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Review

Minireview: nucleotide prodrugs

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Abstract

Nucleotides have shown interesting biological activities in a wide variety of antiviral, antiproliferative, immunomodulatory and other biological assays, and they present promising drug candidates. Because of their negative charge(s) nucleotides suffer from some disadvantages which can be successfully overcome by the utilization of nucleotide prodrugs. Nucleotide prodrugs were successfully used to increase oral absorption of nucleotides in vivo. By taking advantage of intracellular triggers (reducing potential, enzyme activity, pH), nucleotide prodrugs can be used in vitro for the intracellular delivery of the nucleotide resulting in enhanced potency and in some cases enhanced selectivity. Nucleotide prodrugs have also been utilized for tissue specific delivery of the nucleotides in vivo resulting in altered selectivity and reduced toxicity. For nucleotide prodrugs, their ultimate intended use is (in most cases) in vivo for the treatment of a disease. Thus, it is important to incorporate adequate assays and design criteria into any prodrug effort. In vivo systems are complicated because of metabolism, excretion and tissue distribution of the prodrug and the parent. Thus, results of in vitro assays have to be interpreted cautiously because they may be unsuitable predictors of the in vivo situation.

Keywords: Nucleotide prodrug; Intracellular delivery; Increased absorption; Enhanced potency; Enhanced selectivity; Reduced toxicity

Abbreviations and acronyms: ara-C = β-D-arabinofuranosylcytosine; ara-A = β-D-arabinofuranosyladenine; dd = dideoxy; U = uridine; T = thymidine; TK^- = thymidine kinase deficient; p = phosphate; AZT = 3′-azido-3′-deoxythymidine; ACV = acyclovir; FU = 5-fluorouracil; FdU = 5-fluoro-2′-deoxyuridine; GCV = ganciclovir; HSV = herpes simplex virus; HPV = human papilloma virus; cAMP = cyclic adenosine monphosphate; cGMP = cyclic guanosine monophosphate, i.p. = intraperitoneal; HIV = human immunodeficiency virus; SIV = simian immunodeficiency virus; MSV = Maloney sarcoma virus; FIV = feline immunodeficiency virus; CMV = cytomegalovirus; HBV = hepatitus B virus.

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1. Introduction

Nucleoside and nucleotide analogues have great therapeutic potential for the treatment of viral diseases and cancer (Robins, 1984; De Clercq, 1990; Herdewijn, 1992; Chu and Baker, 1993). The area of nucleotide analogues has received a lot of attention recently due to the discovery of nucleotides with potent antiviral activities (Holy, 1993). Since the negative charge(s) on the phosphorous entail(s) nucleotides with short comings (low permeability and bioavailability), increasing work in the literature is focusing on overcoming these difficulties with nucleotide prodrugs, an approach which temporarily masks the negative charges and liberates the parent nucleotide at a specific site.

It is not the intention of the authors to give an exhaustive survey of the field of nucleotide prodrugs, but the current review is intended to summarize and critically survey the work which has been done in the area with specific emphasis on the rationale and mechanism. The aim of this article is to direct the attention of the reader to the field of nucleotide prodrugs, give a flavor of the types and classes of compounds in the literature, and perhaps stimulate further drug design in this area. This review is generally restricted to compounds containing one nucleoside and one phosphorous moiety. In most cases, the full details of the compounds discussed in this review, such as chemical synthesis and biological evaluation, may be gleaned from the original literature.

2. Nucleosides and nucleotides as drug candidates

A vital step in the mode of action of many purine and pyrimidine nucleoside analogues against viral and neoplastic diseases is their metabolic activation by cellular and viral kinases. Such activation is generally mediated through kinases or transferases to yield the mono-, di-, and triphosphate analogues. The biologically active species of many nucleosides is the triphosphate analogue, which inhibits viral or cellular polymerases. Some nucleoside analogues fail to undergo the necessary phosphorylation to the active nucleotide form either because they are poor substrates for the phosphorylating enzymes, e.g. ddU (Hao et al., 1990), or due to the absence of a phosphorylating enzyme. Such an absence can occur with the development of viral resistance, i.e. TK^- HSV mutant is ACV resistant because it lacks the enzyme which converts ACV to ACVp. Another possibility is the temporal or tissue specific absence of the phosphorylating enzyme as in the case of AZT, which is poorly phosphorylated to AZTp in resting cells due to the low level of cellular TK in such cells (Zhu et al., 1990; Gao et al., 1993). In contrast to nucleosides, nucleotides are phosphorylated species and do not require that first step in their metabolic activation. Thus, nucleotides could be expected to show biological activity where the corresponding nucleoside is inactive due to lack of intracellular phosphorylation. Nucleotides will also retain activity against certain nucleoside resistant viral mutants (e.g. TK^- HSV) and finally, nucleotides could show an altered biological profile from nucleosides due to different tissue distribution of the activated metabolite. Another rationale for utilizing nucleotides is in cases where nucleosides are metabolically deactivated in vivo due to the actions of certain enzymes, as in the case of ara-C and ara-A, which are rapidly deaminated in vivo by cytidine

