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Review Article

Novel phosphonate nucleosides as antiviral agents

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CONTENTS

Abstract

The phosphonate nucleosides have been extensively studied as potent antiviral agents since (*S*)-HPMPA was discovered to exhibit a broad spectrum of antiviral activity against DNA viruses, such as herpes simplex virus (HSV), varicella-zoster virus (VZV), human cytomegalovirus (HCMV) and TK^- mutants of HSV and VZV. The phosphonate nucleosides inhibit viral replication via a different mode of action from nucleoside agents such as zidovudine (AZT), lamivudine (3TC), didanosine (ddI), aciclovir (ACV) and penciclovir. In contrast to nucleoside agents, the phosphonate nucleosides are intracellularly metabolized to active forms. Their diphosphates, which are incorporated into viral DNA or RNA during DNA or RNA elongation, result in termination of polymerization. Although the phosphonate nucleosides exhibit a broad spectrum of antiviral activity, they have low cellular permeability and low oral bioavailability due to the negative charges of the phosphonate functionality. Therefore, prodrug approaches to masking the negative charges were essential. Three compounds, cidofovir ([*S*]-HPMPC), tenofovir (PMPA) disoproxil fumarate and adefovir (PMEA) dipivoxil, are currently marketed for the therapy of HCMV infection in HIV patients, HIV and hepatitis B virus (HBV), respectively. In addition, MCC-478 (Mitsubishi, Lilly) and LB-80380 (LG Life Sciences) are currently undergoing clinical trials evaluating their anti-HBV efficacy.

Introduction

The phosphonate nucleosides are widely used therapeutic agents known to have a broad spectrum of antiviral activity and a few have been reported to have antitumor activity as well. In 1989, De Clercq and Holy reported that (*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine [(*S*)-HPMPA] had potent and selective activity against a broad spectrum of DNA viruses, including herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), thymidine kinase-deficient (TK^-) mutants of HSV and VZV, and human cytomegalovirus (HCMV) (1). Subsequently, extensive studies on this molecule, its mode of action, antiviral effects, analogue synthesis and utility as an antiviral agent have been conducted.

The phosphonate nucleosides can be regarded as nucleosides possessing a phosphonate moiety which is equivalent to a phosphate group of the nucleoside monophosphate. Nucleoside antiviral agents must be triphosphorylated intracellularly by viral or cellular kinases to exhibit their antiviral effects. Therefore, the intracellular concentrations of their mono-, di- and triphosphates may be related to their inhibitory effect against viral replication. For example, as the rate of esterase hydrolysis of the nucleoside monophosphates to nucleosides is increased, their antiviral activities would be reduced. The phosphonate nucleosides have a strong covalent bond between the phosphorus atom and the carbon atom, resulting in resistance to hydrolysis by esterases. Furthermore, the nucleoside phosphonates bypass the monophosphorylation step, which may be a critical step in conversion of nucleoside agents to their active metabolites, triphosphates, by viral or host cellular kinases.

A number of new chemical entities have been designed and synthesized in the search for new antiviral agents, of which cidofovir, tenofovir disoproxil fumarate and adefovir dipivoxil have been introduced for the therapy of HCMV, HIV and hepatitis B virus (HBV) infection, respectively.

This review briefly describes mechanistic aspect of the phosphonate nucleosides as antiviral agents, the synthesis of important chemical entities, and prodrug approaches to overcome low permeability and poor oral bioavailability. In addition, two promising compounds disclosed recently, MCC-478 and LB80380, which are in

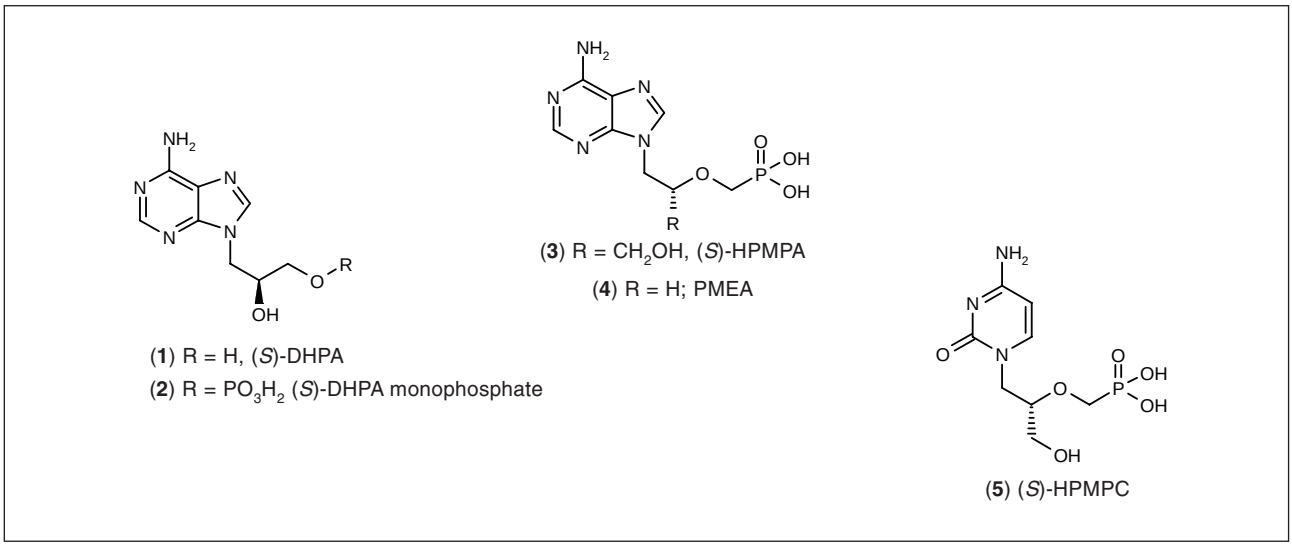


Fig. 1. Structures of (*S*)-DHPA (**1**), (*S*)-DHPA monophosphate (MP) (**2**), (*S*)-HPMPA (**3**), PMEA (adefovir, **4**) and (*S*)-HPMPC (cidofovir, **5**).

the clinical stage of development for the treatment of HBV infection, are discussed.

The discovery of the phosphonate nucleoside (*S*)-HPMPC

(*S*)-9-(2',3'-Dihydroxypropyl)adenine [(*S*)-DHPA, **1** in Fig. 1] is an aliphatic acyclic nucleoside analogue that exhibits a broad spectrum of antiviral activity against DNA and RNA viruses (vaccinia, herpes simplex, vesicular stomatitis and measles virus) (2). However, (*S*)-DHPA monophosphoric ester (**2**) has no significant biological activity *in vivo* due to its dephosphorylation to (*S*)-DHPA (3a).

(*S*)-9-(3-Hydroxy-2-phosphonomethoxypropyl)adenine [(*S*)-HPMPA, **3** in Fig. 1] is the first phosphonate nucleoside and was designed and synthesized to prevent it from intracellular dephosphorylation by esterases. The weak phosphoric acid ester bond ($-CH_2$-O-P(O)$(OH)_2$) in (*S*)-DHPA monophosphate is replaced by the strong phosphonomethyl ether bond ($-O-CH_2$-P(O)$(OH)_2$) in (*S*)-HPMPA (3a). (*S*)-HPMPA has potent and selective inhibitory activities against DNA viruses such as HSV-1 and HSV-2 (EC_{50} = 1-3 µg/ml), VZV (EC_{50} = 0.002 µg/ml), HCMV (EC_{50} = 0.15 µg/ml) and TK^- mutants of HSV-1 (EC_{50} = 0.7 µg/ ml). However, (*S*)-HPMPA shows no effect on normal host cell metabolism at up to > 100 µg/ml (1, 4).

The first synthesis of (*S*)-HPMPA was achieved with (*S*)-DHPA as a starting material, as shown in Scheme 1 (3a-3d). The phosphonomethyl ether bond on 2'-OH of (*S*)-HPMPA was introduced by intramolecular cyclization of its chloromethanephosphonyl ester (**7**).

