 (1987).
8. Notari R. E.: Pharmacol. Ther. *14*, 25 (1987).
9. Canonico P. G., Kende M., Gabrielsen B.: Adv. Virus Res. *35*, 271 (1988).
10. Zimmerman P. T., Domin B. A., Mahony W. B., Prus K. L.: Nucleosides Nucleotides *8*, 765 (1989).

11. Phillips J. W. in: *Adenosine and Adenine Nucleotides as Regulators of Cellular Function* (J. W. Phillips, Ed.). CRC Press, Boca Raton 1991.
12. Hertz L. in: *Adenosine and Adenine Nucleotides as Regulators of Cellular Function* (J. W. Phillips, Ed.), p. 85. CRC Press, Boca Raton 1991.
13. Plagemann P. G. W., Aran J. M.: Biochim. Biophys. Acta *1028*, 289 (1990).
14. Jhun B. H., Rampal A. L., Berenski C. J., Jung C. Y. in: *Adenosine and Adenine Nucleotides as Regulators of Cellular Function* (J. W. Phillips, Ed.), p. 251. CRC Press, Boca Raton 1991.
15. Darnell J., Lodish H., Baltimore D.: *Molecular Cell Biology*, p. 617. Scientific American Books 1986.
16. Plagemann P. G. W., Woffenden C.: Biochim. Biophys. Acta *905*, 17 (1988).
17. Mahony W. B., Zimmerman T. P., Domin B. A., McConnell R. T.: J. Biol. Chem. *263*, 9285 (1988).
18. Cheung A., Keney D.: J. Chromatogr. *506*, 119 (1990).
19. Balzarini J., Cools M., De Clercq E.: Biochem. Biophys. Res. Commun. *158*, 413 (1989).
20. Srinivasan V., Higuchi W. I.: Int. J. Pharm. *60*, 133 (1990).
21. Wileman T.: Biochem. J. *232*, 1 (1985).
22. Doxsley S. J., Sambrook J.: J. Cell Biol. *101*, 19 (1985).
23. Hsu M. C., Choppin P. W.: Proc. Natl. Acad. Sci. U.S.A. *79*, 5862 (1983).
24. Fallon L., Schwartz A. L.: Adv. Drug Delivery Rev. *4*, 49 (1989).
25. Basu S. K.: Biochem. Pharmacol. *40*, 1941 (1990).
26. Vitetta E. S., Fulton R. J.: Science *238*, 1098 (1987).
27. Spitler L.: Cancer Res. *47*, 1717 (1987).
28. Stanislawski M., Rousseau V.: Cancer Res. *49*, 5497 (1988).
29. Jung G., Ledbetter J. A.: Proc. Natl. Acad. Sci. U.S.A. *84*, 4611 (1987).
30. Lin M. A., Nusbaum S. R., Eisen H. N.: Science *239*, 395 (1988).
31. Gordon S., Rabinowitz S.: Adv. Drug Delivery Rev. *4*, 27 (1989).
32. Belshe R. B.: *Textbook of Human Virology*. Moshby Year Book, St. Louis 1991.
33. Bijsterbosch M. K., Ziere G. J.: Mol. Pharmacol. *36*, 484 (1989).
34. Goldstein J. L., Ho Y. K.: Proc. Natl. Acad. Sci. U.S.A. *76*, 333 (1979).
35. Paterson A. R.: Pharmacol. Ther. *12*, 515 (1981).
36. Zimmerman T. P., Mahony W. B., Prus K. J.: J. Biol. Chem. *262*, 5784 (1987).
37. Jones L. A., Moorman A. R., Reynolds J. P., Krenitsky T. A.: J. Med. Chem. *35*, 56 (1992).
38. (S. M. Roberts, Ed.): *Molecular Recognition – Chemical and Biochemical Problems*, Royal Society of Chemistry, London 1989.
