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Review

Antiviral drug discovery and development: Where chemistry meets with biomedicine

Editor's Note: Each year the International Society for Antiviral Research presents the Gertrude Elion Lecture Award, supported by GlaxoSmithKline, to an outstanding scientist who has made considerable contributions in the field of antiviral research. This year the award was given to Professor Erik De Clercq at the 18th International Conference on Antiviral Research in Barcelona, Spain on 11 April 2005. The following is the lecture presented by Prof. De Clercq describing his prodigious contributions to the field. (George J. Galasso, Review Editor.)

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Abstract

The successful development of antiviral drugs is highly dependent on a close interaction and collaboration between the chemist and the biologist (biomedic). This is illustrated by a number of representative examples: *S*-adenosylhomocysteine (SAH) hydrolase inhibitors which display broad-spectrum antiviral activity, bromovinyldeoxyuridine (BVDU) and derivatives thereof, that are highly selective inhibitors of varicella-zoster virus (VZV), (dideoxy)nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) which are now widely used in the treatment of HIV infections (AIDS), the bicyclams (i.e. AMD3100) which were originally discovered as anti-HIV agents, then found to be potent CXCR4 antagonists and now being pursued for a number of indications such as stem cell mobilization, and the acyclic nucleoside phosphonates which have heralded a new strategy for the treatment of various DNA virus (herpes-, adeno-, pox-, papillomavirus) infections (cidofovir), hepatitis B (adefovir) and AIDS (tenofovir).

Keywords: Antivirals; HIV (AIDS); HBV (Hepatitis B); HSVC (Herpes Simplex); VZV (Herpes Zoster); DNA viruses

Contents

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1. Introduction

The most successful experiment, in retrospect, that I ever did was that in 1976 when I endeavored at travelling to a meeting in Göttingen, organized by Karl-Heinz Scheit (Fig. 1), to affront, as a biomedical scientist, a unique mix of outstanding organic/medicinal chemists who had come down to Göttingen to attend the Symposium on Synthetic Nucleosides, Nucleotides and Polynucleotides at the Max-Planck-Institut für Biophysikalische Chemie (Fig. 1). I travelled from Leuven to Göttingen together with Paul F. Torrence, and, at Göttingen, I got in contact with such important chemists as John Montgomery, Nelson Leonard, John Moffatt, Maciej Wiewiórowski, Willi Guschlbauer, Helmut Vorbrüggen, Wolfgang Pfleiderer, David Shugar, Fritz Eckstein (the latter two I had known and collaborating with before), and Anthonin Holý and Dick Walker, who, as would appear later, greatly influenced, and even determined my further career in antiviral research.

I will now describe (some of) the antiviral drug research programs I have been involved with and which to a great extent emanated from the contacts made at the 1976 Göttingen Symposium (where Paul F. Torrence presented our joint work on double-stranded polynucleotides as interferon inducers and I spoke on the antiviral activity of nucleoside analogues). The different lines of research reviewed here are (i) *S*-adenosylhomocysteine hydrolase inhibitors starting from (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA), as broad-spectrum antiviral agents, (ii) (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) as a highly potent inhibitor of varicella-zoster virus (VZV) replication, (iii) 2′,3′-dideoxynucleosides (ddNs) as NRTIs (nucleoside reverse transcriptase inhibitors) for the treatment of human immunodeficiency virus (HIV) infections, (iv) NNRTIs (non-nucleoside reverse transcriptase inhibitors) for the treatment of HIV infections, (v) the bicyclams, from an impurity to a potent anti-HIV agent and stem cell mobilizing agent, and (vi) the acyclic nucleoside phosphonates cidofovir, adefovir and tenofovir, representing a new dimension for the treatment of DNA virus and retrovirus infections.

2. DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine] → *S*-adenosylhomocysteine hydrolase inhibitors

Within two years after the beginning of our collaboration, Anthonin Holý and I discovered DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine] (Fig. 2) as a broad-spectrum antiviral agent, that showed a distinct, albeit relatively weak, potency, against a number of RNA and DNA viruses (De Clercq et al., 1978). The description of the antiviral activity of DHPA came very shortly (within a few months) after acyclovir, like DHPA an acyclic nucleoside analogue, had been described as a selective anti-herpesvirus agent, depending for its antiviral activity on a specific phosphorylation by the herpes simplex virus (HSV)-encoded thymidine kinase (Elion et al., 1977; Schaeffer et al., 1978). DHPA would later be marketed, in the Czechoslovak Republic under the name of Duvira gel (Fig. 2) for the topical treatment of herpes labialis ("cold sores").

Within the 25 years following the original description of DHPA as an antiviral agent, it was demonstrated that DHPA,

Fig. 1. Participants in the Symposium on Synthetic Nucleosides, Nucleotides and Poly-nucleotides, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany, 3–5 May 1976.

Fig. 2. (*S*)-9-(2,3-Dihydroxypropyl)adenine (DHPA), previously marketed in the Czechoslovak Republic as Duvira gel.

and a variety of other acyclic and carbocyclic adenosine analogues, owe their antiviral activity to an inhibition of *S*-adenosylhomocysteine (SAH) hydrolase (De Clercq, 2004a) and could therefore be termed SAH hydrolase inhibitors. As shown in Fig. 3, SAH hydrolase inhibitors block the cleavage of *S*-adenosylhomocysteine (SAH) into its two components homocysteine (Hcy) and adenosine (Ado), which itself can be further metabolized into three components (AMP, adenine and inosine). As a consequence of the SAH hydrolase inhibition, *S*-adenosylhomocysteine accumulates, and as SAH is both a product and an inhibitor of the reaction leading from *S*-adenosylmethionine (SAM) to SAH, this reaction will be suppressed. SAM is essential for the methylation (i.e. capping) of viral mRNAs, which means that if the methylation reaction(s) starting with SAM as the methyldonor are blocked, maturation of viral mRNAs will be suppressed, and so will be the production of progeny virus particles (De Clercq, 2004a).

SAH hydrolase inhibitors may be expected to be effective against a broad spectrum of viruses: rhabdoviruses (i.e., vesicular stomatitis, rabies), filoviruses (i.e., Ebola, Marburg), arenaviruses (i.e., Junin, Tacaribe), reoviruses (i.e., rota), paramyxoviruses [i.e., parainfluenza, mumps, measles, respiratory syncytial virus (RSV)], retroviruses (i.e., HIV), herpesviruses [i.e., cytomegalovirus (CMV)] and poxviruses (i.e., variola, vaccinia, monkeypox) (De Clercq, in press).

Various acyclic and carbocyclic adenosine analogues have been described (De Clercq, in press), among which carbocyclic 3-deazaadenosine (C-c^{3}Ado) and 3-deazaneplanocin A (Fig. 4) have received particular attention. C-c^{3}Ado was found to fully protect BALB/c mice against a lethal challenge with Ebola (Zaire) virus when a single injection of the compound (80 mg/kg) was given on day 0, 1 or 2 with respect to the infection (Bray et al., 2000). A similar protective effect was achieved with 3-deazaneplanocin A (Bray et al., 2002), and, concomitantly, 3-deazaneplanocin A was found to induce massively increased amounts of interferon in the Ebola virus-infected mice, but not in uninfected mice. It can be postulated that 3-deazaneplanocin A, through its

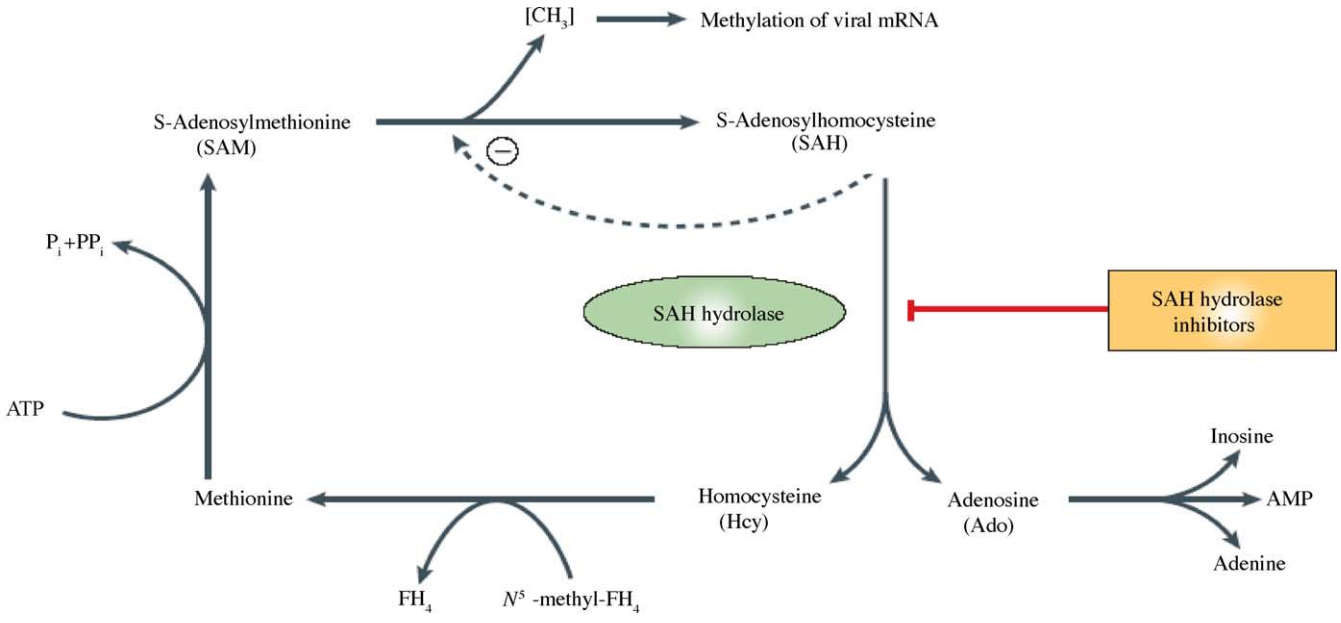
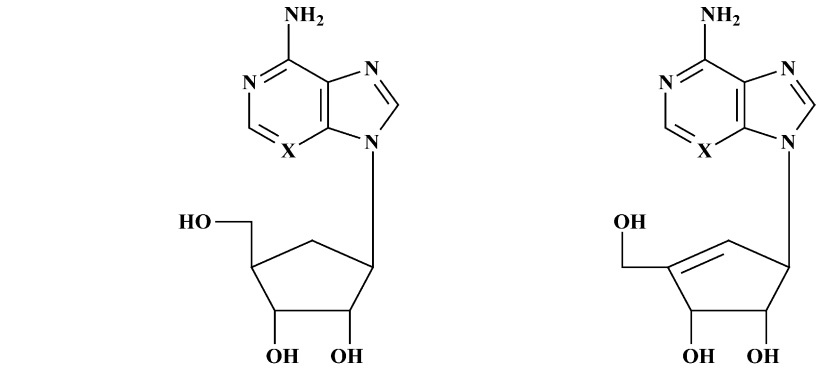


Fig. 3. Mechanism of action of adenosine analogues such as carbocyclic 3-deazaadenosine and 3-deazaneplanocin A (De Clercq, 2004a).