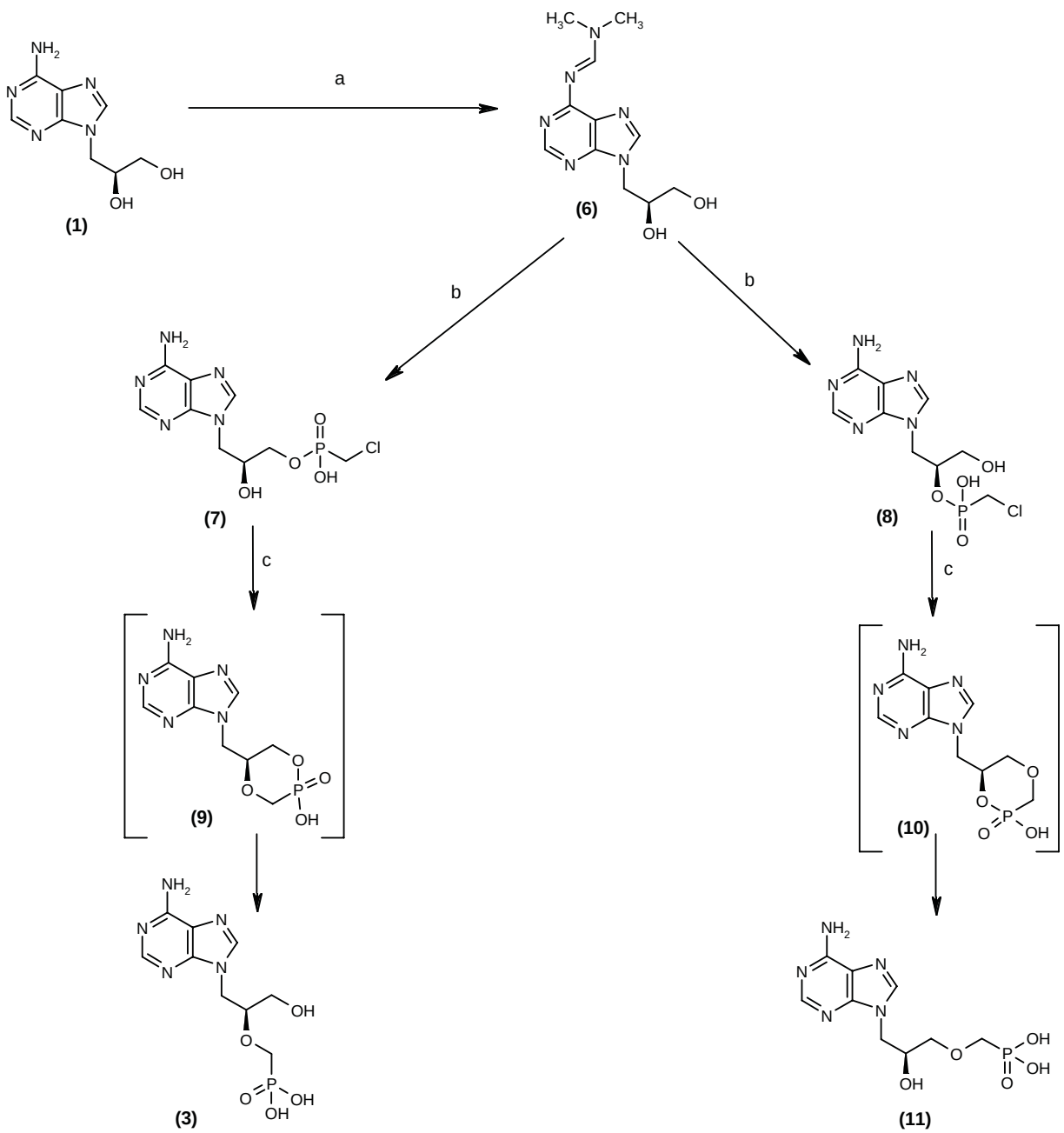
N^6-Protected (*S*)-DHPA (**6**) was esterified with $ClCH_2P(O)Cl_2$, followed by hydrolysis with ammonia, to afford compound **7** as the major product, along with the isomeric compound **8**. After separation, **7** and **8** were subjected to intramolecular cyclization, followed by alkaline hydrolysis, to produce (*S*)-HPMPA (**3**) and the 3'-isomer (**11**), respectively. The isomeric compound **11** was much less active against various viruses than (*S*)-HPMPA. The optimal process for the synthesis of (*S*)-HPMPA and (*S*)-HPMPC (cidofovir, **5**) was established later by Martin *et al.* (9a, 9b).

In the next phase, the hydroxymethyl moiety at the 2'-carbon of (*S*)-HPMPA (**3**) was replaced by a proton to give 9-(2-phosphonomethoxyethyl)adenine (PMEA, adefovir, **4**) (Fig. 1). The related 9-(2-phosphonomethoxyethyl)guanine (PMEG) and PME base analogues were reported by Holy and Rosenberg (5a, 5b). PMEA and PMEG have antiviral activities against DNA viruses, as well as retroviruses and hepadnaviruses (6, 7).

PMEA was prepared by a straightforward route from readily available 2-acetoxyethoxymethyl chloride (**12**), as shown in Scheme 2. The key intermediate, diethyl 2-bromoethylmethoxyphosphonate (**15**), was coupled with adenine in the presence of sodium hydride, followed by hydrolysis to afford PMEA (**4**).

As shown in Table I, (*S*)-HPMPA and (*S*)-HPMPC show a similarly broad spectrum of antiviral activity against DNA viruses, including herpesviruses, adenoviruses, hepadnaviruses and papovaviruses. In addition, they exhibit notable antiviral effects against TK^- HSV, with equal efficacy compared to against wild-type HSV-1. However, they do not inhibit the replication of human immunodeficiency virus (HIV). (*S*)-HPMPC (cidofovir; Gilead), marketed for the treatment of HCMV infection in HIV patients, exhibits excellent antiviral effects against wild-type HCMV (EC_{50} = 0.7 µM), ganciclovir-resistant (EC_{50} = 0.94 µM against UL mutants, EC_{50} = 7.3 µM

Scheme 1. **Synthesis of (*S*)-HPMPA** (**3**).

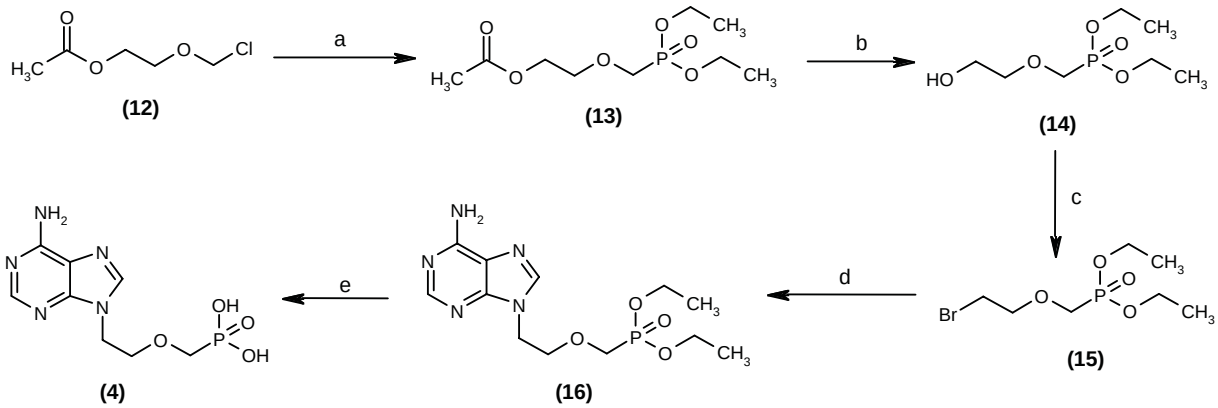


(a) Dimethylformamide dimethylacetal; (b) 1. $ClCH_2P(O)Cl_2$, 2. NH_4OH; (c) heating, NaOH

against other mutants) and forscarnet-resistant HCMV (EC_{50} = 0.6 μM) (11).

In contrast to (*S*)-HPMPA and (*S*)-HPMPC, PMEA shows excellent antiviral activity against HIV and HBV (EC_{50} = 0.2-2.0 μM), but mild to weak activity against HSV-1, HSV-2, human herpesvirus type 6 (HHV-6), HCMV and adenoviruses (7, 8, 10). PMEA dipivoxil (adefovir dipivoxil, **17**, Fig. 2) has been investigated in clinical studies in patients with HIV and HBV infection as an oral prodrug of PMEA. It significantly reduced HIV viral load at 120 mg once daily for 6 months but it was associated with nephrotoxicity (elevated serum creatinine level, hypophosphatemia) upon longer periods of treatment (12). In 2002, adefovir dipivoxil (Hepsera®; Gilead) was

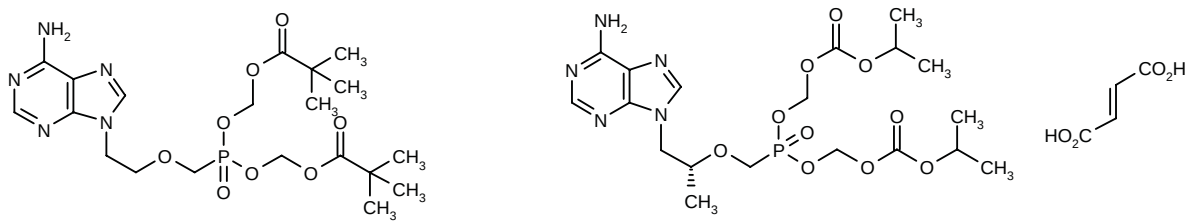
Scheme 2. Synthesis of PMEA (**4**).