39. Muehldorf A. V., Van Engen D., Warner J. C.: J. Am. Chem. Soc. *110*, 6561 (1988); Hamilton A. D., Little D. J.: J. Chem. Soc., Chem. Commun. *1990*, 297; Rebek J.: Angew. Chem., Int. Ed. *29*, 225 (1990).
40. Furuta H., Furuta K., Sessler J. L.: J. Am. Chem. Soc. *113*, 4706 (1991).
41. Bundgaard H.: *Design of Prodrugs*. Elsevier, New York 1985.
42. Elgavish A., Elgavish G. A.: Biochim. Biophys. Acta *812*, 595 (1985).
43. Martin J. C., Ed.: *Nucleotide Analogues as Antiviral Agents*, ACS Symposium Series *401* (1989).
44. De Clercq E.: Biochem. Pharmacol. *42*, 963 (1991).
45. Foster S. A., Cerny J., Cheng Y. C.: J. Biol. Chem. *266*, 238 (1991).
46. Palu G., Stefanelli S., Rassu M., Parolin C., Balzarini J., De Clercq E.: Antiviral Res. *16*, 115 (1991).
47. De Clercq E.: Microbiologica (Bologna) *13*, 165 (1990).
48. *a*) Meier C., Neumann J. M., Andre F., Henin Y., Huynh-Dinh T.: J. Org. Chem. *57*, 7300 (1990); Meier C., Huynh-Dinh T.: Bioorg. Medicinal. Chem. Letter *1*, 527 (1991); *b*) Farquhar D.,

Srivastava D. N., Kuttesch N. J., Saunders P. P.: J. Pharm. Sci. *72*, 324 (1983); Srivastava D. N., Farquhar D.: Bioorg. Chem. *12*, 118 (1984); Iyer A., Phillips L. R., Egan W.: Tetrahedron Lett. *30*, 7141 (1989).
49. Starrett J. E., Tortolani D. R., Hitchcock M. J. M., Martin J., Mansuri M. M.: Antiviral Res. *19*, 267 (1992); Srinivas R. V., Robbins B. L., Connelly M. C., Gong Y. F., Bischofberger N., Fridland A.: Antimicrob. Agents Chemother. *37*, 2247 (1993).
50. *a*) Freeman S., Irwin J. W., Mitchell G. A., Nicholls D., Thomson W.: J. Chem. Soc., Chem. Commun. *1991*, 875; Meier C.: Angew. Chem. Int. Ed. *32*, 1704 (1993); *b*) Glazier A., Kwong C., Rose J., Buckheit R., Korba B., Abou-Donia M., Smith E., Wright G. E.: Antiviral Res. *17*, Suppl. 1, 77 (1992); *c*) Glazier A., Buckheit R., Yanachkova M., Yanachkov I., Wright G. E.: Antiviral Res. *23*, Suppl. 1, 65 (1994).
51. Farquhar D., Smith R.: J. Med. Chem. *26*, 1153 (1983); Jones A., McGuigan C., Walker R. T., Balzarini J., De Clercq E.: J. Chem. Soc., Perkin Trans. 1 *1984*, 1471; Hunston R. N., Jones A., McGuigan C., Walker R. T.: J. Med. Chem. *27*, 440 (1984); Farquhar D., Smith R.: J. Med. Chem. *28*, 1358 (1985).
52. Tietze L., Beller M., Fischer R.: Angew. Chem., Int. Ed. *29*, 782 (1990).
53. Kumar A., Coe P. L., Jones A. S., Walker R. T., Balzarini J., De Clercq E.: J. Med. Chem. *33*, 2368 (1990).
54. Farrow S. N., Jones A. S., McGuigan C., Balzarini J., De Clercq E.: J. Med. Chem. *33*, 1400 (1990).
55. Hunston R. N., Jones A. S., McGuigan C.: J. Med. Chem. *27*, 440 (1984).
56. Iglesias F. G., Neumann J. M., Huynh-Dinh T.: Tetrahedron Lett. *28*, 3581 (1987); Gouyette C., Neumann J. M., Fauve R.: Tetrahedron Lett. *30*, 6019 (1989); Neumann J. M., Herve M., Debouzy J. C., Guerra F. I., Gouyette C., Dupraz B.: J. Am. Chem. Soc. *111*, 4270 (1989); Debouzy J. C., Herve M., Neumann J. M., Gouyette C., Dupraz B.: Biochem. Pharmacol. *39*, 1657 (1990).
