Antiviral agents

Viruses and viral diseases

- Viruses are non-cellular, infectious agents which take over a host cell in order to survive and multiply.
- Viruses can be transmitted in a variety of ways.
- Those responsible for diseases such as influenza (flu), chicken pox, measles, mumps, viral pneumonia, rubella, and smallpox can be transmitted through the air by an infected host sneezing or coughing.
- Some viruses are unable to survive for long outside the host and are transmitted through physical contact. The viruses responsible for AIDS, cold sores, the common cold, genital herpes, certain leukaemias, and rabies are examples of this kind.
- Finally, food- or water-borne viruses can lead to hepatitis A and E, poliomyelitis, and viral gastroenteritis.

Structure of viruses

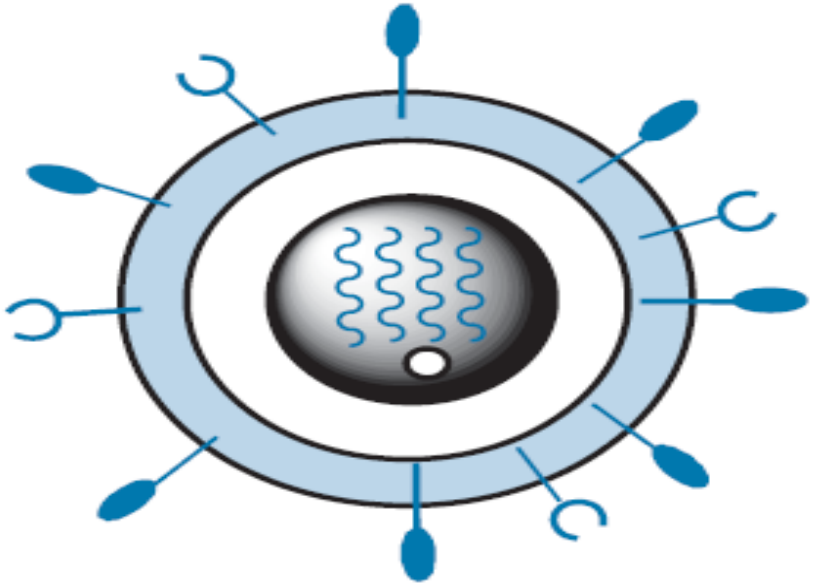
- viruses can be viewed as protein packages transmitting foreign nucleic acid between host cells.
- All viruses contain one or more molecules of either RNA or DNA, but not both. They can, therefore, be defined as **RNA or DNA viruses .**
- **Most RNA viruses contain** single-stranded RNA (**ssRNA), but some viruses contain** double-stranded RNA (**dsRNA).**
- If the base sequence of the RNA strand is identical to viral mRNA, it is called the positive (+) strand. If it is complementary, it is called the negative (−) strand.
- **Most DNA viruses** contain double- stranded DNA (**dsDNA), but a small number contain** single-stranded DNA (**ssDNA).**

Structure of viruses

- The viral nucleic acid is contained and protected within a protein coat called the **capsid** .
- **Capsids are usually** made up of protein subunits called **protomers which** are generated in the host cell and can interact spontaneously to form the capsid in a process called **self-assembly** .
- Once the **capsid** contains the viral nucleic acid, the whole assembly is known as the **nucleocapsid** .
- **In some viruses,** the nucleocapsid may contain viral enzymes which are crucial to its replication in the host cell. For example, the flu virus contains an enzyme called **RNA-dependent RNA polymerase within its nucleocapsid.**

Structure of viruses

- Additional membranous layers of carbohydrates and lipids may surround the nucleocapsid, depending on the virus concerned. These are usually derived from the host cell, but they may also contain viral proteins which have been coded by viral genes.
- The complete structure is known as a **virion** and this is the form that the virus takes when it is outside the host cell.



Nucleic acid ((–)ssRNA)

Capsid

Nucleocapsid

Membranous layer

Haemagglutinin (HA)

Neuraminidase (NA)

