1438 J. Med. Chem. 2010, 53, 1438–1450
DOI: 10.1021/jm900932g

Journal of Medicinal Chemistry Perspective

Highlights in the Discovery of Antiviral Drugs: A Personal Retrospective

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Received June 24, 2009

Introduction

In 1959 Bill Prusoff described the synthesis of idoxuridine (5-iodo-2′-deoxyuridine, IDU[a]),[1] later to become the first antiviral drug to be licensed for clinical use. This marked the birthday of the antiviral drug era, which has now grown to about 50 licensed antiviral drugs, half of which have been approved for the treatment of human immunodeficiency virus (HIV) infections.[2] In retrospect, I was attracted to microbiological research by my mentor, Prof. Piet De Somer who had first pioneered, in the post Second World War years, on the mass production of penicillin before tackling in the 1950s the polio vaccine and then persuaded me (at the time my professor of microbiology) to start a career on interferon. This molecule had been discovered by Isaacs and Lindenmann in 1957[3] and was expected to be the panacea for the treatment of virus infections. Of pivotal importance was the discovery by Maurice Hilleman's group, in 1967, that interferon could be induced by double-stranded RNA, such as poly(I)·poly(C).[4] The fact that poly(I)·poly(C) was such a potent inducer of interferon-β allowed us, in a collaborative effort with Walter Fiers and Jean Content, to get the interferon-β cloned and expressed in prokaryotic systems.[5,6]

While waiting for interferon to fulfill its premises as an antiviral drug, I became more interested in small molecules as potential antiviral agents. The year 1976 was of crucial importance in this regard for a number of reasons: (i) for the first description of the successful systemic use of an antiviral drug, adenine arabinoside, for the therapy of herpes zoster,[7] and (ii) for a meeting that I attended in Göttingen, Germany (Symposium on Synthetic Nucleosides, Nucleotides and Polynucleotides, Max-Planck-Institut für Biophysikalische Chemie, May 3−5, 1976) where I met with a number of the leading experts in the nucleoside chemistry field, including John Moffatt, Helmut Vorbrüggen, Wolfgang Pfleiderer, David Shugar, and Fritz Eckstein, and where my collaboration started with Dick Walker and Tony Holý. My collaboration with Dick Walker would lead to the discovery of BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] and that with Tony Holý to the discovery of a totally new class of antiviral agents, i.e., the acyclic nucleoside phosphonates, which have now expanded to a whole array of marketed compounds (adefovir, cidofovir, tenofovir).

In 1976, the number of known (low molecular weight) antiviral compounds was limited to a handful compounds: IDU and TFT (trifluridine), which, as demonstrated by Herbert Kaufman[8,9] in 1962 and 1964, could be used against HSV (herpes simplex virus) infections in the eye; ara-A, first shown to be active as an anti-HSV agent by Privat de Garilhe and de Rudder;[10] amantadine, first reported as an antiviral active against influenza by Davies et al.;[11] and ribavirin, first reported by Sidwell et al.[12] as a broad spectrum antiviral agent.

Acyclovir, announced in December 1977 by Trudy Elion and her colleagues[13] and in March 1978,[14] was greeted as the first specific antiviral because it took advantage, for its activation, by a specific virus-induced enzyme, thymidine kinase (TK). A few months after acyclovir was described as the first acyclic guanosine analogue to be specifically active against herpesviruses encoding for a specific viral TK (i.e., HSV), we described the acyclic adenosine analogue (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] as a broad-spectrum antiviral agent.[15] Its target of action, as demonstrated later, appeared to be a cellular enzyme, *S*-adenosylhomocysteine hydrolase.

Preview

Acyclovir [9-(2-hydroxyethoxymethyl)guanine], while still be considered today as the gold standard for the treatment of HSV infections, served as the starting point for the synthesis of a number of acyclic guanosine analogues, as well as prodrugs thereof, i.e., aminoacyl esters,[16,17] one of which, valaciclovir, the valine ester of acyclovir, has now largely replaced acyclovir for the oral treatment of HSV infections.

Within 1−2 years after acyclovir, we described BVDU as a potent and selective anti-HSV agent.[18] Like acyclovir, BVDU owes its selective antiviral activity to a specific phosphorylation by the virus-encoded TK, but unlike acyclovir, BVDU is much more active against HSV-1 than HSV-2 (which, of course, limits its therapeutic use). However, BVDU is much more active than acyclovir against varicella-zoster virus (VZV), and new congeners of BVDU, i.e., Cf 1743 (**12**), are still more potent inhibitors of VZV replication.[19]

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[a] Abbreviations: IDU, 5-iodo-2′-deoxyuridine; HIV, human immunodeficiency virus; BVDU, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine; HSV, herpes simplex virus; TK, thymidine kinase; (*S*)-DHPA, (*S*)-9-(2,3-dihydroxypropyl)adenine; HPMPA, (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; HBV, hepatitis B virus; (*R*)-PMPA, (*R*)-9-(2-phosphonylmethoxypropyl)adenine; TDF, tenofovir disoproxil fumarate; PMEG, 9-(2-phosphonylmethoxyethyl)guanine; cPrPMEDAP, 9-(2-phosphonomethoxyethyl)-N^6-cyclopropyl-2,6-diaminopurine; HEPT, 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine; TIBO, tetrahydroimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; NDP, nucleoside diphosphate; BVU, (*E*)-5-(2-bromovinyl)uracil; PML, progressive multifocal leukoencephalopathy; HPV, human papilloma virus; HDP, hexadecyloxypropyl; ODE, octadecyloxyethyl; ITU, iminothiourea; DATA, diaryltriazine; DAPY, diarylpyrimidine.

 Published on Web 10/27/2009

In 1986 we described a totally new approach to inhibit DNA virus and retrovirus infections, namely, that based on the acyclic nucleoside phosphonates with (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) as the prototype.[20] (*S*)-HPMPA could be viewed as the hybrid of (*S*)-DHPA with a known antiviral agent, phosphonoformic acid (PFA, foscarnet).

Not (*S*)-HPMPA, but its cytosine counterpart cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine], first described in 1987,[21] was further developed. It was formally approved in 1996 for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients. Adefovir [9-(2-phosphonylmethoxyethyl)adenine, PMEA], which had been mentioned in the same paper as (*S*)-HPMPA,[20] was eventually (in 2002) licensed as its oral prodrug form, adefovir dipivoxil, for the treatment of hepatitis B virus (HBV) infections. Its methyl derivative tenofovir [(*R*)-9-(2-phosphonylmethoxypropyl)adenine, (*R*)-PMPA] was first described in 1993 for its antiretroviral properties.[22] Eight years later, in 2001, it was approved as its oral prodrug form (TDF, tenofovir disoproxil fumarate) for the treatment of HIV infections and has ever since remained an essential component in anti-HIV drug cocktails.