(a) $P(OC_2H_5)_3$; (b) Dowex 50xs; (c) CBr_4/Ph_3P; (d) Adenine/NaH; (e) TMSBr.

Table I: Biological activity of PMEA, (S)-HPMPA and (S)-HPMPC.

	EC_{50} (uM)				EC_{50} (uM)		
Virus	PMEA	(*S*)-HPMPA	(*S*)-HPMPC	Virus	PMEA	(*S*)-HPMPA	(*S*)-HPMPC
Retrovirus				*Hepadnavirus*			
HIV-1	0.2-2.0	> 125		Human HBV	0.2-2.0	1.5	14
Herpesvirus				Duck HBV	0.14		
HSV-1	26	6.6	7.2	*Poxvirus*			
TK^- HSV-1	26	6.6	7.2	Vaccinia	>500	2.3	14
HSV-2	26	13	36	*Papovavirus*			
VZV	27	0.14	0.6	Human polyomavirus	>180	15	17
EBV	1.1	0.08	0.03	*Iridovirus*			
HHV-6	30	10	14	African swine fever virus	18	0.03	3.6
Adenovirus							
Human Ad5	> 400	0.17	0.54				
Human Ad8	> 400	0.08	0.06				



(**17**) PMEA dipivoxil

(**18**) (*R*)-PMPA disoproxil fumarate

Fig. 2. Structures of PMEA dipivoxil (**17**) and (*R*)-PMPA disoproxil fumarate (**18**).

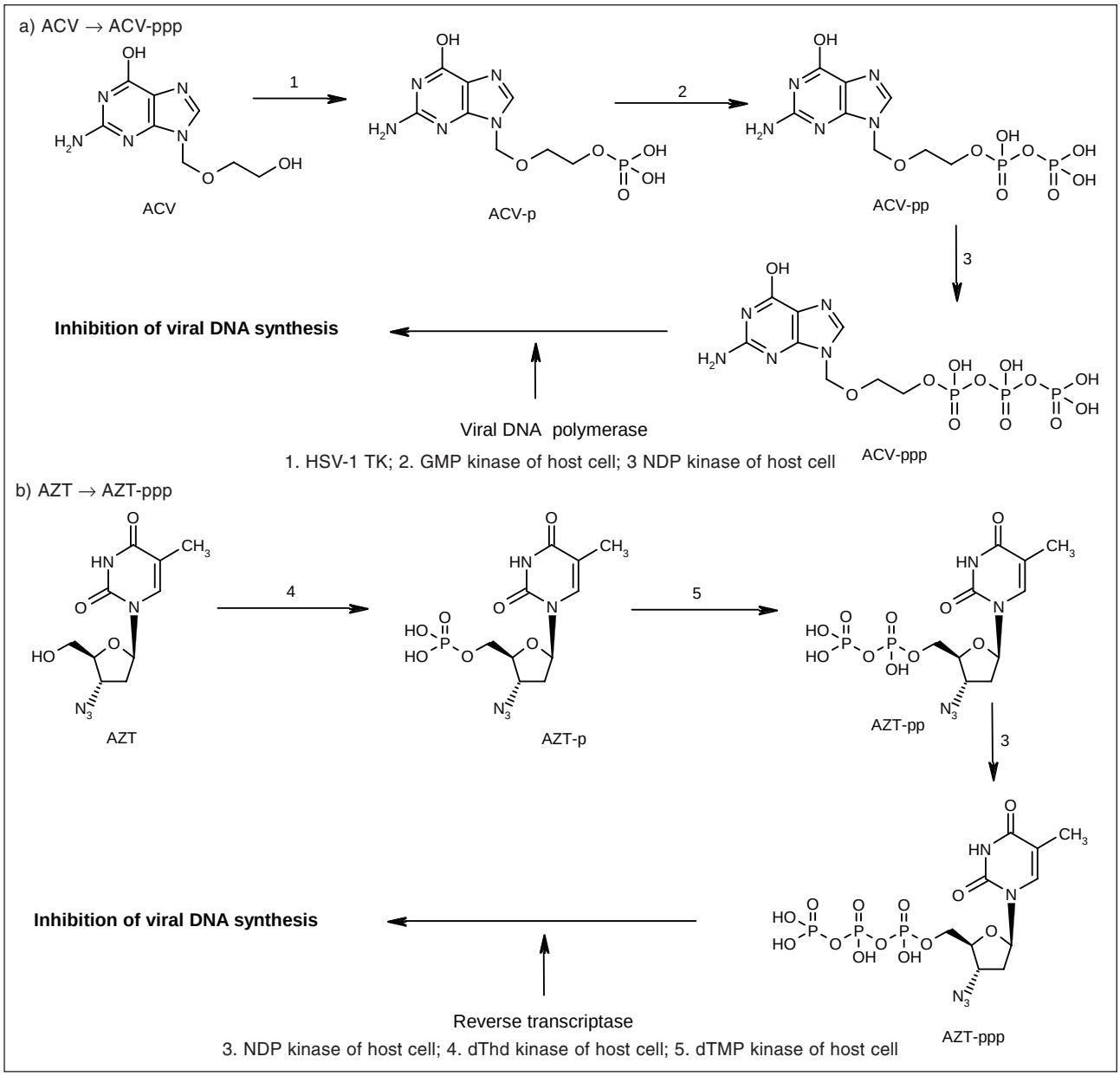


Fig. 3. Conversion of aciclovir (ACV) and zidovudine (AZT) to their respective active triphosphate metabolites ACV-ppp and AZT-ppp.

approved for the treatment of HBV infection, with 10 mg taken orally once daily being the recommended dose.

Tenofovir (PMPA) disoproxil fumarate (tenofovir DF, **18**, Fig. 2) is an oral prodrug of PMPA that is rapidly converted to PMPA following absorption. Tenofovir DF was approved in 2001 for the treatment of HIV-1 infection at a dose of 300 mg taken orally once daily. PMPA has demonstrated activity against both HIV and HBV.

Mechanistic aspects of phosphonate nucleosides

To exhibit antiviral effects, nucleoside agents, including acyclic nucleosides, must be phosphorylated intracellularly to their triphosphate forms, active metabolites. The triphosphates are incorporated into viral DNA or RNA during DNA or RNA elongation and terminate the synthesis of DNA or RNA, resulting in inhibition of viral replication (13).

Acyclic nucleoside agents, such as aciclovir, ganciclovir and penciclovir, are active against DNA viruses, HSV, VZV and HCMV. The first phosphorylation to their monophosphate is achieved by the HSV/VZV-encoded thymidine kinase or by the HCMV-encoded protein kinases. Subsequent di- and triphosphorylations are completed by host cellular kinases to produce the active forms, their triphosphates (Fig. 3a). In the case of retroviruses (HIV) and hepadnaviruses (HBV), all three steps

of phosphorylation of AZT (anti-HIV drug) or 3TC (anti-HIV and anti-HBV drug) are achieved by host cellular kinases such as a nucleoside kinase, a nucleoside 5'-monophosphate kinase or a 5'-diphosphate kinase, since HIV and HBV do not have viral-encoded kinases (Fig. 3b).

In contrast to nucleosides, the phosphonate nucleosides bypass the first phosphorylation step and the final two steps are accomplished by host cellular kinases to form the diphosphates, which are equivalent to nucleoside triphosphates. With the advantage of skipping the first phosphorylation, (*S*)-HPMPA shows equally potent inhibitory activities against both wild-type and mutant TK^- HSV and TK^- HCMV. The mutants are unable to convert acyclic nucleosides to their monophosphates due to the lack of a specific viral thymidine kinase or protein kinase, and they are therefore resistant to acyclic nucleoside drugs (aciclovir, penciclovir, ganciclovir and prodrugs).