RNA polymerase

Viral proteins

Life cycle of viruses

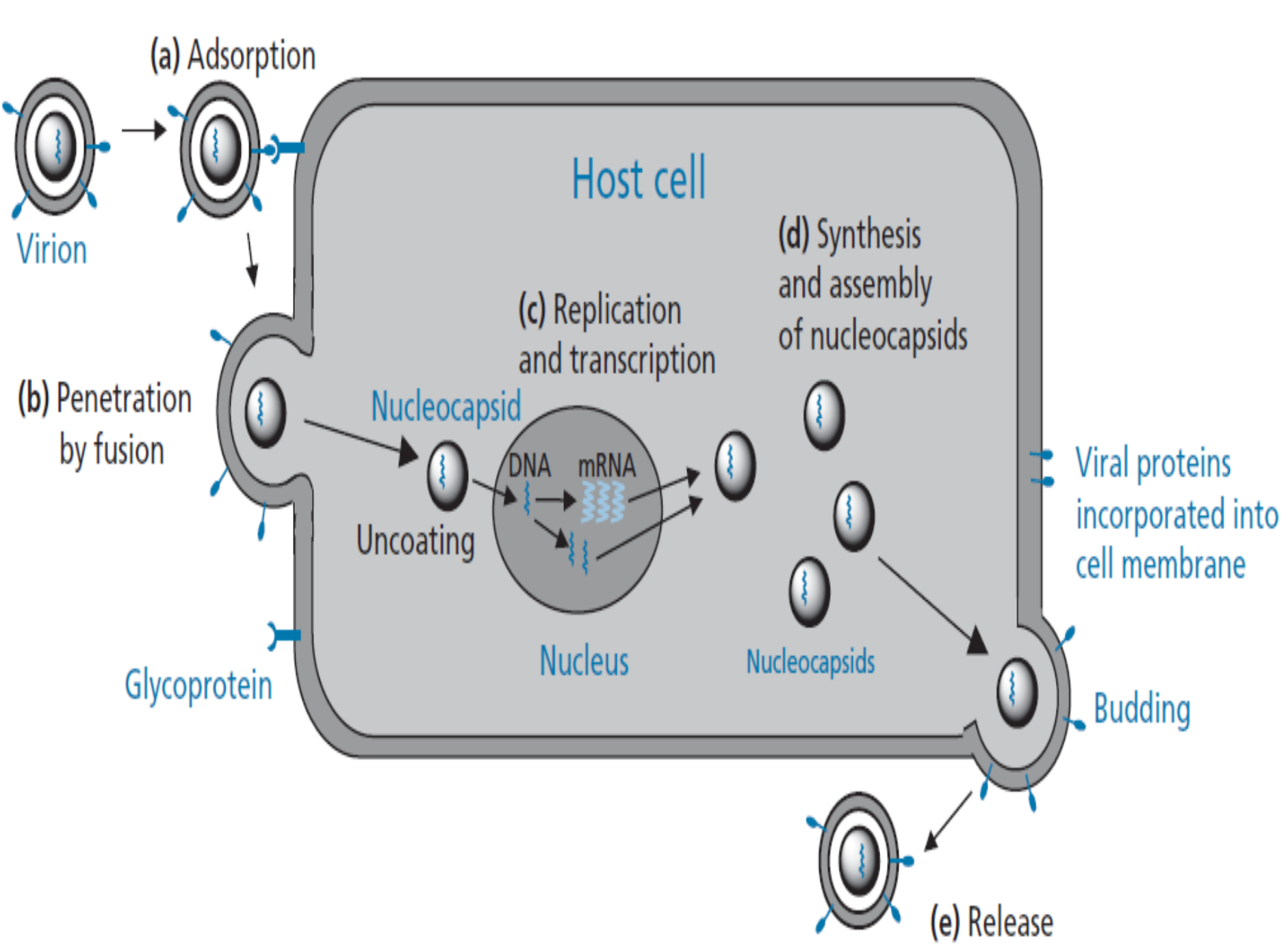
- The various stages involved in the life cycle of a virus are as follows:-
- **Adsorption** : a virion has to first bind to the outer surface of a host cell. This involves a specific molecule on the outer surface of the virion binding to a specific protein or carbohydrate present in the host cell membrane.
- **Penetration and uncoating**: different viruses introduce their nucleic acid into the host cell by different methods. Some inject their nucleic acid through the cell membrane; others enter the cell intact and are then uncoated.

Replication and transcription

- Viral genes can be defined as *early or late*. *Early genes take over the host* cell such that viral DNA and/or RNA is synthesized.
- The mechanism involved varies from virus to virus. For example, viruses containing negative ssRNA use a viral enzyme called RNA-dependent RNA polymerase (or transcriptase) to synthesize mRNA which then codes for viral proteins.
- **Synthesis and assembly of nucleocapsids**: late genes direct the synthesis of capsid proteins and these self assemble to form the capsid. Viral nucleic acid is then taken into the capsid to form the nucleocapsid.

Virion release

- **Virion release : naked virions (those with no outer layers** round the nucleocapsid) are released by cell lysis where the cell is destroyed.
- In contrast, viruses with envelopes are usually released by a process known as **budding.**
- Viral proteins are first incorporated into the host cell's plasma membrane. The nucleocapsid then binds to the inner surface of the cell membrane and, at the same time, viral proteins collect at the site and host cell proteins are excluded. The plasma membrane containing the viral proteins then wraps itself round the nucleocapsid and is pinched off from the cell to release the mature virion


(a) Adsorption
Virion
Host cell
(d) Synthesis
and assembly
of nucleocapsids
(c) Replication
and transcription
(b) Penetration
by fusion
Nucleocapsid
DNA
mRNA
Uncoating
Viral proteins
incorporated into
cell membrane
Nucleus
Nucleocapsids
Glycoprotein
Budding
(e) Release

Vaccination

- Vaccination is the preferred method of protection against viral disease and has proved extremely successful against childhood diseases such as polio, measles, and mumps.
- Vaccination works by introducing the body to foreign material which bears molecular similarity to some component of the virus, but which lacks its infectious nature or toxic effects. The body then has the opportunity to recognize the molecular fingerprint of the virus (i.e. specific **antigens) and the immune system is primed to attack** the virus should it infect the body. Usually a killed or weakened version of the virus is administered so that it does not lead to infection itself. Alternatively, fragments of the virus (subunit vaccines) can be used if they display a characteristic antigen.

Vaccination

- there are difficulties surrounding the HIV and flu viruses, because rapid gene mutation in these viruses results in constant changes to the amino acid composition of glycoproteins normally present on the viral surface. Because these glycoproteins are the important antigens that trigger the immune response, any changes in their structure 'disguise' the virus, and the body's primed immune system fails to recognize it.
- Another problem concerning vaccination relates to patients with a weakened immune response. The main categories of patients in this situation are cancer patients undergoing chemotherapy, patients undergoing organ transplants (where the immune system has been deliberately suppressed to prevent organ rejection), and AIDS patients. Vaccination in these patients is less likely to be effective because of the weakened immune response.