Concomitantly with (*S*)-HPMPA, PMEA, and (*R*)-PMPA, their 2,6-diaminopurine counterparts were described: (*RS*)-HPMPDAP, PMEDAP,[21] and (*R*)-PMPDAP.[22] Although these compounds themselves were not developed as antiviral drugs, they could be considered as isosteric to the "open chain" 6-[2-(phosphonomethoxy)alkoxy]pyrimidines (*R*)-HPMPO-DAPy, PMEO-DAPy, and (*R*)-PMPO-DAPy congeners,[23,24] all yielding attractive potential as anti-DNA virus or antiretrovirus agents or both. Also yielding great potential for the treatment of DNA virus infections at large are the 5-azacytosine counterpart of cidofovir, (*S*)-HPMP-5-azaC, its cyclic analogue, and alkoxyalkyl [i.e., octadecyloxyethyl (ODE) and hexadecyloxypropyl (HDP)] esters thereof.[25]

In 1987 we also described 9-(2-phosphonylmethoxyethyl)-guanine (PMEG).[21] Being too cytotoxic, PMEG was not further pursued for its antiviral properties. Yet its prodrug, 9-(2-phosphonomethoxyethyl)-N^6-cyclopropyl-2,6-diaminopurine (cPrPMEDAP) was later described as a cytostatic agent,[26] and GS-9131, the bis(phosphonoamidate) of cPrPMEDAP, which could thus be considered as a prodrug of the prodrug of PMEG, appeared to specifically target the lymphoid cells, thus ensuring its potential for the treatment of lymphomas such as NHL (non-Hodgkin's lymphoma).

The years 1989 and 1990 date the first description of the specific activity against HIV-1 of 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT)[27,28] and tetrahydroimidazo-[4,5,1-*jk*][1,4]benzodiazepin-2(1H)-one (TIBO).[29] This discovery heralded the advent of the non-nucleoside reverse transcriptase inhibitors (NNRTIs), which, with four licensed drugs [nevirapine, delavirdine, efavirenz, and etravirine and a fifth one forthcoming (rilpivirine)],[2] have now acquired an established position in the treatment of HIV-1 infections.

A final topic dealt with in this review concerns the bicyclam(s) originally discovered as an impurity in a commercially available monocyclam preparation. The compound (AMD3100) was first shown to have potent anti-HIV activity before it appeared to act as a mobilizer of leukocytes (particularly, stem cells) because of its specific interaction with the chemokine receptor, CXCR4. AMD3100 was recently (December 2008) licensed, as Mozobil, for mobilization of hematopoietic stem cells for autologous transplantation in patients with hematopoietic malignancies such as NHL or multiple myeloma (MM).[30]

This "retrospective" article does not aim to be exhaustive or comprehensive in its approach; it is only meant to highlight some antiviral drug discoveries from a personal viewpoint. It does not look too much into the future or too far back in the past. For a more historical perspective, those interested may read my article "Looking Back in 2009 at the Dawning of Antiviral Therapy Now 50 Years Ago: An Historical Perspective".[31]

In this retrospective viewpoint I will address seven subjects: (1) acyclovir derivatives, (2) IDU, TFT, and BVDU derivatives, (3) (*S*)-HPMPA and (*S*)-HPMPC derivatives, (4) PMEA and (*R*)-PMPA derivatives, (5) PMEG prodrugs, (6) NNRTIs (HEPT and TIBO derivatives), and (7) bicyclam derivatives (AMD3100).

Acyclovir Derivatives

The potential of acyclovir (**1**) for the treatment of HSV infections was quickly realized after it had been introduced by Elion et al.[13] and its antiviral activity further substantiated by Schaeffer et al.[14] Because of its limited solubility in water, we (at the Rega Institute) endeavored to synthesize a number of aminoacyl (i.e., glycyl and alanyl) (**2**, **3**) esters, which, while retaining the antiviral potency of the parent compound (acyclovir), showed markedly increased solubility in aqueous medium, thus allowing for administration of **1**, for example, in the format of eye drops or parenterally (intramuscularly/subcutaneously) injectable solutions.[16,17] Of these amino acid esters of **1**, the valine ester (i.e., valaciclovir) (**4**) (Figure 1) was finally selected as the anti-HSV drug of choice because it had a better oral absorption profile than **1**.[32] Compound **4** (Figure 1) is now the standard (oral) drug for the treatment for HSV infections.

Following **1**, numerous acyclic guanosine analogues were described as being effective against HSV, the most prominent of these **1** derivatives being ganciclovir (**5**) (and its oral prodrug valganciclovir (**6**)), penciclovir (**7**) (and its oral prodrug famciclovir (**8**)) (Figure 1), and a number of other acyclic or carbocyclic analogues, i.e., A-5021, synguanol, cyclohexenylguanine, and H2G [(−)2HM-HBG], which I described previously.[33] Following **1**, **4** has now become the gold standard for the treatment of HSV infections. Compound **5** and its oral prodrug **6**, which are (both) highly active against HSV infections, have been primarily used for the treatment of CMV infections. Compound **8**, as the prodrug of **7** (Figure 1) is primarily used for the treatment of HSV and secondarily for the treatment of VZV infections. Various other guanosine analogues such as A-5021 and those cited above have not been fully explored to ascertain their potential in the treatment of HSV or other herpesvirus infections. The success obtained with **1** in the treatment of HSV infections has, to some extent, dampened or discouraged further development in the area of anti-HSV drug research, but this is not entirely justified, given the amplitude of opportunities still existing for the treatment of herpesvirus infections at large.

BVDU Derivatives

In contrast with IDU (**9**) and TFT (**10**) (Figure 2), which are not specifically phosphorylated by viral enzymes, BVDU (**11**) (Figure 2), like **1**, is specifically phosphorylated by the HSV-1 thymidine kinase (TK) and even more so by the VZV-encoded TK, thus explaining its exquisite activity against VZV.[34]