X = N : Carbocyclic adenosine (C-Ado)
X = CH : Carbocyclic 3-deazaadenosine (C-c^{3}Ado)

X = N : Neplanocin A
X = CH : 3-Deazaneplanocin A

Fig. 4. Representative examples of *S*-adenosylhomocysteine (SAH) hydrolase inhibitors.

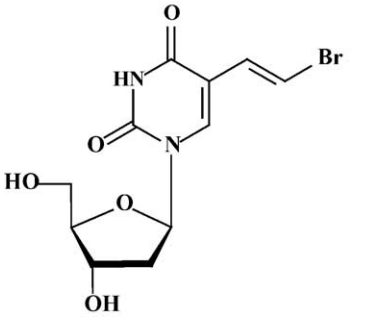
inhibitory effect on the methylation (i.e. capping) of viral mRNAs, prevents the release of the mRNAs, thus favoring complex formation of the latter with the (−)RNA template, thereby leading to an accumulation of double-stranded RNA complexes, which have been known for almost four decades to be excellent inducers of interferon.

3. BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] → highly specific VZV inhibitors

The collaboration I started with Richard ("Dick") Walker in 1976 led after a couple of years to the discovery of BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine], a highly selective antiviral agent (De Clercq et al., 1979), which proved specifically active against herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) (De Clercq, 2004b). BVDU (brivudin) has been marketed in several European counties (i.e., Germany and Italy under the trade name Zostex® and Brivirac®, respectively) (Fig. 5) for the treatment of herpes zoster (shingles, zona). It is administered orally at a dose of 125 mg/day for a total period of 7 days.

From a structure–activity relationship (SAR) viewpoint, the (*E*)-5-(2-bromovinyl) substituent in the C-5 position of the uracil moiety (whereby *E* stands for "Entgegen" or "trans", referring to the positions of the hydrogens at C_1 and C_2 of the vinyl group) is the crucial determinant for the specific antiviral activity of BVDU. Many congeners of BVDU have been described, the best known being BVaraU (sorivudine), which, like BVDU, exhibit specific activity against HSV-1 and, especially, VZV (Fig. 6) (De Clercq, 2004b).

The selective activity of BVDU against HSV-1 and VZV is dependent on a specific phosphorylation of the compound by the HSV-1- or VZV-encoded thymidine kinase (TK) which convert BVDU subsequently to its 5′-monophosphate (BVDU-MP) and 5′-diphosphate (BVDU-DP) (Fig. 7) (De



(*E*)-5-(2-Bromovinyl)-2'-deoxyuridine
BVDU

Fig. 5. (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU, brivudin), now marketed in several European countries [i.e., Germany (Zostex®) and Italy (Brivirac®)].

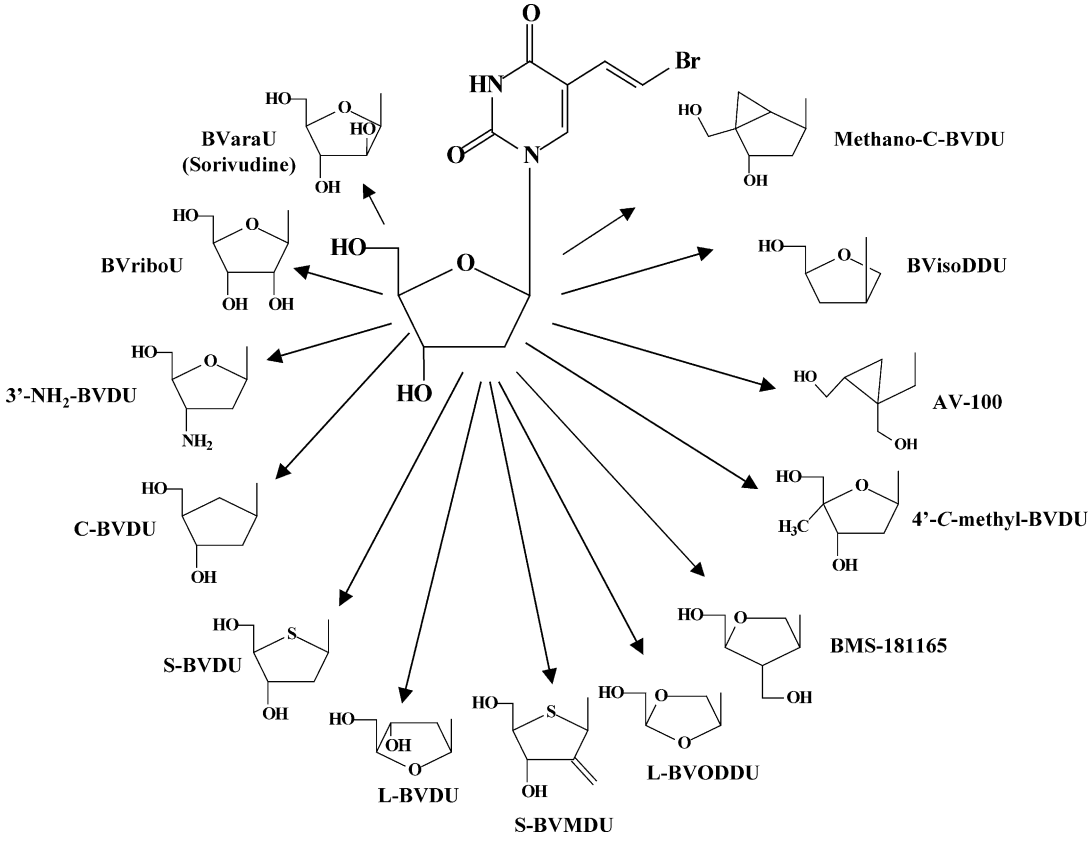


Fig. 6. The "BVDU connection": BVDU and related compounds all containing the BVU [(*E*)-2-bromovinyl] substituent in the C-5 position of the uracil moiety (De Clercq, 2004b).

Clercq, 2004b). The latter is then converted to the 5′-triphosphate BVDU-TP by a nucleoside diphosphate (NDP) or other cellular kinase, whereupon BVDU-TP enters in competition with the natural substrate dTTP for the viral DNA polymerase. It can inhibit the incorporation of dTTP into the viral DNA or, as an alternate substrate it can be itself incorporated, thus leading to the formation of a structurally and functionally disabled viral DNA (Fig. 7) (De Clercq, 2004b).

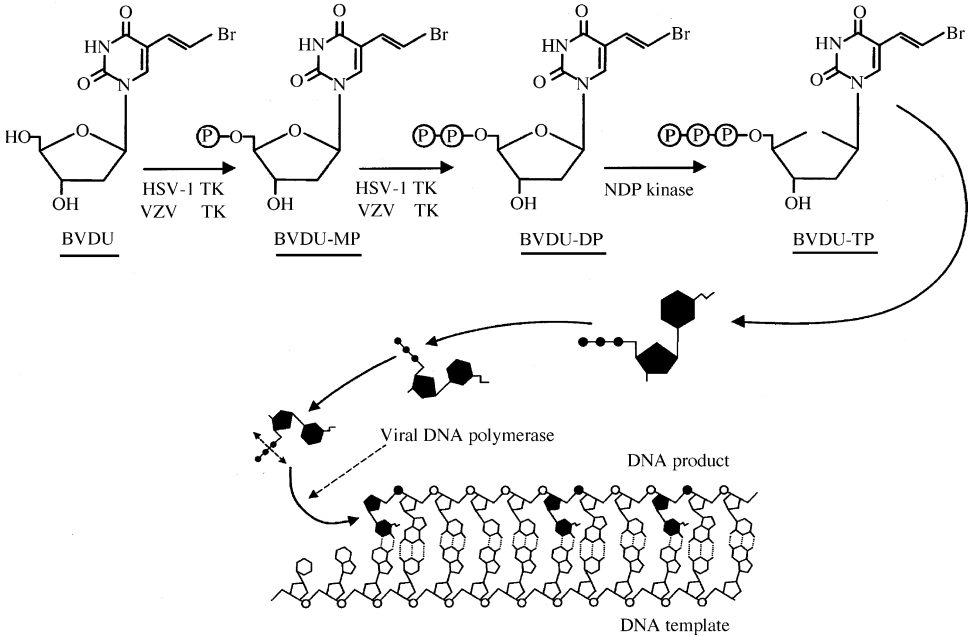


Fig. 7. Mechanism of action of BVDU (De Clercq, 2004b).

In contrast with HSV-1 and VZV, herpes simplex virus type 2 (HSV-2) is much less sensitive to the inhibitory action of BVDU, and this can be ascribed to the fact that HSV-2 TK, in contrast with HSV-1 TK and VZV TK, is unable to convert BVDU from its 5′-mono- to its 5′-diphosphate form.

Several double-blind studies have indicated that oral BVDU is superior to either intravenous or oral acyclovir in the therapy of herpes zoster in either immunocompromised patients (Wutzler et al., 1995) or immunocompetent patients (Wassilew and Wutzler, 2003), as based upon the cessation of new lesion formation as primary endpoint. Of note, in this context, is that BVDU can be given as a single oral dose of 125 mg daily, whereas acyclovir has to be administered five times daily (each time four capsules of 200 mg), which brings the total dose for acyclovir to 4 g daily and the total daily pill burden for acyclovir to 20 (as compared to a single daily pill for BVDU).

There is a problem with BVDU, however; it can be readily converted by thymidine phosphorylase to its free base, BVU [(*E*)-5-(2-bromovinyl)uracil], which is a potent inhibitor of dihydropyrimidine dehydrogenase (DPD), the enzyme that is responsible for the degradation of thymine and uracil. In patients receiving 5-fluorouracil (e.g. for a malignant disease), DPD also takes care of the degradation of 5-fluorouracil. Therefore, BVDU should not be given to patients under 5-fluorouracil treatment, as through the release of BVU, it may enhance the toxicity of 5-fluorouracil in these patients (De Clercq, 2003a).