Antiviral drugs: general principles

- Good drug targets are proteins which are likely to have the following characteristics :
- they are important to the life cycle of the virus such that their inhibition or disruption has a major effect on infection;
- they bear little resemblance to human proteins, thus increasing the chances of good selectivity and minimal side effects.
- they are common to a variety of different viruses and have a specific region which is identical in its amino acid composition. This makes the chances of developing a drug with broad antiviral activity more likely;

Antiviral drugs used against DNA viruses

- Most of the drugs which are active against DNA viruses have been developed against herpes viruses to combat diseases such as
- cold sores,
- genital herpes,
- chicken pox,
- shingles,
- eye diseases,
- mononucleosis,
- Burkitt's lymphoma, and
- Kaposi's sarcoma.
- Nucleoside analogues have been particularly effective

Inhibitors of viral DNA polymerase

- Aciclovir has a nucleoside-like structure and contains the same nucleic acid base as deoxyguanosine.
- However, it lacks the complete sugar ring.
- In virally infected cells, it is phosphorylated in three stages to form a triphosphate which is the active agent, and so aciclovir is a prodrug.
- Nucleotide triphosphates are the building blocks for DNA replication where a new DNA strand is constructed using a DNA template—a process catalysed by the enzyme **DNA polymerase.**

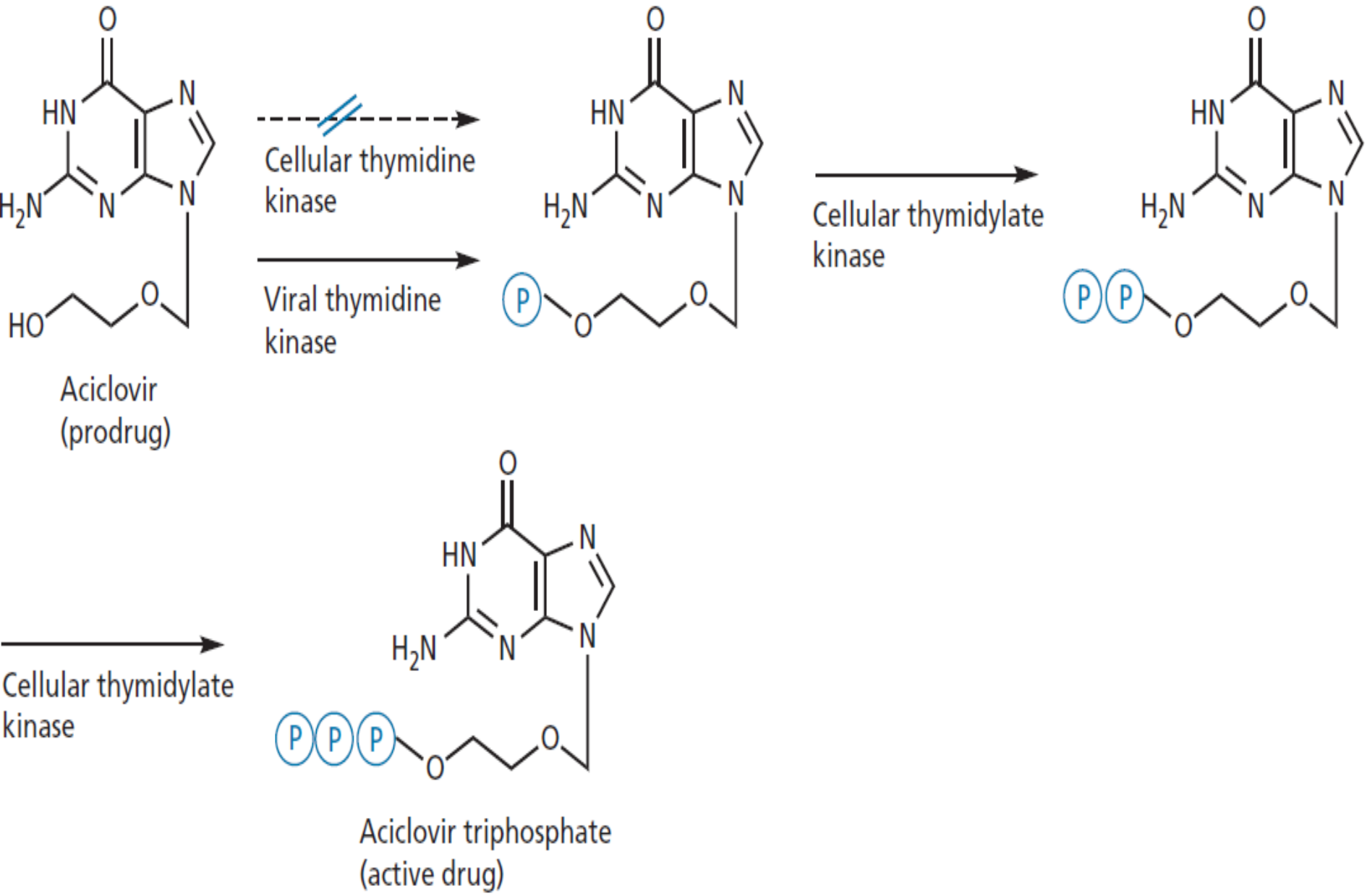
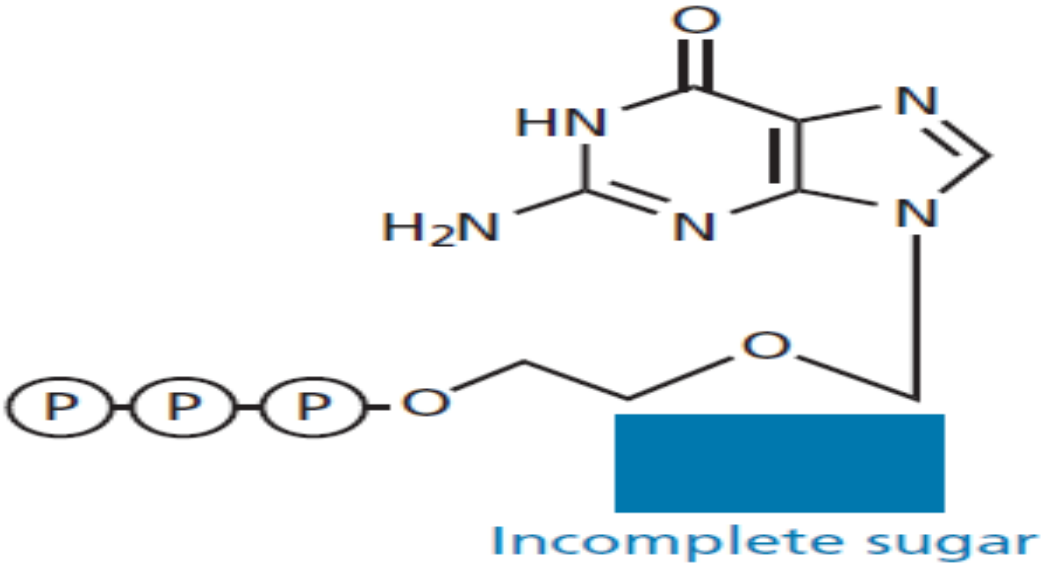