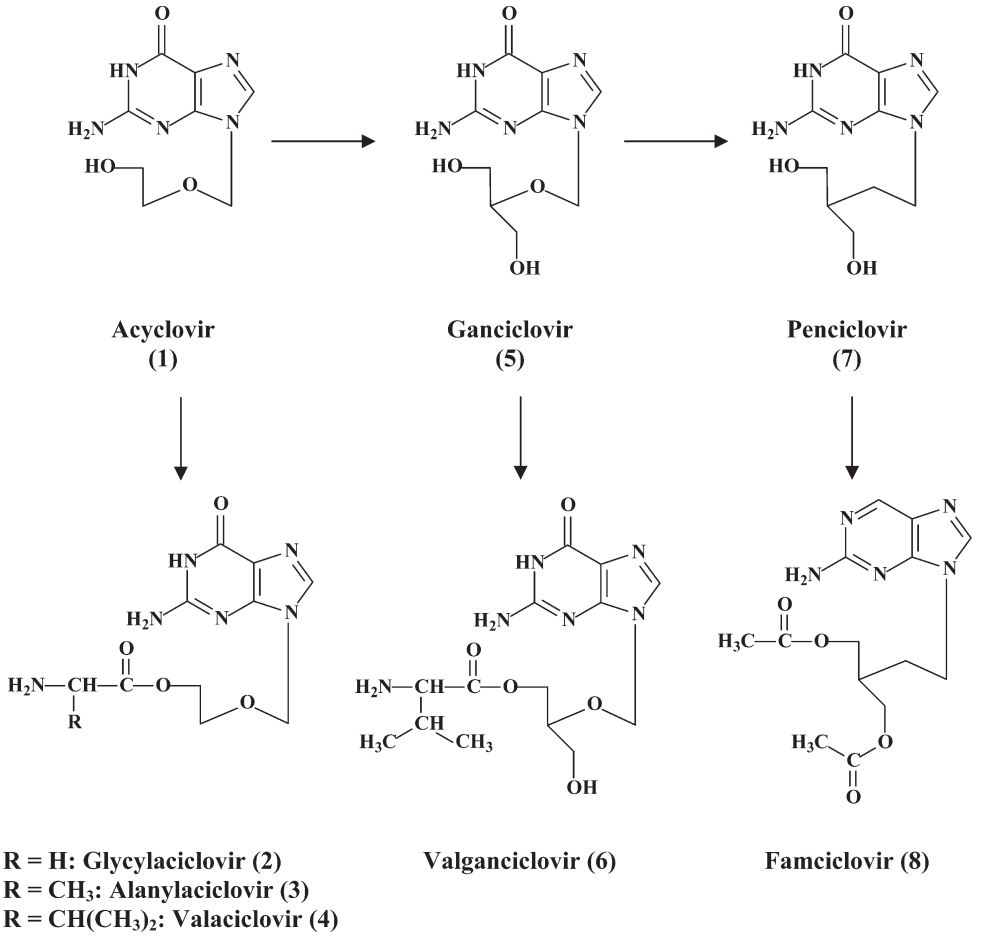

Figure 1. Acyclovir derivatives. Arrows represent development timelines.

The VZV-encoded TK phosphorylates **11** successively to the 5′-mono- and 5′-diphosphate, and after further phosphorylation, i.e., by nucleoside diphosphate (NDP) kinase, to the 5′-triphosphate, the latter acts as a competitive inhibitor/alternate substrate with respect to the natural substrate (dTTP) in the viral DNA polymerase reaction.[35] The efficacy of **11** in the treatment of VZV infections, i.e., herpes zoster,[36,37] was originally mentioned in 1980, long before **1** was licensed for the treatment of herpes zoster. Compound **11** was then shown to be superior to **1** in the treatment of VZV infections in both immunocompromised patients[38] and immunocompetent patients.[39] Compound **11** has been marketed in several European countries, including Germany, Italy, and Belgium, at a convenient dosage (of 125 mg as a single daily tablet for 7 days). A specific warning is that **11** should not be given to patients under therapy with 5-fluorouracil (or prodrugs thereof) because (*E*)-5-(2-bromovinyl)uracil (BVU), the degradation product of **1**, has been shown to interfere with dihydropyrimidine dehydrogenase (DPD), the enzyme that is responsible for the first step in the catabolic pathway of pyrimidines including 5-fluorouracil, and thus BVU may be expected to increase the half-life and toxicity of 5-fluorouracil.[34]

If, however, the pyrimidine ring of **1** is converted to a furo[2,3-*d*]pyrimidine carrying an alkyl or, even better, an arylalkyl side chain, as in **12** (Figure 2),[19,40,41] the potency against VZV is further increased: **12** proved to be active against VZV at subnanomolar concentrations[42] while not showing activity against other viruses, not even HSV, and furthermore, **12**, unlike **11**, did not prove susceptible to degradation by thymidine phosphorylase that may otherwise release the free aglycone; but even if the aglycone would be released by any other means, it was reassuring that it did not inhibit DPD and therefore did not carry the risk of increasing the toxicity of 5-fluorouracil.[40] As had been done with success for **1** and **5** (see previous section) to increase their oral bioavailability, **12** has been converted to its valine ester FV-100 (**13**).[43] Compound **13** (FV standing for FermaVir) has already passed phase I clinical trials and should soon move to phase II clinical trials for the treatment of herpes zoster.

(*S*)-HPMPA Derivatives

While (*S*)-HPMPA (**14**) served as the prototype of acyclic nucleoside phosphonates with broad-spectrum anti-DNA virus activity against a wide variety of DNA viruses,[20,44,45] it was not further developed as an antiviral drug. Neither was its 2,6-diaminopurine (DAP) derivative (*S*)-HPMPDAP (**15**) (Figure 3), despite the therapeutic potential of these compounds for the treatment of, in particular, orthopoxvirus infections[46] and parapoxvirus infections.[47] Both **14** and **15** should, if not further developed for clinical use in human medicine, be seriously considered for potential use in veterinary virology.

A further candidate along this line is (*R*)-HPMPO-DAPy (**16**) (Figure 3) which was shown more effective than postexposure smallpox vaccination in a lethal model of monkey poxvirus infection in cynomolgus monkeys.[48] The 6-[2-(phosphonomethoxy)alkoxy]2,4-diaminopyrimidines

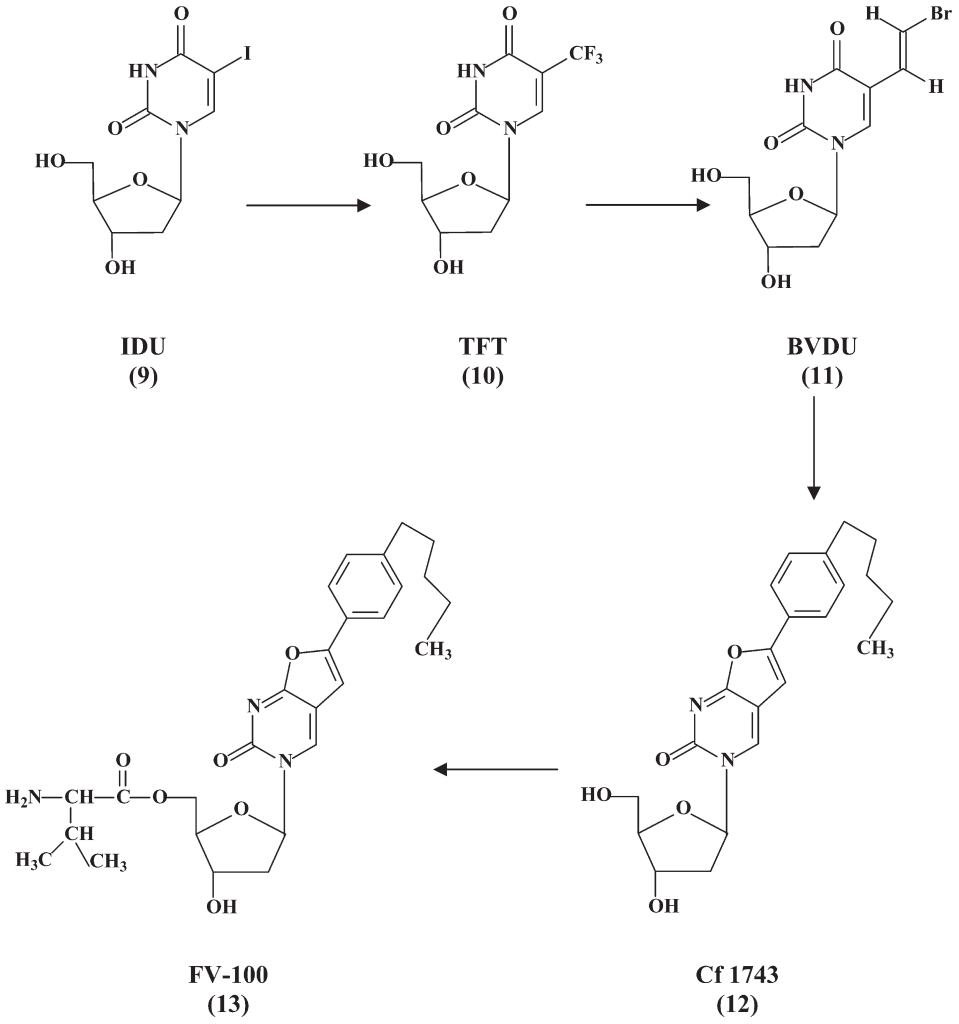

Figure 2. IDU, TFT, BVDU, and its derivative **12**. Arrows represent development timelines.