57. Henseleit U., Plasa G., Haest C.: Biochim. Biophys. Acta *1029*, 127 (1990).
58. Henin Y., Gouyette C., Schwartz O., Debouzy J. C., Neumann J. M., Huyn-Dinh T.: J. Med. Chem. *34*, 1830 (1991).
59. Namane A., Gouyette C., Fillion M. P., Fillion G., Huynh-Dinh T.: J. Med. Chem. *35*, 3039 (1992).
60. Namane A., Gouyette C., Fillon M. P., Huynh-Dinh T.: J. Med. Chem. *35*, 3039 (1992).
61. Bodor N., Brewster M. E.: Pharmacol. Ther. *19*, 337 (1983).
62. Chu C. K., Bhadti V. S., Doshi K. J., Etse T. J., Gallo J. M., Boudinot D. F., Schinazi R. F.: J. Med. Chem. *33*, 2188 (1990).
63. Palomino E., Kessel D., Horwitz P.: J. Med. Chem. *32*, 622 1989); Morin K. W., Wiebe L. I., Knaus E. E.: Carbohydr. Res. *249*, 109 (1993); Kumar R., Knaus E. E., Wiebe L. I.: Nucleosides Nucleotides *12*, 895 (1993).
64. Fauci A. S.: Science *239*, 617 (1988).
65. Brewster M. A., Pop E., Braunstein A. J., Pop A. C., Druzgala P., Dinculescu A., Anderson W., El Koussi S., Bodor N.: Pharm. Res. *10*, 1356 (1993).
66. Tabushi I., Kobuke Y., Imuta J.: J. Am. Chem. Soc. *102*, 1744 (1980).
67. Hamilton A. D., Pant N., Muehldorf A.: Pure Appl. Chem. *60*, 533 (1988).
68. Goswani S., Hamilton A., Van Engen D.: J. Am. Chem. Soc. *111*, 3425 (1989).
69. Rebek J. in: *Molecular Recognition* (S. M. Roberts, Ed.), p. 211. Royal Society of Chemistry, London 1989.
70. Kemp D. S., Petrakis K. S.: J. Org. Chem. *46*, 5140 (1981).
71. Benzing T., Tjivikua T., Wolfe J., Rebek J.: Science *242*, 266 (1988).

72. Furuta H., Magda D., Sessler J. L.: J. Am. Chem. Soc. *113*, 978 (1991).
73. *a*) Furuta H., Cyr J. M., Sessler J. L.: J. Am. Chem. Soc. *113*, 6677 (1991); *b*) Kral V., Sessler J. L., Furuta H.: J. Am. Chem. Soc. *114*, 8704 (1992); *c*) Sessler J. L., Furuta H., Kral V.: Supramol. Chem. *1*, 209 (1993); *d*) Sessler J. L., Cyr M., Furuta H., Kral V., Mody T., Morishima T., Shionoya M., Weghorn S.: Pure Appl. Chem. *65*, 393 (1993).
74. Hosseini M. W., Lehn J. M., Jones K. C., Mertes M. P.: J. Am. Chem. Soc. *111*, 6330 (1989).
75. Hosseini M. W., Blacker A. J., Lehn J. M.: J. Am. Chem. Soc. *112*, 3896 (1990).
76. Otoski R. M., Wilcox C. S.: Tetrahedron Lett. *29*, 2615 (1988).
77. Heifetz A., Keenan R. W.: Biochemistry *18*, 2186 (1979).
78. Suhadolnik R. J.: *Nucleosides as Biological Probes*. Wiley, New York 1979.
79. Molema G., Jansen R., Pauwels P., De Clercq E.: Biochem. Pharmacol. *40*, 2603 (1990).
80. Fiume L., Buzi C., Mattioli A., Spinelli C.: Naturwissenschaften *1989*, 74.
81. Ouchi T., Banba T.: J. Bioact. Compat. Polym. *4*, 363 (1989).
82. Parrot H., Djedaini F., Perly B.: Tetrahedron Lett. *31*, 1999 (1990).
83. Midoux P., Negre E., Roche A. C., Mayer R., Monsigny M., Balzarini J., De Clercq E.: Biochem. Biophys. Res. Commun. *167*, 1044 (1990).