The mode of action of (*S*)-HPMPC slightly differs from that of PMEA. Both compounds are metabolized to their diphosphates and then incorporated into viral DNA. As shown in Figure 4a, (*S*)-HPMPC is converted to (*S*)-HPMPC-pp by pyrimidine nucleoside mono- and diphosphate kinases. (*S*)-HPMPC-pp is incorporated into viral DNA and is also possibly converted to (*S*)-HPMPC-p-

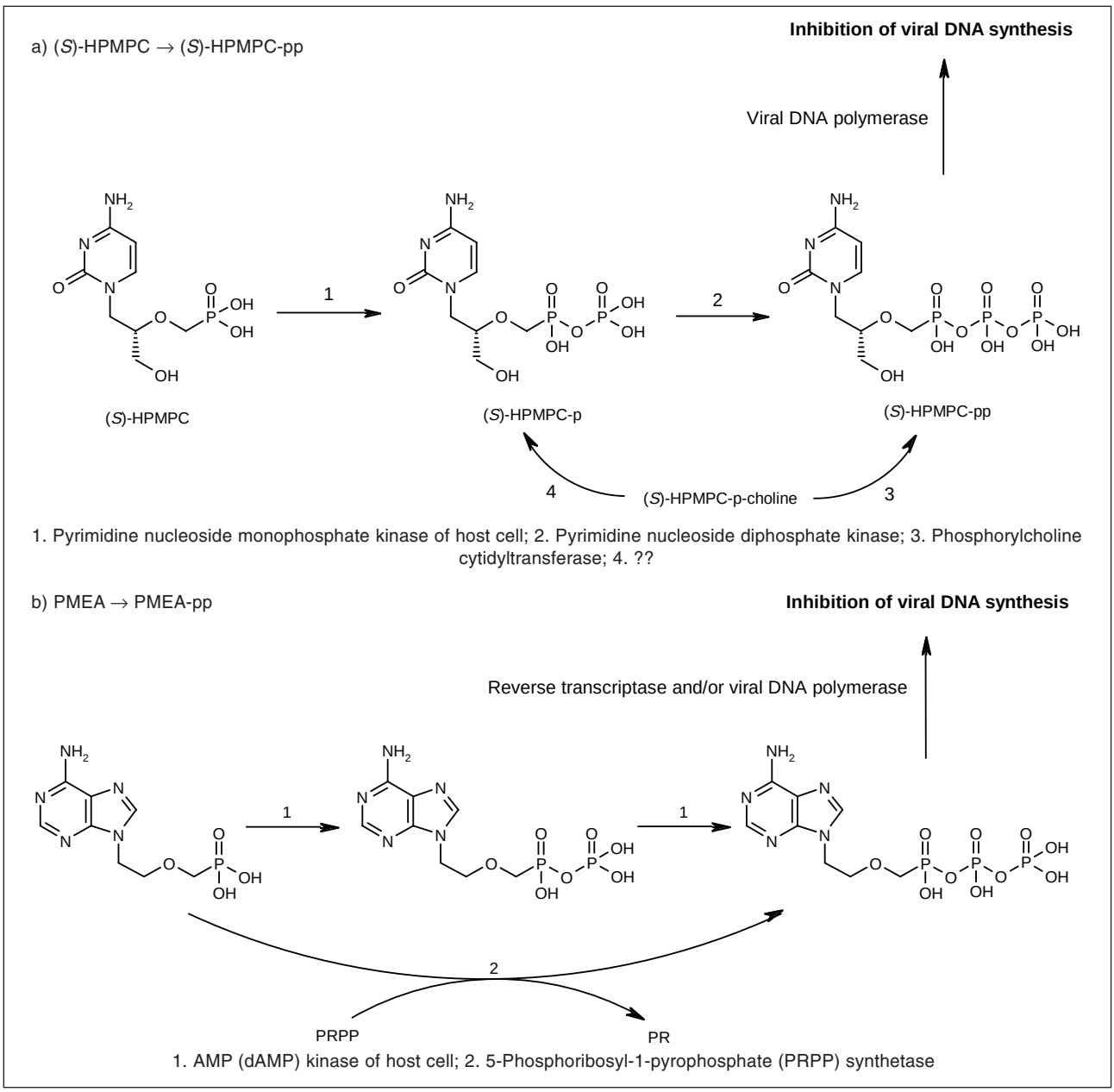
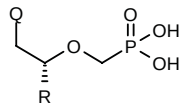


Fig. 4. Conversion of (*S*)-HPMPC and PMEA to their respective active diphosphate metabolites.



		Q	R
(*R*)-PMPG	(**19**)	Guanine	CH_3
(*R*)-PMPA	(**20**)	Adenine	CH_3
(*R*)-	(**21**)	Guanine	CH_2N_3
(*R*)-FPMPA	(**22**)	Adenine	CH_2F
(*R*)-	(**23**)	Guanine	$CH{=}CH_2$

Fig. 5. Structures of 2'-modified PMEA derivatives.

choline, which could function as an intracellular reservoir of (*S*)-HPMPC. That may explain the longer lasting antiviral effects of (*S*)-HPMPC *in vivo* (13, 14a, 14b).

On the other hand, it is not yet clear how PMEA is converted to its active metabolite PMEA-pp, although this may involve two routes: a two-step process involving monophosphatase (AMP kinase) or a one-step process involving 5-phosphoribosyl-1-pyrophosphate synthetase (PRPP synthetase), as shown in Figure 4b. PMEA-pp is incorporated into retroviral DNA by reverse transcriptase and has a relatively longer intracellular half-life compared to the nucleoside triphosphates AZT-ppp and d4T-ppp. This longer intracellular half-life may explain the longer lasting activity of PMEA against retroviruses (13, 15a-15c).

Chemical modification of (*S*)-HPMPA and PMEA

2'-Modified-[2-(phosphonomethoxy)ethyl] analogues

The broad spectrum of antiviral activity of (*S*)-HPMPA and PMEA has led medicinal chemists to synthesize the 2'-modified analogues and examine their antiviral activities. Although PMEG exhibits excellent inhibitory activity against various viruses, including HIV, HBV, HSV and HCMV (EC_{50} = 0.2, 0.2, 1.1 and 0.09 μM, respectively) (16a, 18), it also shows severe cytotoxicity (CC_{50} = 0.2 μM). Of the 2'-modified PME analogues, 2'-methyl PME analogues ([*R*]-PMP analogues) exhibit excellent antiretroviral activity against HIV and HBV and a good toxicity profile. (*R*)-PMPG (**19**) (Fig. 5) shows potent anti-HIV activity (EC_{50} = 1.0 μM; CC_{50} > 350 μM) and also exhibits moderate to weak activity against HCMV and HSV (EC_{50} = 16 and 82 μM, respectively) (16b). (*R*)-9-(2-Phosphonomethoxylpropyl)adenine ([*R*]-PMPA, **20**) exhibits excellent inhibition of HIV replication (EC_{50} = 0.04-5.9 μM depending on the cell line) and no significant cytotoxicity (CC_{50} > 300 μM) (17). PMPA was transformed to the oral prodrug tenofovir DF (**18**) (Fig. 2) by masking the phosphoric dianionic charges with a lipophilic group.

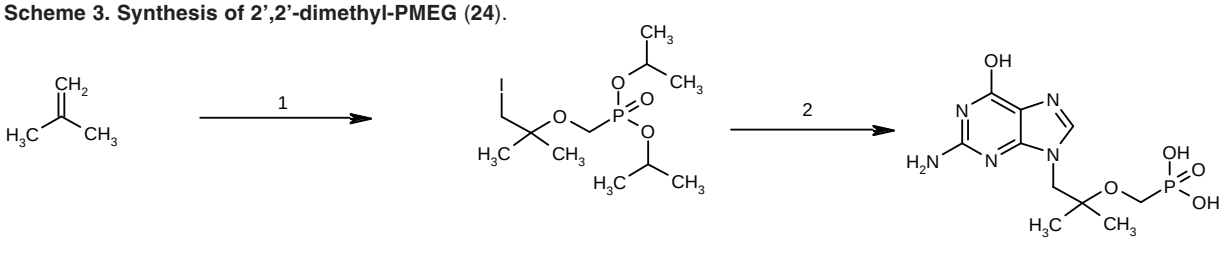
Other typical 2'-substituted analogues having antiviral activity are shown in Figure 5 (18). In general, the (*S*)-enantiomers of 2'-modified analogues have relatively weak antiviral activity compared to the (*R*)-enantiomers. Enantioselective synthesis of 2'-substituted analogues was mainly achieved by using known enantiomeric-enriched chemicals as starting materials (16b).

The chemically interesting 2',2'-dimethyl-PMEG (**24**) was prepared by a different route (Scheme 3). Reaction of isobutene and diisopropyl phosphonylmethanol in the presence of IBr as an electrophile afforded the phosphonate, which was converted to the desired 9-[2-methyl-2-(phosphonomethoxy)propyl]guanine (16a). Compound **24** shows a comparable anti-HBV effect (EC_{50} = 2.6 μM) to (*R*)-PMPG (**19**) but no activity against HCMV and HSV-2 at concentrations below 300 μM.

Isosteres of (S)-HPMPC and PMEA

Kim and other groups have worked extensively on isosteres of (*S*)-HPMPC and PMEA to identify the structural limitations for antiviral activity. Variation of the length of the carbon chain between the purine and the phosphonomethoxy functionality demonstrated that 2 carbon atoms are optimal for antiviral activity against HSV-1, HSV-2 and HIV-1 (compounds **25**, **28, 31**, Fig. 6) (20a). Replacement of the 3'-oxygen with a carbon or sulfur atom resulted in a total loss of anti-HSV and anti-HIV-1 activity (compounds **26, 27, 29**) (20a). Introduction of a double bond to the alkyl chain significantly reduced antiviral activity against HIV and HSV (compound **30**) (23). On

Scheme 3. Synthesis of 2',2'-dimethyl-PMEG (**24**).