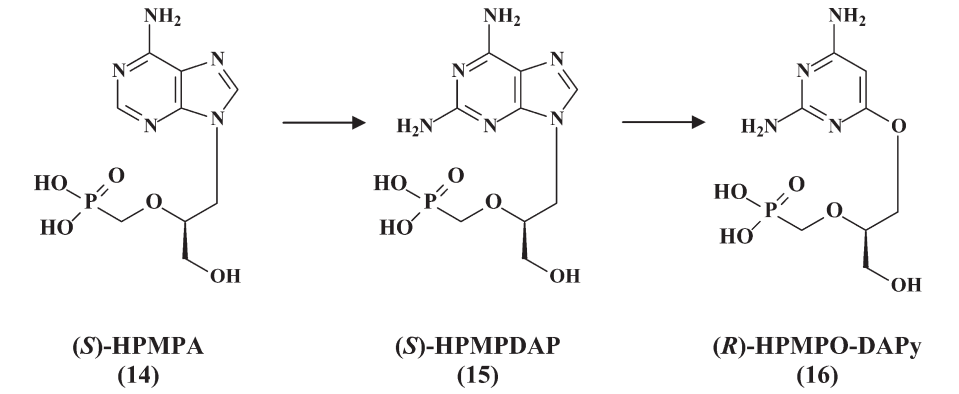

Figure 3. HPMPA derivatives. Arrows represent development timelines.

were first described by Holý and his colleagues in 2002.[23,49] Their potential for the treatment of a broad variety of DNA virus infections has been duly reviewed,[24] but notwithstanding the still unexplored potential of (**16**) in the treatment of both human and veterinary pox and other DNA virus infections, they offer an interesting study object from a purely fundamental viewpoint.

Compound **16** (Figure 3), being a pyrimidine analogue but conformationally isosteric to its 2,6-diaminopurine counterpart **15**, could theoretically behave as both a purine analogue and a pyrimidine analogue. Then the question arises of whether **16** could serve as a chain terminator and/or, like **14**, be incorporated into the template DNA strand.[50]

(*S*)-HPMPC Derivatives

While formally licensed for the treatment of CMV retinitis in AIDS patients, (*S*)-HPMPC (cidofovir) (**17**) has proven efficacious and is used "off-label" for the treatment of various other DNA virus infections, particularly herpesvirus infections (i.e., TK-deficient HSV and VZV infections resistant to **1**),

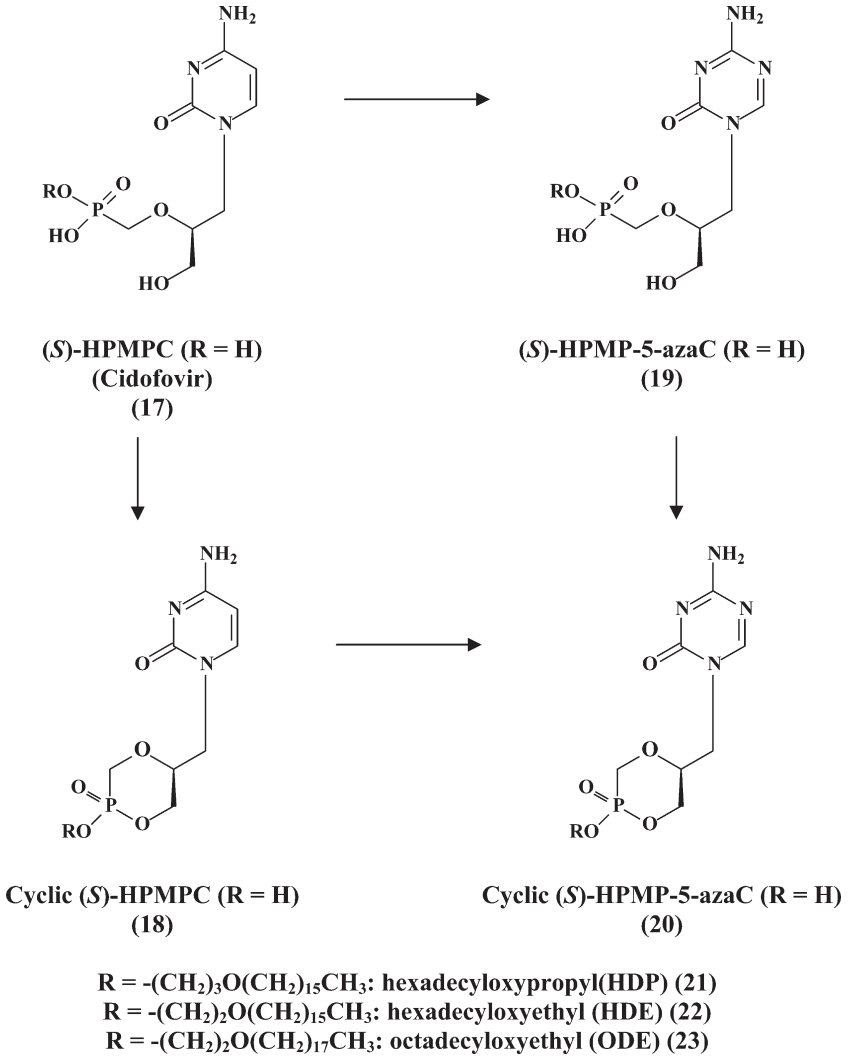

Figure 4. HPMPC derivatives. Arrows represent development timelines.

polyomavirus infections [i.e., progressive multifocal leukoencephalopathy (PML)], human papilloma virus (HPV) infections (i.e., warts, papillomatous lesions in general), adenovirus infections and poxvirus infections [orthopox (i.e., vaccinia), parapox (i.e., orf), and molluscipox (i.e., molluscum contagiosum)].[51−59] The case of orf in an immunocompromised patient treated successfully with **17** cream[60] and the case of a hypopharyngeal papilloma successfully treated with topical **17** injection[61] are illustrative of the clinical efficacy of **17**. Equally convincing is the efficacy demonstrated by **17** in a murine (experimental) model of disseminated progressive vaccinia.[62]

The problem with **17** and other acyclic nucleoside phosphonates is that they have poor, if any, oral bioavailability. For **24** and **28**, this problem has been overcome by the development of the oral **24** and **28** prodrugs, **25** and **31**, respectively. For **17** (and its cyclic derivative, **18**), alkoxyalkyl derivatives [i.e., hexadecyloxypropyl (HDP) and octadecyloxyethyl (ODE) esters] (Figure 4) have been constructed. These derivatives, as a rule, proved highly active in the oral treatment of a variety of DNA virus infections (that are intrinsically sensitive to **17**),[63] such as the orthopoxvirus infections (cowpox, vaccinia, and ectromelia).[64−66]

Compared to **17** (and **18**), the triazine analogues,[67] i.e., (*S*)-HPMP-5-azaC (**19**) (and its cyclic derivative, **20**) (Figure 4), have distinct advantages in that they display greater potency and selectivity, they are more extensively phosphorylated intracellularly and more extensively incorporated into DNA,[68] and in particular, the alkoxyalkyl ester prodrugs (**21**, **22**, **23**) of (cyclic) **19** (Figure 4)[25] have proved highly promising as potent antiviral agents. Their full potential in the treatment of the various DNA virus infections of both human and veterinary importance remains to be further explored.

