The Medicinal Chemistry of Antibiotics

Introduction

The development of antibiotics over the past eight decades has been one of medicinal chemistry's greatest success stories. However, on a cautionary note, the pathogens are fighting back and we humans are locked in a never-ending arms race with these microscopic adversaries. While deaths from bacterial infections have declined markedly in the developed world, deaths from bacterial infections are sill relatively common in the developing world. The World Health Organisation (WHO) has estimated that tuberculosis (TB) was responsible for around 2 million deaths in 2002, and that in 2000 1.9 million children died of respiratory infections, with 70% of the deaths occurring in Africa and Asia. In the developed world, multiple-resistant Staphylococcus aureus (MRSA) is a growing problem, with most new infections acquired in hospitals. Deaths from MRSA infections are becoming more common among elderly or immunocompromised patients, and this has attracted widespread publicity.

The topic of antibiotics is extensive, and so in this course we shall focus on two main classes; the sulfonamides, and the β-lactams. The latter include the penicillins and cephalosporins, which are still widely used today despite the growing problem of resistance, as bacteria evolve effective biochemical defences against these drugs.

Bacterial pathogens

Bacteria are single-cell microorganisms that were first observed by Anton van Leeuwenhoek in the 1670s, using the microscope, which he had developed. In comparison with plant and animal cells, they are relatively simple in structure. Bacterial cells lack clearly defined nuclei and organelles which animal cells possess. The bacterial cell also has a quite distinct biochemistry; possessing enzymes, which enable it to synthesize essential vitamins which animal cells, can obtain directly from food. Bacterial cells have cell membranes and cell walls, whereas animal cells have only membranes. The cell wall is crucial to the bacterial cell's survival, enabling them to colonise a very wide range of environments and osmotic pressures. The cell wall prevents the uncontrolled flow of water into the cell, and provides protection against a myriad of hostile environmental factors such as heat, cold, acidity, alkalinity, salinity and radiation.

Bacteria can be characterised by a staining technique, which allows them to be defined as Gram positive or Gram negative. The staining technique involves the addition of a purple dye followed by washing with acetone. Bacteria with a thick cell wall (20-40nm) absorb the dye and are stained purple and are classed as Gram positive. Bacteria which possess a thin cell wall (<10nm) absorb only a small amount of the dye, which is washed out by acetone. They are stained pink by a second dye and are defined as Gram negative. These latter also possess an outer membrane, made up of liposaccharides, which is similar to the cell wall. Such differences in cell walls play a key role in the targeting of both types of bacteria by antibacterial agents.

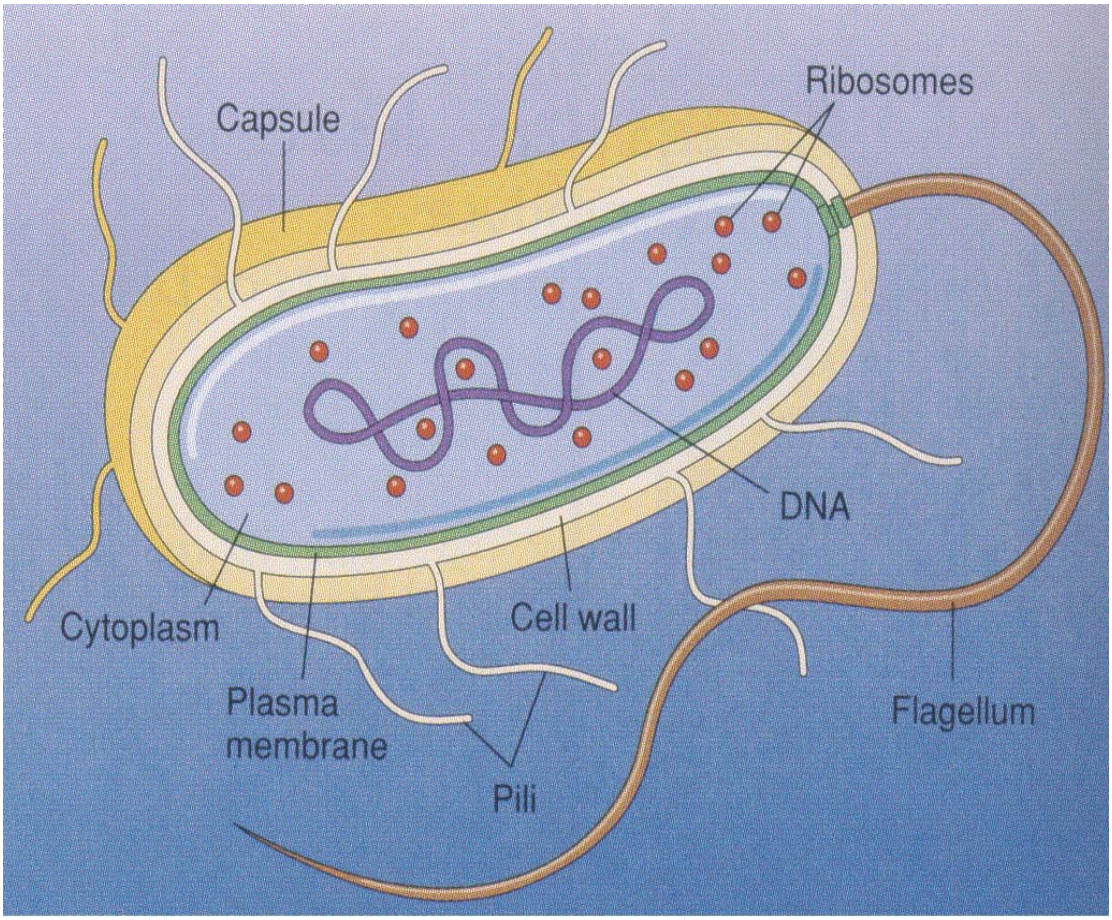


Fig 1 The structure of a bacterial cell

Mechanisms of Antibacterial action

There are five principle mechanisms by which antibiotics act:

- *Inhibition of cell wall synthesis*
  This results in the construction of faulty cell walls, which are unable to control the flow of water and nutrients in/out of cell. Lysis and cell death results.
  Examples include *penicillins*, *cephalosporins* and *vancomycin*.