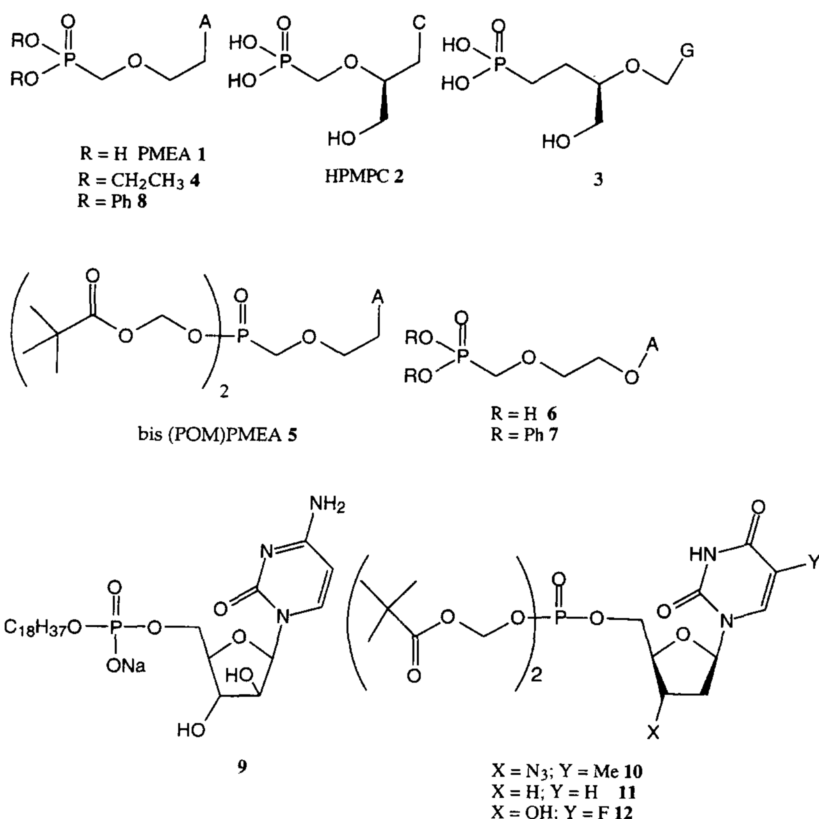

Fig. 1.

deaminase (Camiener and Smith, 1965; Ho, 1973) and adenosine deaminase (Cohen and Plunkett, 1975), respectively. Also, nucleoside analogues are often substrates for phosphorylase-induced glycosidic bond cleavage (Birnie et al., 1963; Saffhill and Hume, 1986).

Cohen and Plunkett were the first to unequivocally show that the nucleotide ara-Ap could enter cells directly (Cohen and Plunkett, 1975). The field of nucleotides started to flourish with the discovery by Holy and De Clercq of phosphonomethylether nucleosides, a class of nucleotide analogues with interesting biological activities. Two prototype examples in this series are PMEA (**1**) and HPMPC (**2**) (Fig. 1) (Holy, 1993). PMEA has antiretroviral and antiherpesvirus activity and has shown efficacy in retroviral animal models (MSV, FIV, SIV) (Tsai et al., 1994) and as a parenteral agent in human clinical trials against HIV (Collier et al., 1993; Walker et al., 1993). HPMPC is a broad spectrum antiherpes virus agent and is currently undergoing evaluation as a treatment for CMV, HSV and HPV infections in humans (De Clercq, 1993).

Another nucleotide analogue, the 5′-methylene phosphonate of GCV (**3**) was found to have activity against CMV and it is currently undergoing preclinical evaluation (Smee et al., 1994; Prisbe et al., 1986).