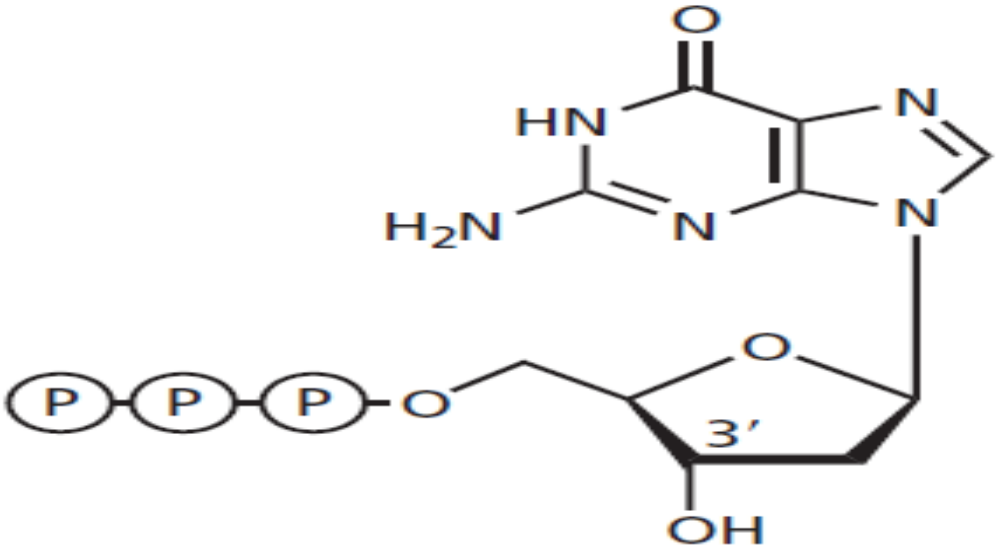
FIGURE 20.3 Activation of aciclovir. (P) represents phosphate groups.

Inhibitors of viral DNA polymerase

- Aciclovir triphosphate prevents DNA replication in two ways.
- Firstly, it is sufficiently similar to the normal deoxyguanosine triphosphate building block that it can bind to DNA polymerase and inhibit it.
- Secondly, DNA polymerase can catalyse the attachment of the aciclovir nucleotide to the growing DNA chain. As the sugar unit is incomplete and lacks the required hydroxyl group normally present at position 3' of the sugar ring, the nucleic acid chain cannot be extended any further. Thus, the drug acts as a **chain terminator**
- However, what is to stop aciclovir triphosphate inhibiting DNA polymerase in normal, uninfected cells?



Aciclovir triphosphate



Deoxyguanosine triphosphate

FIGURE 20.4 Comparison of aciclovir triphosphate and deoxyguanosine triphosphate.

Inhibitors of viral DNA polymerase

- The answers lies in the fact that aciclovir is only converted to the active triphosphate in infected cells.
- The explanation for this lies in the first phosphorylation reaction catalysed by the enzyme thymidine kinase . Although this enzyme is present in host cells, the herpes virus carries its own version.
- It turns out that viral thymidine kinase is 100 times more effective at converting aciclovir to its monophosphate than host cell thymidine kinase.
- Once formed, the monophosphate is converted to the active triphosphate by cellular enzymes.

Inhibitors of viral DNA polymerase

- Therefore, in normal, uninfected cells, aciclovir is a poor substrate for cellular thymidine kinase and remains as the prodrug. This, along with the fact that there is a selective uptake of aciclovir by infected cells, explains its excellent activity and much reduced toxicity relative to previous drugs.
- Another feature which enhances its safety is that aciclovir triphosphate shows a 50-fold selective action against viral DNA polymerases relative to cellular polymerases.
- The oral bioavailability of aciclovir is quite low (15– 30%).
- To overcome this, various prodrugs were developed to increase water solubility.