- *Targeting of plasma membrane*
  The membrane becomes permeable, resulting in cell death.
  Examples include *polymyxins* and *tyrothricin*.

- *Antimetabolites*
  Selectively target bacterial-enzyme catalysis, impeding bacterial growth.
  The best examples are the *sulfonamides.*

- *Inhibition of protein synthesis*
  Selectively block synthesis of essential proteins and enzymes.
  Examples include *chloramphenicol* and *tetracyclines.*

- *Inhibition of nucleic acid functions*
  Selectively target transcription and replication, which impede cell division.
  Examples include intercalators such as *proflavine*.

Sulfonamides

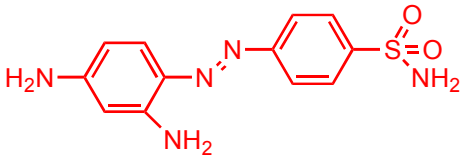


Figure 2.0 Prontosil (red dye)

Also known as *sulfa drugs*, the sulfonamides have been around since 1935 when it was discovered that a red dye called prontosil had antibacterial properties *in vivo*, when fed to laboratory animals, but, somewhat surprisingly, no effects *in vitro*. Eventually it was determined that prontosil was metabolised by intestinal bacteria to yield *sulfanilamide*, which was the real antibacterial agent. Sulfanilamide was synthesized in the laboratory and became the first synthetic antibiotic to be prescribed for the treatment of a myriad of bacterial infections. Medicinal chemists then began to synthesize many analogues of sulfanilamide, enabling them to draw up an accurate *structure-activity* profile, which led to the following conclusions:

- The *para*-amino group is essential for activity and must be unsubstituted.
- The aromatic ring and sulfonamide functional group are essential.
- The aromatic ring can only be *para*-substituted.
- The sulfonamide nitrogen can only be primary or secondary, and the only region that can be varied is the R-group appended to the sulfonamide nitrogen.

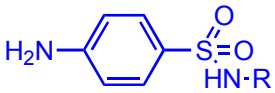


Figure 3.0 The essential structure of antibiotic sulfanilamides; only the R group can be varied.

How do they work?
The figure below shows the biochemical pathway utilized by bacteria to synthesize tetrahydrofolate, an essential enzyme co-factor that provides one-carbon units for the synthesis of the pyrimidine bases (thymine and cytosine) in DNA.

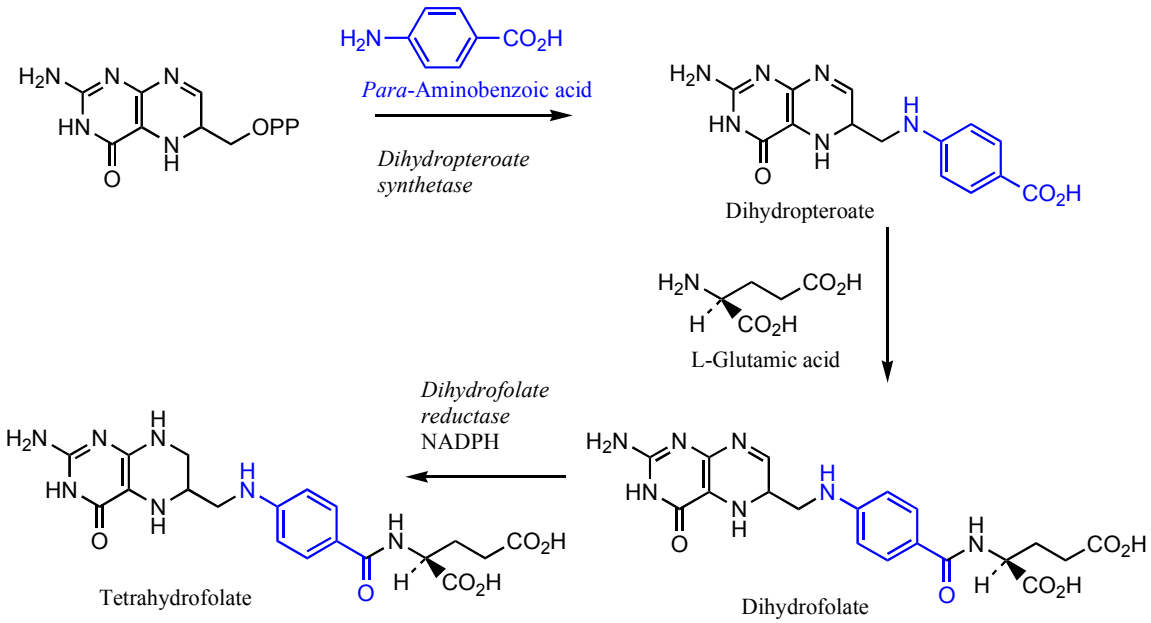


Fig 4.0 Bacteria synthesize the essential co-factor tetrahydrofolate using *para*-aminobenzoic acid (PABA).