PMEA Derivatives

PMEA (adefovir) (**24**) has an antiviral activity spectrum that is fundamentally different from that of **17** in that **24** is primarily active against retroviruses, including HIV but also feline immunodeficiency virus (FIV) and hepadnaviruses (i.e., HBV) which are not particularly sensitive to **17**.[69,70] Compound **24** was initially pursued as a potential antiretroviral agent for the treatment of HIV infections, but it proved to be nephrotoxic at the dosage needed to treat HIV infections (125 or 62.5 mg/day).[57] Meanwhile, the inhibitory effects of **24** on HBV replication became known,[69−71] and **24**, as its oral prodrug form, adefovir dipivoxil (**25**) (Figure 5), would eventually be licensed for the treatment of HBV infections. The clinical efficacy of **25** (at an oral dosage of 10 mg daily) in the treatment of chronic hepatitis B has been well documented.[72−74]

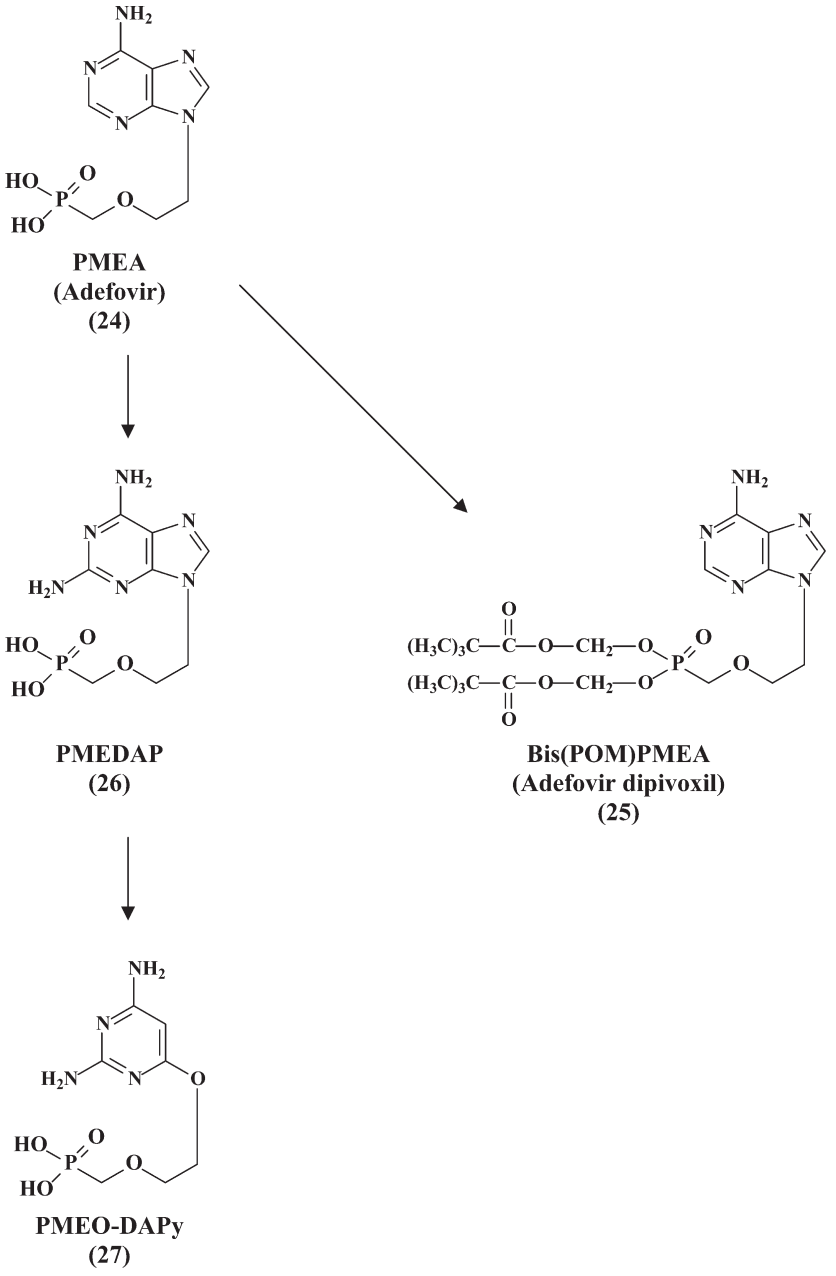

Figure 5. PMEA derivatives. Arrows represent development timelines.

In addition to **24**, its 2,6-diaminopurine derivative PMEDAP (**26**) (Figure 5) was also from the start found to be highly effective in inhibiting HBV replication,[69,70] and similarly, the 2,4-diaminopyrimidine derivative PMEO-DAPy (**27**) (Figure 5) and 5-substituted derivatives thereof[75] were found to offer attractive potential as antiretrovirus and antihepadnavirus agents.[24,76] However, no follow-up studies on **27** have recently appeared.

PMPA Derivatives

(*R*)-PMPA (tenofovir) (**28**) and its 2,6-diaminopurine counterpart (*R*)-PMPDAP (**29**) (Figure 6) were first described as potent antiretrovirus agents in 1993[22] and, 1 year later, as potent anti-HBV agents.[77] Against both HIV and HBV, **29** was found to be (about 10-fold) more potent than **28**. While **28** has become (one of) the most important component(s) in anti-HIV drug cocktails, **29** was not further developed clinically for treatment of either HIV or HBV infections. Also, despite its potential for the treatment of HIV and HBV infections, the 2,4-diaminopyrimidine derivative (Figure 6) (*R*)-PMPO-DAPy (**30**)[24] has not received much further attention.

Compound **28**, however, in its oral prodrug form, tenofovir disoproxil fumarate (TDF) (**31**) (Figure 6), has in recent years emerged as the drug of choice for the treatment of HIV infections, either as such (Viread) or in a fixed-dose combination with emtricitabine (Truvada) or in a fixed-dose combination with both emtricitabine and efavirenz (Atripla).[78] In their mode of action, **28** and **24** follow a similar strategy:[79,80] following intracellular phosphorylation to their diphosphorylated derivative, the latter acts as an analogue of the normal substrate (dATP) at the RNA-dependent DNA polymerase (reverse transcriptase) level, and once incorporated as an alternative substrate into the DNA chain, it prevents further DNA elongation, thus functioning as an obligate chain terminator. Since they are attached by a phosphonate linkage rather than a phosphate linkage, **24** and **28**, once incorporated