Recently, we have described a new class of highly specific and potent anti-VZV agents, namely the bicyclic furo[2,3-*d*]pyrimidine nucleoside analogues (BCNAs, Fig. 8). These compounds are only inhibitory to VZV (McGuigan et al., 1999, 2000). They are not inhibitory to HSV or any other (herpes)viruses (De Clercq, 2003a). The most potent among the BCNAs are Cf 1742 and Cf 1743. They inhibit the replication of VZV (laboratory strains and clinical isolates) at subnanomolar concentrations [EC_{50} (50% effective concentration): 0.1–1 nM], that is about 10-fold lower than the EC_{50} of BVDU, and 10,000-fold lower than the EC_{50} required for acyclovir and penciclovir to inhibit VZV replication (Andrei et al., 2005a).

How do the BCNAs act? Their mechanism of anti-VZV action has only partially been resolved (Fig. 9) (De Clercq, 2003a). It has been unequivocally shown that for these compounds to inhibit VZV replication, they must be phosphorylated by the VZV-encoded thymidine kinase (TK). The VZV TK may, as previously shown for BVDU, phosphorylate the BCNA consecutively to the 5′-mono- and 5′-diphosphate, and the NDP kinase (or other cellular kinases) may then phosphorylate the BCNA 5′-diphosphate to its 5′-triphosphate (BCNA-TP). Whether the latter acts, as has been shown for BVDU-TP, as a competitive inhibitor and/or alternate substrate for the viral DNA synthesis, can only be surmised, and thus needs to be further corroborated.

What has been clearly shown, however, is that the BCNAs do not serve as a substrate for thymidine phosphorylase (TPase), and, unlike BVDU, are not able to release the free (heterocyclic) base following exposure to TPase, and even if the free base would be released (by other means), this would not matter, as the free base of the BCNAs was found not to inhibit DPD, and, consequently, did not interfere with the degradation of 5-fluorouracil, and did not enhance the plasma levels of 5-fluorouracil in vivo (De Clercq, 2003a). This implies that the BCNAs could eventually be used in the therapy of VZV infections in patients whether or not under 5-fluorouracil treatment.

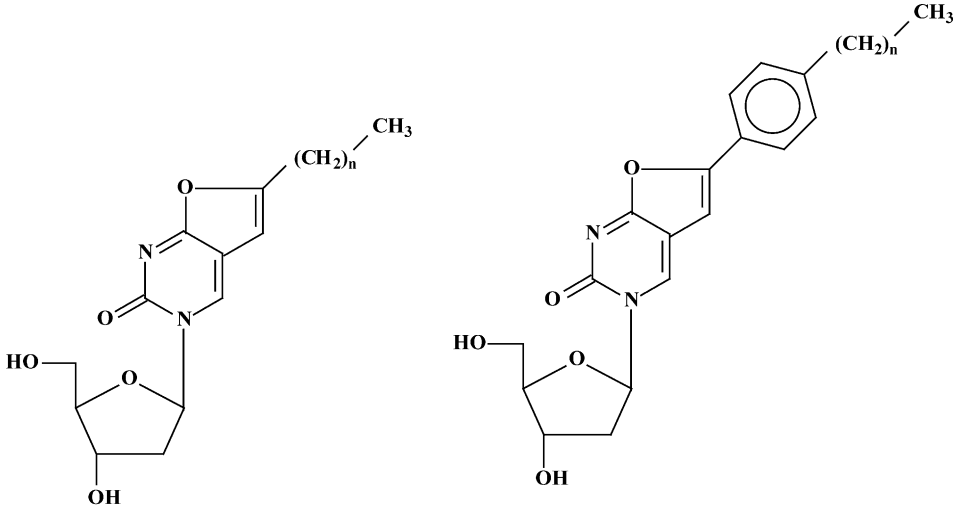


Fig. 8. Bicyclic furo[2,3-*d*]pyrimidine nucleoside analogues (BCNAs).

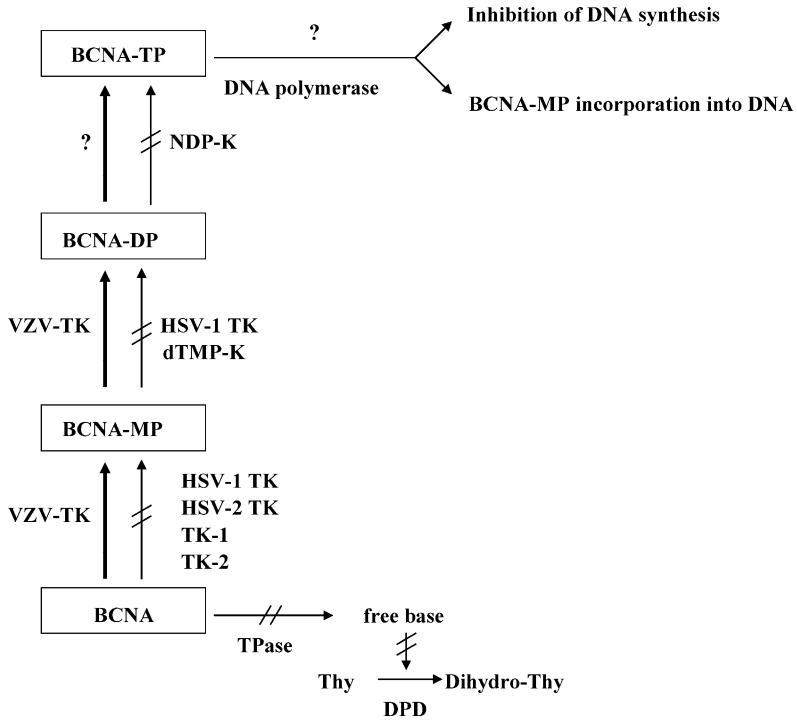


Fig. 9. Mechanism of action of BCNAs (De Clercq, 2003a).

4. Nucleoside reverse transcriptase inhibitors (NRTIs)

There are, at present, seven 2′,3′-dideoxynucleoside analogues [also referred to as NRTIs (nucleoside reverse transcriptase inhibitors)] that are formally approved for the treatment of HIV infections: zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC) and emtricitabine ((−)FTC) (Fig. 10). The anti-HIV activity of AZT, ddI and ddC was first described by Mitsuya et al. (1985), and Mitsuya and Broder (1986). We were the first to describe the anti-HIV properties of d4T (2′,3′-didehydro-2′,3′-dideoxythymidine) (Baba et al., 1987), which later on would be marketed under the trade name of Zerit®. In the late eighties we also reported that various other 2′,3′-dideoxynucleoside analogues such as 2′,3′-didehydro-2′,3′-dideoxycytidine (d4C) (Balzarini et al., 1986), and the 3′-fluoro derivatives 3′-fluoro-2′,3′-dideoxythymidine (FLT) and 3′-fluoro-2′,3′-dideoxyguanosine (FLG) (Herdewijn et al., 1987; Balzarini et al., 1988) (Fig. 11) had potent anti-HIV activity. The 5-fluoro-substituted d4C [also known as Reverset™ (β-D-Fd4C) (Schinazi et al., 2002)], FLT [also known as MIV-310, alovudine (FddThd)] and the valine-lactic acid ester of FLG (known as MIV-210) are being further pursued as anti-HIV drug candidates, and so are a number of other 2′,3′-dideoxynucleoside analogues, including racemic (±)FTC (FdOTC, Racivir®), AVX754 (SPD-754, (−)dOTC), elvucitabine (ACH-126443, β-L-Fd4C), amdoxovir (DAPD, diaminopurine dioxolane), 1-(β-D-dioxolane)thymine (DOT), and 4′-*C*-ethynyl-2-*F*-2′-deoxyadenosine.

In their mechanism of action, all 2′,3′-dideoxynucleoside (ddN) analogues follow the same strategy as originally established for AZT (Fig. 12) (De Clercq, 2002). Following three phosphorylation steps which convert the parent compound successively to its 5′-monophosphate, 5′-diphosphate and 5′-triphosphate, the latter acts as a competitive inhibitor/alternate substrate for its target enzyme, the HIV-encoded reverse transcriptase. If incorporated into the DNA chain (as ddNMP, following removal of the β- and γ-phosphate groups), the ddNMP inevitably leads to termination of the DNA chain elongation, as it does not provide the 3′-hydroxyl function that is needed for formation of the ester linkage with the α-phosphate group of the next nucleotide.

5. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Whereas the NRTIs, following their phosphorylation to the 5′-triphosphates (ddNTPs), interact with the normal substrates (dNTPs) at the substrate binding site of the HIV reverse transcriptase (RT), the NNRTIs (non-nucleoside reverse transcriptase inhibitors) interact directly with an allosteric “pocket” binding site, located at a short distance (∼15 Å) from the catalytic site. Through this interaction, NNRTIs interfere in a non-competitive fashion with the binding of the normal substrates at the HIV-1 RT. Of