If this pathway is impeded, bacterial DNA synthesis stops and they can no longer proliferate. Sulfonamides inhibit this pathway by acting as mimics of p-amino benzoic acid. Note the similarity in structure between sulfonamides and PABA in the figure below. The bacterial *dihydropteroate synthetase* accepts the sulfonamides into its active site, and once bound, it prevents PABA from binding and so dihydropteroate synthesis is inhibited. Sulfonamides act as competitive inhibitors of PABA.

Fig 5.0 Sulfonamides act as competitive inhibitors of PABA, and stop bacterial proliferation.

Higher animals, including humans, synthesize tetrahydrolate co-factor from folic acid (obtained in food) using a very different biochemical pathway. The folic acid is carried across animal cell membranes by transport proteins. Animal cells lack the enzyme *dihydropteroate synthetase* and so are unaffected by sulfonamides.

Because sulfonamides inhibit bacterial growth, they do not actively kill the pathogens, but rather prevent them from actively multiplying and growing and enable the bodies' own defence mechanisms (hunter-killer CD8 cells) to eliminate the microbial invaders. They are referred to as *bacteriostatic* rather than *bacteriocidal*. They are not suitable for use in immuno-compromised patients, like those suffering from AIDS, or who have just undergone chemotherapy and organ transplant. Examples of sulfonamide drugs used in the clinic today are shown below.

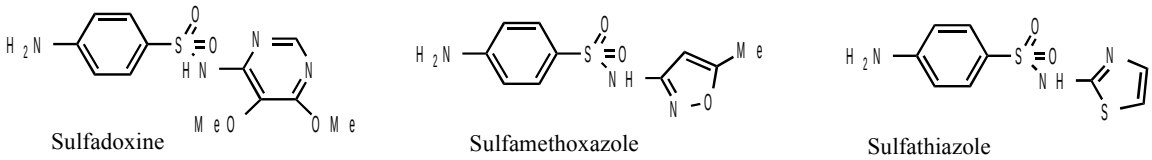


Fig 6.0 Examples of sulfonamide drugs that are currently used.

They are useful in treating infections of the eyes, mucous membranes, gastrointestinal and urinary tracts.

Sulfonamide Resistance
Bacteria can obtain resistance to sulfonamides by synthesizing more PABA. Higher concentrations of PABA can compete better with sulfonamides for the target enzyme's active site. The dosage of sulfonamide drug must then be increased in order to restore competitive inhibition. Some bacteria express resistance through mutations in the target enzyme, whereby it has a lower affinity for the drug, and through changes in the cell membrane, which prevent uptake of the drug.

Inhibitors of cell wall synthesis: The β-lactam antibiotics
This group of antibiotics is the best known and most widely used, and may be divided into two main groups; the penicillins and the cephalosporins. The groups differ only in the nature of the rings appended to the β-lactam moiety. These drugs were at the forefront of the healthcare revolution of the 20th century, which saw previously incurable and debilitating diseases remedied and controlled. However the rapid emergence of resistance, particularly in more recent years, has rendered these former wonder drugs less efficacious, and new classes of antibacterials, which act through different means, had to be developed in order to stay ahead in the never ending struggle with microbial pathogens.

Structure of penicillins
Penicillin molecules all contain a highly strained 4-membered β-lactam ring fused to a 5-membered thiazolidene ring. The β-lactam ring is unstable and is primarily responsible

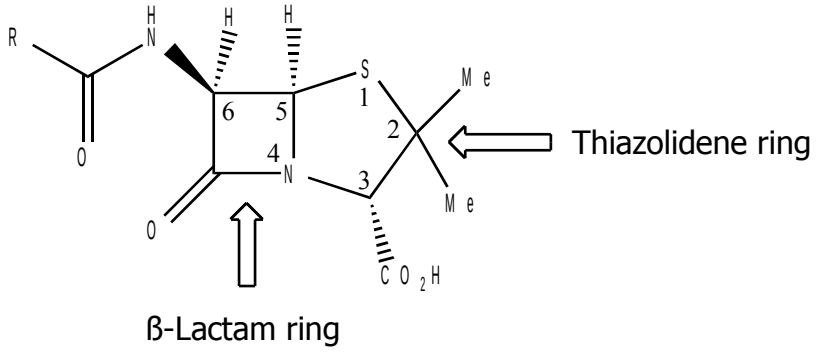


Fig 7.0 The structure of penicillins

for the antibiotic potency of these molecules. To appreciate this, recall the nature of normal amides. The lone pair on the nitrogen atom overlaps with the carbonyl group,

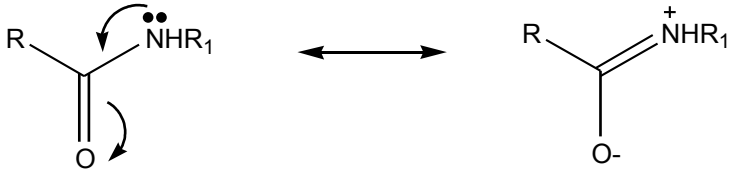


Fig 8.0 In normal amides, the nitrogen lone pair overlaps with the carbonyl system.

resulting in a double bond between the nitrogen and the carbonyl carbon, and a positive charge on the nitrogen and a negative charge on the oxygen. This overlap between the nitrogen lone pair and the carbonyl system has two consequences. Firstly, it renders the carbon atom less electrophilic, and therefore less susceptible to

nucleophilic attack in comparison with aldehydes, ketones and esters. Secondly, it makes the oxygen atom the nucleophilic centre of an amide.