84. Leonetti P., Ragner B.: Gene *1990*, 323.
85. Blume G.: Biochim. Biophys. Acta *1029*, 91 (1990).
86. Wenstein J. N., Leserman L. D.: Pharmacol. Ther. *24*, 207 (1983).
87. Clarkson B. D.: Cancer *30*, 1572 (1972).
88. Gish D., Kelly R., Camiener G.: J. Med. Chem. *14*, 1159 (1971).
89. Hamamura E. K., Prysztasz M., Verheyden J. P.: J. Med. Chem. *19*, 667 (1976).
90. Wechter W., Johnson M., Hall C.: J. Med. Chem. *19*, 1017 (1976).
91. Ho D. W. H., Neil G. L.: Cancer Res. *37*, 1640 (1977).
92. Neil G. L., Wiley P. F.: Cancer Res. *30*, 1047 (1970).
93. Aoshima M., Kobayashi H.: Cancer Res. *36*, 2726 (1976).
94. Nakamura K., Eisum Y., Kamura K.: J. Med. Virol. *31*, 141 (1990).
95. Schott H., Heb W., Leitner B., Schwendener R. A.: Nucleosides Nucleotides *7*, 721 (1988).
96. Raetz C. R., Chu M. Y., Srivastava S. P., Turcotte J. G.: Science *196*, 303 (1977).
97. Turcotte J. C., Srivastava S. P., Stein J., Chu M. Y.: Biochim. Biophys. Acta *619*, 619 (1980).
98. *a*) Hong Ch., Nechaev A., West R. Ch.: J. Med. Chem. *33*, 1380 (1990); *b*) Hostetler K. Y., Richman D. D., Carson D. A., Stuhmiller L. M., Van Wijk G. M. T., Van den Bosch H.: Antimicrob. Agents Chemother. *36*, 2025 (1992); Vanwijk G. M. T., Hostetler K. Y., Suurmeijer C. N. S. P., Van den Bosch H.: Biochim. Biophys. Acta *1165*, 45 (1992); Hong C. I., Nechaev A., Kirisits A. J., Vig R., West C. R.: J. Med. Chem. *36*, 1785 (1993); Hong C. I., Kirisits A. J., Buchheit D. J., Nechaev A., West C. R.: J. Med. Chem. *33*, 1380 (1990); Hong C. I., West C. R., Bernacki R. J., Tebbi C. K., Berdel W.: Lipids *26*, 1437 (1991); *c*) Hostetler K. Y., Parker S., Sridhar C. N., Martin M. J., Li L. M., Stuhmiller L. M., Vanwijk G. M. T., Van den Bosch H., Gardner M. F., Aldern K. A., Richman D. D.: Proc. Natl. Acad. Sci. U.S.A. *90*, 11835 (1993).
99. Lier J. E., Kan G., Nigan V.: Nature *267*, 522 (1977).
100. Hong C. I., Nechaev A., West C. R.: J. Med. Chem. *22*, 1428 (1978).
101. Hong C. I., Nechaev A., West C. R.: Biochem. Biophys. Res. Commun. *94*, 1169 (1980); Hong C. I., Nechaev A., West C. R.: J. Med. Chem. *29*, 2038 (1986).