(24)

1. $(i\text{-}OPr)_2POCH_2OH$/IBr; 2. a) 6-Cl-Guanine, b) TMSBr, 2,4,6-lutidine, c) 1 N HCl.

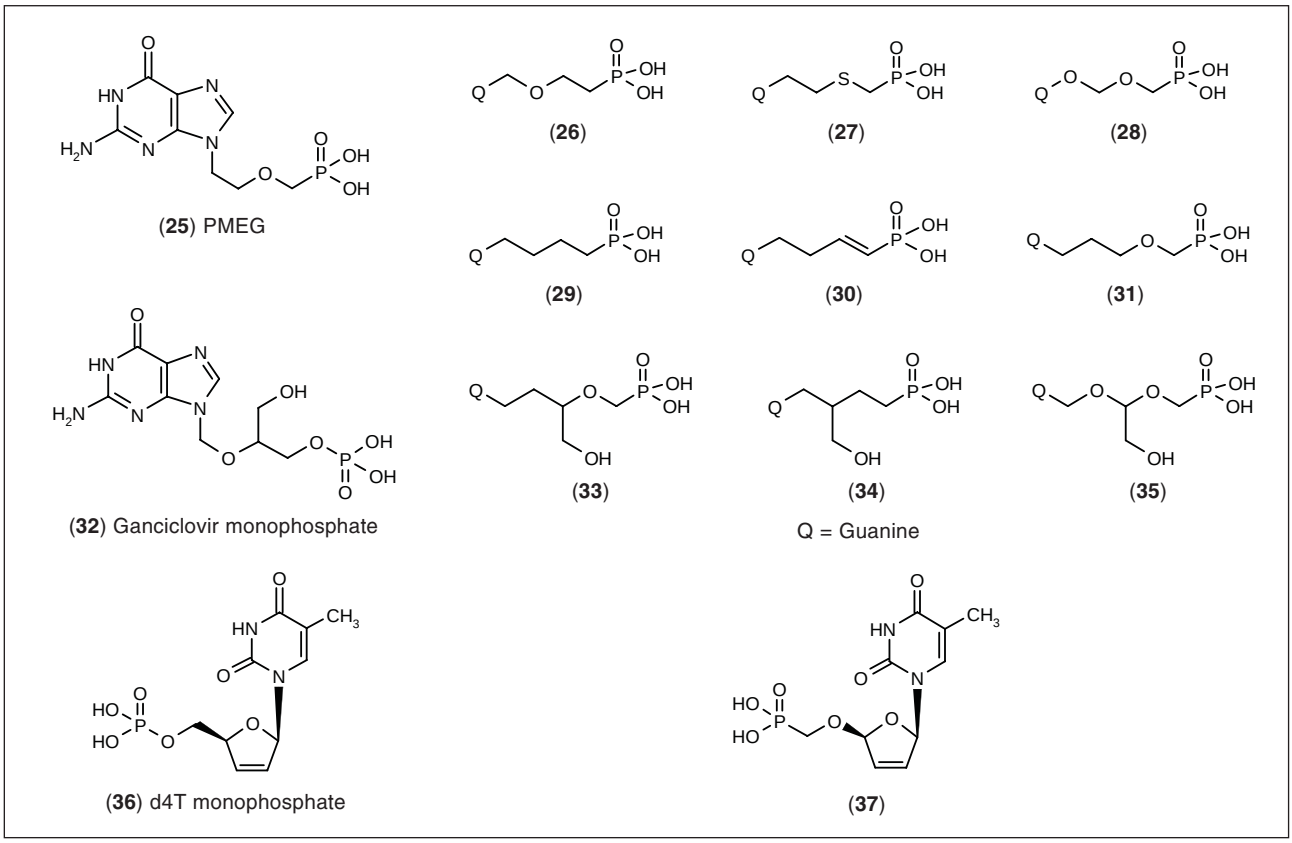


Fig. 6. Representative structures of isosteres of PMEA and (*S*)-HPMPC.

the other hand, isosteres of d4T (stavudine) monophosphate (**36**) exhibited potent anti-HIV activity comparable to that of d4T (compound **37**) (20d). Isosteres of ganciclovir monophosphate (**32**) exhibited significantly reduced activity against HSV-1, HSV-2 and HCMV compared to PMEG (compounds **33**, **35**) (20b, 20c, 21, 22).

8-Azaguanine analogues and others

Compared to PMEA and (*S*)-HPMPA, PMPG and (*S*)-HPMPG exhibited more potent antiviral activities against DNA and/ or retroviruses, but they showed higher cytotoxicity to the host cells. To reduce the cytotoxicity while retaining antiviral activity, 8-aza analogues of PMEG and (*R*)-PMPG were synthesized and evaluated against HIV-1 and HIV-2 (19a). PME and (*R*)-PMP 8-azaguanine analogues exhibited low antiviral activity (19b). 8-Azaguanine analogues **38** and **39** (Fig. 7) showed improved cellular toxicity, but also reduced antiviral activity

Recently, Holy reported PME and PMP pyrimidine analogues and their isomers. PME modified purine analogues were also synthesized and evaluated for antiviral activity (24a, 24b, 25).

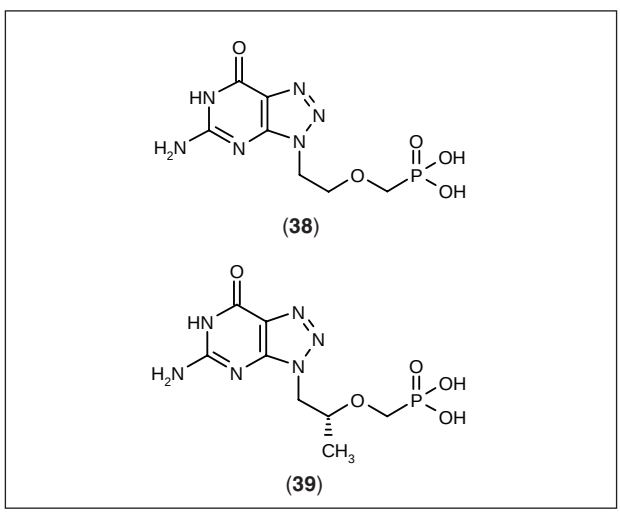


Fig. 7. Structures of 8-azaguanine analogues **38** and **39**.

Prodrugs of PMEA and PMPA

Work to overcome the shortcomings of the phosphonate nucleosides such as low cellular permeability and oral bioavailability has focused on the prodrug approach, which involves masking the negative charges on the phosphonate functionality and releasing the parent drug

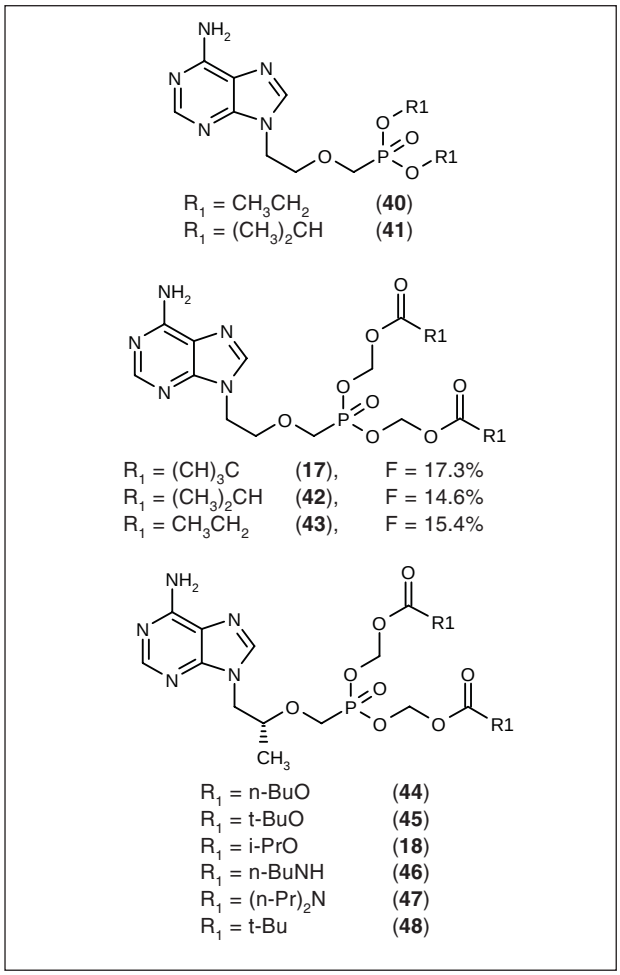


Fig. 8. Prodrugs of PMEA and (*R*)-PMPA.

at the targeted site (29a, 29b). The prodrugs should have suitable stability for formulation and storage and appropriate durability in the gastrointestinal tract.

