ACYCLIC NUCLEOSIDE PHOSPHONATES: AN UNFINISHED STORY

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While cidofovir, adefovir and tenofovir are the three acyclic nucleoside phosphonates (ANPs) that have been licensed for clinical use (the latter as a single-, double- and triple-drug combination), there are many more ANPs that await their application for medical or veterinary use: (*S*)-HPMPA, (*S*)-HPMPDAP, cPrPMEDAP, (*R*)-HPMPO-DAPy, PMEO-DAPy, 5-X-PMEO-DAPy, (*R*)-PMPO-DAPy, (*S*)-HPMP-5-azaC, and cyclic (*S*)-HPMP-5-azaC, and alkoxyalkyl prodrugs thereof.

Keywords: ANPs; (*S*)-HPMPA; Cidofovir; Adefovir; Tenofovir; Phosphonate; Veterinary; Prophylactic; Nucleotides; Nucleosides; Viruses.

1. INTRODUCTION

This year, 2011, marks a few anniversaries, including the 25th anniversary of the discovery of the antiviral nucleoside phosphonates (ANPs) with the description of (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)-adenine] as a novel selective broad-spectrum anti-DNA virus agent[1], the 35th anniversary of the first encounter between Antonín Holý and myself on the 6th May 1976 in Göttingen (Germany) at the Symposium on Synthetic Nucleosides, Nucleotides and Polynucleotides (organized by the late Karl-Heinz Scheit), thus unleasing 10 years later, in 1986, the birth of the ANPs [2]. In previous reviews, I have depicted the past, present and future of the ANPs, thereby referring to the importance of the phosphonate bridge[3,4]. Here, I want to emphasize some hallmarks of the still evolving story of the ANPs, in commemoration of Dr. Antonín Holý's 75th birthday on the 1st September 2011, and evoke the ANPs as the ninth uncompleted Symphony of Ludwig Beethoven.

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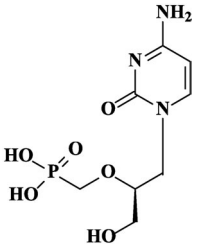
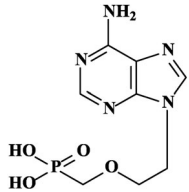
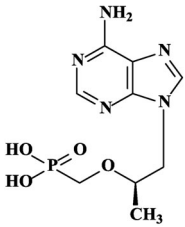


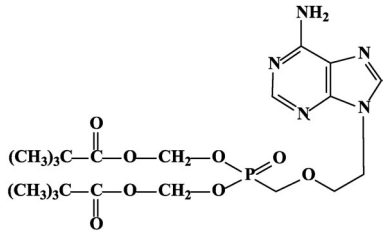
(*S*)-HPMPC (Cidofovir)



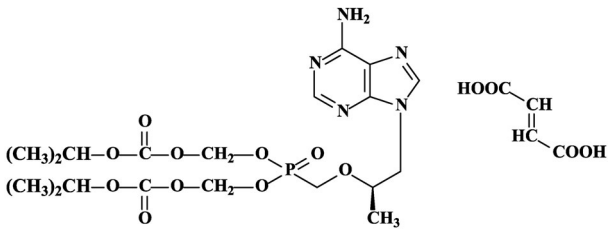
PMEA (Adefovir)



(*R*)-PMPA (Tenofovir)



Adefovir dipivoxil



Tenofovir disoproxil fumarate

There are, at present, three ANPs on the market, cidofovir (Vistide®), adefovir dipivoxil (Hepsera®) and tenofovir disoproxil fumarate (TDF) (Viread®); the latter two are marketed for the treatment of HIV (human immunodeficiency virus) and HBV (hepatitis B virus) infections, and for the treatment of HIV infections TDF is also available, in combination with emtricitabine, as Truvada®, and in combination with both emtricitabine and efavirenz, as Atripla®. Forthcoming combinations are those of Truvada® with rilpivirine (TMC 278) and Truvada® with elvitegravir and cobicistat (to boost the activity of elvitegravir, an HIV integrase inhibitor, which, like

raltegravir, represents a new dimension in the treatment of HIV infections (AIDS)).