102. Tokunaga Y., Iwaso T., Kagagana A.: Chem. Pharm. Bull. *36*, 4060 (1988).
103. Schrier W., Nagyvary J.: Biochem. Pharmacol *26*, 2375 (1977).
104. Wilkinson D. S., Tlsty T. D., Hanas R. J.: Cancer Res. *35*, 3014 (1975).
105. Hiller S. A., Zhuk R. A., Lidak M.: Dokl. Akad. Nauk SSSR *176*, 332 (1967).

106. Au J. L., Sadee W.: Cancer Res. *40*, 2814 (1980).
107. Yasumoto M., Hashimoto S.: J. Med. Chem. *21*, 738 (1978).
108. Ozaki S., Ike Y., Mori N.: Bull. Chem. Soc. Jpn. *50*, 2406 (1977).
109. Hoshi A., Ioshida M.: Chem. Abstr. *110*, 185493 (1986).
110. Pischel H., Holy A., Vesely J., Wagner G., Cech D.: Collect. Czech. Chem. Commun. *47*, 2806 (1982).
111. Pischel H., Holy A., Vesely J., Wagner G.: Collect. Czech. Chem. Commun. *49*, 2541 (1984).
112. Pischel H., Holy A., Vesely J., Wagner G., Cech D.: Collect. Czech. Chem. Commun. *52*, 2061 (1987).
113. Yamamoto A., Lee V.: J. Control. Rel. *14*, 43 (1990).
114. Yamamoto A.: Chem. Pharm. Bull. *37*, 2861 (1989).
115. Kingsbury W., Boehm J., Mehta R.: J. Med. Chem. *27*, 1447 (1984).
116. Bowen D., Fumigbokan M., Diasio R.: Proc. Am. Assoc. Cancer Res. *23*, 219 (1982).
117. Ninomira Y., Miwa M., Eda H., Sahara H.: Jpn. J. Cancer Res. *81*, 188 (1990); Ninomira Y., Miwa M., Eda H., Sahara H.: Chem. Pharm. Bull. *38*, 998 (1990); Nio Y., Kimura H., Tsubono M., Tseng C. C., Kawabata K., Masai Y., Hayashi H., Araya S., Meyer C., Fukumoto M.: Anti-Cancer Drug *3*, 387 (1992).
118. Au J. L., Sadee W.: Cancer Res. *39*, 4289 (1979).
119. Holy A., Konig J., Vesely J., Cech D., Votruba I., DeClercq E.: Collect. Czech. Chem. Commun. *58*, 1589 (1987).
120. Osawa T.: *Sialic Acid, Proc. Jpn-Ger. Symp. 214 (1988*).
121. Jones A., McGuigan C., Walker R. T.: J. Chem. Soc., Perkin Trans. 1 *1984*, 1471; Hunston R. N., Jones A., McGuigan C., Walker R. T.: J. Med. Chem. *27*, 440 (1984); Farquhar D., Smith R.: J. Med. Chem. *28*, 1358 (1985).
122. *a*) Waalkes M., Borssum V., Scherphof B.: Sel. Cancer Ther. *1990*, 15; *b*) Halmos T., Moroni P., Antonakis K., Uriel J.: Biochem. Pharmacol. *44*, 149 (1992).
123. Ji Y., Moog Ch., Schmitt G., Bischoff B.: J. Med. Chem. *33*, 2264 (1990).
124. Krenitsky T. A., Beauchamp L. M., De Miranda P., Schaeffer H. J., Whiteman P. D.: Proc. Natl. Acad. Sci. U.S.A. *81*, 3209 (1984); Beauchamp L. M., Orr G. F., De Miranda P., Burnette T., Krenitsky T. A.: Antiviral Chem. Chemother. *3*, 157 (1992).
125. Blum M. R., Soul-Lawton J., Smith C. M., On N. T., Posner R., Rolan P. E.: Antiviral Res. *23*, Suppl. I, 74 (1994).