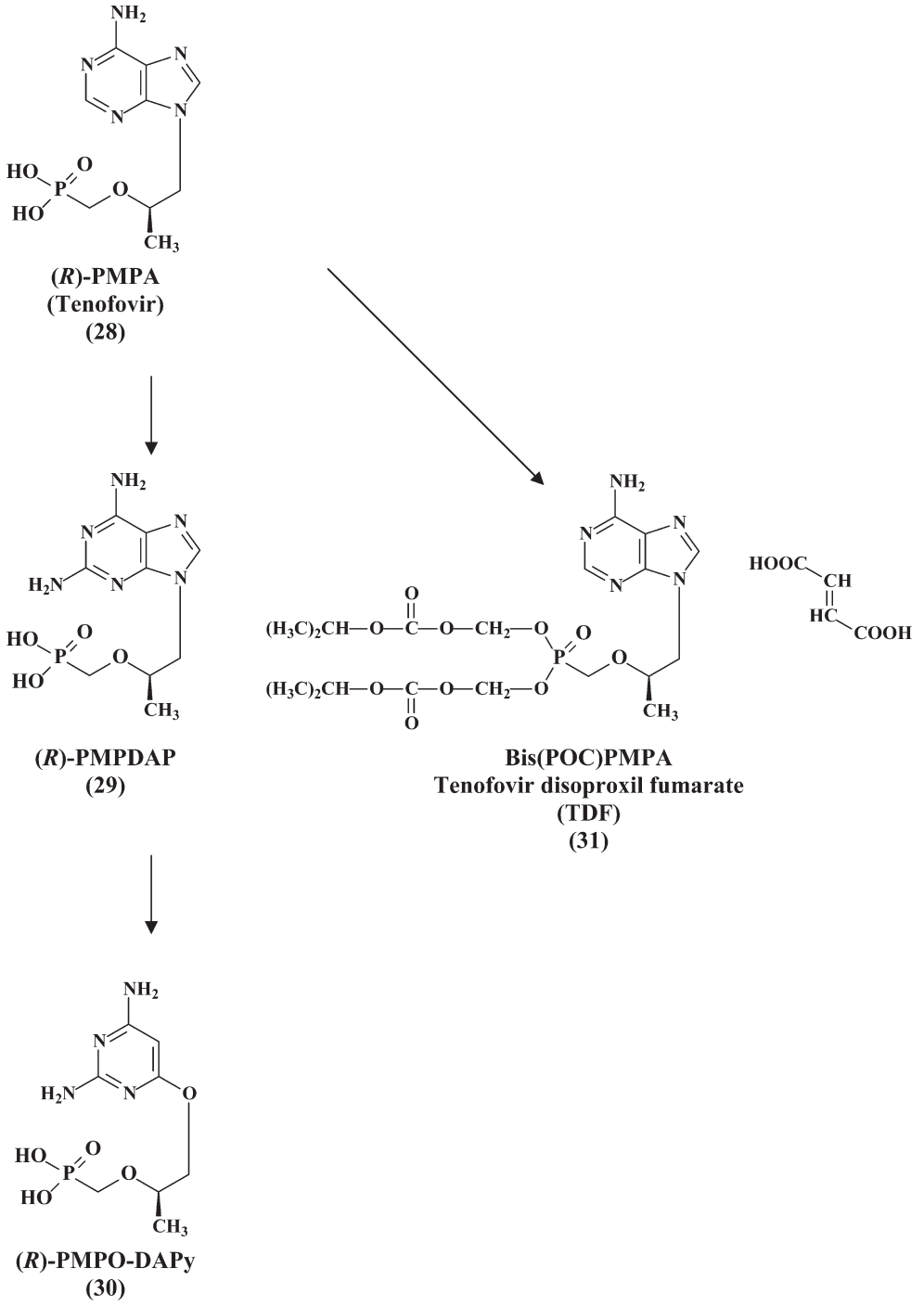

Figure 6. PMPA derivatives. Arrows represent development timelines.

as obligate chain terminators, cannot easily be excised again (which may contribute to the relative lack of resistance development toward these compounds).

The clinical use of **31** has been recently extended to the treatment of chronic hepatitis B,[57] where it has proven more efficacious than **25**[81] (which is not surprising, considering the dosages used: 300 mg versus 10 mg daily). Where **31** holds the greatest promise, however, is its prophylactic use, in the pre-exposure prophylaxis of HIV infections (whether transmitted by the parenteral, sexual, or perinatal route).[58]

PMEG Prodrugs

Featured among the phosphonylmethoxyalkyl purine and pyrimidine derivatives described in 1987 for their antiviral activity was PMEG (**32**).[21] Yet this compound was, from the beginning, looked upon as a potential antitumor agent: its antitumor activity was demonstrated in mice engrafted with murine leukemia P388 cells or murine melanoma B16 cells.[82] Compound **32** was from the beginning assumed to act via the inhibitory effect on cellular DNA polymerase(s) of its diphosphate (PMEG-DP) formed intracellularly from **32**. In this sense, **32** could be considered as a merely cytotoxic agent, inhibiting indiscriminately the proliferation of normal and tumor cells.

In 1999, however, we described a prodrug of **32**, namely, cPrPMEDAP (**33**), which had a more specific antitumor activity than **32**, against a number of tumor cells (i.e., K562 and L1210)[26] and, more strikingly, tumor formation in vivo,

i.e., choriocarcinoma tumor development in rats.[83] In contrast with **26** (which is not deaminated intracellularly to **32** but phosphorylated to its 5-diphosphate metabolite), **33** is first deaminated to **32** by an adenylate deaminase-like enzyme.[84] The enzyme responsible for this deamination has been characterized as N^6-methyl-AMP aminohydrolase.[85]

As a prodrug of **32** and in agreement with the original observations of Kreider et al.,[86] we confirmed that **33** specifically inhibits the proliferation of HPV-harboring cell lines as compared to HPV-negative cell lines.[87] This observation has led to the recognition of **33** as a potential drug for the treatment of HPV-associated proliferative diseases. As a prodrug of **32**, **33** can as such be used as a potential antitumor agent, but to direct it specifically to tumor cells from the lymphoid lineage, it should be further derivatized to its bis(phosphonoamidate) prodrug GS-9219 (diethyl-*N*,*N*-[({2-[2-amino-6-cyclopropylamino-9-*H*-purin-9-yl]ethoxy}methyl)-phosphonoyl]di-L-alanilate) (**34**) (Figure 7).

Thus, the bis(phosphonoamidate) **34** may be considered as a prodrug of **33** (to which it may be converted by cathepsin A), which then acts as a prodrug of **32** (to which it is converted by N^6-methyl-AMP aminohydrolase). In this sense, **34** acts as a prodrug of **33**, which by itself acts as a prodrug of **32** (Figure 7). Compound **34** could thus be termed a "pro-prodrug" of **32**. Compound **34** has been recently shown by Reiser et al.[88] to elicit, following a single intravenous injection, a remarkable regression of spontaneous, advanced-stage non-Hodgkin's lymphomas in beagle dogs, and phase clinical trials with **34** in humans with hematological malignancies have been initiated.