In the 4-membered ring β-lactam system, however, the bond angles are 90° instead of 120°, which results in 30° ring strain. This prevents the overlap of the nitrogen lone pair with the adjacent carbonyl system, and thus the β-lactam carbonyl is much more electrophilic than a normal amide, and is therefore susceptible to nucleophilic attack. The lone pair on the nitrogen is readily protonated under acidic conditions, making the β-lactam ring sensitive to strong acids. Thus, the β-lactam ring system in penicillins and cephalosporins is very susceptible to ring-opening under strongly acidic and basic conditions, and by strong nucleophiles.

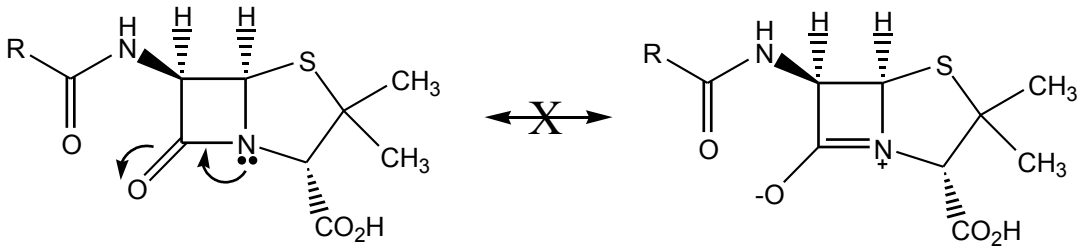


Fig 9.0 Ring strain prevents the normal amide resonance from taking place in β-lactam rings.

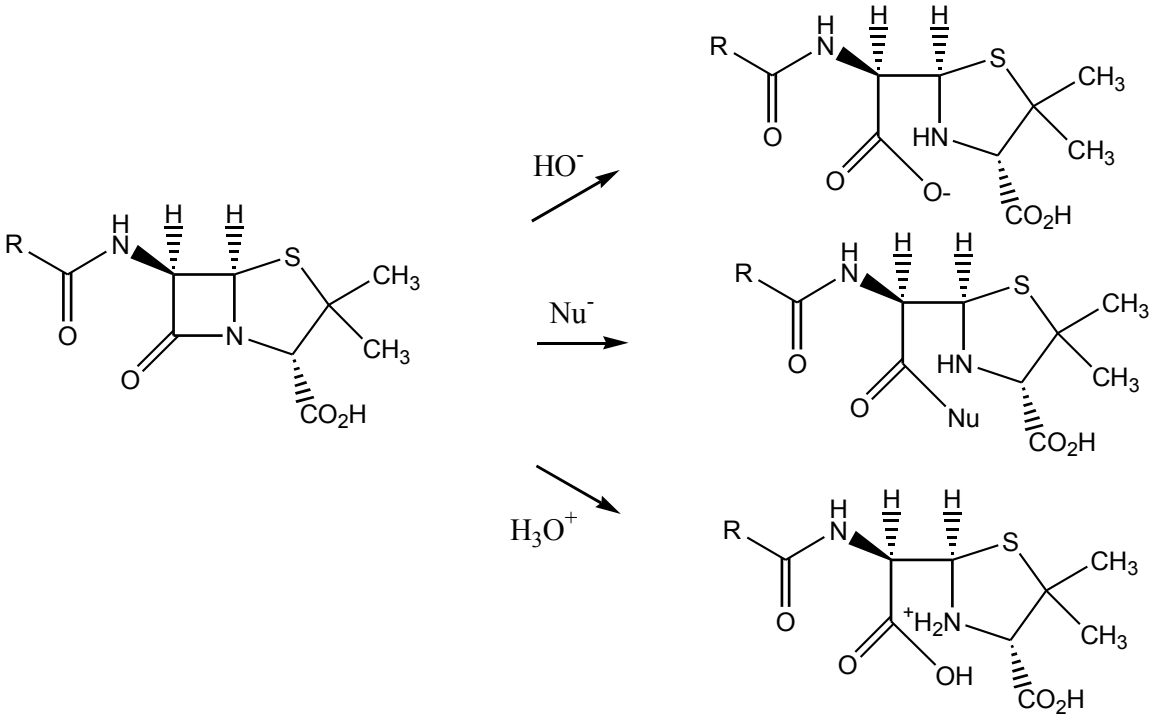
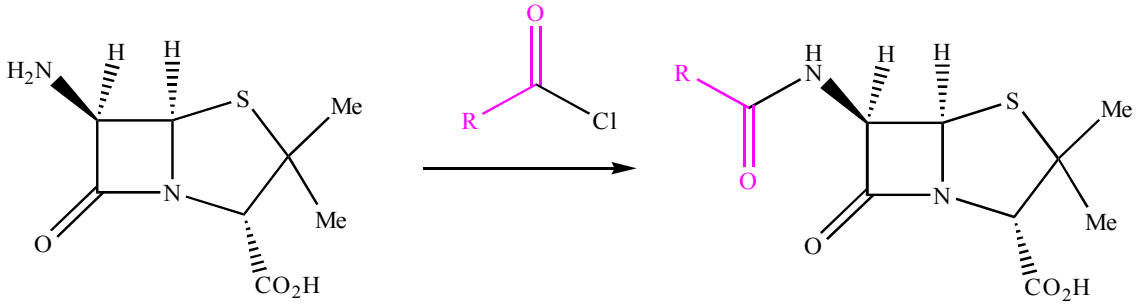


Fig 10.0 The β-lactam ring in penicillins readily opens in the presence of acids, bases and strong nucleophiles.