At previous occasions, in my stories on Antiviral Drug Discovery I have emphasized the role of ANPs as both antiviral and antitumoral drugs[5–9]. Here, for the Collect. Czech. Chem. Commun. I want to report that the ANPs still offer unexplored and untapped opportunities (both prophylactic and therapeutic) for the future.

2. PROPHYLACTIC USE OF TENOFOVIR

In 2006, I strongly endorsed the use of tenofovir disoproxil fumarate (TDF) (as a single daily pill) for the pre- and post-exposure prophylaxis of HIV infections in humans[10]. Keynote observation for this proposal was that of Tsai et al.[11] reporting full protection from parenteral SIV (simian immunodeficiency virus) infection in rhesus macaques treated with tenofovir starting 24 h post infection. Later observations extended the potential of tenofovir in the prophylaxis of intravaginal HSV-2 [12] as well as perinatal SIV infection[13] in macaques. The efficacy of tenofovir as an HIV-1 microbicide for pre-exposure prophylaxis of HIV-1 infection was further corroborated by the experimental findings of Parikh et al.[14] and Rohan et al.[15].

The effectiveness and safety of tenofovir gel, as an antiretroviral microbicide, for the prevention of HIV infection in women was unequivocally demonstrated in a landmark paper in Science by Abdool Karim et al.[16]. That tenofovir, in the format of Truvada®, would be effective and useful in the pre-exposure (PrEP) chemoprophylaxis of HIV infection was demonstrated in men who have sex with men[17].

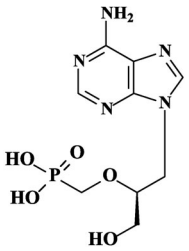
Ongoing studies should ascertain whether Viread® and/or Truvada® would also be effective in the PrEP of HIV infection in intravenous drug users, discordant couples, and heterosexual men and women[18,19]. Earlier studies have pointed to the efficacy of TDF (Viread®) in the chemoprophylaxis of HIV infection in women[20] and simian human immunodeficiency virus (SHIV) infection in rhesus macaques[21].

In principle, the chemoprophylaxis of HIV infection, in the abeyance of an effective HIV vaccination, may be based upon either Viread® or Truvada®, or any other acyclic (or cyclic) nucleoside phosphonate-containing medicine. Among the ANPs, the most to be considered might be (*R*)-PMPDAP, whereas the 5-X-PMEO-DAPy derivatives and (*R*)-PMPO-DAPy may also seem equally promising (see Section 6).

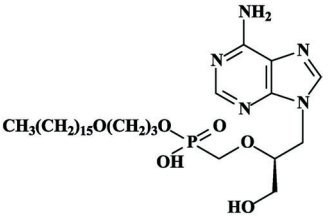
3. (*S*)-HPMPA AND (*S*)-HPMPDAP ALKOXYALKYL PRODRUGS

(*S*)-HPMPA (9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine) can be considered as the prototype of the ANPs [1,2]. Despite its therapeutic potential as a widely applicable anti-DNA virus agent[1], it has not been commercialized for any medical (or veterinary) indication. This oral therapeutic applicability of (*S*)-HPMPA is significantly enhanced by its alkoxyalkyl prodrugs (i.e. hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE))[22], and so is the potential applicability of the 3-deaza counterpart of (*S*)-HPMPA, particularly as an anti-poxvirus agent[23]. In fact, not only the 3-deaza counterpart of (*S*)-HPMPA, (*S*)-HPMP-3-deaza A, but also (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine [(*S*)-HPMPDAP], would emerge as attractive drug candidates for treatment of poxvirus infections, especially as their alkoxyalkyl (ODE and HDP) ester prodrugs[24].

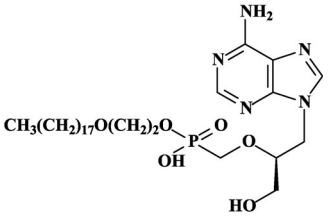
Twenty-five years after its discovery, (*S*)-HPMPA still looks for a therapeutic applicability, in the medical or veterinary sector, and so do its 3-deaza- and 2,6-diaminopurine counterparts, and their alkoxyalkyl esters, in the treatment of DNA virus infections, as diverse as pox-, herpes-, papilloma-, adeno- and polyomaviruses.