Both adefovir (PMEA) and cidofovir ([*S*]-HPMPC) are dianionic charges at physiological pH due to low pK_a values (26). The dianionic charges of the phosphonate cause low cell permeability and low oral bioavailability, which limits the use of these drugs to chronic diseases such as HIV and HBV. The oral bioavailability of adefovir was low in animals (10% in mice, 11% in rats, < 1% in monkeys), as well as in humans (16.4 ± 16%) (26, 28a).

This low oral bioavailability necessitates the design and synthesis of oral prodrugs intended to enhance intestinal absorption. A series of PMEA prodrugs were synthesized and evaluated for oral bioavailability in rats (27a,b). Although bis(alkyl)PMEA (**40**, **41**) (Fig. 8) afforded more than 40% oral bioavailability in rats, the prodrugs were incompletely converted to the parent compound PMEA, resulting in low efficacy *in vivo*. Bis[(acyloxy)alkyl]PMEA (**17, 42, 43**) increased oral bioavailability of PMEA up to 17% in rats and the oral prodrug was efficiently cleaved to free PMEA. Among the bis[(acyloxy)alkyl]PMEA series, the most promising prodrug, bis[(pivaloyloxy)methyl]PMEA (**17**), revealed 17% and 30% oral bioavailability in rats and monkeys, respectively (28a, 28b).

In the case of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA), it showed low oral bioavailability in animals, presumably for the same reason as PMEA: the dianionic charges. A prodrug approach involving masking the phosphonate moiety has been studied by synthesizing and evaluating methylcarbonate and methylcarbamate prodrugs. The pivaloyloxyalkyl ester prodrug (**48**), like PMEA dipivoxil, was excluded, because the molecule generates pivalic acid during the release of free PMEA (Fig. 9).

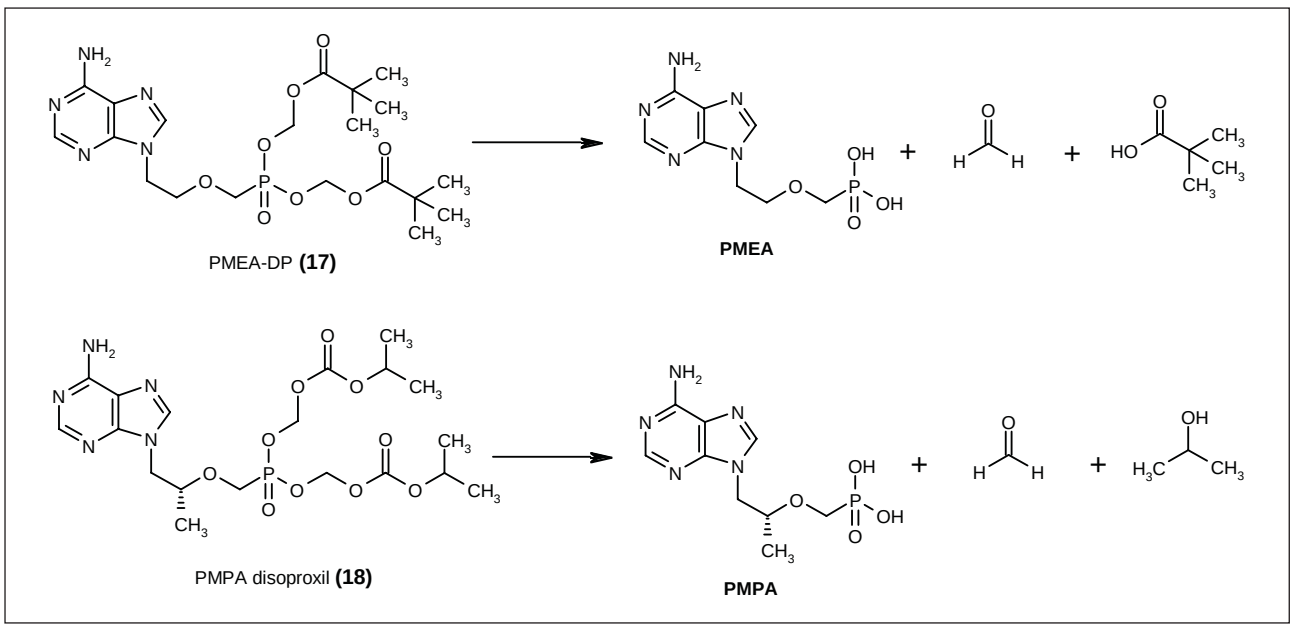


Fig. 9. Conversion of PMEA diphosphate (PMEA-DP) and PMPA disoproxil to their parent molecules.

Pivalic acid may decrease carnitine levels in serum at high doses (12, 30a).

At pH 2.2 and 7.7, values associated with the gastrointestinal tract, the chemical stability of methylcarbonate prodrugs of PMPA (**18, 44**) (Fig. 8) was comparable to that of PMEA dipivoxil. Oral bioavailabilities of the oral carbonate PMPA prodrugs **18, 44** and **45** were 20-30% in dogs, and metabolites other than PMPA were not found. Methylcarbamate prodrugs (**46**, **47**) (Fig. 8) of PMPA showed 8.8% oral bioavailability of parent PMPA and about 10% of intact prodrugs (30a). Methylcarbamate prodrugs were so stable under physiological conditions that they were incompletely metabolized to the parent drug in an appropriate time period, resulting in reduced efficacy *in vivo*.

As shown in Figure 9, PMEA dipivoxil and PMPA disoproxil may generate formaldehyde and pivalic acid as by-products from PMEA dipivoxil, and formaldehyde and isopropyl alcohol from PMPA disoproxil in the metabolic process during release of PMEA and PMPA. However, the generation of toxic formaldehyde may not be of concern at the therapeutic doses.

Recently developed analogues MCC-478 and LB-80380

Recently, two new phosphonate nucleosides (Fig. 10), MCC-478 (**49**) (Mitsubishi, Lilly) and LB80380 (**50**) (LG Life Sciences), entered clinical studies for the treatment of HBV infection.

MCC-478 was designed to reduce the severe cytotoxicity of PMEG (CC_{50} = 6.5 μM in HB611 cells), although PMEG showed higher anti-HBV efficacy than PMEA (18b). Introduction of an arylthio group into the 6-position

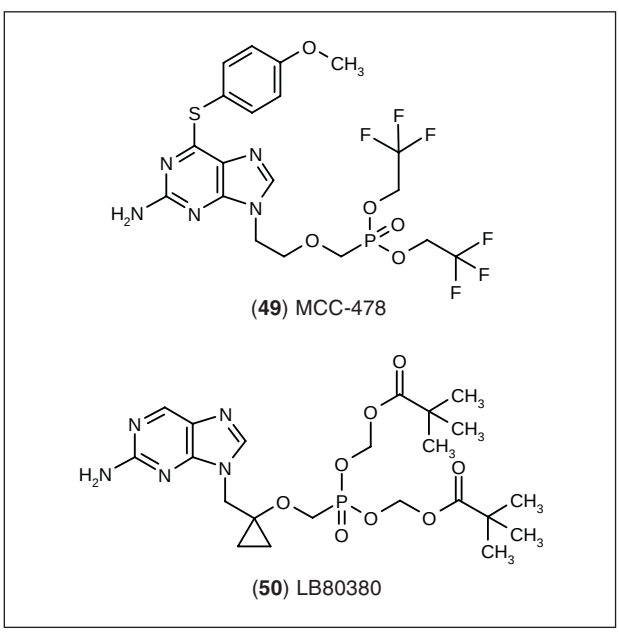


Fig. 10. Structures of MCC-478 and LB80380.