Synthesis of penicillin analogues

Penicillin analogues were originally synthesized by a fermentation process, in which different carboxylic acid derivatives were added to yield penicillins containing different 6-amido derivatives. The principle disadvantage of this approach was that not all carboxylic acids were biologically acceptable, and thus only a limited number of analogues could be prepared.



6-Aminopenicillanic acid (6-APA)

Fig. 11 6-APA and all possible acyl chlorides are used to prepare novel penicillin derivatives by organic chemical synthesis.

The semi-synthetic approach relies on the isolation of 6-aminopenicillanic acid (6-APA) from fermentation media. A large number of 6-amido derivatives can then be prepared by chemical reaction with acyl chlorides.

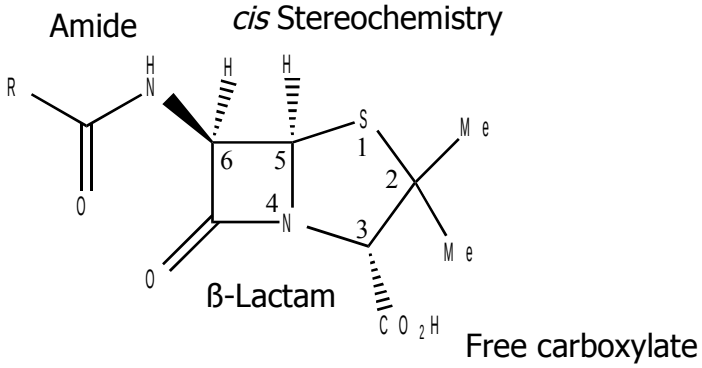


Fig. 12 Structural features essential for activity in penicillins

Figure 12 above displays the structural requirements essential in penicillins. The bicyclic ring system containing the β-lactam is crucial, as are the cis relationship between the two hydrogens at positions 5 and 6, a free 3-carboxylate and a 6-amide. Changing one or more of these results in a loss of activity.

How do they work?

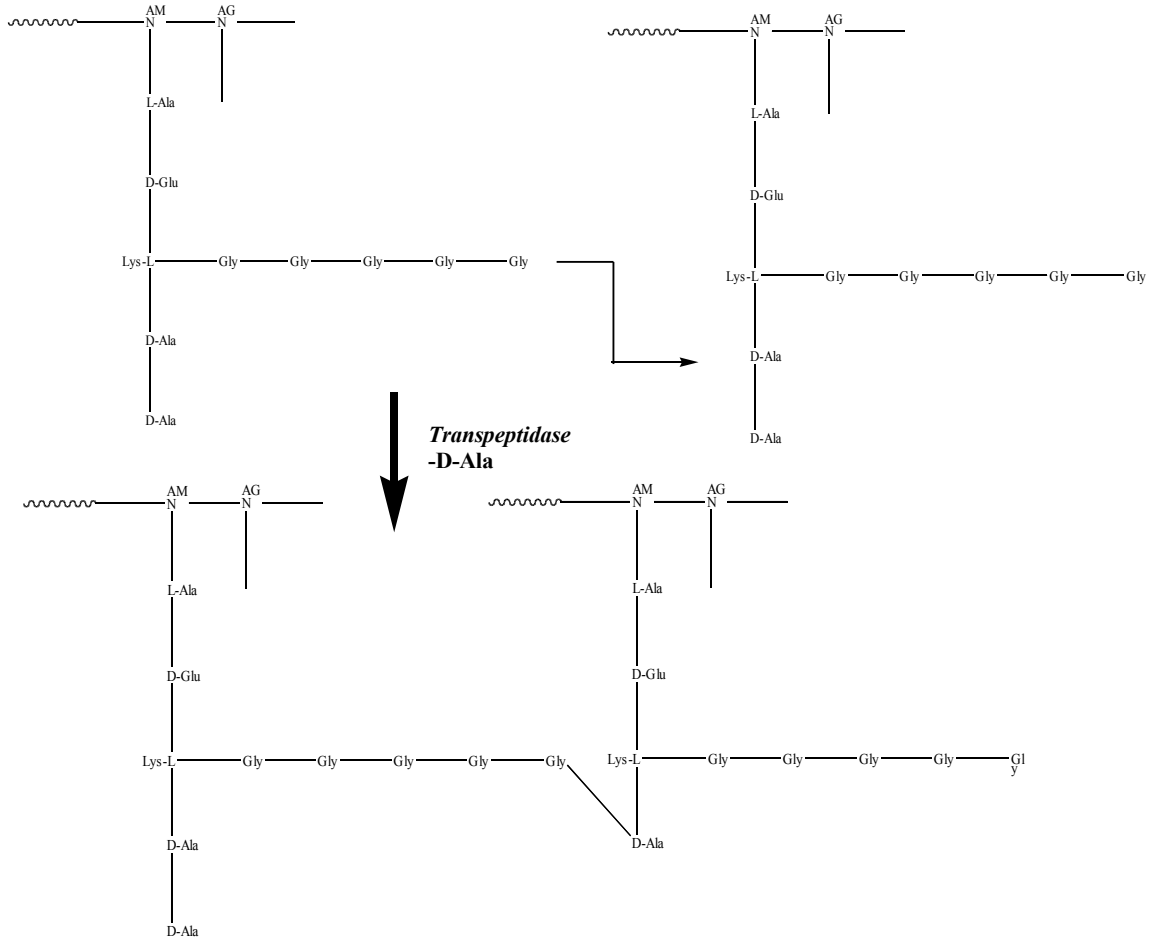


Fig. 13 Bacterial transpeptidase enzymes are essential for building cell walls by cross-linking peptide chains together, with the loss of one D-alanine residue.