126. Good S. S., De Miranda P.: J. Pharmacol. Exp. Ther. *27*, 644 (1983).
127. Winkler J., Winkelman E., Rolly H.: Arzneim.-Forsch. *1988*, 1545.
128. Reardon J. E., Spector T.: J. Biol. Chem. *264*, 7405 (1989).
129. Ogilvie K. K., Cherigan O., Kennel W. V.: Can. J. Chem. *60*, 3005 (1982).
130. Germershausen J., Bostedor R., Tolman R. L.: Antimicrob. Agents Chemother. *29*, 1025 (1986).
131. Martin J. C., Tippie M. A., McGee P. D.: J. Pharm. Sci. *76*, 180 (1987).
132. Harnden M.: J. Med. Chem. *32*, 1738 (1989).
133. Winkelmann E., Winkler I., Rolly H., Rosner M., Jahne G.; Arzneim.-Forsch./Drug. Res. *38*, 1545 (1988).
134. Bacon T. H., Shinazi R. F.: Antiviral Chem. Chemother. *4*, Suppl. 1, 25 (1993).
135. Bacon T. H., Boyd M. R.: Antiviral Res. *23*, Suppl. 1, 99 (1994).
136. Pue M. A., Bennett L. Z.: Antiviral Chem. Chemother. *4*, Suppl. 1, 47 (1993).
137. Portnoy J.: Antiviral Res. *23*, Suppl. 1, 98 (1994).
138. Hanessian S.: J. Med. Chem. *16*, 290 (1973).
139. Baker D. C., Haskell T. H., Sloan B. J.: J. Med. Chem. *22*, 273 (1979).
140. Shannon W. M., Westbrook L., Kumar S.: J. Pharm. Sci. *74*, 1157 (1985).

141. Winkler K.: Nucleic Acids Res. *1989*, 6065.
142. Machida H., Sakata S.: Antiviral Res. *4*, 135 (1984).
143. Kano F., Ijichi K., Ashida N., Watanabe Y., Sakata S., Machida H.: Antiviral Chem. Chemother. *5*, 74 (1994); Baraldi P. G., Bazzanini R., Manfredini S., Simoni D., Robins M. J.: Tetrahedron Lett. *34*, 3177 (1993).
144. Koff W. C., Pratt R. D., Elm J. L.: Antimicrob. Agents Chemother. *24*, 134 (1983).
145. Reardon J. E., Spector T.: J. Biol. Chem. *264*, 7405 (1989).
146. Furman P. A., Fyfe J. A., Weinhold K.: Proc. Natl. Acad. Sci. U.S.A. *83*, 8333 (1986).
147. Kawaguchi T., Ishikawa K., Seki T., Juni K.: J. Pharm. Sci. *79*, 531 (1990); Kawaguchi T. T., Hasegawa T., Seki T., Juni K., Morimoto Y., Miyakawa A.: Chem. Pharm. Bull. Tokyo *40*, 1338 (1993); Hasegawa T. T., Seki T., Juni K., Saneyoshi M., Kawaguchi T.: J. Pharm. Sci. *82*, 1232 (1993); Kerr S. G., Kalman T. I.: J. Med. Chem. *35*, 1996 (1992).
148. Aggarwal S. K., Goju S. R., Rangan S. R. S., Aggarwal K. C.: J. Med. Chem. *33*, 1505 (1990).
149. Hanson R. N., Hasan M.: J. Med. Chem. *30*, 29 (1987).
150. Bodor N., Farag H. H.: J. Med. Chem. *26*, 313 (1983); Gogu S., Aggarwal S., Rangan S., Agrawal K.: Biochem. Biophys. Res. Commun. *160*, 656 (1989); Chu C., Bhadti V., Doshi V., Etse J., Gallo J., Boudinot F., Schinazi R.: J. Med. Chem. *33*, 2188 (1990); Little R., Bailey D., Brewster M., Estes K., Clemmons R., Saab A., Bodor N.: J. Biopharm. Sci. *1*, 1 (1990); Doshi K. J., Islam Q., Gallo J. M., Boudinot F. D., Hsieh L., Qin Y., Schinazi R. F., Chu C. K.: Antivir. Chem. Chemother. *4*, 263 (1993).