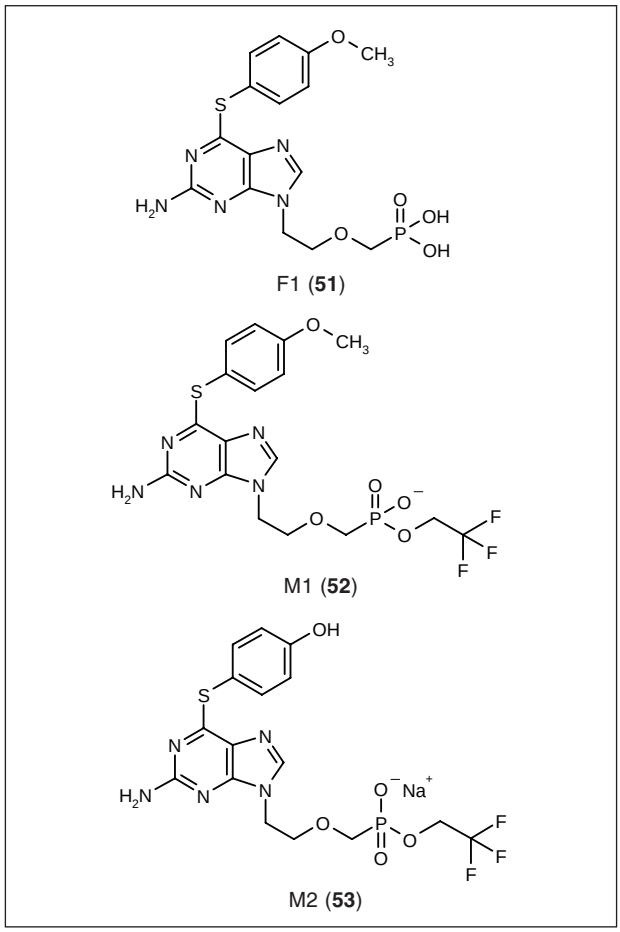


Fig. 11. Major metabolites of MCC-478 *in vivo*.

of the guanine ring of PMEG retained anti-HBV activity and exhibited toxicological changes. In addition, the dianionic phosphoric acid functionality was masked with the bis(2,2,2-trifluoroethyl) group to increase oral bioavailability (31a, 31b). MCC-478 and certain related compounds showed high anti-HBV activity and low cytotoxicity, but no significant anti-HIV and anti-HSV-1 activities at concentrations of up to 34 μM. In particular, MCC-478 shows 10-fold higher (IC_{50} = 0.03 μM) anti-HBV activity than PMEA and is also active against different lamivudine-resistant mutants, although its potency against the mutants was decreased by 100-fold (IC_{50} = 2.6 μM) compared to wild-type strains (IC_{50} = 0.03 μM) (32).

Some dialkyl prodrugs of PMEA were not converted *in vivo* to PMEA, and the prodrugs showed reduced anti-HBV activity compared to PMEA *in vivo*. Also, MCC-478 was metabolized in rats and monkeys to produce two major metabolites –M1 (**52**) and M2 (**53**)– in addition to the free parent compound F1 (**51**) (Fig. 11). Unlike alkyl ester prodrugs of PMEA, the metabolites M1, M2 and F1 show anti-HBV activity which is comparable to that of MCC-478. Therefore, the anti-HBV activity of the metabolites could contribute to the *in vivo* efficacy of MCC-478. Further study on the metabolism and mode of action of

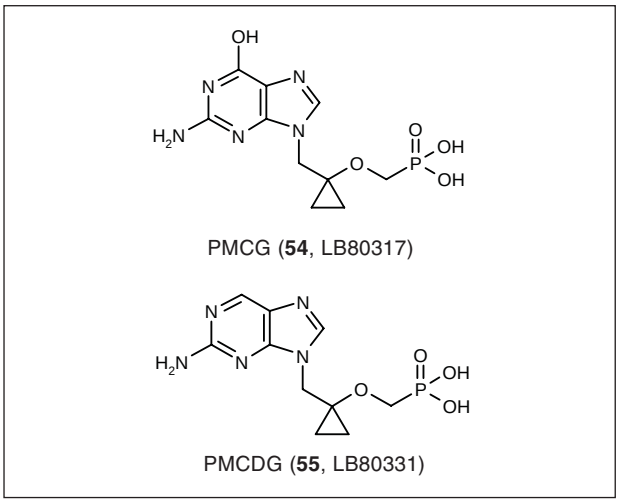


Fig. 12. Structures of PMCG (LB80317) and PMCDG (LB80331).

MCC-478 suggested that intracellular diphosphorylation may not be required to exert anti-HBV activity, unlike PMEA (33).

LB80380 (**50**) (Fig. 10) was recently discovered as an anti-HBV agent by Choi et al. (LG Life Sciences) and is currently undergoing phase II clinical studies in HBV patients. LB80380 is an orally active prodrug of LB80317 [9-[1-(phosphonomethoxylcyclopropyl)methyl]-guanine (PMCG), **54**] (Fig. 12), representative of a novel class of nucleoside phosphonates, and shows highly potent, specific and selective anti-HBV activity (34a, 34b). The parent compound LB80317 blocks HBV replication with excellent potency (EC_{50} = 0.5 μM) in primary cultures of Hep G2 2.2.15 cells. It does not exhibit significant cytotoxicity in several human cell lines up to 1.0 mM and does not inhibit the replication of HIV-1 and HSV-1 at concentrations below 30 μM. Compared to PMEA, which was used as a positive control (EC_{50} = 2.0 μM), LB80317 is 4 times more potent against HBV *in vitro*.

LB80317 has a cyclopropane ring at the 2'-position of PMEG (**25**) (Fig. 6). The characteristic cyclopropane moiety at the 2'-position might play a critical role in the global conformation of LB80317. The extremely high potency of anti-HBV activity and therapeutic selectivity might be attributable to the conformation of LB80317. Compared to PMEG, LB80317 favors the *cis* arrangement between the guanine and the phosphorous atom, as shown in the X-ray structure (Fig. 13).

The *cis* arrangement between the guanine and the phosphorous atom may be conformationally similar to that of natural guanosine monophosphate having a ribose ring. This *cis* arrangement would allow efficient phosphorylation of LB80317 by human kinases to produce the active form PMCG diphosphate, which then might be properly incorporated into DNA during viral replication, resulting in termination of polymerization.

The compounds LB80317 and LB80380 were synthesized as shown in Scheme 4. The key intermediate, cyclopropanol (**56**), was prepared by titanium-mediated Kulinkovich cyclopropanation in over 80% yield as a white solid. Etherification of **56** with diisopropyl bromomethylphosphonate, followed by sequential desilylation, mesylation and coupling reaction with 6-chloroguanine, gave compound **60**. Finally, hydrolysis of compound **60** in the presence of TMSBr afforded LB80317.

LB80331 [9-[1-(phosphonomethoxylcyclopropyl)-methyl]deoxyguanine (PMCDG, **55**] (Fig. 12) was much more potent than LB80317 in an *in vivo* efficacy study in transgenic mice treated subcutaneously. This difference is probably due to the higher cell permeability of LB80331 (35). It is thought that LB80331 may easily permeate the cell membrane and be metabolized intracellularly to yield LB80317 in the liver. Like PMEA and PMPA, LB80331