The bacterial cell wall consists of sugar and peptide units (referred to as *peptidoglycans*) joined together in a specific manner. The exact structure comprises a parallel series of sugar backbones to which peptides are appended. Two different sugars are present; *N*-acetylmuramic acid (NAM), and *N*-acetylglucosamine (NAG). The peptide chains are bound only to the NAM sugars. These peptide chains contain amino acids with the D-stereochemistry. Throughout nature on planet Earth, L-amino acids prevail almost universally (in proteins and enzymes in all organisms), but there are some notable exceptions, like the peptide components of bacterial cell walls. Bacteria contain racemase enzymes that can convert L-amino acids into D-amino acids, a biochemical feature that is absent in higher organisms like mammals. Bacterial cell wall biosynthesis is completed when the peptide chains appended to the sugar backbones are cross-linked together. This occurs when the terminal D-alanine in one chain is displaced by a glycine in another. The enzyme responsible for this cross-linking is known as *transpeptidase*. The exact mechanism is shown in Figure 14 below. An activated serine hydroxyl group attacks the carbon of the amide bond between the two terminal D-alanine residues, resulting in the nucleophilic displacement of one D-alanine and the formation of a peptide chain-transpeptidase enzyme complex. This complex is linked by an ester bond that is rapidly attacked by the amino terminus of the pentaglycine chain, releasing the enzyme and completing the cross-linking between the two peptide chains.

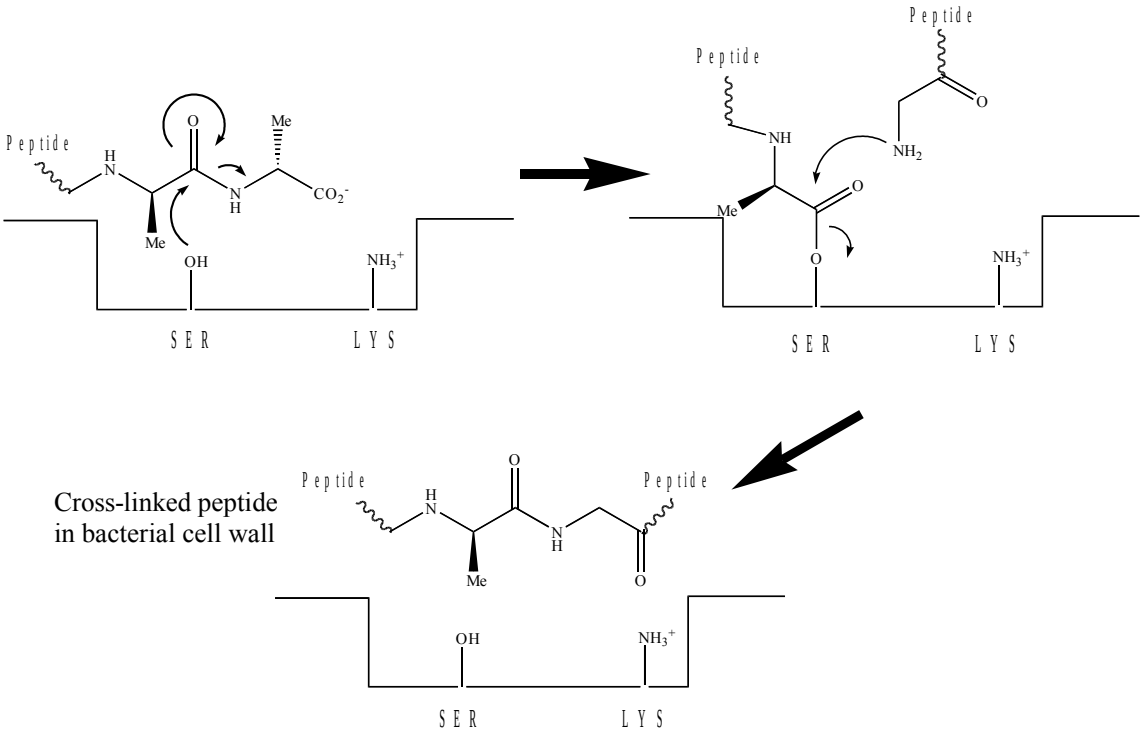


Fig. 14 The mechanism of transpeptidase catalyzed cross-linking of peptide chains in bacterial cell wall synthesis.

Penicillins have a structural resemblance to two D-alanine residues linked together, and are mistaken by the transpeptidase enzyme for D-Ala-D-Ala, and thus incorporated into the active site. Once bound, the β-lactam carbonyl is attacked by the serine hydroxyl, and ring opening occurs to leave the penicillin covalently bound to the enzyme. The bulky thiazolidene ring now blocks access to the active site by either a pentaglycine chain or water. As a result the penicillin becomes irreversibly bound to the transpeptidase enzyme, preventing it from functioning properly. This results in incomplete cell walls that are much more fragile and porous, and eventually lead to swelling followed by cell lysis and death.