Inhibitors of viral DNA polymerase

- **Valaciclovir** is an l-valyl ester prodrug absorbed from the gut far more effectively than aciclovir. However, the prodrug has similar polarity and ionization to aciclovir, and so the prodrug is no more able to cross the cell membranes of the gut wall by passive diffusion than aciclovir.
- Moreover, poorer absorption is observed if d-valine is used for the prodrug instead of l-valine, suggesting that a specific binding interaction is involved in the absorption process.
- This implies that the prodrug is actively transported by transport proteins in the gut, and that the valine allows the prodrug to be recognized and bound by these proteins.

Inhibitors of viral DNA polymerase

- Transport proteins normally responsible for transporting dipeptides across the cell wall have been implicated in this process, i.e. the **human intestinal proton-dependent oligopeptide transporter-1 (hPEPT-1) and human intestinal di/tripeptide transporter-1 (HPT-1).**
- **Once** valaciclovir is absorbed, it is hydrolysed to aciclovir in the liver and gut wall.
- **Desciclovir is a prodrug of** aciclovir which lacks the carbonyl group at position 6 of the purine ring and is more water soluble.
- Once in the blood supply, metabolism by cellular **xanthine oxidase** oxidizes the 6-position to give aciclovir.
- **Ganciclovir is an analogue of aciclovir and** bears an extra hydroxymethylene group; **valganciclovir** acts as a prodrug for this compound

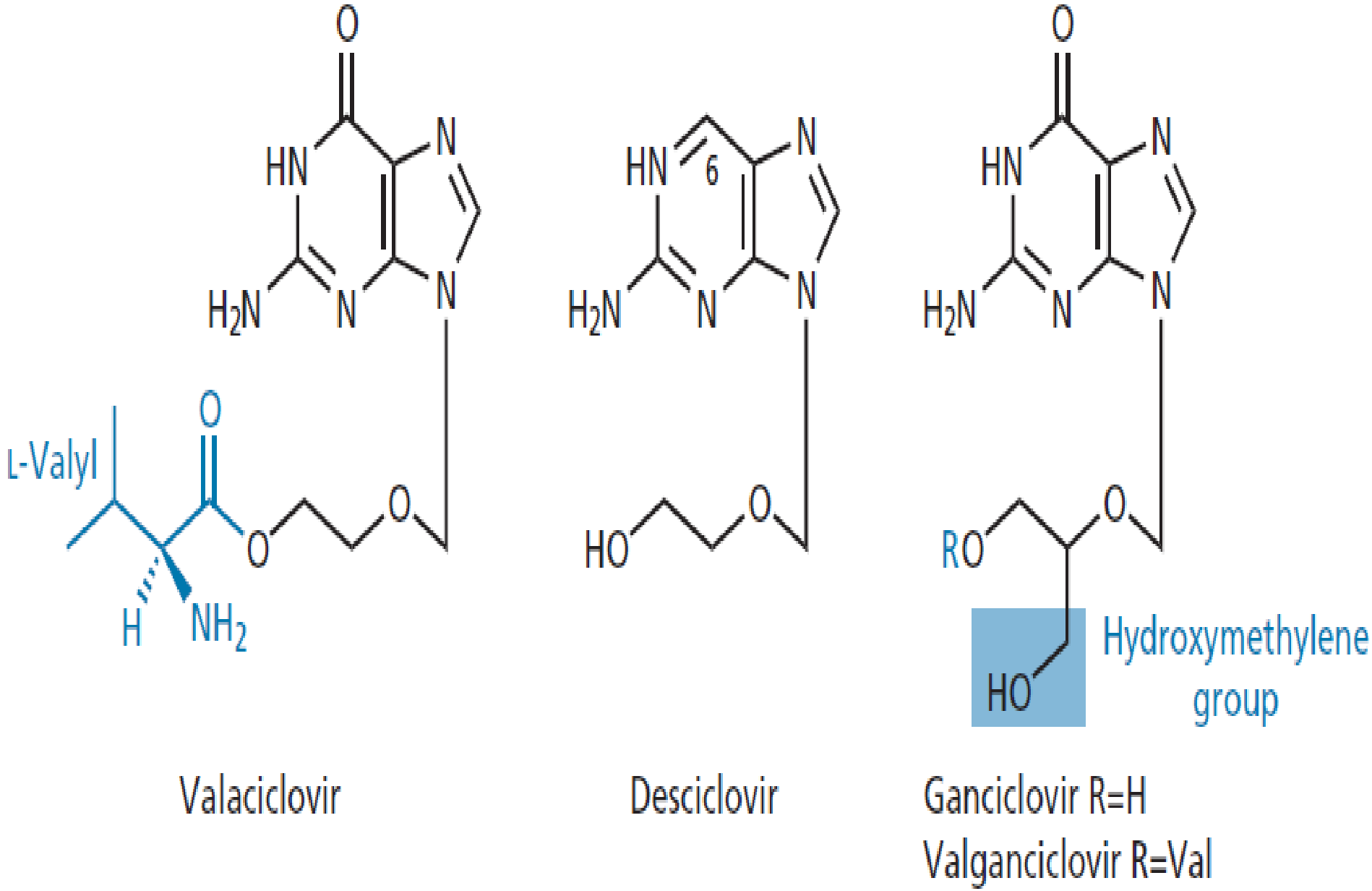


FIGURE 20.5 Prodrugs and analogues of aciclovir.