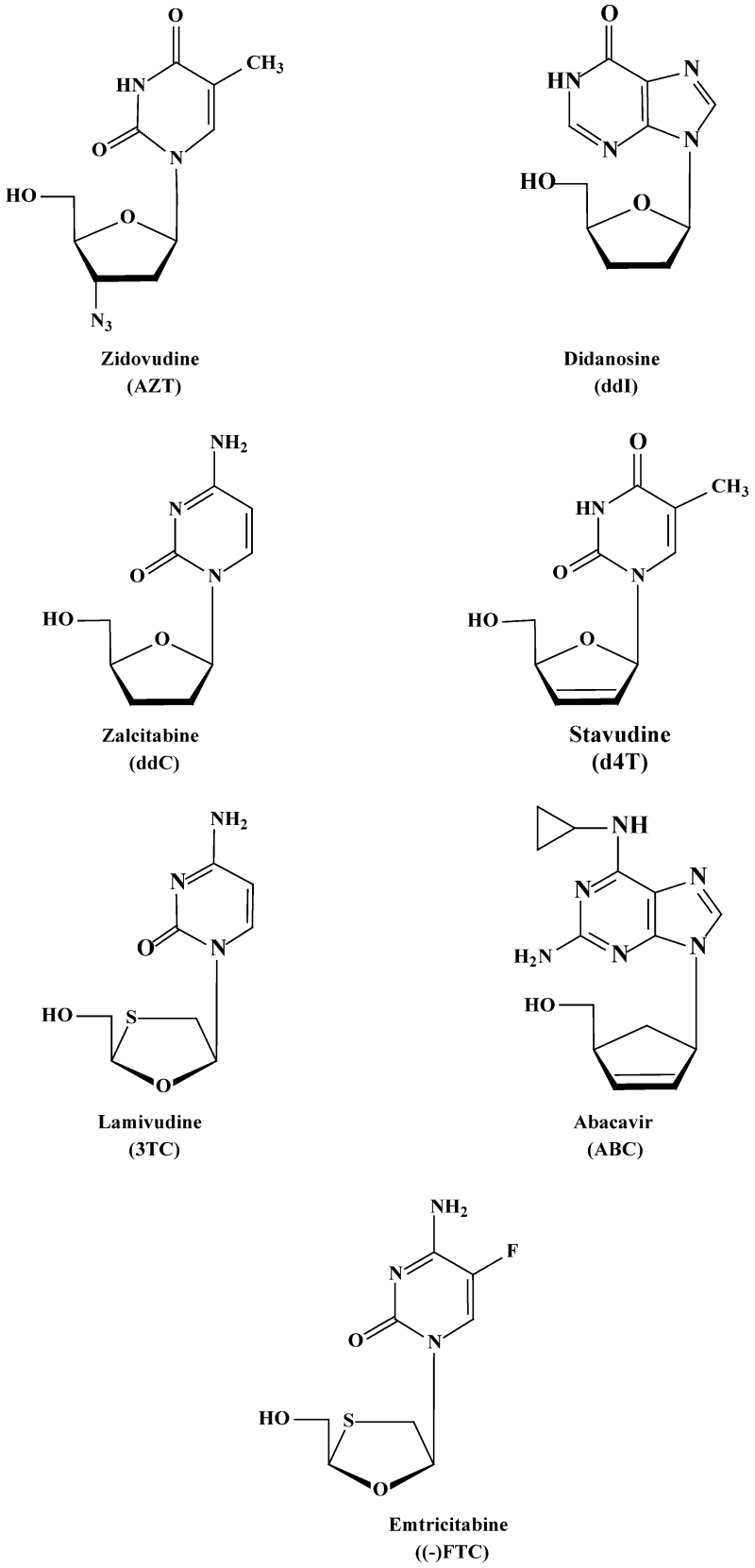


Fig. 10. Nucleoside reverse transcriptase inhibitors (NRTIs) that have been approved for the treatment of HIV infections.

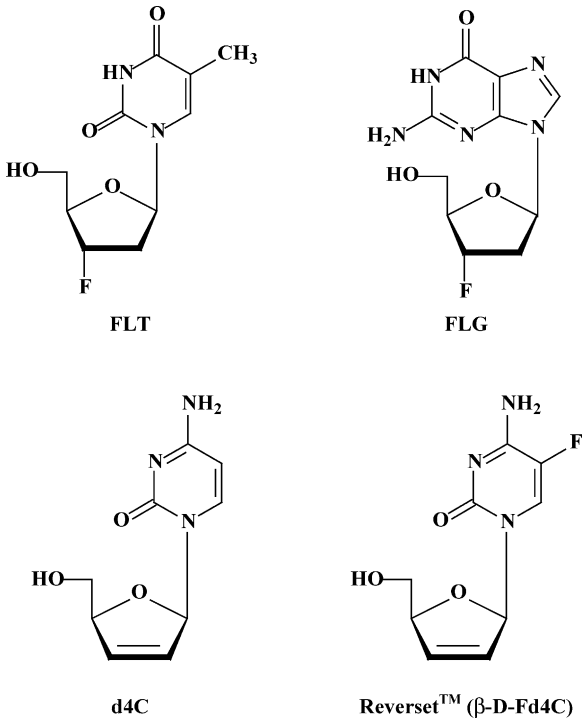


Fig. 11. 3′-Fluoro-2′,3′-dideoxythymidine (FLT), 3′-fluoro-2′,3′-dideoxyguanosine (FLG), 2′,3′-didehydro-2′,3′-dideoxycytidine (d4C) and the 5-fluoro-substituted derivative thereof (β-D-Fd4C).

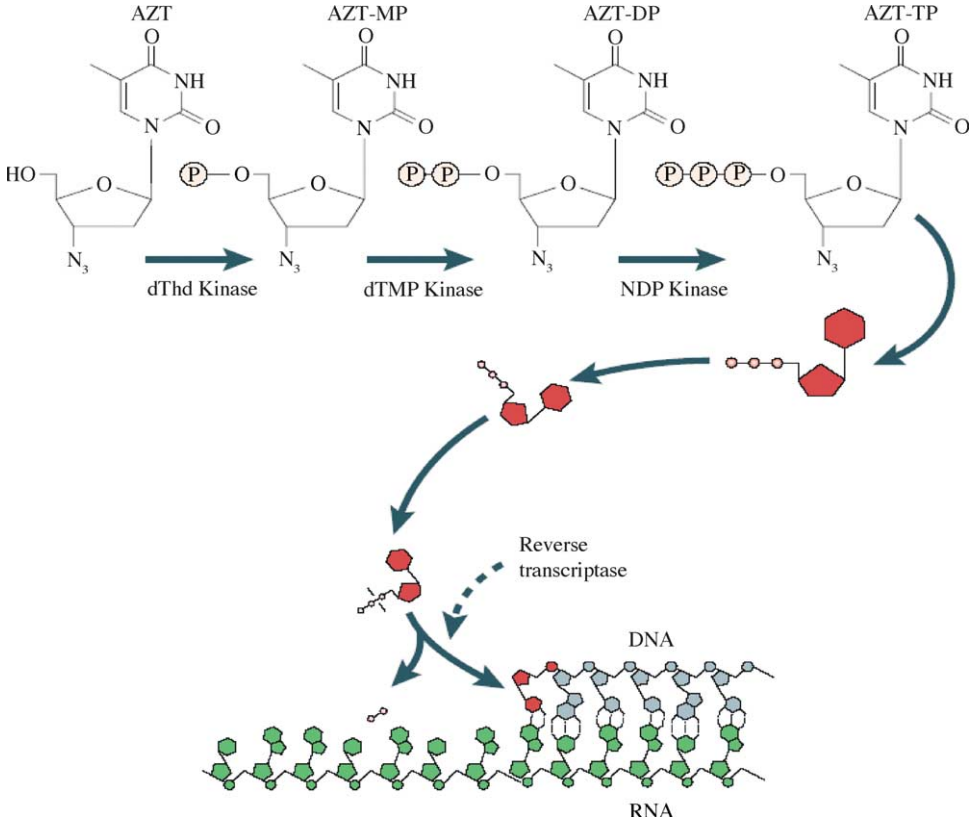


Fig. 12. Mechanism of action of AZT (De Clercq, 2002).

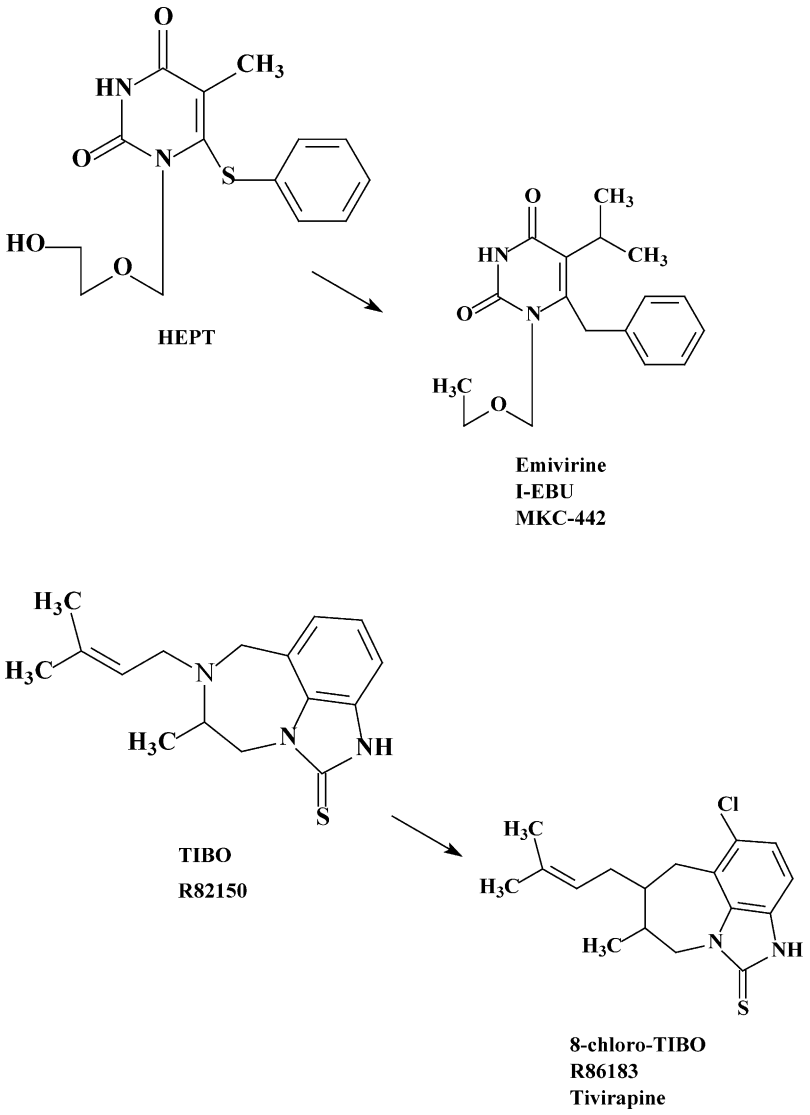


Fig. 13. The first non-nucleoside reverse transcriptase inhibitors (NNRTIs) HEPT and TIBO.

the retroviruses, only HIV-1 is able to accommodate the NNRTIs in the RT "pocket" binding site, and thus, the antiviral activity spectrum of the NNRTIs is restricted to HIV-1, and in principle, does not extend to HIV-2 or other retroviruses.