Nucleotides, however, have some disadvantages over nucleosides. Because of their charge, they enter cells very poorly and show generally low or no activity in vitro. For example, only ~ 0.01% of exogenously supplied ara-Ap was found to enter cells as the intact nucleotide after 4 h of incubation (Cohen and Plunkett, 1975). Similarly, radiolabeled uptake experiments showed that only 1% of extracellularly applied HPMPC permeated into the cell (Connelly et al., 1993). The charges on the nucleotide are also responsible for their low oral bioavailability. It was shown that the oral bioavailability of both PMEA and HPMPC in rats was less than 5% (Bischofberger et al., 1993). Another disadvantage which pertains specifically to nucleoside phosphate analogues is their low stability in biological media, due to rapid dephosphorylation by phosphatases (Cohen and Plunkett, 1975).

Nucleotide prodrugs can potentially overcome these difficulties. Masking the negative charge(s) on the phosphorous by suitable functionalities, which can be converted chemically or biologically to the parent nucleotide, can make nucleotides orally bioavailable, increase intracellular delivery, and alter pharmacokinetics/tissue distribution/metabolism resulting in improved efficacy and target organ specificity.

3. Rationale for nucleotide prodrugs

3.1. Oral bioavailability

In order for a prodrug to fulfill the requirements necessary to deliver the parent nucleotide D into the systemic circulation, the prodrug PD should be stable to the intestinal environment, it should be permeable to cross the intestinal wall and finally, once in the systemic circulation, has to be labile to be converted back to the parent nucleotide (Fig. 2). Because of these seemingly contradictory properties (intestinal stability/permeability/systemic lability) the choice of functionalities to achieve optimized properties is very limited.

Prodrugs of PMEA (**1**) (Fig. 1) were evaluated in an attempt to increase the oral bioavailability of the parent PMEA (Shaw et al., 1994; Starrett et al., 1994). Promising candidates were preselected utilizing a number of assays, including chemical stability and stability in biological media (rat intestinal wash, rat and human intestinal homogenate, rat and human plasma and human liver homogenate). As a measure for the

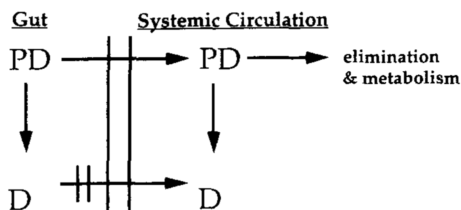

Fig. 2. Prodrug (PD) utilized to increase oral bioavailability of parent drug (D). PD should be stable in the gut, permeate across the intestinal wall, and, once in the systemic circulation, should be converted efficiently to D.

permeability of the prodrugs, the permeation across a cell monolayer (Caco-2 cell culture) was determined in vitro. PMEA prodrugs were also administered orally to rats, and the systemic levels of PMEA were determined by measuring the concentrations of PMEA in the urine. Monoesters exhibited low bioavailability, dialkyl esters were absorbed efficiently; however, their conversion to PMEA was very low: administration of diethyl PMEA (**4**) resulted in the appearance of 40% of **4** in the urine with no parent PMEA being detectable. Bis(acyloxymethyl) esters, first utilized by Farquhar as phosphate prodrugs (Farquhar et al., 1983, 1991), demonstrated improved bioavailability. Particularly, bis(pivaloyloxymethyl) PMEA (bis(POM)PMEA (**5**)) (Fig. 1) showed oral bioavailability of 18% in rats. Subsequent studies showed that **5** is chemically relatively stable (half life $t_{\frac{1}{2}}$ at 40°C/pH 5 = 33 h), but labile in biological media ($t_{\frac{1}{2}}$ in human plasma = 3 min). More detailed pharmacokinetic studies in monkeys showed that **5** has an oral bioavailability of 22–27% in various formulations and that no intact **5** could be detected in plasma (Cundy et al., 1994). Compound **5** is currently undergoing clinical evaluation as an anti-HIV agent in humans.

Similarly, oxo-PMEA **6** (Fig. 1), a compound closely related to PMEA, has low bioavailability in mice (1–2%). Following evaluation of a large number of prodrugs, the diphenyl ester **7** was selected as the preferred prodrug. It showed 50% oral bioavailability and corresponding efficacy on oral administration in mice (Perkins et al., 1993).