151. Yamamoto N., Nakashima H.: Aids Res. *2*, Suppl. 1, 183 (1986).
152. Haines D. R., Fuller R. W., Marquez V. E.: J. Med. Chem. *30*, 542 (1987).
153. Vistica D. T.: Blood *56*, 427 (1980).
154. McGuigan Ch., Nicholls S. R., O'Connor T., Kinchington P.: Antiviral Chem. *1990*, 25; McGuigan C., Nickson C., Petrik J., Karpas A.: FEBS Lett. *310*, 171 (1992); McGuigan C., Pathirana R. N., Balzarini J., De Clercq E.: J. Med. Chem. *36*, 1048 (1993).
155. *a*) McGuigan Ch., Pathirana R. N., Mahmood N., Hay A. J.: Bioorg. Med. Chem. Lett. *2*, 701 (1992); *b*) Thomson W., Nicholls D., Irwin W. J., Almushadani J. S., Freeman S., Karpas A., Petrik J., Mahmood N., Hay A. J.: J. Chem. Soc., Perkin Trans. 1 *1993*, 1239.
156. Murata M., Achiwa K.: Chem. Pharm. Bull. *38*, 836 (1990).
157. Pochet S., Kansal V., Deshonesse F., Sarfati S. R.: Tetrahedron Lett. *31*, 6021 (1990).
158. *a*) Stein Y. M., Neto C., Truncotte J. G.: Biochim. Biophys. Res. Commun. *171*, 451 (1990); Vanwijk G. M. T., Hostetler K. Y., Schlame M., Vandenbosch H.: Biochim. Biophys. Acta *1086*, 99 (1990). *b*) Turcotte J. G., Srivastava S. D., Rizkalla B. H.: Biochim. Biophys. Acta *619*, 604 (1980); *c*) Hostetler K., Lentig H., Bosh H., Ridman D.: J. Biol. Chem. *265*, 6112 (1990).
159. McGuigan C., Bellevergue P., Jones B. C. N. M., Mahmood N., Hay A. J., Petrik J., Karpas A.: Antiviral Chem. Chemother. *5*, 271 (1994).
160. Puech F., Gosselin G., Lefebvre I., Pompon A., Aubertin A.-M., Kirn A., Imbach J.-L.: Antiviral Res. *22*, 155 (1993).
161. Lambert R. W., Martin J. A., Thomas G. J., Duncan I. B., Hall M. J.: J. Med. Chem. *32*, 367 (1989).
162. Stein Y. M., Netto C.: Biochem. Biophys. Res. Commun. *171*, 458 (1990).
163. Rosovsky A., Satra J., Farely F.: Biochem. Biophys. Res. Commun. *172*, 288 (1990).
164. Zemlicka J., Holy A.: Collect. Czech. Chem. Commun. *34*, 2449 (1969).
165. Hanessian S.: J. Med. Chem. *16*, 290 (1973).
166. Kalman T. I., Reddy A. R. V.: Ann. N. Y. Acad. Sci. *616*, 540 (1992).
167. Narukar M. M., Mitra A. K.: Pharm. Res. *6*, 887 (1989).

168. Uhlman E., Peyman A.: Chem. Rev. *90*, 553 (1990).
169. Presova M., Smrt J.: Collect. Czech. Chem. Commun. *54*, 487 (1989); Kabanov A. V., Vinogradov S. V., Ovcharenko A. V.: Collect. Czech. Chem. Commun. *55*, 587 (1990); Zhang G.: Diss. Abstr. Int., B *51*, 221 (1990).
170. Leonetti J. P., Machy P., Leblen B.: Proc. Natl. Acad. Sci. U.S.A. *87*, 2448 (1991).
171. Leonetti J. P., Ragner B., Gagnon L., Milhaud P.: Gene *1988*, 323.
172. Lesiak K., Torrence P. F., De Clercq E.: Nucleosides Nucleotides *1989*, 1387.

Translated by the author (A. H.).