The era of the NNRTIs started in 1989/1990 with the discovery of the HEPT (Baba et al., 1989, 1994) and TIBO (Pauwels et al., 1990) derivatives (Fig. 13). Following HEPT and TIBO, numerous other chemical entities have been described as NNRTIs: i.e. nevirapine, delavirdine and efavirenz, that were eventually commercialized for the treatment of HIV-1 infections, and capravirine (S-1153, AG1549), thiocarboxanilide (UC-781), dapivirine (TMC-120, R-147681), etravirine (TMC-125, R-165335), and rilpivirine (R-278474) that are currently in (clinical) development as candidate anti-HIV-1 drugs. The discovery of rilpivirine (R278474), the most potent NNRTI ever described (EC_{50}: 0.1 nM), following the continued efforts of lead optimization over a 16 year period (Fig. 14), is a beautiful example of modern drug design through classical medicinal chemistry (Janssen et al., 2005).

Although most of the NNRTIs that have been described over the past 15 years markedly differ in chemical structure, they share a common, "butterfly" type of conformation with a central polar part and two lateral hydrophobic wings. Recognition of this "butterfly" conformation has allowed a first, rather artisanal attempt at the superposition of tivirapine on emivirine (Fig. 15A) (De Clercq, 2004c), and in a more elegant manner, the superposition of nevirapine on etravirine (Fig. 15B) (Pauwels, 2004). How an NNRTI would fit within its binding "pocket" at the HIV-1 RT is depicted in Fig. 16, where etravirine (TMC125) is positioned at its binding site relative to the amino acid residues that surround this binding site (Pauwels, 2004).

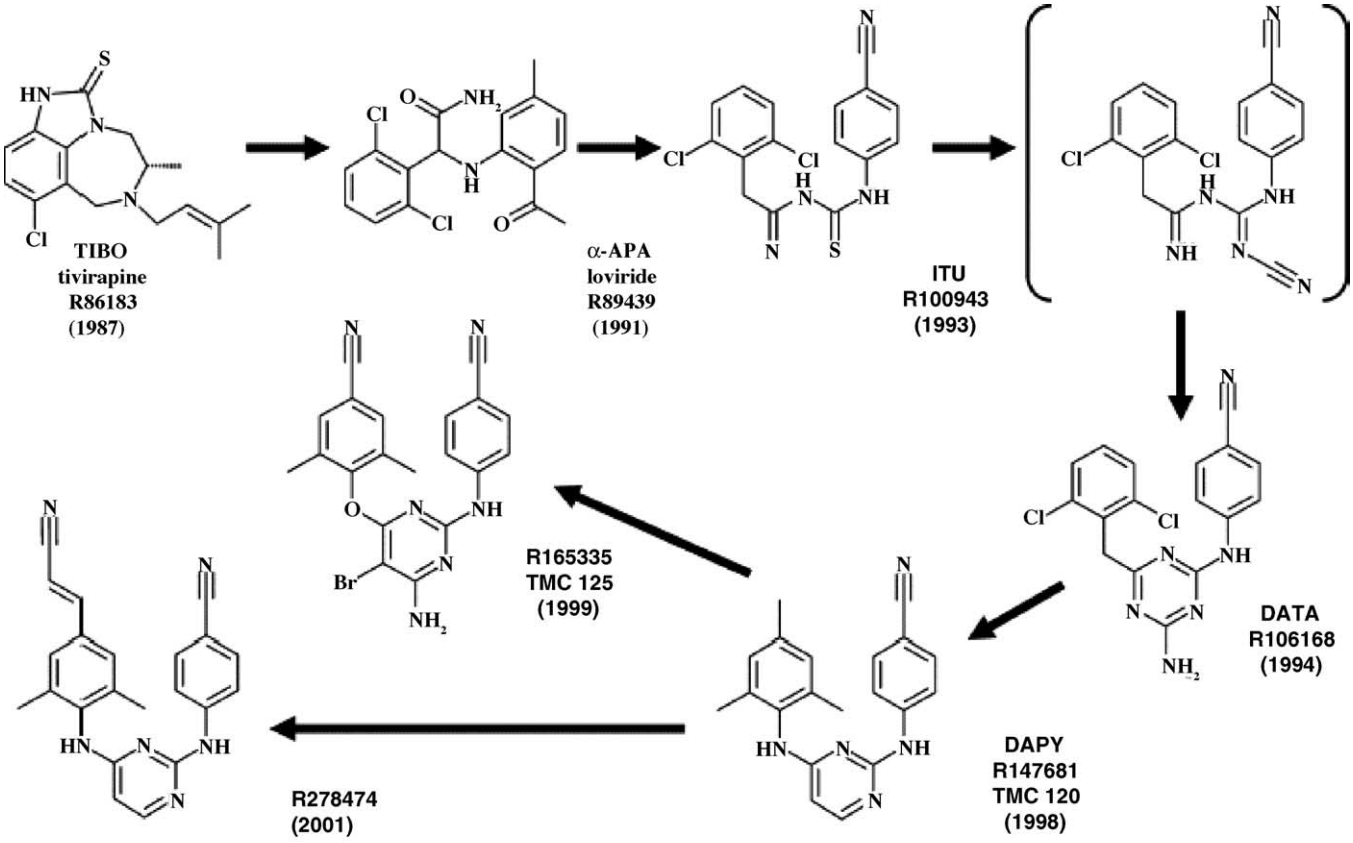


Fig. 14. Chemical evolution from TIBO (R86183) to rilpivirine (R278474), starting in 1987 (Janssen et al., 2005).

6. Bicyclams

The "bicyclam" project actually started as a "monocyclam" project with the aim to construct metal-cyclam complexes that, in analogy with polyoxometalate complexes such as $H_4SiW_{12}O_{40}$ (JM1493) (De Clercq, 2003b), might exert an inhibitory effect on HIV replication. As a prelude to this work, several commercial preparations of monocyclams were evaluated (before any attempts were made to insert the required metals) to ensure that the monocyclam by itself had no anti-HIV activity. To our surprise, one commercial cyclam preparation turned out to be quite effective in inhibiting the replication of HIV, and on closer inspection, this turned out to be due to the presence of an impurity in this preparation: the impurity was characterized as a bicyclam (JM1493) in which the two cyclam moieties were tethered by a direct carbon-carbon bridge (Fig. 17) (De Clercq et al., 1992). As it proved too difficult to resynthesize this particular "impurity", a program was started whereby hundreds of bicyclams were synthesized, containing either an aliphatic bridge, as in JM2763 (De Clercq et al., 1992), or an aromatic bridge, as in JM3100 (later renamed AMD3100) (De Clercq et al., 1994).

As compared to JM2763 (De Clercq et al., 1992), AMD3100 (De Clercq et al., 1994) proved about 100-fold more potent in its anti-HIV activity: with an EC_{50} of 1–5 nM and a selectivity index of 10^5 it stands out as one of the most potent and selective anti-HIV agents ever described. Its mode of action remained mysterious for quite some time. While the presence of drug-resistance mutations in the viral envelope glycoprotein gp120 pointed to the (indirect) role of gp120 in the mode of action of the compound, it was not until the chemokine receptors CCR5 and CXCR4 were identified as co-receptors for the cell entry of macrophage (M)-tropic (R5) and T-lymphocyte (T)-tropic (X4) HIV strains, respectively, that CXCR4 could be unequivocally implicated as the target for the action of AMD3100 against T-tropic viruses (Schols et al., 1997; Donzella et al., 1998) (Fig. 18). Following AMD3100, various bicyclam derivatives [for example, AMD3465 (Fig. 17)] have been found to be comparable to AMD3100 in both CXCR4 antagonization and anti-HIV (X4) activity (Hatse et al., 2002). A pyridinylmethyl monocyclam derivative (AMD3451, Fig. 17) was recently reported as a dual CCR5/CXCR4 antagonist, exhibiting activity against both X4 and R5 HIV strains (Princen et al., 2004).

The bicyclam AMD3100 highly specifically interacts with CXCR4, the natural receptor for stromal-derived factor 1 (SDF-1), and not with any other chemokine receptors that were evaluated for a potential interaction with AMD3100 (Hatse et al., 2003). Certain aspartic acid residues, i.e. those located at positions 171 and 262, have proven crucial in the interaction of CXCR4 with AMD3100 (Fig. 19) (De Clercq, 2003b). As a potent and selective CXCR4 antagonist,

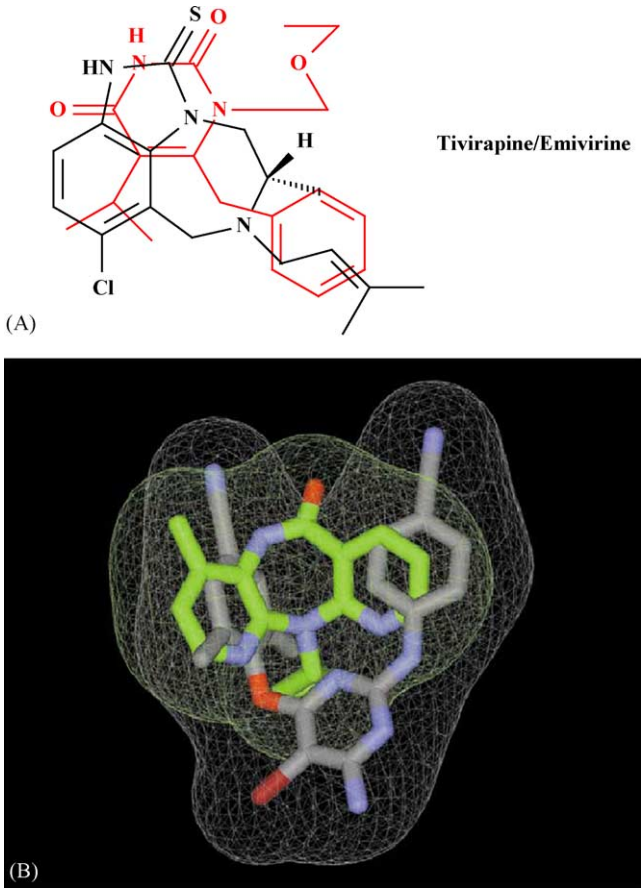


Fig. 15. (A) Superposition of TIBO (tivirapine) with HEPT (emivirine) (De Clercq, 2004c). (B) Superposition of nevirapine (C-atoms coloured in green) with etravirine (TMC125) (C-atoms coloured in grey) (Pauwels, 2004).