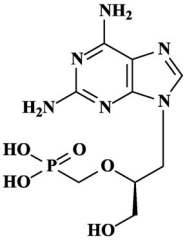
(*S*)-HPMPA



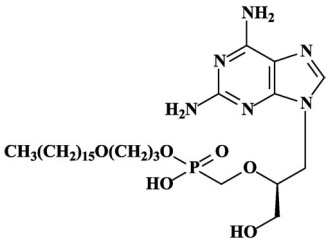
HDP-(*S*)-HPMPA



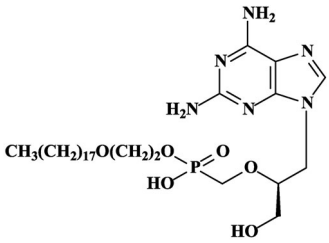
ODE-(*S*)-HPMPA



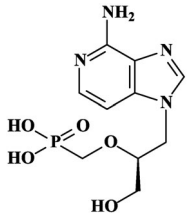
(*S*)-HPMPDAP



HDP-(*S*)-HPMPDAP



ODE-(*S*)-HPMPDAP



3-deaza-(*S*)-HPMPA

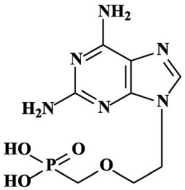
4. CIDOFOVIR [(*S*)-HPMPC] AND CYCLIC CIDOFOVIR ALKOXYALKYL PRODRUGS

Cidofovir was the first ANP to be licensed (in 1996) for clinical use, i.e. for intravenous treatment of human cytomegalovirus (HCMV) retinitis in AIDS patients. The disease HCMV retinitis has virtually disappeared due to the efficient therapy of HIV infection. Yet, cidofovir has found, albeit off-label, other niches, i.e. for the treatment of (i) human papilloma virus (HPV) infections (reviewed by De Clercq[8]) (such as hypopharyngeal papillomatosis[25], laryngeal papillomatosis[26], cervical papillomatosis[27] and HPV-associated skin lesions in transplant recipients[28]); (ii) polyomavirus (JC and BK)-associated infections such as progressive multifocal leukoencephalopathy (PML), hemorrhagic cystitis (HC) and BK virus nephropathy (BKN) (reviewed by De Clercq[9]) (for an illustrative case of a dual infection with polyomavirus BK and acyclovir-resistant herpes simplex virus (HSV) in a bone marrow transplant recipient, successfully treated with cidofovir, see Andrei et al.[29]); and (iii) poxvirus infections[30], including orthopox (i.e. vaccinia, cowpox, monkeypox), parapox (i.e. orf) and molluscipox (i.e. molluscum contagiosum) (cidofovir has proven highly efficacious in a murine model of disseminated progressive vaccinia[31], and is clearly more effective than smallpox vaccination upon lethal monkeypox virus infection[32]).

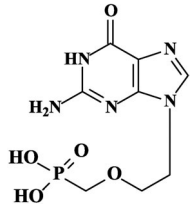
Cidofovir and cyclic cidofovir, and their alkoxyalkyl (i.e. hexadecyloxypropyl (HDP), hexaoctyloxyethyl (HDE)) prodrugs offer great potential for the treatment of not only herpesvirus infections, but also pox, polyoma and papilloma virus infections.

5. cPrPMEDAP, AS A PRODRUG OF PMEG, AND ITS PRODRUGS GS-9191 AND GS-9219

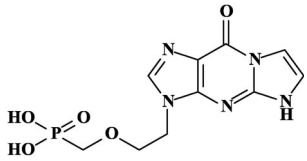
In 1999, we reported the antitumor potential of cPrPMEDAP [9-(2-phosphonomethoxyethyl)-N^6-cyclopropyl-2,6-diaminopurine], a prodrug of PMEG [9-(2-phosphonomethoxyethyl)guanine][33]. The antitumor potential of cPrPMEDAP was further demonstrated in a choriocarcinoma model in rats[34]. The enzyme responsible for the conversion of cPrPMEDAP to PMEG was shown to be an hitherto unknown N^6-methyl-AMP aminohydrolase[35].