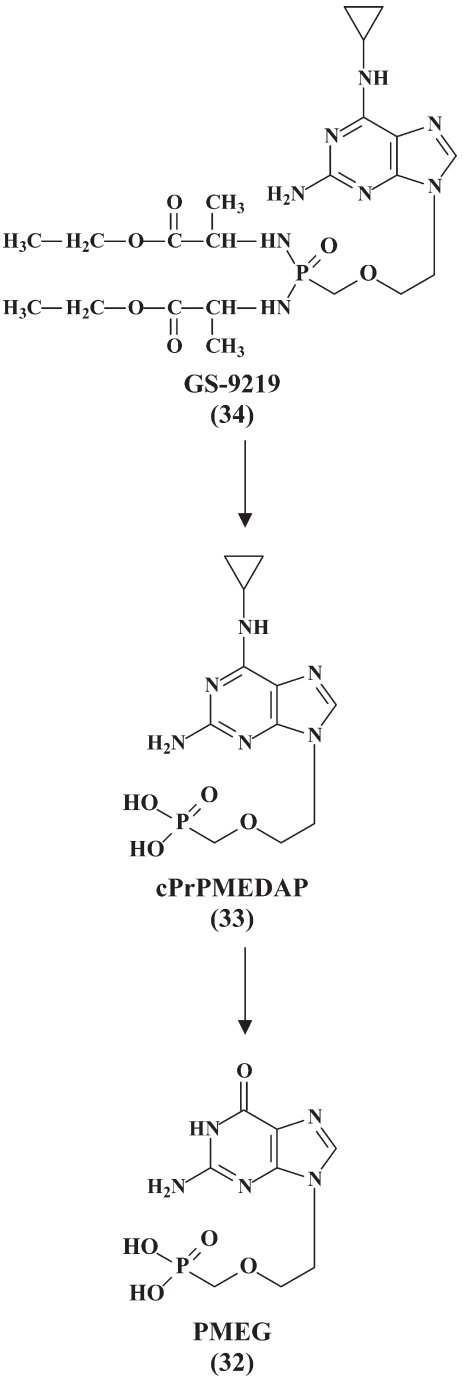

Figure 7. PMEG pro- and pro-prodrugs. Arrows represent development timelines.

NNRTIs (HEPT and TIBO Derivatives)

From its 2-hydroxyethoxymethyl side chain (Figure 8) [the same as in **1** (Figure 1)] it could be deduced that HEPT (**35**) was originally conceived to be an antiherpetic agent. Like several other 1-(2-hydroxyethoxymethyl)pyrimidine derivatives, however, **35** was inactive against HSV, but when evaluated for its anti-HIV activity, it proved (unexpectedly) quite active.[27,28] In retrospect, **35** was the first NNRTI ever described that is a non-nucleoside reverse transcriptase (RT) inhibitor that was specifically targeted at the HIV-1 RT at (as it would be shown later) an allosteric binding site.[89−92] Further structure−activity relationship (SAR) studies led to the identification of MKC-442 (emivirine) (**36**) (Figure 8) as the clinical drug candidate.[93] Compound **36** (registered as Coactinon) progressed to advanced phase III clinical trials before it was eventually abandoned for further development.[55] Its three successors, nevirapine, delavirdine, and efavirenz, however, were successfully launched as anti-HIV-1 drugs[2] [although delavirdine is virtually no longer used for the treatment of HIV-1 infections].

Concomitantly with, but independently from, **35**, the TIBO derivatives were discovered as specific anti-HIV agents resulting from a collaborative project we had initiated with the late Dr. Paul Janssen (stories told at previous occasions[55,57]). Of the TIBOs the first clinical drug candidate was R86183 (tivirapine) (**37**) (Figure 8).[29] A variety of TIBO derivatives were synthesized,[94,95] but as their chemical synthesis proved too cumbersome, simpler scaffolds were searched for. This led to the identification of the α-anilinophenylacetamide class of compounds.[96] However, the prototype compound loviride (R89439) (**38**) (Figure 8) did not have the desired pharmacokinetics to be further pursued as a drug (candidate).

Further research carried out by Janssen and his colleagues led from α-APA to the ITU (iminothiourea) analogues (i.e., R100943) (**39**) (Figure 8),[97] the DATA (diaryltriazine) analogues (i.e., R106168) (**40**) (Figure 8),[98] and finally, the DAPY (diarylpyrimidine) analogues (i.e., R147681) (**41**) (Figure 8).[99] The first of the DAPY compounds **41** corresponds to dapivirine (TMC120) and is being pursued for its microbicidal potential. The second DAPY compound (R165335) (**42**) corresponds to etravirine (TMC125) (Figure 8),[100] which has been recently approved (Intelence) for clinical use, and the third DAPY compound (R278474) (**43**) corresponds to rilpivirine (TMC278) (Figure 8),[101] which is expected to be approved soon for clinical use.

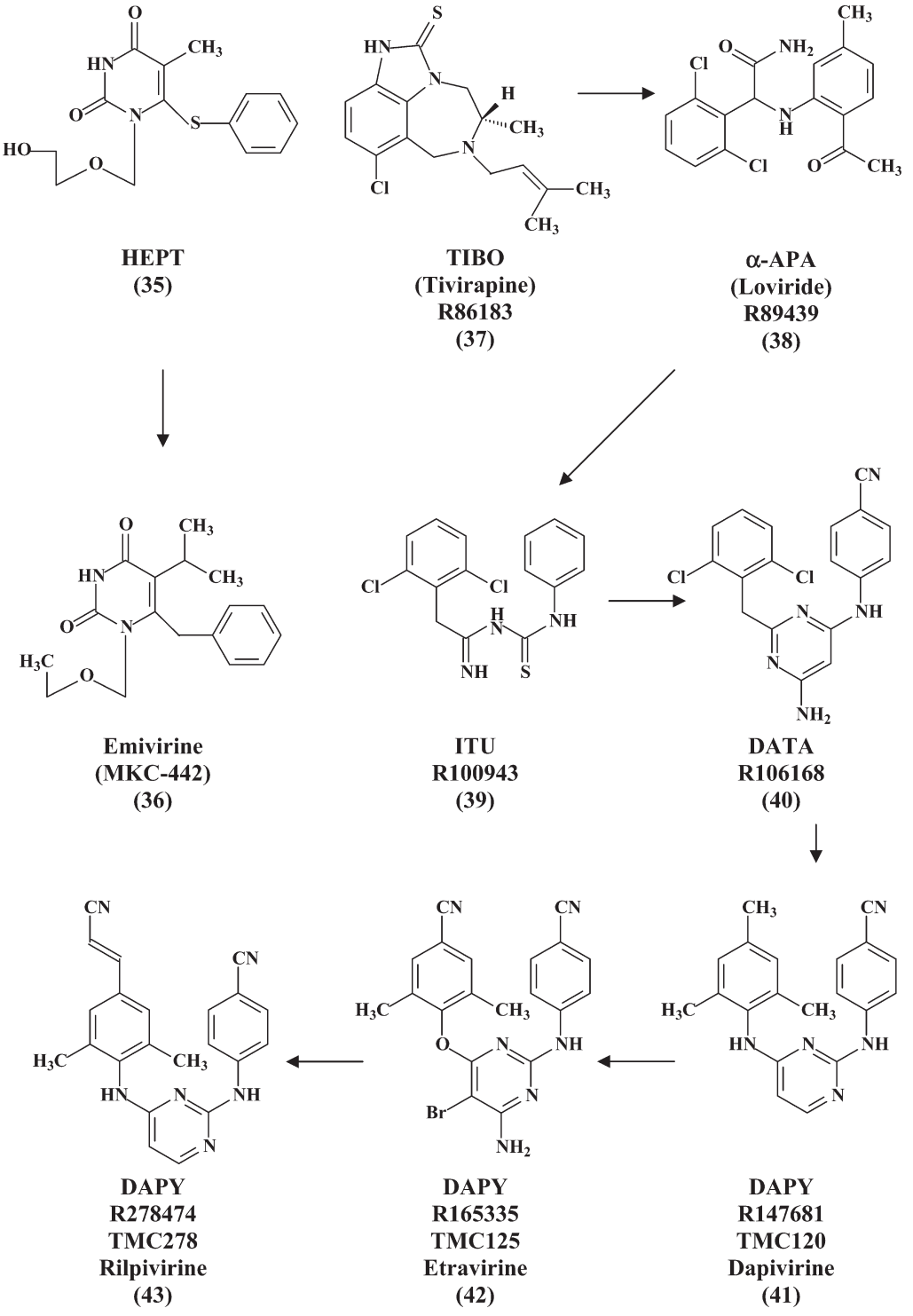

Figure 8. HEPT and TIBO derivatives. Arrows represent development timelines.