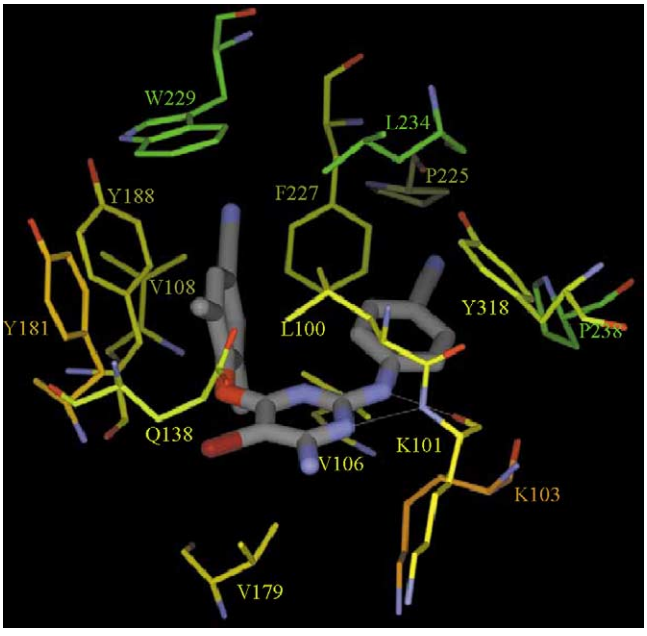


Fig. 16. Etravirine (TMC125) in the NNRTI binding site on HIV-1 RT (Pauwels, 2004).

AMD3100 may have potential applications that go far beyond X4 HIV infections (De Clercq, 2003b). In principle, it may be pursued for its therapeutic role of a number of diseases in which the CXCR4/SDF-1 axis plays an important role such as malignant processes, rheumatoid arthritis, and other allergic diseases. AMD3100 has recently entered phase III clinical trials as a stem cell mobilizing agent for transplantation of stem cells in patients with non-Hodgkin's lymphoma or multiple myeloma. AMD3100 is also being considered for preserving myocard function through the mobilization of stem cells (De Clercq, 2003b). The first indication for this unexpected potential of an anti-HIV agent was when during phase I clinical trial AMD3100 was found to increase the peripheral leukocyte counts following single-dose intravenous AMD3100 administration (Hendrix et al., 2000). As it turned out, this was mainly due to the mobilization of hematopoietic ($CD34^+$) stem cells from the bone marrow into the blood circulation.

7. Acyclic nucleoside phosphonates

7.1. Cidofovir

The acyclic nucleoside phosphonates can be conceived as originating from the hybridization of (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA) with phosphonoformic acid (PFA), thus resulting in the formation of (*S*)-9-(3-hydroxy-2-phosphonylmethoxy-propyl)adenine (HPMPA) (Fig. 20), the first acyclic nucleoside phosphonate (nucleotide analogue) to be accredited with broad-spectrum antiviral activity against DNA viruses (De Clercq et al., 1986). Soon after HPMPA, HPMPC (which structurally differs from HPMPA only by the substitution of a cytosine for adenine as the heterocyclic ring moiety) was described as a broad-spectrum antiviral agent active against various DNA viruses (De Clercq et al., 1987). Within 9 years after it was described as an antiviral agent active against cytomegalovirus (CMV) and other DNA viruses, cidofovir has been licensed (in 1996) for clinical use, under the trade name of Vistide® (Fig. 21), for the treatment of CMV retinitis in AIDS patients.

Cidofovir exhibits a remarkably broad antiviral activity spectrum encompassing papovaviridae (murine and human polyomaviruses, human papilloma viruses), adenoviridae (human adenoviruses), herpesviridae [thymidine kinase-positive (TK^+ and TK^-) HSV-1, ($TK^+$and TK^-) HSV-2, (TK^+ and TK^-) VZV, Epstein–Barr virus (EBV), protein kinase-positive (PK^+ and PK^-) CMV, human herpesvirus-6 (HHV-6), HHV-7 and HHV-8], and poxviridae (vaccinia, variola, cowpox, monkeypox, camelpox, molluscum contagiosum and orf virus) (De Clercq, 2003c,d).

To accomplish its antiviral action, cidofovir (HPMPC) must first be phosphorylated to its monophosphate (HPMPCp) and, subsequently, its diphosphate (HPMPCpp), which can be considered as the active metabolite of HPMPC (Fig. 22) (De Clercq, 2004a). These two phosphorylation steps are carried out by cellular enzymes. Specifically, the

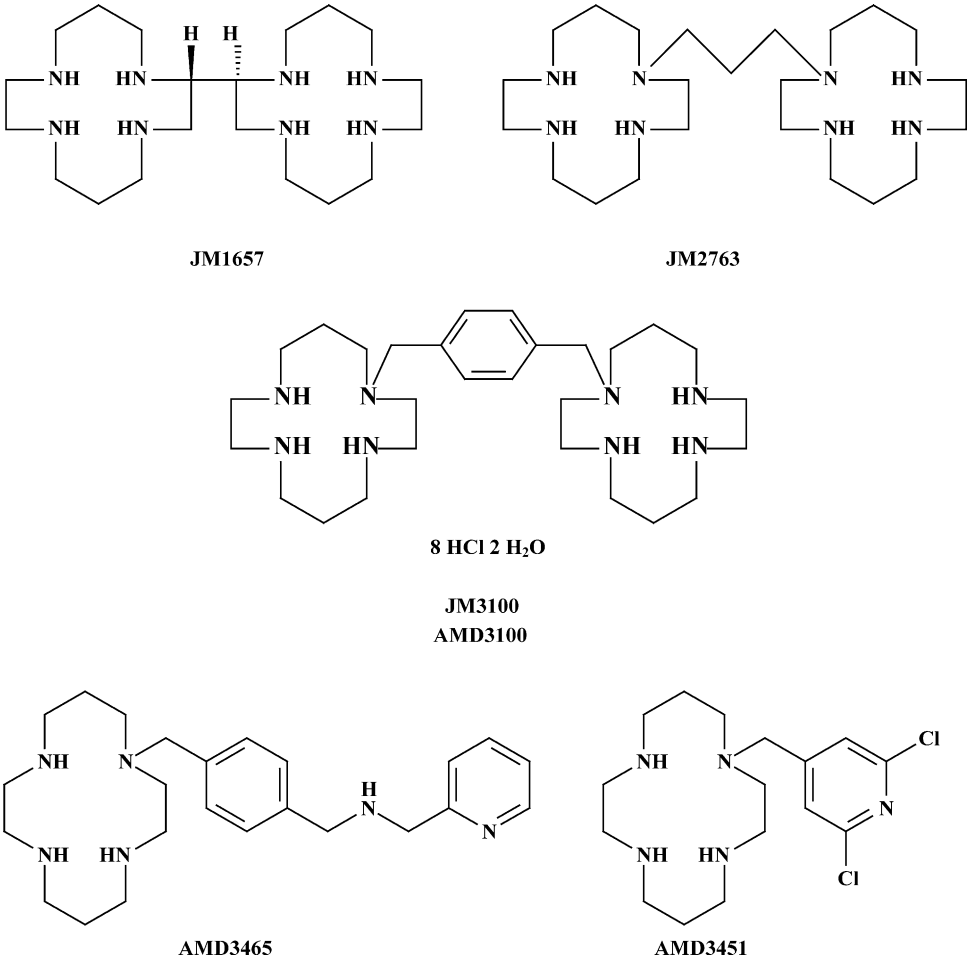


Fig. 17. Bicyclams (JM1657, JM2763, AMD3100) and derivatives thereof (AMD3465, AMD3451).

intracellular phosphorylation of HPMPC is independent of the HSV- or VZV-encoded thymidine kinase (TK) or CMV-encoded protein kinase (PK), which explains why HPMPC is active against TK^- and PK^- virus strains. At the viral DNA polymerase level, HPMPCpp acts as a competitive inhibitor/alternate substrate with respect to dCTP. If incorporated (after removal of the diphosphate group), HPMPC will terminate DNA chain elongation: as has been established with CMV DNA polymerase, two consecutive incorporations of HPMPC (in front of two consecutive dGMP nucleotides in the template chain) are required to definitely stop further DNA chain elongation (Fig. 22) (De Clercq, 2004a).

Cidofovir, administered by the intravenous (i.v.) route, has been approved for the treatment of CMV retinitis in patients with AIDS, but has also been used with success in the treatment of HSV-1, HSV-2 and VZV infections (i.e. those that are resistant to acyclovir), CMV, EBV, HHV-6, HHV-7 and HHV-8 infections, polyomavirus infections [i.e. progressive multifocal leukoencephalopathy (PML)], papillomavirus infections (i.e. disseminated respiratory papillomatosis), adenovirus infections, and poxvirus infections (i.e. vaccinia, monkeypox, smallpox, molluscum contagiosum, ...). When given topically (gel, cream, injection, ...), cidofovir has also proved beneficial in the treatment of mucocutaneous HSV-1 and HSV-2 infections (particularly those that are resistant to acyclovir), HPV-associated papillomatous lesions [i.e. recurrent laryngeal papillomas, anogenital warts, cervical intraepithelial neoplasia (CIN) grade III] and poxvirus infections (i.e. molluscum contagiosum, orf, ...) (De Clercq, 2003c).

7.2. Adefovir

Simultaneously with HPMPA, 9-(2-phosphonylmethoxyethyl)adenine (PMEA) was reported as an antiviral agent (De Clercq et al., 1986). This compound in its bis(pivaloyloxymethl)prodrug form [bis(POM)PMEA] was originally developed as an anti-HIV candidate drug, at a dose of 120 mg per day, but as it proved too (nephro)toxic at this dose, it was abandoned for this indication, and, subsequently, it was pursued, and successfully implemented at a much lower dose (10 mg daily) for the treatment of chronic hepatitis B, under the trade name of Hepsera® (Fig. 23) (De Clercq, 2003c).