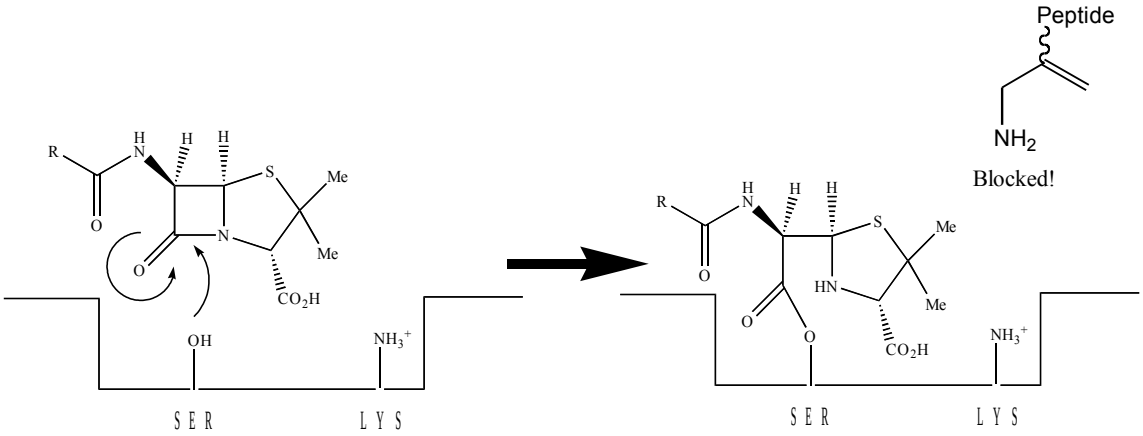


Fig. 15 The mechanism of penicillin inhibition.

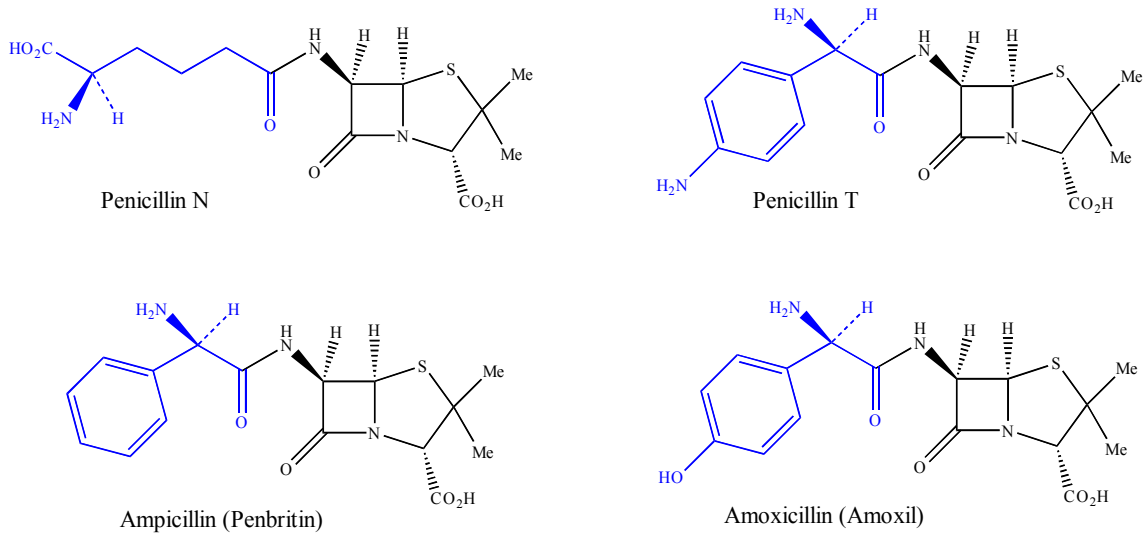


Fig 16 Penicillin derivatives containing electron withdrawing acyl side chains can be administered orally.

Amino penicillins

These penicillin derivatives have proved to be superior clinically in comparison with simpler derivatives like penicillin G (benzylpenicillin). They were among the first to become available in tablet form owing to their improved acid stability. They showed great selectivity towards microbes and relatively little toxicity to mammalian hosts. They have been used to successfully treat bronchitis, pneumonia, typhoid, gonorrhoea and urinary tract infections. However, the same amino group that enhanced their stability in acidic conditions also results in their relatively poor absorbance through the walls of the gastrointestinal tract, and as a result diarrhoea is a common side effect, as they will act upon and diminish the numbers of essential bacteria present in the intestines.

For penicillin derivatives in general it has been found that hydrophobic side chains confer good activity against Gram positive bacteria, but poor activity against Gram negative strains. For penicillins containing hydrophilic side chains the reverse is true; good activity is observed against Gram negative bacteria and relatively poor activity against Gram positive species.

Overcoming penicillin resistance

Penicillin resistance first became a serious problem in the early 1960s. Overuse of penicillins (particularly penicillin G) led to rapid emergence of penicillin resistant bacterial strains which contained an enzyme, β-lactamase which deactivated the drug by cleaving the β-lactam ring.

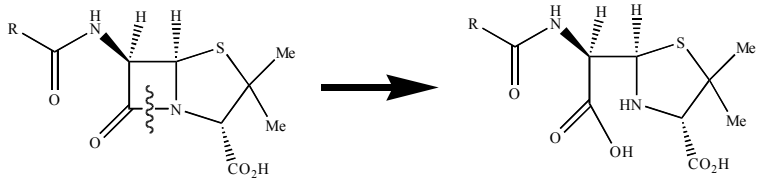


Fig 17 Penicillin resistant bacteria possess β-lactamase enzymes which deactivate the drug by opening the β-lactam ring.

Fortunately, it was discovered that penicillin derivatives containing bulky groups on the side chains were poor substrates for β-lactamases. However, such additional

bulk could also make the penicillins less efficacious as substrates for the target transpeptidase enzyme. Thus, a great deal of work needed to be done to find the appropriate blocking groups that would prevent binding with the β-lactamase enzyme while enabling interaction with the target enzyme. Two examples of these blocking penicillins are methicillin and nafcillin. Both must be administered intravenously, as their side chains are electron releasing, making them too acid sensitive for oral administration.