Inhibitors of viral DNA polymerase

- **Penciclovir and its** prodrug **famciclovir are analogues of ganciclovir.**
- In famciclovir, the two alcohol groups of penciclovir are masked as esters making the structure less polar, resulting in better absorption.
- Once absorbed, the acetyl groups are hydrolysed by esterases and the purine ring is oxidized by **aldehyde oxidase in the liver to generate** penciclovir.

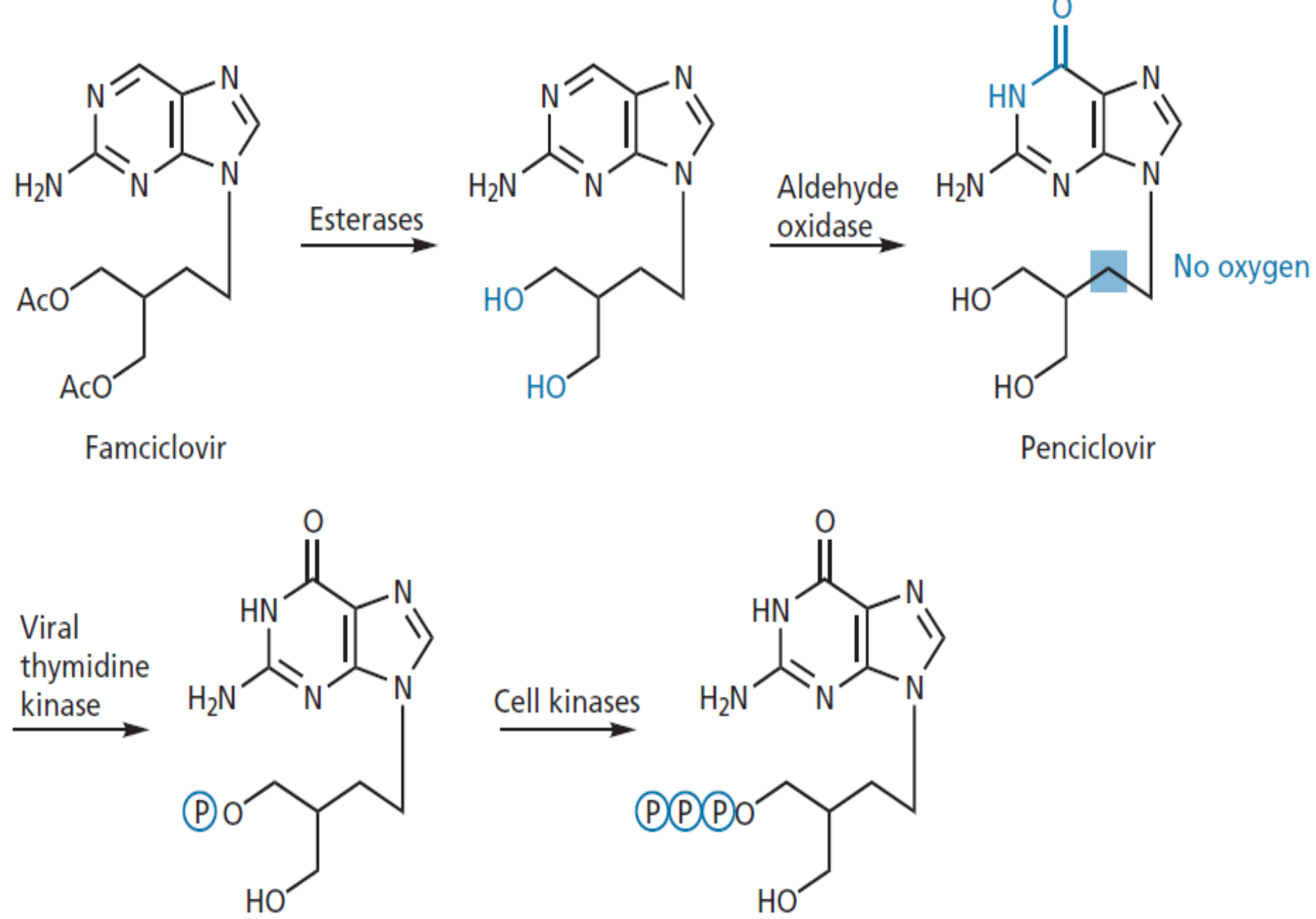


FIGURE 20.6 Penciclovir and famciclovir. (P) represents a phosphate group.

Inhibitors of viral DNA polymerase

- Some viruses are immune from the action of these antiviral agents because they lack the enzyme thymidine kinase. As a result, phosphorylation fails to take place.
- **Cidofovir was designed to combat this problem** . It is an analogue of **deoxycytidine 5-monophosphate where the sugar and phosphate** groups have been replaced by an acyclic group and a phosphonomethylene group respectively.
- **The latter group acts as a bioisostere for the phosphate group and is used because the phosphate group itself would be susceptible to enzymatic hydrolysis**.
- As a phosphate equivalent is now present, the drug does not require thymidine kinase to become activated. Two more phosphorylations can now take place catalysed by cellular kinases, to convert cidofovir to the active ‘triphosphate’.