PMEDAP

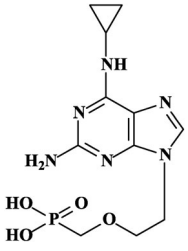


PMEG

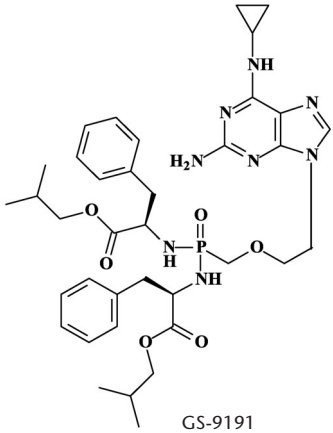


Compound 8
2-(9-Oxo-5,9-dihydro-3*H*-imidazo[1,2-*a*]purin-3-yl)ethoxymethylphosphonic acid

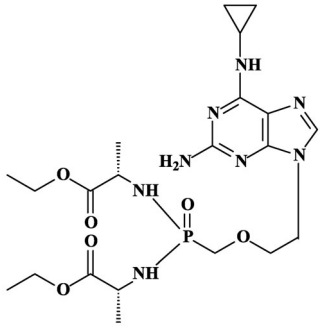
From cPrPMEDAP, two prodrugs, GS-9191 and GS-9219, respectively, were designed. Both GS-9191 and GS-9219 can be considered as "propro drugs" of PMEG, their active metabolite being PMEG diphosphate acting as competitive substrate of dGTP for the cellular DNA polymerase. GS-9191 has been further pursued for its utility in the topical treatment of HPV-associated lesions such as genital warts[36], whereas GS-9219, upon intravenous administration, has potent anti-neoplastic activity in dogs with spontaneous non-Hodgkin's lymphoma (NHL)[37]. GS-9191 and GS-9219 are now under further development for topical treatment of warts by Graceway Pharmaceuticals and intravenous treatment of lymphoma by Vet DC, respectively. (It should be emphasized that Vet DC is developing GS-9219 for veterinary use only.)



cPrPMEDAP



GS-9191



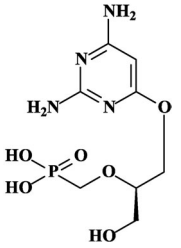
GS-9219

From both PMEG and PMEDAP tricyclic etheno derivatives have been synthesized[38]. These compounds did not exhibit any significant cytostatic activity, but proved markedly active against HCMV and VZV (varicella-zoster virus) (particularly 2-(9-oxo-5,9-dihydro-3*H*-imidazo[1,2-*a*]purin-3-yl)ethoxymethylphosphonic acid, compound **8** (ref.[38])). This work is reminiscent of the 1,*N*-2-ethenoguanine-based acyclovir and ganciclovir synthesized by Bozenna Golankiewicz and Jerzy Boryski[9] and should be worth further pursuing.

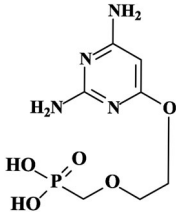
6. (*R*)-HPMPO-DAPy, PMEO-DAPy, 5-X-PMEO-DAPy AND (*R*)-PMPO-DAPy

In 2002, Antonín Holý et al. introduced a new class of ANPs, that of the 6-[2-(phosphonomethoxy)alkoxy]pyrimidines, with (*R*)-HPMPO-DAPy, PMEO-DAPy and (*R*)-PMPO-DAPy as the prototypes[39,40]. As I reviewed pre-

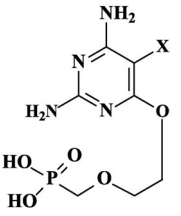
viously[41]. This class of DAPys could be easily discerned in two classes, (i) that of (*R*)-HPMPO-DAPy with an antiviral activity spectrum similar to that of (*S*)-HPMPA and (*S*)-HPMPC (cidofovir) and (ii) that of PMEO-DAPy, 5-X-PMEO-DAPy and (*R*)-PMPO-DAPy.



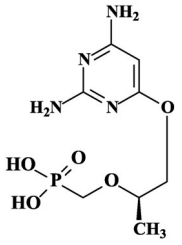
(*R*)-HPMPO-DAPy



PMEO-DAPy



5-X-PMEO-DAPy
(X = CH_3, CN, ...)