Interestingly, in the case of PMEA, the corresponding diphenylester **8** was promising based on in vitro assays (stability, permeability), and it also showed high absorption on oral administration in rats. However, PMEA was only found at low levels in plasma (3%), the major product was an unknown metabolite (Shaw et al., 1994).

The octadecanolester of ara-Cp **9** was shown to be orally active in mice with a minimum effective dose only twice the i.p. dose. The prodrug was found in the plasma of the rats with a t_{max} of 1 h. Additionally, this prodrug was not hydrolyzed by cytidine deaminase after 2 h in plasma (Saneyoshi et al., 1980).

3.2. Intracellular delivery

As mentioned, nucleotides cross cellular membranes very inefficiently and are generally not very potent in vitro (Cohen and Plunkett, 1975). Thus, increasing the rate of cellular permeation by the prodrug approach and delivering the nucleotide to the inside of a cell would increase the potency of nucleotides. Intracellular delivery of the nucleotide is especially important for nucleoside phosphates where the phosphate is required to overcome the lack of phosphorylation of the nucleoside by cellular or viral kinases. In this case, the extracellular metabolism of the prodrug or the nucleotide to the nucleosides competes with the uptake into the cell (Lichtenstein et al., 1960).

The efficient intracellular delivery of nucleotides using a prodrug approach necessitates the existence of triggers or different rates of conversion of the prodrug to the drug intracellularly vs extracellularly (k_2 has to be bigger than k_1) (Fig. 3).

The intracellular triggers that have been utilized and exploited are the differences in reducing potential, enzyme activity (especially esterases), and pH. One class of compounds are dithioethyl- and *S*-acyl-2-thioethyl (SATE) esters (Fig. 4). The disulfide group takes advantage of the greater reducing potential within the cell to target the

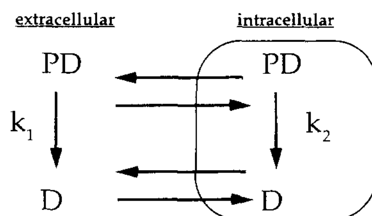

Fig. 3. Prodrug (PD) utilized to deliver the parent drug (D) to the inside of a cell. The conversion of PD to D has to be faster intracellularly than extracellularly ($k_2 > k_1$)

cytosol. Dithioethyl esters of ddUp and AZTp were hydrolyzed 30 times faster in cell extracts than in culture medium. The ddUp ester derivative was active in vitro whereas ddU and ddUp were not. The AZTp ester derivative was active in TK^- cells in which AZT itself was inactive (Puech et al., 1993).

S-Acyl-2-thioethyl derivatives can be hydrolyzed enzymatically by carboxyesterases which may be more prevalent intracellularly. The 2-thioethyl moiety collapses to episulfide and the nucleotide. However, episulfide is reported to be toxic on acute and chronic exposure in mice and rats (Pugaeva et al., 1969) and toxic and mutagenic in vitro (Luethy et al., 1981) which may limit the practical applications of this approach. The bis(SATE) ester of ddAp (Fig. 4) is reported to be more stable in serum complemented culture medium ($t_{\frac{1}{2}} = 9$ h) than in CEM cell extracts ($t_{\frac{1}{2}} = 10$ min), and displays little or no drop off in activity in CEM TK^- cells (Benzaria et al., 1994).

Similarly, the acyloxylmethyl group has been attached to both nucleoside phosphates and phosphonates with interesting biological activity in the resulting compounds. Specifically, bis(POM)PMEA (**5**) (Fig. 1) was found to be ~ 100-fold more potent in vitro due to increased cellular permeation (Starrett et al., 1992). Once inside the cell, the molecule is converted by esterases to PMEA. Radiolabel uptake experiments also showed that the intracellular levels of PMEA and its metabolites were 100 × higher when **5** was added to cells than when PMEA was applied (Srinivas et al., 1993).

Similarly, the bis(POM) derivatives of AZTp **10** (Pompon et al., 1994), ddUp **11** (Sastry et al., 1992) and FdUP **12** (Farquhar et al., 1994) were synthesized (Fig. 1).