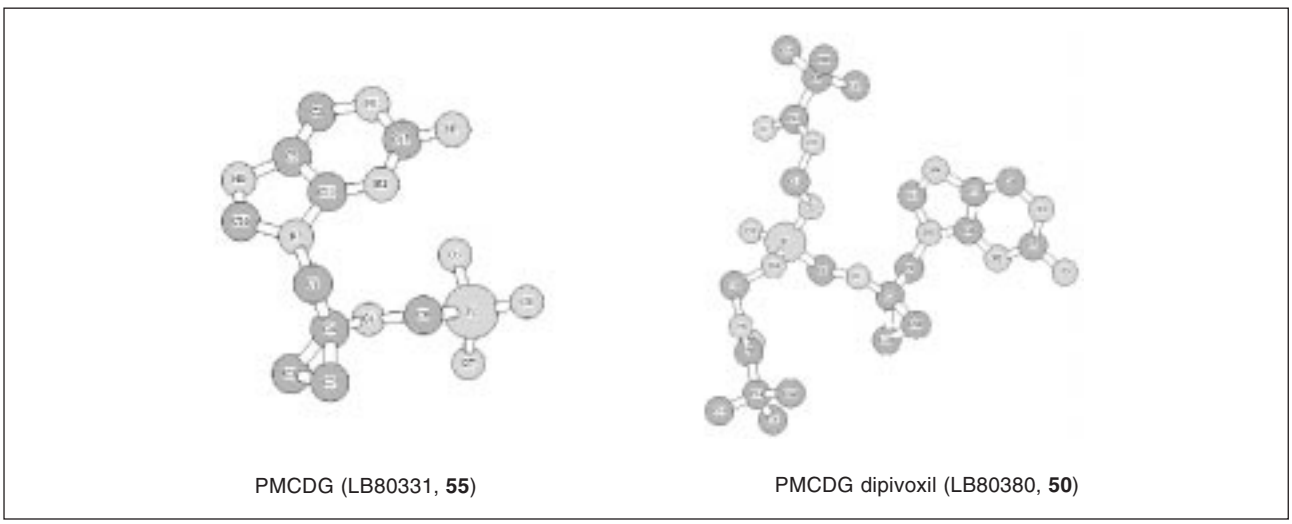
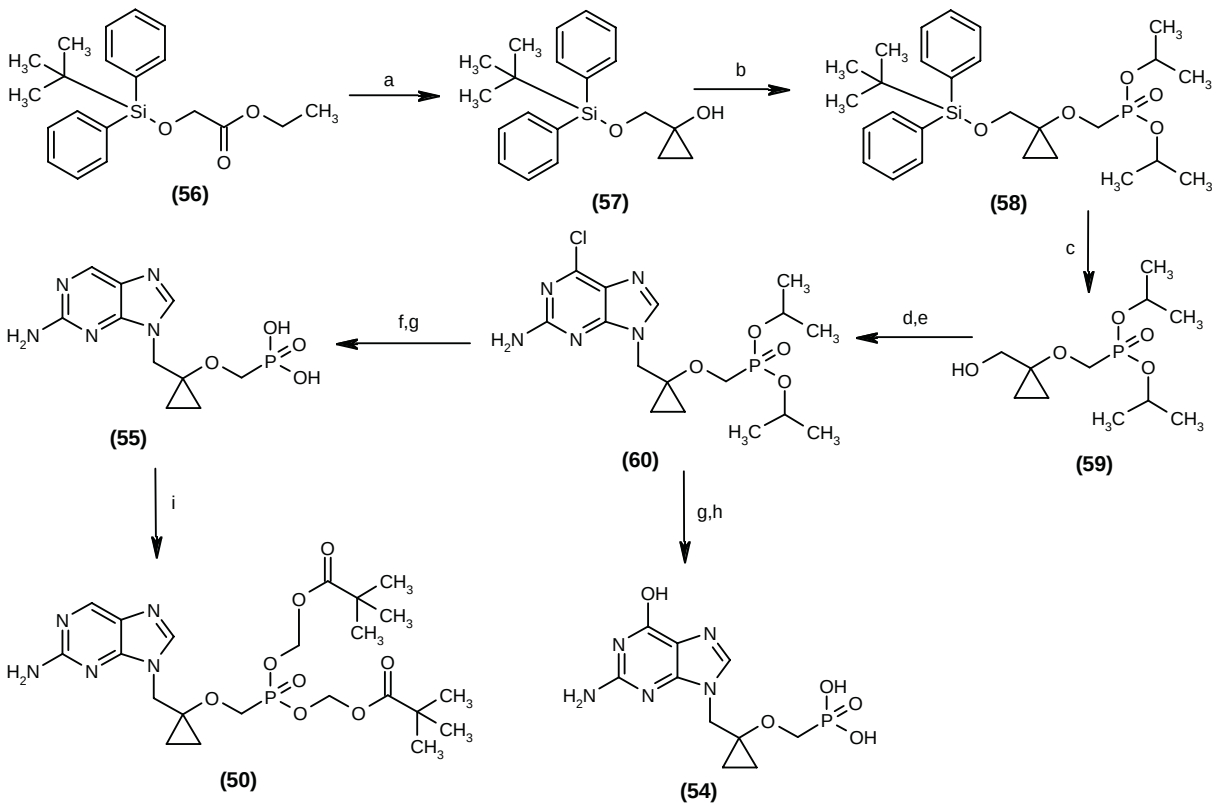


Fig. 13. Molecular structures of PMCDG dipivoxil (LB80380) and PMCDG (LB80331) from X-ray diffraction (hydrogen atoms are omitted for clearance).

Scheme 4. Synthesis of PMCG (54), PMCDG (55) and PMCDG dipivoxil (50).



(a) CH_3CH_2MgBr, Ti(*i*-PrO)$_4$ (0.25 equiv.); (b) $BrCH_2P(O)$(*i*-PrO)$_2$; (c) NH_4F; (d) MsCl; (e) 6-Chloroguanine; (f) H_2, 5% Pd/C; (g) TMSBr; (h) 2N HCl; (i) Chloromethyl pivalate.

also showed low oral bioavailability (> 5% in rats) due to the dianionic charges. LB 80331 was modified to the oral prodrug LB80380 by masking the polar phosphoric acid functionality with pivalolyoxymethyl (dipivoxil). No molecule other than the parent molecule LB80331 was detected in plasma after oral administration of LB80380 to rats, dogs and monkeys, with respective oral bioavailabilities of 25%, 64% and 15% (36). Efficacy evaluation of LB80380 *in vivo* was conducted at oral doses of 5 and 15 mg/kg for 4 weeks in woodchuck hepatitis B virus (WHBV)-infected woodchucks. The DNA titers of WHBV were reduced by 10^8 after 4 weeks of treatment at both doses and slowly rebounded up to 12 weeks after stopping LB80380 treatment, as shown in Figure 14.

Compared to PMEA dipivoxil (**17**), which was used as a positive control, LB80380 (**50**) showed much better efficacy *in vivo*. LB80380 has successfully completed phase I studies and is currently undergoing phase II testing.

Summary and outlook

The phosphonate nucleosides are an example of mechanism-based drug design to prevent dephosphorylation of the acyclic nucleoside monophosphates to their parent acyclic nucleosides. They exhibit a broad spectrum of antiviral activity against DNA and RNA viruses. Unlike nucleoside agents, they skip the monophosphorylation step to form active metabolites, the diphosphates, which incorporate into viral DNA or RNA during elongation to terminate viral replication. Some of the diphosphates are only incorporated into viral DNA or RNA, and do not incorporate into host cell DNA, thereby affording excellent therapeutic selectivity. A prolonged elimination *in vivo* allows infrequent dosing to maintain efficacy: intravenous administration every 2 weeks for cidofovir and once-daily oral dosing for adefovir dipivoxil, tenofovir disoproxil fumarate and LB80380. The shortcoming of low oral bioavailability due to the dianionic charges of the phosphonate has been overcome with prodrug approaches masking the dianionic charges. Adefovir dipivoxil is a promising anti-HBV agent, although nephrotoxicity is a concern with extended treatment. Tenofovir disoproxil fumarate is also a potent once-daily anti-HIV agent and is expected to enhance the cure rate of combination therapy with other HIV drugs. LB80380 is much more potent than adefovir dipivoxil in animal efficacy studies and

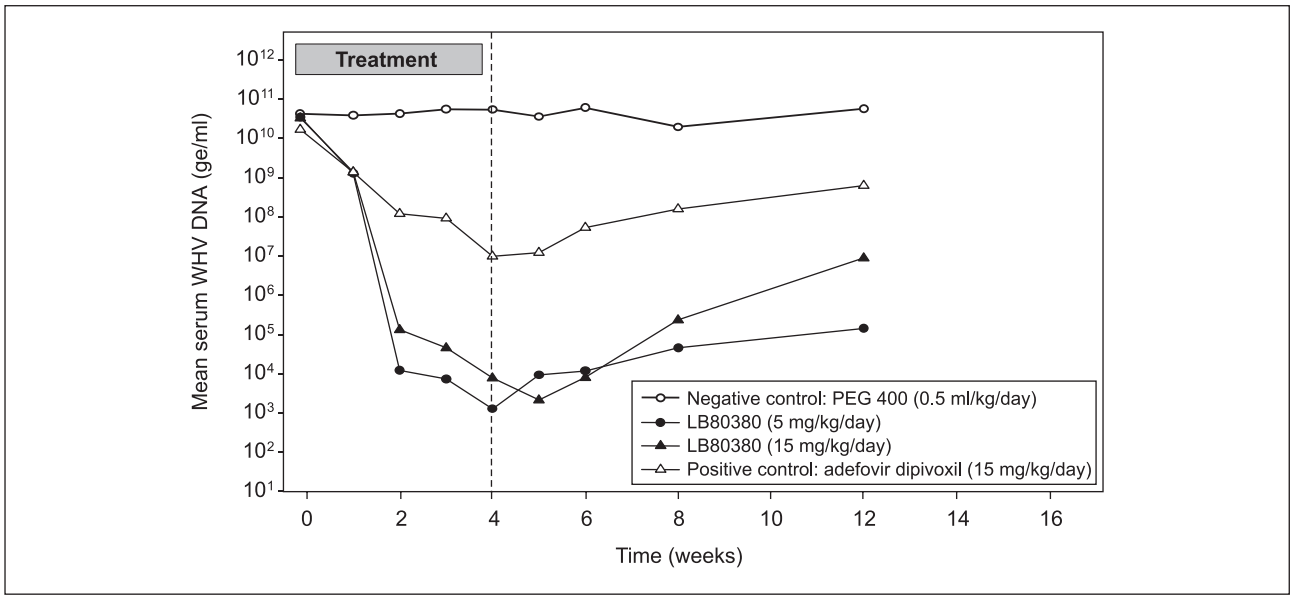


Fig. 14. *In vivo* efficacy of LB80380 at 5 and 15 mg/kg/day p.o. for 4 weeks, with 12-week follow-up.

appears to be a promising anti-HBV drug with a good toxicity profile and no emergence of drug-induced resistance. The new class of phosphonate nucleosides are therefore important agents for addition to the pool of antiviral agents, including the acyclic nucleosides. In addition, studies on the phosphonate nucleosides are expected to lead to the discovery of safer and more therapeutically effective antiviral drugs, especially for hepatitis C virus (HCV) infection.

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