(*R*)-PMPO-DAPy

The major therapeutic potential of (*R*)-HPMPO-DAPy lies in the same area as for cidofovir [(*S*)-HPMPC], and these indications have not been (fully) explored, i.e. adenovirus infections[42] and poxvirus infections[32]. (*R*)-HPMPO-DAPy has a far-reaching therapeutic potential, encompassing, in principle, that of cidofovir, thus, herpes-, polyoma-, papilloma-, adeno- and all poxvirus infections, whether ortho-, para- or molluscipox. (*R*)-HPMPO-DAPy derivatives, including its alkoxyalkyl esters, therefore, offer, as yet to be unexplored opportunities for further investigation and development.

Unlike (*R*)-HPMPO-DAPy, which resembles (*S*)-HPMPC in antiviral activity spectrum, PMEO-DAPy (and its 5-substituted derivatives), and (*R*)-PMPO-DAPy share a similar activity spectrum as PMEA (adefovir) and (*R*)-PMPA (tenofovir). PMEO-DAPy and (*R*)-PMPO-DAPy exhibit primarily antiretrovirus activity, against HIV [43,44] and HBV [45]. The 5-X-substituted

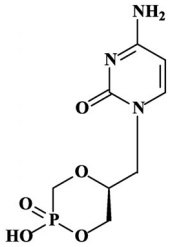
PMEO-DAPys, i.e. the 5-cyano-2,4-diamino-6-[2-(phosphonomethoxy)-ethoxy]pyrimidine derivatives[46], should be further explored for their antiretroviral activity, as this is comparable to that of the reference drugs adefovir and tenofovir.

What makes PMEO-DAPy even more attractive, that is that it is incorporated more efficiently than (*R*)-PMPA by the K65R HIV-1 reverse transcriptase (RT) mutant and is not as efficiently excised as (*R*)-PMPA by HIV-1 RT containing thymidine analog mutations[47]. As expected, PMEO-DAPy was shown to act as a purine nucleotide mimetic in the HIV-1 RT DNA polymerization. Whether (*R*)-HPMPO-DAPy and 5-X-substituted PMEO-DAPy derivatives also behave as purine nucleotide mimetics in the viral DNA polymerization reaction remains to be demonstrated.

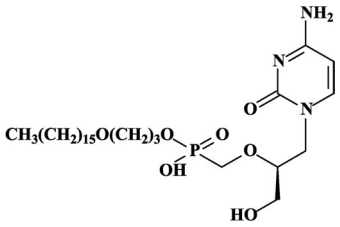
7. (*S*)-HPMP-5-azaC AND CYCLIC (*S*)-HPMP-5-azaC, ALKOXYALKYL PRODRUGS

The antiviral activity of triazine analogues of (*S*)-HPMPC (cidofovir), related compounds and their (alkoxyalkyl) prodrugs were reported by Krečmerová et al.[48,49]. The therapeutic potential of (*S*)-5-aza-HPMPC and its (alkoxyalkyl) prodrugs has not been fully explored, although they are favorably positioned from a pharmacologic viewpoint[50]. HPMP derivatives containing a 5-azacytosine moiety (i.e. HDE (hexadecyloxyethyl)ester of cyclic (*S*)-HPMPC) possess remarkable activity against polyoma viruses. This by itself deserves due attention as there are at present no ANPs considered for further use in the treatment of polyomavirus infections[51].

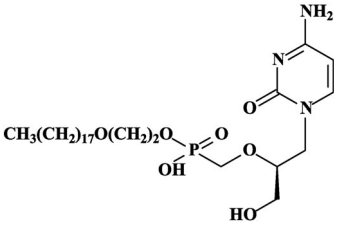
Currently, HDE-cHPMP-5-azaC has also been identified as a potential ANP analog for the treatment of camelpox virus (CMLV) infections, an orthopox virus that is the most closely related to the prototype of the poxvirus infections, variola virus, the causative agent of smallpox[52].