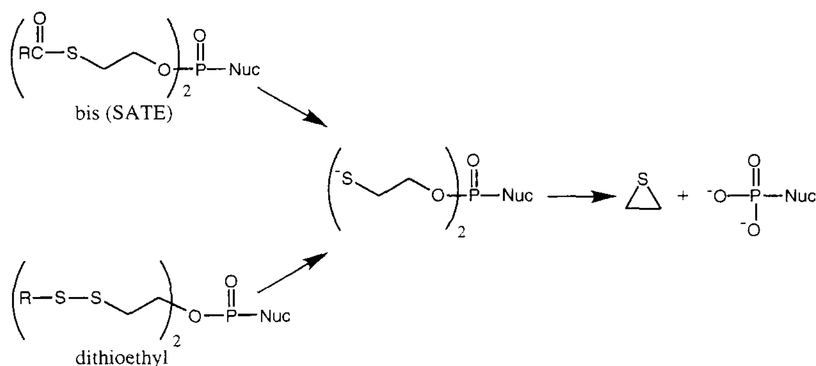

Fig. 4. Decomposition pathways of *S*-acyl-2-thioethyl (SATE) and dithioethyl esters.

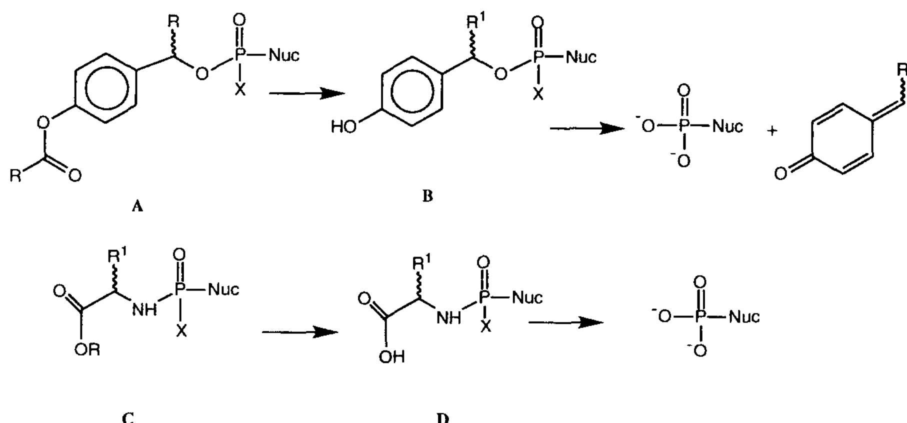

Fig. 5. Decomposition pathways of acyloxybenzyl esters **A** and amino acid ester amidates **C**.

Incubation of TK$^-$ CEM cells with **10** gave rise to intracellular AZTp, AZTpp and AZTppp, whereas no intracellular nucleotides were formed with AZT. From kinetic studies it was shown that the first step in the metabolism of **10** was catalyzed by a carboxyesterase. Compound **11** was effective in vitro against HIV in wild-type and CEM TK$^-$ cells, whereas the parent molecule ddU, which is not phosphorylated by TK (Hao et al., 1990), was not. Compound **12** was found to have growth inhibitory potency in vitro against a cell line which was resistant to FU. It also retained therapeutic activity in vivo when dosed i.p. in mice against a P 388 leukemia line resistant to FU, indicating that **12** was an effective membrane permeable prodrug of 5-fluoro-dUp.

The acyloxybenzyl derivatives **A** (Fig. 5) also rely on in vivo activation for activity. After chemical or enzymatic deacylation, the phenolic moiety **B** is oxidized or hydrolyzed to the *p*-quinonemethide to release the nucleotide (Thomson et al., 1993). However, the *p*-quinonemethides are very reactive alkylating agents (Filar and Weinstein, 1960). Acyloxybenzyl prodrugs of AZTp were prepared which showed comparable in vitro antiviral activity against HIV-1 and SIV to AZT, although they were more toxic than the parent (Thomson et al., 1993; Glazier et al., 1992). Similarly, acyloxybenzyl esters of PMEA were prepared and were found to be more potent in vitro than PMEA itself (Glazier et al., 1994).