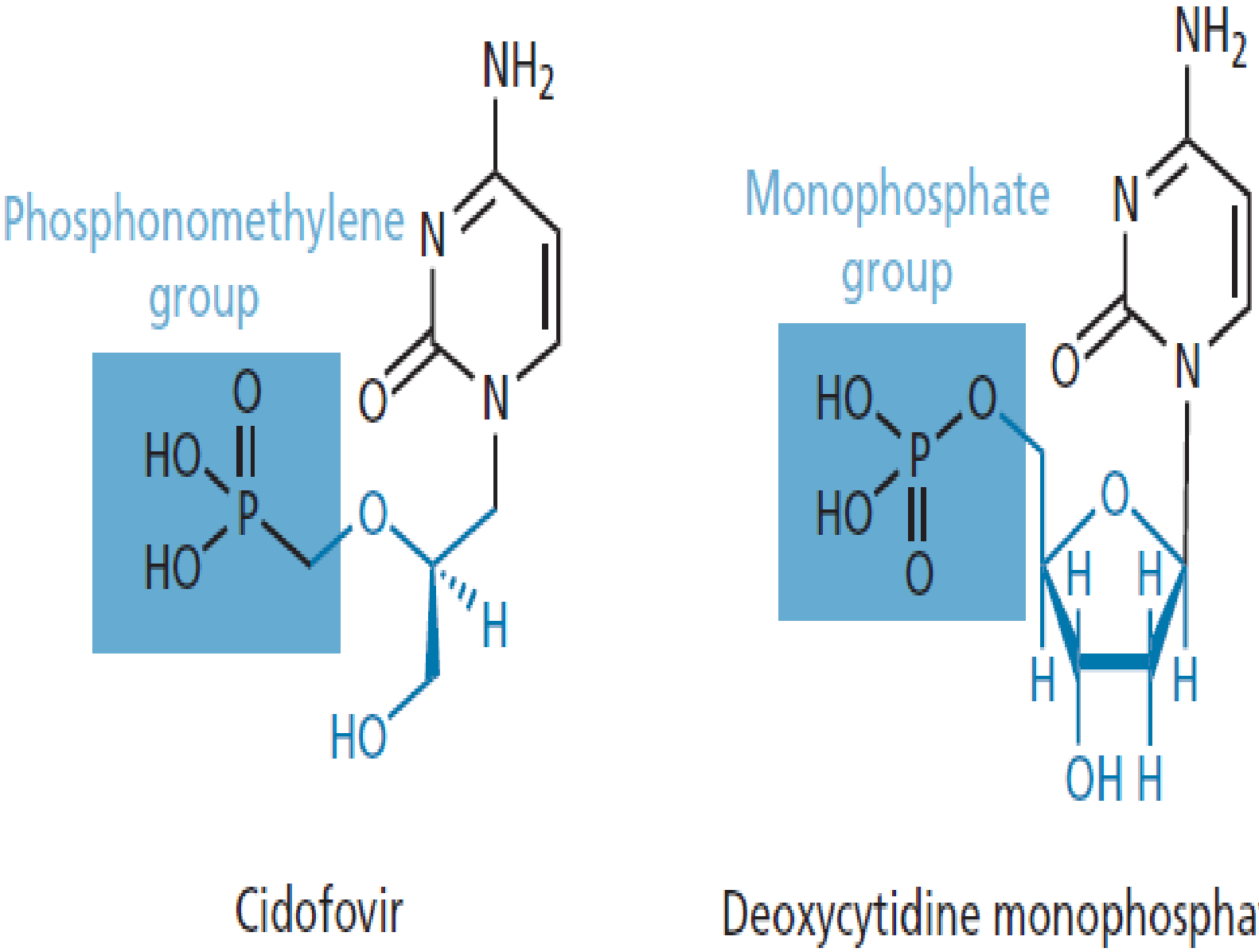
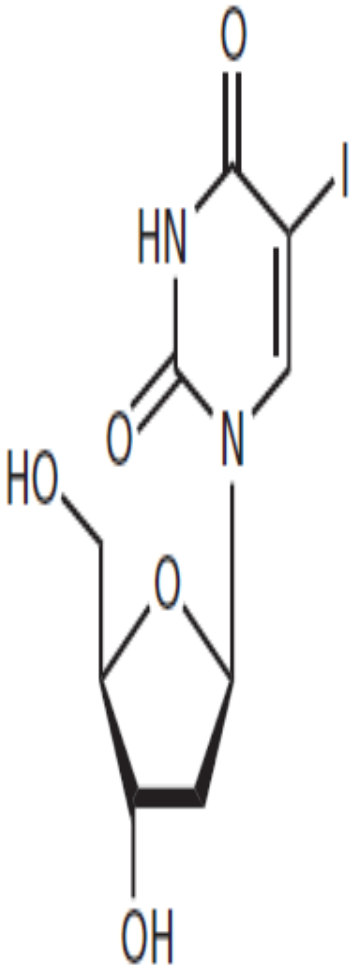


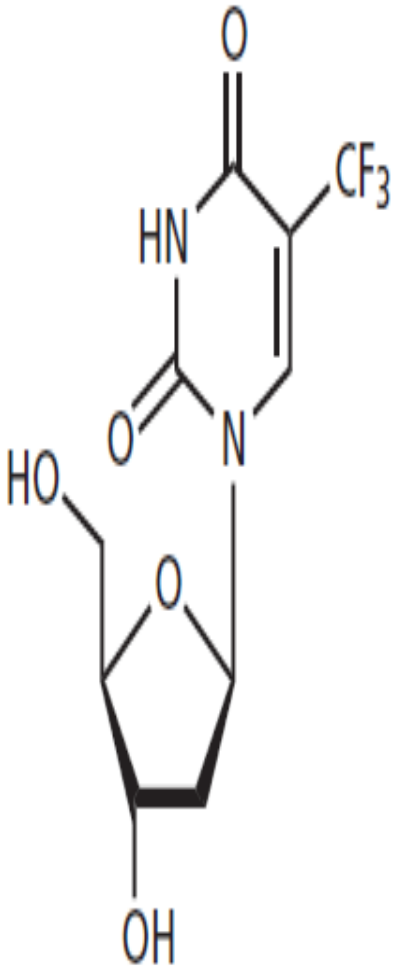
FIGURE 20.7 Comparison of cidofovir and deoxycytidine monophosphate.