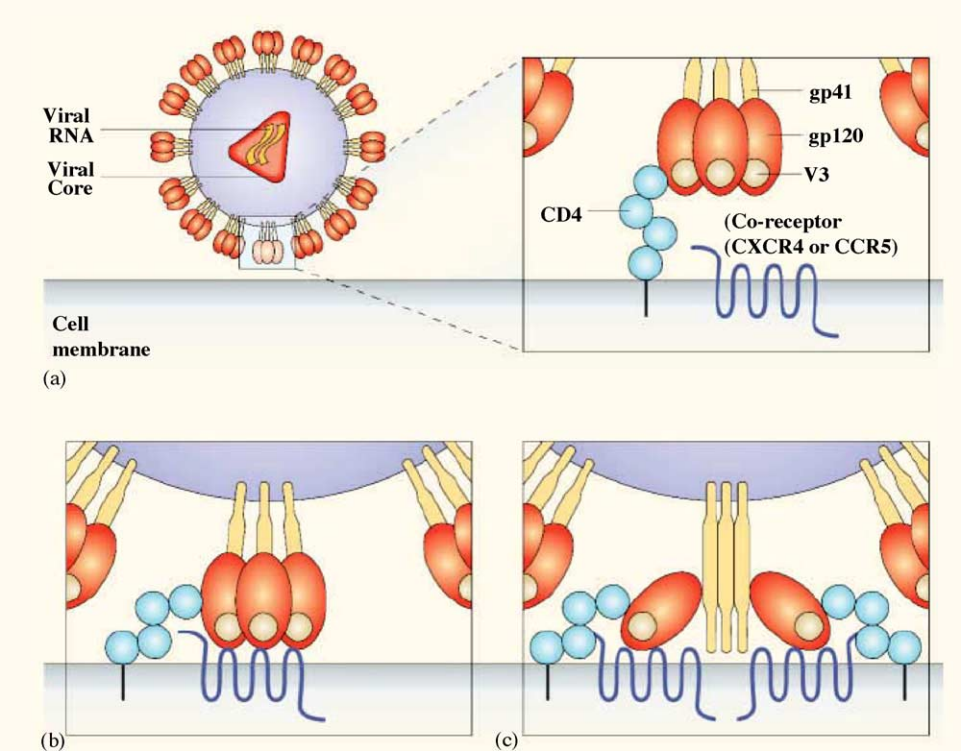


Fig. 18. Mechanism of HIV entry into the host cells (De Clercq, 2003b). During the viral adsorption process, (a) the viral envelope glycoprotein gp120 interacts with the CD4 receptor at the cell membrane. (b) Subsequently, gp120 interacts with the co-receptor CXCR4 for T-tropic (X4) HIV strains, whereupon (c) the viral glycoprotein gp41 anchors into the cell membrane. The bicyclams block the interaction between gp120 and CXCR4.

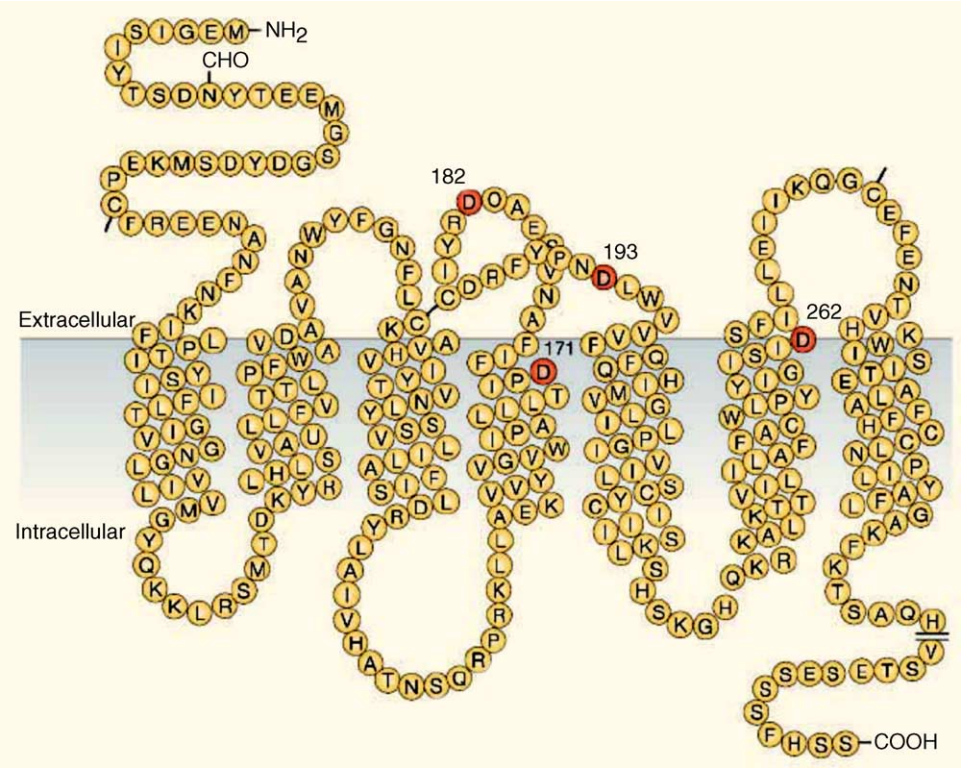


Fig. 19. The CXCR4 receptor: crucial aspartic acid residues (at positions 171, 182, 193 and 262) involved in the interaction of CXCR4 with AMD3100 are highlighted (De Clercq, 2003b).

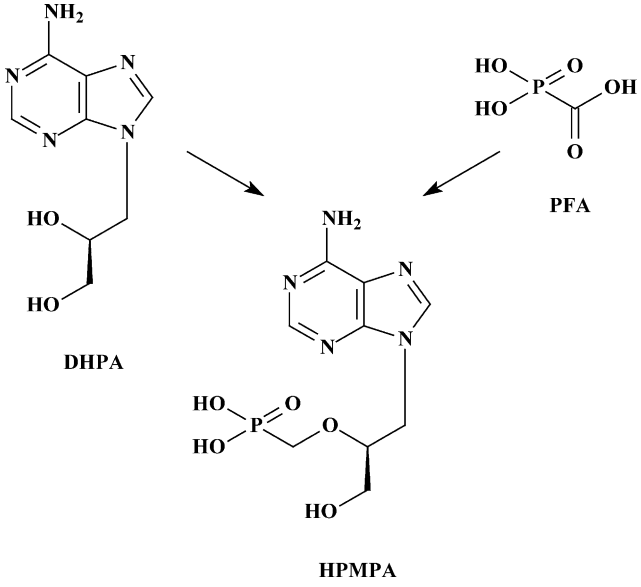


Fig. 20. HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] conceived as an hybrid between DHPA [(*S*)-2,3-dihydroxypropyl)adenine] and PFA (phosphonoformic acid).

The antiviral activity spectrum of adefovir encompasses herpesviridae (HSV-1, HSV-2, VZV, EBV, CMV, TK^- HSV, TK^- VZV), hepadnaviridae [human hepatitis B virus (HHBV) and duck hepatitis B virus (DHBV)], and retroviridae [HIV-1, HIV-2, simian immunodeficiency virus, feline immunodeficiency virus, visna/maedi virus, feline leukemia virus, LP-BM5 (murine AIDS) virus and Moloney (murine) sarcoma virus].

Adefovir (PMEA) has to be converted to its diphosphate (PMEApp) to be antivirally active. This metabolic conversion can be achieved (theoretically) in one step by PRPP synthetase using PRPP (5-phosphoribosyl-1-pyrophosphate) as the diphosphate donor or, practically, in two steps by AMP kinase with ATP as the phosphate donor (Fig. 24)

Fig. 21. (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, cidofovir) marketed (worldwide) as Vistide®.

(De Clercq, 2004a). PMEApp, after removal of the diphosphate group, than acts as an obligatory chain terminator of the HIV (or HBV) reverse transcriptase reaction. Although active against the replication of both HIV and HBV replication, PMEA in its oral prodrug form (adefovir dipivoxil) is currently used primarily for the treatment of HBV infections.

7.3. Tenofovir

For tenofovir, the anti-HIV properties were first described in 1993 (Balzarini et al., 1993). Eight years later, the compound was licensed for clinical use for the treatment of HIV infections (Fig. 25). Like adefovir, tenofovir is not sufficiently bioavailable by the oral route. Therefore, an oral prodrug has been developed, namely tenofovir disoproxil, corresponding to the bis(isopropyloxycarbonyloxymethyl) ester of PMPA, or bis(POC)PMPA, which has been formulated as its salt, tenofovir disoproxil fumarate (TDF) or Viread® (Fig. 25). After its formal approval in 2001 Viread® has now become a cornerstone in the therapy of HIV infections (De Clercq, 2004a).

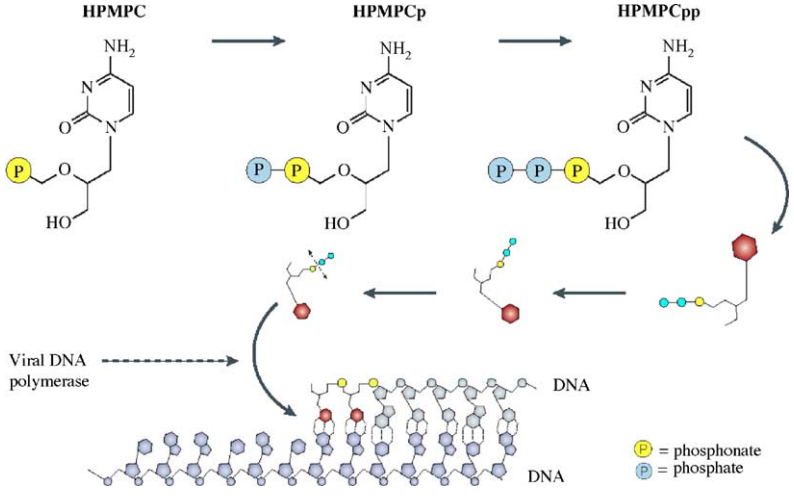


Fig. 22. Mechanism of action of cidofovir (De Clercq, 2004a).

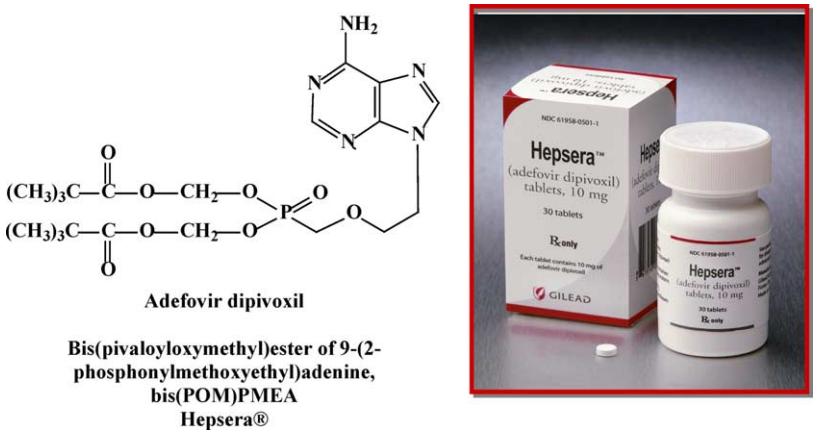


Fig. 23. Bis(pivaloylmethyl)ester of 9-(2-phosphonylmethoxyethyl)adenine [bis(POM)PMEA, adefovir dipivoxil] marketed (worldwide) as Hepsera®.