Another class of nucleotide prodrugs are phosphoramidates which have one or two nitrogens attached to the phosphorous. The chemical hydrolysis of phosphoramidates proceeds rapidly at lower pH and, therefore, the compounds may utilize an intracellular pH trigger to target endosomes, lyzosomes, or some tumor cells where the pH is lower than the normal physiological pH (Ross, 1961). Amino acid ester phosphoramidates **C** (Fig. 5) can also be hydrolyzed by esterases to form the free amino acid phosphoramidate **D** which is unstable and undergoes hydrolysis to yield the free nucleotide (Juodka and Smrt, 1974). A wide variety of amines and amino acids have been utilized as prodrug moieties (McGuigan et al., 1990a, 1991, 1992; Gabrielsen et al., 1994). The phosphoroamidate formed between AZTp and alanine methyl ester (**13**) (Fig. 6) was found to be active against HIV in a cell line restrictive to the activity of AZT due to

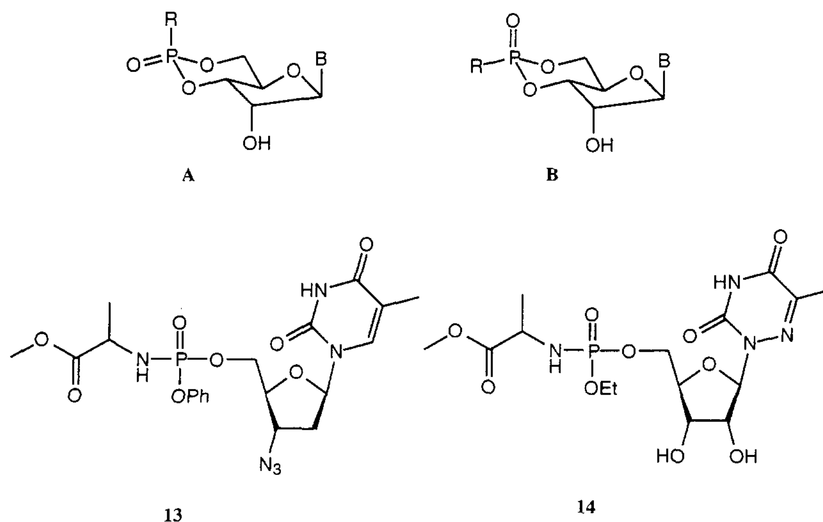

Fig. 6.

poor phosphorylation (McGuigan et al., 1992). The two substituents on the phosphorous have been connected by linkers to give cyclic phosphoramidates (Farquhar et al., 1983; Jones et al., 1984; Kumar et al., 1990). Amidates of cAMP and cGMP (**A**,**B**) (Fig. 6) have been prepared as diastereomeric mixtures (Russell and Moffatt, 1969; Meyer et al., 1973; Bentrude and Tomasz, 1984; Beres et al., 1985a,b; Bottka and Tomasz, 1985, 1988; Beres et al., 1986; Baraniak and Stec, 1987; Tomasz et al., 1987; Curley et al., 1990; Kinchington et al., 1992). The amidate prodrug ($R=NH_2$) (Fig. 6) is reported to hydrolyze under acidic conditions to a mixture of products, while the dialkylamidate ($R=NMe_2$) yields mainly cAMP (Meyer et al., 1973; Bottka and Tomasz, 1985; Tomasz et al., 1987). Therefore, the *N*,*N*-dialkyl phosphoroamidates may be useful prodrug moieties for cAMP although the behavior of these compounds in biological systems has not been investigated.

In order to completely mask the negative charge on phosphorous, a wide variety of triester prodrugs have been made of ara-Cp (Colin et al., 1989), ara-Ap (McGuigan et al., 1989), AZTp (Gouyette et al., 1989; Devine et al., 1990; McGuigan et al., 1990b; 1992; 1993a; Le Bec and Huynh-Dinh, 1991), ddCp (McGuigan et al., 1990b), Tp (Chawla et al., 1984), 3′-substituted Tp (Russell and Moffatt, 1969; McGuigan et al., 1990c), and 5-substituted dUp (Shuto et al., 1987; Shuto et al., 1988; Hostetler et al., 1990; Henin et al., 1991). The trihaloethyl and aryl esters have shown some good in vitro activity in CEM TK^- cells (McGuigan et al., 1991, 1992, 1993b). The R groups are sometimes connected by linkers to give cyclic derivatives (i.e. substituted 1,3-propandiols) (Farquhar et al., 1983; Hunston et al., 1984). In general, simple alkyl triesters are too stable to be useful as prodrugs and the resulting phosphorous esters are inactive; however, aryl esters and activated alkyl esters are capable of functioning as nucleotide prodrugs.

Alkylesters of AZT-5′-hydrogen phosphonates were evaluated as potential prodrugs of AZTp; however, they appear to act as depot forms of AZT rather than AZTp (McGuigan et al., 1994).