Inhibitors of viral DNA polymerase

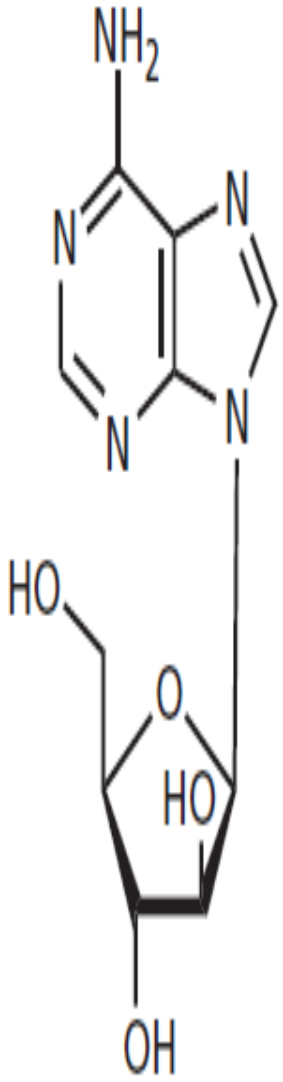
- In contrast to aciclovir, **idoxuridine , trifluridine , and vidarabine are phosphorylated equally well** by viral and cellular thymidine kinases, and so there is less selectivity for virally infected cells. As a result, these drugs have more toxic side effects. **Idoxuridine**, like trifluridine, is an analogue of deoxythymidine and was the first nucleoside-based antiviral agent licensed in the USA. The triphosphate inhibits viral DNA polymerase, as well as thymidylate synthetase.
- **Vidarabine** contains an arabinoside sugar ring and was developed from a natural product isolated from a marine sponge.
- **Foscarnet** inhibits viral DNA polymerase. However, it is nonselective and toxic. It also has difficulty crossing cell membranes due to its high charge.



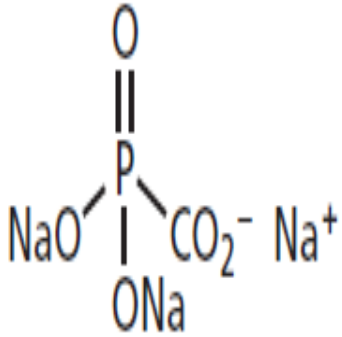
Idoxuridine



Trifluridine



Vidarabine



Foscarnet

FIGURE 20.8 Miscellaneous antiviral agents.

Inhibitors of tubulin polymerization

- The plant product **podophyllotoxin has** been used clinically to treat genital warts caused by the DNA virus **papilloma virus , but it is not as effective as imiquimod . It is a powerful inhibitor of** tubulin polymerization.

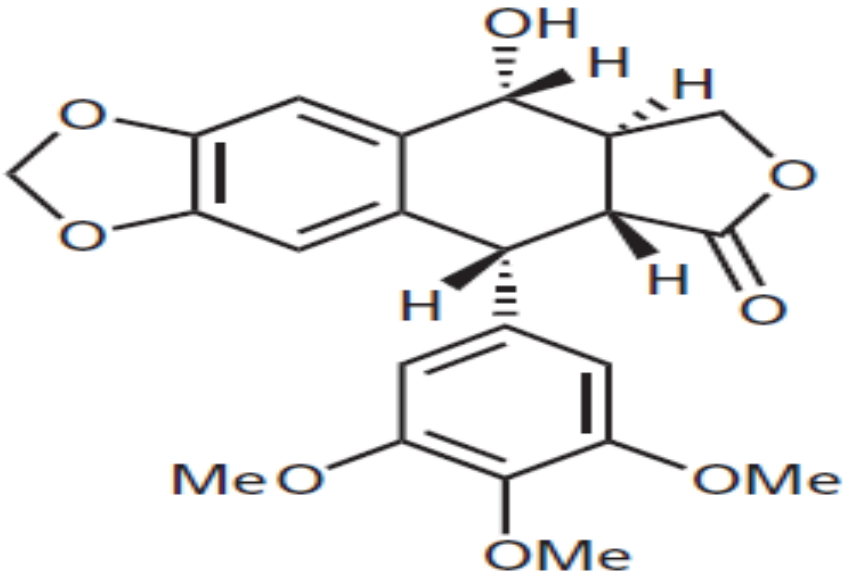


FIGURE 20.9 Podophyllotoxin.

Antisense therapy

- **Fomivirsen is the first, and so far the only,** DNA antisense molecule that has been approved as an antiviral agent. It consists of 21 nucleotides with a phosphonothioate backbone rather than a phosphate backbone to increase the metabolic stability of the molecule.
- The drug blocks the translation of viral RNA and is used against retinal inflammation caused by CMV in AIDS patients. Because of its high polarity, it is administered as an ocular injection (**intravitreal injection).**

d(*P*-thio)(G-C-G-T-T-T-G-C-T-C-T-T-C-T-T-C-T-T-G-C-G)

FIGURE 20.10 Fomivirsen.