The antiviral activity spectrum of tenofovir differs from that of adefovir in that it is restricted to hepadnaviridae (i.e. HBV) and retroviridae (i.e. HIV) and does not encompass herpesviridae (HSV, VZV, CMV, ...). Like adefovir, tenofovir is active against all retroviruses that have been looked at (i.e. human, simian, feline, murine, ovine, etc.). Its mechanism of action is similar, if not identical, to that of adefovir (De Clercq, 2003d). TDF (Viread®) has been approved for the treatment of HIV infections (AIDS), and has proven to be effective in the treatment of HBV infections (chronic hepatitis B) as well. Recently, Viread® (300 mg) has become available in a fixed dose combination with emtricitabine [(−)FTC, Emtriva® (200 mg)], under the trade name of Truvada™ (Fig. 26), as a once-daily pill for the treatment of AIDS.

8. Outlook for new nucleoside phosphonates

HPV-associated diseases whether benign (i.e. warts) or malignant (i.e. cervix carcinoma) represent an important group of viral infections that are in the highest need of therapeutic intervention. A variety of acyclic nucleoside phosphonates, including PMEG [9-(2-phosphonylmethoxyethyl)guanine] and cPrPMEDAP [N^6-cyclo-propyl-9-(2-phosphonyl-methoxyethyl)-2,6-diaminopurine] (Fig. 27) are

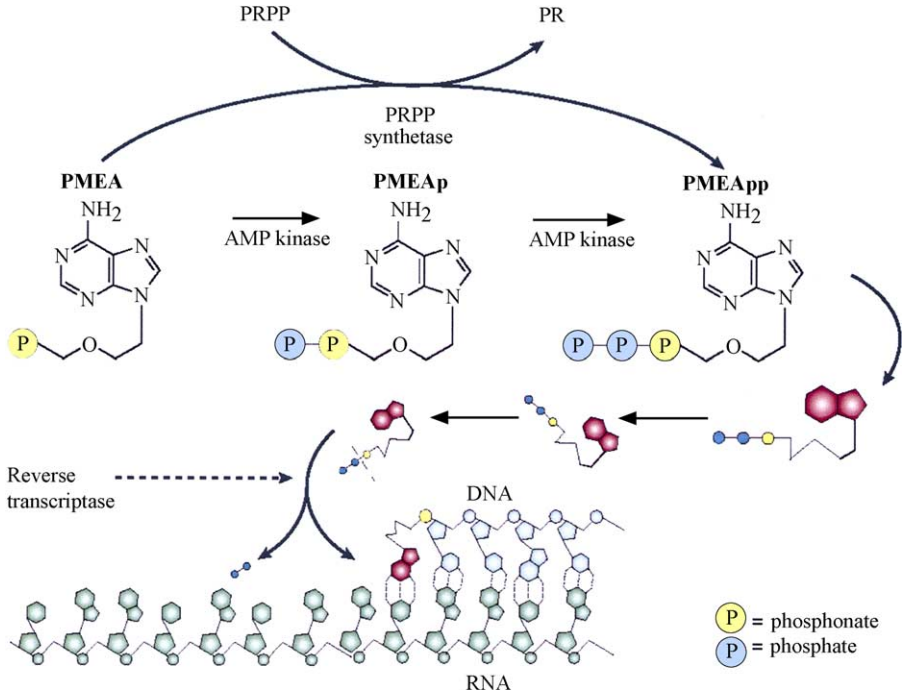
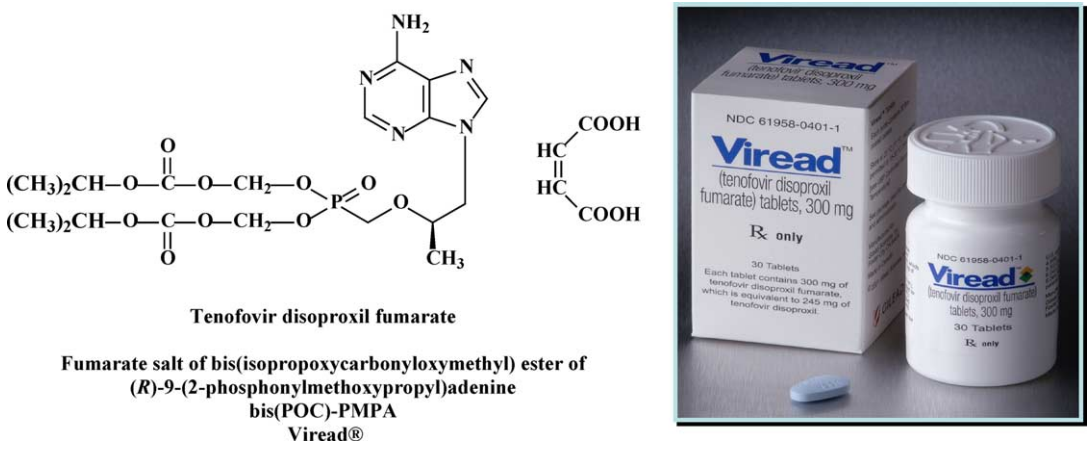


Fig. 24. Mechanism of action of adefovir (De Clercq, 2003d).



Tenofovir disoproxil fumarate

Fumarate salt of bis(isopropoxycarbonyloxymethyl) ester of (*R*)-9-(2-phosphonylmethoxypropyl)adenine bis(POC)-PMPA Viread®

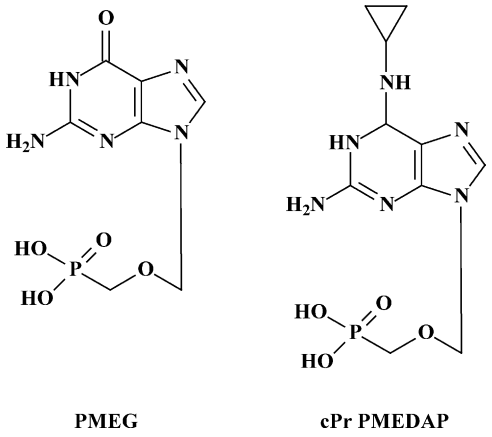
Fig. 25. Tenofovir disoproxil fumarate. Fumarate salt of bis(isopropoxycarbonyloxymethyl) ester of (*R*)-9-(2-phosphonylmethoxypropyl)adenine, bis(POC)-PMPA, marketed worldwide as Viread®.

currently being evaluated for their potential in the treatment of papillomatous lesions (Andrei et al., 2005b).

Recently, a new class of acyclic nucleoside phosphonates has been described: the 6-[2-phosphonylmethoxy)alkoxy]-2,4-diaminopyrimidines (Fig. 28) (De Clercq et al., in press). These compounds fall into two categories with as prototypes HPMPO-DAPy, on the one hand, and PMEO-DAPy and (*R*)-PMPO-DAPy, on the other hand. The activity spectrum of HPMPO-DAPy is similar to that of cidofovir, and, therefore, HPMPO-DAPy should be further explored for its potential in the treatment of papilloma-, adeno-, herpes- and poxvirus infection. The activity spectrum of PMEO-DAPy and (*R*)-PMPO-DAPy is similar to that of adefovir and tenofovir, and, thus, PMEO-DAPy and (*R*)-PMPO-DAPy deserve to be further evaluated against HIV and HBV infections (De Clercq et al., in press).

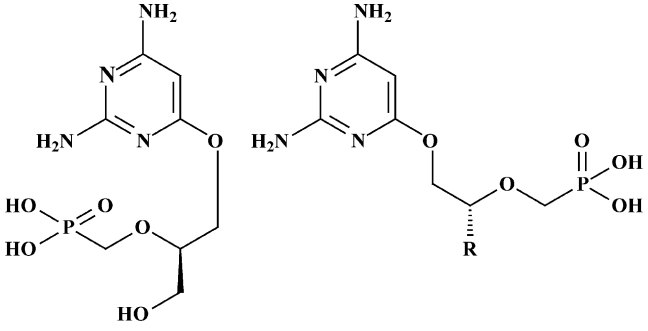
Of a very recent vintage is the class of the deoxythreosyl phosphonate nucleosides (Fig. 29) (Wu et al., 2005). Of the phosphonomethoxydeoxythreosyl (PMDT) derivatives two compounds, containing either adenine (PMDTA) or thymine (PMDTT), were identified as potent anti-HIV agents, targeted, as are PMEA and PMPA, at the viral reverse transcriptase (Wu et al., 2005). It would seem worth pursuing these compounds, and structurally related analogues thereof, not only for their potential against HIV and HBV infections, but also other virus infections.

Fig. 26. Truvada™: combination of emtricitabine (Emtriva®) with tenofovir disoproxil fumarate (Viread®).



PMEG cPr PMEDAP

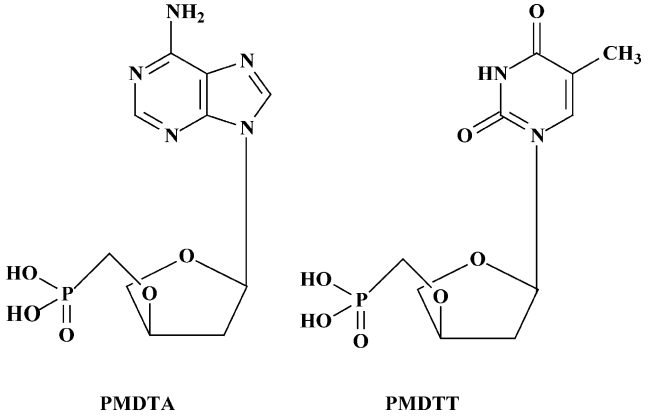
Fig. 27. Acyclic nucleoside phosphonates PMEG and cPr PMEDAP.



HPMPO-DAPy

R = CH_3 : (*R*)-PMPO-DAPy
R = H : PMEO-DAPy

Fig. 28. 6-[2-(Phosphonomethoxy)alkoxy]-2,4-diaminopyrimidines.



PMDTA PMDTT

Fig. 29. Deoxythreosyl phosphonate nucleosides: 1-(adenin-9-yl)-2-deoxy-3-*O*-(phosphonomethyl)-L-threose (PMDTA) and 1-(thymin-1-yl)-2-deoxy-3-*O*-(phosphonomethyl)-L-threose (PMDTT).

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