Compound **43** comes close to fulfilling the postulated requirements for an ideal anti-HIV drug: (1) high antiviral potency against wild-type and mutant HIV variants (while having itself a high genetic barrier to resistance development); (2) high oral bioavailability, allowing once-daily (or even less frequently) dosing; (3) minimal side effects; and (4) easy synthesis and formulation.[101]

Bicyclam Derivatives (AMD3100)

The bicyclam AMD3100 story[30,102] starts with a description of the anti-HIV activity of the bicyclam JM1657 (**44**) (Figure 9).[103] In fact, **44** was discovered as an impurity in a commercial cyclam preparation. The (mono)cyclam itself had little, if any, anti-HIV activity, but the impurity characterized by two cyclam rings tethered by a direct carbon−carbon linkage (i.e., **44**) appeared to be responsible for the marked anti-HIV activity of the crude cyclam preparation. As it did not prove feasible to resynthesize **44**, a program was launched to synthesize bicyclam derivatives with the cyclam rings tethered by an aliphatic bridge, the bicyclam derivative with the propyl bridge (i.e., JM2763, **45**) (Figure 9) being about as active as **44**.[103]

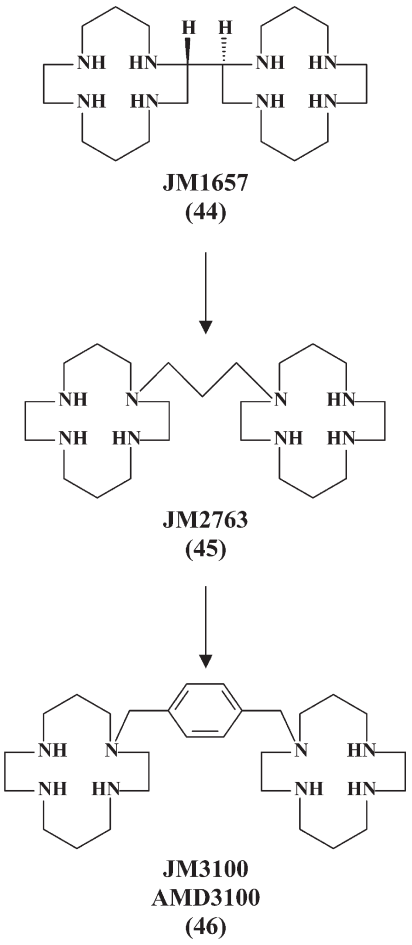

Figure 9. Bicyclam derivatives. Arrows represent development timelines.

A 100-fold increase in potency was noted upon replacing the aliphatic (i.e., propyl) bridge by an aromatic [i.e., 1,4-phenylene-bis(methylene)] bridge, as in JM3100 (**46**) (later renamed AMD3100).[104] Compound **46** was found to inhibit HIV-1 and HIV-2 within 1−10 nM while not being toxic to the host cells at concentrations up to 500 μM, thus achieving a selectivity index of approximately 100 000. The target of action was initially thought to be the viral envelope glycoprotein gp120. It appeared only to be the indirect target. The direct target turned out to be the coreceptor CXCR4 used by T-lymphotropic HIV strains (now referred to as X4 strains) to enter the cells.[105−107]

Initial (phase I) clinical trials undertaken with **46**, as a prelude to its development as a candidate anti-HIV drug for the treatment of AIDS, showed an unexpected side effect: an increase in the white blood cell count (WBC).[108] The WBC-mobilizing effect was dose-dependent over a dosage range of 10−240 μg/kg,[108,109] and on closer inspection, the white blood cells mobilized by **46** appeared to carry the marker CD34 and could thus be characterized as hematopoietic stem cells.[110] Apparently, **46** specifically mobilized $CD34^+$ hematopoietic stem cells from its reservoir (primarily the bone marrow) into the peripheral bloodstream. Stromal-derived factor 1 (SDF-1), through its interaction (as the natural ligand for CXCR4), retains the stem cells in the bone marrow (a process characterized as "homing"). Compound **46** specifically antagonizes this interaction.

Compound **46** in combination with granulocyte colony stimulating factor (G-CSF) resulted in the collection of more progenitor cells than G-CSF alone.[111−114] At present, the major indication for the clinical use of **46** is the mobilization of hematopoietic stem cells from the bone marrow into the peripheral blood for transplantation in patients with hematological malignancies such as non-Hodgkin's lymphoma or multiple myeloma.[30]

Conclusion

The approaches reviewed here focus on both antiviral and antitumoral strategies, particularly antiviral toward herpes simplex virus (HSV), varicella-zoster virus (VZV), poxvirus (variola, vaccinia, etc.), hepatitis B virus (HBV), human immunodeficiency virus (HIV), and human papilloma virus (HPV) infections and antitumoral toward non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM). Prominent among the antiviral approaches for HSV and VZV are the acyclovir and BVDU derivatives, respectively. For the treatment of poxvirus infections and DNA virus infections at large, HPMPA, HPMPC, and their derivatives should be recommended. PMEA and PMPA derivatives yield particular promise for the treatment of HIV and HBV infections. The HEPT and TIBO derivatives should be mentioned for their potential in the treatment of HIV-1 infections. Of particular importance for the treatment of non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) are **33** and AMD3100, first, because it serves as the prodrug of the cytotoxic agent PMEG and, second, because it stimulates the production of stem cells to be used in the autologous transplantation of hematopoietic stem cells in patients afflicted by either NHL or MM.

Acknowledgment. I am very much indebted to Christiane Callebaut for her never-waning, dedicated, and proficient editorial assistance.

Biography

Erik De Clercq, M.D., Ph.D., is President of the Rega Foundation and a Director of the Belgian (Flemish) Royal Academy of Medicine, a member of the Academia Europaea, and Fellow of the American Association for the Advancement of Science. He is an active Emeritus Professor of Katholieke Universiteit Leuven (Belgium). He is an honorary doctor of the Universities of Ghent (Belgium), Athens (Greece), Ferrara (Italy), Shandong (Jinan, China), Charles (Prague, Czech Republic), and Jihoceska (Budëjovice, Czech Republic). In 2008 he was elected European Inventor of the Year. He is the (co)inventor of a number of antiviral drugs used for the treatment of HSV (valaciclovir, Valtrex, Zelitrex), VZV (brivudin, Zostex, Brivirac, Zerpex), CMV (cidofovir, Vistide), HBV (adefovir dipivoxil, Hepsera), and HIV infections (AIDS) (tenofovir disoproxil fumarate, Viread).

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