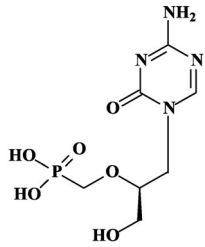
(*S*)-cHPMPC



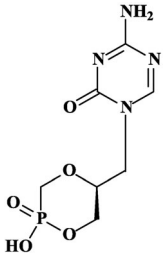
HDP-(*S*)-HPMPC



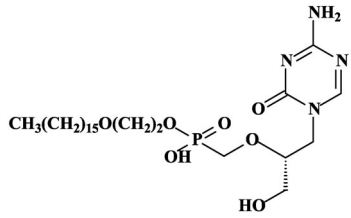
ODE-(*S*)-HPMPC



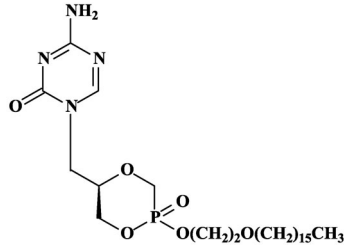
(*S*)-HPMP-5-azaC



(*S*)-cHPMP-5-azaC



HDE-(*S*)-HPMP-5-azaC



HDE-(*S*)-cHPMP-5-azaC

8. VETERINARY USE OF ANPs

The potential usefulness of the ANPs in the therapy and/or prophylaxis of veterinary virus infections has remained largely unexplored. Yet, their potential to treat virus infections in the veterinary sector is enormous, encompassing not only poxvirus infections, as described above, but also herpesvirus and retrovirus infections, i.e. EHV-1 (equine herpesvirus-1)[53], FHV-1 (feline herpesvirus-1)[54] and caprine herpesvirus type 1 [55].

The 2,6-diaminopurine derivatives, such as (*S*)-HPMPDAP, should be further explored as anti-poxvirus agents, and so should be an abundance of

HPMPO-DAPy, PMEO-DAPy and (*R*)-PMPO-DAPys for their potential against HIV and HBV infections. FLV (feline leukemia virus) and FIV (feline immunodeficiency virus) infections in cats require primary attention, and so are the koi herpesvirus (KHV) or cyprinid herpesvirus-3, the cause of mass mortality among common carp (*Cyprinus carpio carpio*). ANPs can interfere with KHV replication, and as they persist intracellularly for a prolonged time, they allow to exert an antiviral action for an extended time period.

Major indications for the veterinary use of ANPs are FIV, FLV and KHV, where both the "old" ANPs, adefovir, tenofovir, cidofovir and the newer ones, HPMPO-DAPy, PMEO-DAPy and (*R*)-PMPO-DAPy could be of particular utility. For veterinary use, the applicability of ANPs remains unequalled.

9. ARE ANPs ACTIVE AGAINST RNA VIRUSES?

Some reports have pointed to the activity of some ANPs against RNA viruses: (i) Wyles et al. reported that the ODE-(*S*)-HPMPA would potently and selectively inhibit the replication of hepatitis C virus (HCV)[56], while (*S*)-HPMPA as well as (*R*)-HPMPA were inactive; (ii) data originating from our Laboratory indicated that the isopropyl ester of 6-methyl-HPMP-5-azaC would be active (at 20 μg/ml) against Sindbis virus, although it was not assessed whether this activity extended to other viruses and/or other compounds[57]; (iii) (*S*)-HPMPC and PMEDAP, but not (*R*)-PMPA (tenofovir) or PMEA (adefovir), showed a moderate antiviral effect on the single-stranded (ss)RNA virus Turnip yellow mosaic virus (TYMV)[58].

The potential of ANPs in the elimination of plant viruses should be further explored. Eight years ago we reported that the ANPs, adefovir, tenofovir and PMEDAP were efficient in the elimination of banana streak virus, a double-stranded (ds)DNA virus, from banana (Musa spp.)[59]. This observation has not been further exploited, however.

10. CONCLUSIONS

Various acyclic nucleoside phosphonates (ANPs), whether "old" (such as (*S*)-HPMPC (cidofovir) or (*S*)-HPMPA) or "new" (such as (*R*)-HPMPO-DAPy or (*S*)-HPMP-5-azaC) have yet to be fully explored for the treatment of pox-, herpes-, papilloma-, polyoma- and adenovirus infections. This also extends to the new ANPs PMEO-DAPy, 5-X-PMEO-DAPy and (*R*)-PMPO-DAPy with regard to their potential for the treatment of HIV and HBV infections, where adefovir (HBV) and tenofovir (HIV, HBV) have acquired a leading

position. What needs to be further evaluated is the veterinary usefulness of the ANPs, including (*S*)-HPMPDAP and PMEDAP, in addition to those mentioned above, and of cPrPMEDAP for the treatment of non-Hodgkin's lymphoma (NHL). Another lead is that of the use of ANPs for eradication of plant viruses, banana streak virus having proved the way in this direction.

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