Phosphate esters of cAMP and cGMP have also been prepared (Nagyvary et al., 1973; Gohil et al., 1974; Engels and Pfleiderer, 1975a,b; Engels and Schlaeger, 1977; Engels and Reidys, 1978; Engels, 1979; Nargeot et al., 1983; Nerbonne et al., 1984; Kataoka et al., 1986, 1989, 1991; Nelson et al., 1987). These compounds were obtained as a diastereomeric mixture of axial (**A**) and equitorial (**B**) isomers at phosphorous (Fig. 6), with the axial isomer being thermodynamically favored (Engels and Schlaeger, 1977). Nucleophilic attack by thiols on the benzyl or methyl esters gives cAMP exclusively while alkaline hydrolysis predominantly affords 5′-AMP via ring opening (Gohil et al., 1974). The benzyl esters of cAMP and cGMP, especially the *o*-nitrobenzyl esters (Nargeot et al., 1983) are useful tools for studying the various intracellular roles these secondary messengers play (Postemark, 1974). The benzyl esters were shown in vitro to penetrate into cells and induce cAMP responses in guinea pig myocardium. The photolabile *o*-nitrobenzyl esters upon irradiation produce intracellular ‘concentration jumps’ of cAMP or cGMP, leading to cellular responses and changes in the strength and frequency of heartbeats in isolated bullfrog hearts (Korth and Engels, 1979; Nargeot et al., 1983; Nerbonne et al., 1984).

The intracellular delivery of nucleotides through prodrugs in vivo is complicated by differences in distribution, (e.g. uptake of the prodrug preferentially into certain tissues and organs), metabolism (e.g. preferential conversion of the prodrug to the nucleotide in the liver) and excretion (e.g. rapid excretion of the prodrug through the kidney). Such differences would be expected to lead to a different toxicity/efficacy profile of the prodrug. An example of the complexity with intracellular delivery of a nucleotide in vivo is the phosphoramidate prodrug of 6-aza-Up **14** (Fig. 6) which exhibited antiviral activity in vitro similar to 6-azaU except that 300- to 450-fold higher concentrations of **14** were required to attain comparable activity. However, in an in vivo model in mice, **14** showed enhanced activity when administered i.p. along with reduced toxicity. These results could imply that **14** delivered 6-aza-Up intracellularly in vivo, but it is also possible that the observed effects are due to differences in distribution or pharmacokinetics (Gabrielsen et al., 1994). Such differences could also be highly dependent on the corresponding animal model, which make any extrapolation to humans difficult. Another example is **5** which was shown to deliver high levels of the parent PMEA into cells resulting in 100x higher potency in vitro (Srinivas et al., 1993). However, in vivo, no intact **5** could be detected on oral application of **5** to monkeys and thus, **5** apparently in vivo merely delivers the parent PMEA to the blood but not intracellularly (Cundy et al., 1994). Thus, it is clear that tissue culture antiviral assays of prodrugs are poor predictors of prodrug function in vivo.

3.3. Altered pharmacokinetics, target organ specificity, tissue distribution and metabolism

Prodrugs can be utilized to change the pharmacokinetics of a nucleotide. This is warranted, for example, in cases where the nucleotide is excreted rapidly. In such cases, a prodrug can act as a slow release form. Prodrugs can also be utilized to change the

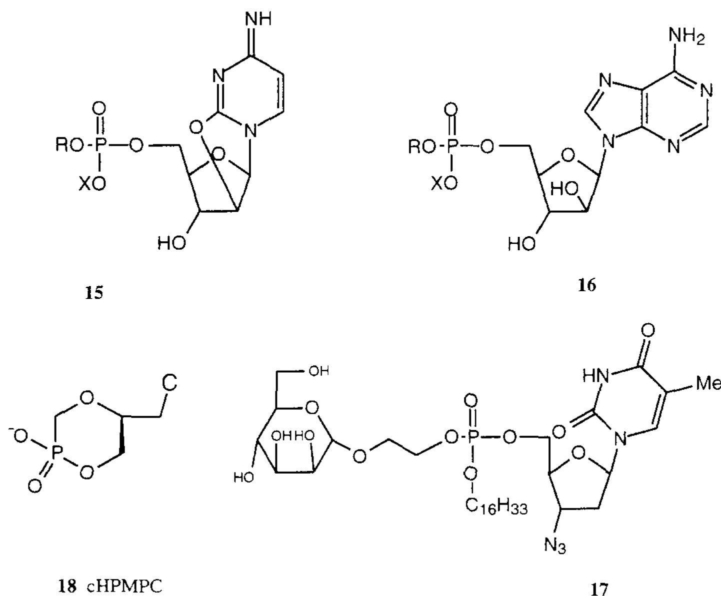

Fig. 7.

target organ specificity and tissue distribution. The objective of this approach can be to deliver the nucleotide to a site of action (e.g. the brain) or to decrease delivery to a site of toxicity (e.g. the kidney).

Historically, the first major work for nucleotide prodrugs was with ara-Cp. The objective was not only to increase the bioavailability of ara-C, but also circumvent the deactivating actions of cytidine deaminase (Camiener and Smith, 1965). Numerous negatively charged lipophilic mono-esters of ara-Cp have been prepared with various ester groups attached including: alkyls and aryls (Saneyoshi et al., 1980; Rosowsky et al., 1982), steroids (Hong et al., 1979a, 1979b, 1980, 1985), sugars, 1,2-diacylglycerol 1,2-diamidoalkyl, oxyalkyl and thioalkyl ether glycerols, cyclic and long-chain aliphatic alcohols (Ryu et al., 1982). The ester **9** (Fig. 1) has recently been approved in Japan for the treatment of various leukemias via both parental and oral administration, and has been shown to slowly release ara-C in the liver. After a 5-day oral administration of 300 mg/day of the prodrug, ara-C concentrations in the blood corresponded to low-dose (15–20 mg/day) continuous infusion (Ohno et al., 1991; Kodama et al., 1989). Similar compounds have also been prepared of the 2,2′-anhydro ara-Cp **15** (Fig. 7), ara-Ap **16** (Ji et al., 1990), AZTp (Hostetler et al., 1991; Piantadosi et al., 1991), as well as other nucleosides (Shuto et al., 1987, 1988; Hostetler et al., 1990; Ji et al., 1990).

Dioleoyl phosphatidyl ddC was shown to be less toxic in human HBV-infected hepatoma cells, and when administered to mice, the levels of ddC in the liver were 40 × greater than when ddC was administered (Hostetler et al., 1994). This may result in an improved therapeutic index for ddC in vivo in the treatment of HBV and this strategy may also be useful to target drugs to lymphoid tissues, important reservoirs of HIV infection (Hostetler et al., 1994b).

Ara-Ap has been conjugated with fetuin and lactosaminated serum albumin to target hepatocytes for the treatment of HBV virus (Fiume et al., 1980, 1981, 1984, 1986). The serum albumin lysosomotropic drug-carrier complex was stable in mouse blood after parental administration, was specifically released in the liver, and did not display any recognizable sign of acute bone marrow toxicity inherent in the unconjugated ara-A, although it was not determined whether ara-Ap or just ara-A was being delivered.

Targeting the blood–brain barrier has been classically addressed by applying Bodor's nicotinic acid/dihydropyridine prodrug carriers (Bodor and Farag, 1983) to nucleosides (Torrence et al., 1988; Gogu et al., 1989; Palomino et al., 1989; Chu et al., 1990). For nucleotides, a glucosyl phosphotriester prodrug of AZTp (**17**) (Fig. 7) has been reported to improve the uptake of AZT in mouse brain. Oral dosing of **17** provided a 100-fold increase of AZT derivatives in the plasma and 1000-fold increase in the brain over AZT itself, with AZTp as the primary metabolite (Henin et al., 1991; Namane et al., 1992). This prodrug concept was borrowed from the glucosyl dolichol phosphate (Neumann et al., 1989) and could allow efficient delivery of nucleotides to the systemic circulation and the brain.

An example where the toxicity of the nucleotide was reduced by a prodrug approach is in the case of cyclic HPMPC (cHPMPC) **18** (Fig. 7) which was found to be an intracellular prodrug for HPMPC. Compound **18** is stable chemically and also in biological media, but gets converted intracellularly to the active parent compound, HPMPC. Compound **18** has similar in vitro and in vivo potency to HPMPC, but it displays much reduced nephrotoxicity in the rat. The reason for this reduced nephrotoxicity is presumably the fact that **18**, because of its reduced charge, does not get transported into the proximal convoluted tubular cells by the anion-specific transport system in the kidney as efficiently as HPMPC itself (Bischofberger et al., 1994).

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