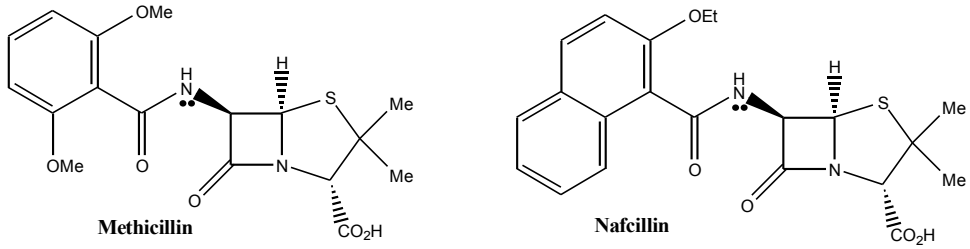


Fig 18 The bulky groups on methicillin and nafcillin shield them from the β-lactamase active site.

Cephlasporins

Cephalosporins were the second major group of β-lactam antibiotics to be discovered.

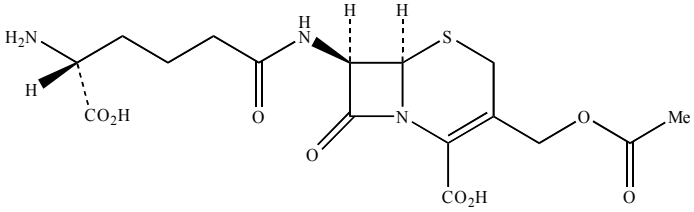


Fig 19 The structure of cephalosporin C

Cephalosporins contain a β-lactam ring, like the penicillins. The main difference being that while the latter contain a 5-membered thiazolidene ring, cephalosporins have a 6-membered dihydrothiazine.

Their mode of action against bacteria is essential the same as that of penicillins. These molecules impede the transpeptidase enzymes, causing bacteria to construct faulty cell walls which rupture, causing death.

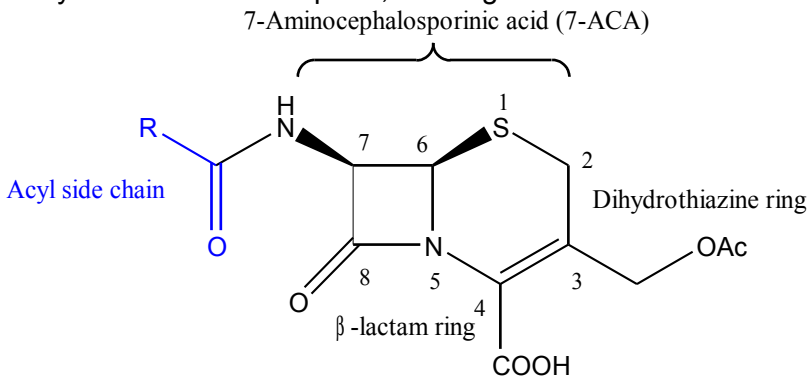


Fig 20 Structural features of cephalosporins that are essential for biological activity.

Fig 20 above shows the structural moieties of cephalosporins that are critical for antimicrobial activity. They include the β-lactam ring, the dihydrothiazine ring

containing a C3-C4 double bond, the presence of a C4-carboxyl and a C7-amino side chain. The only features that can be varied are the 7-acylamino side chain, the 3-acetoxymethyl side chain, and additional substitution on C7.

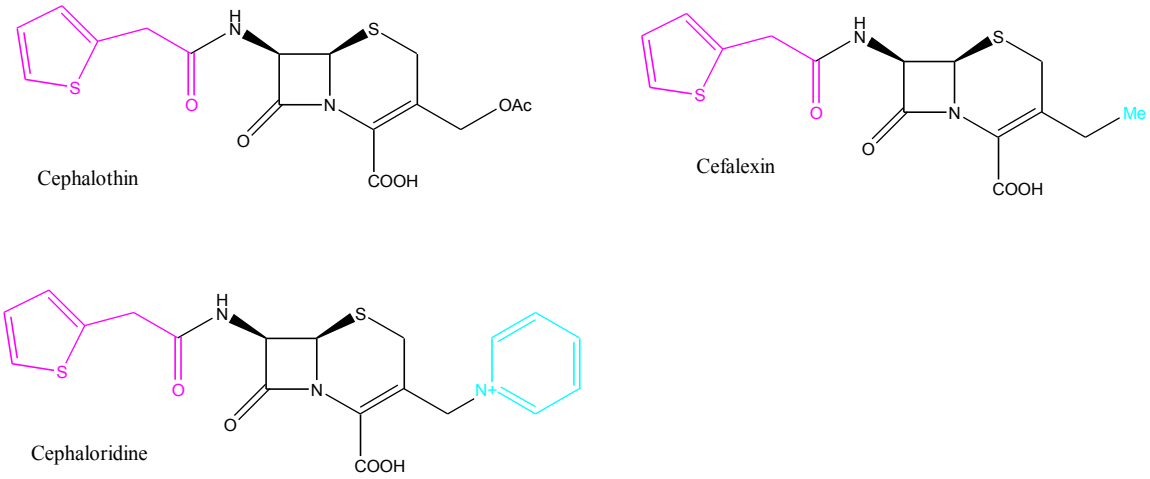


Fig 21 Names and structures of some first-generation cephalosporins

First-generation cephalosporins
The names and structures of three first generation cephalosporins are shown in Fig 21 above. In general, these drugs have a lower antibiotic activity than penicillins, but a greater range. While their best activity is against Gram-positive bacteria, they also display better activities against Gram-negative infections. They show poorer bioavailability than penicillins and are generally administered intravenously.

Cephalothin can be deactivated by host esterases which cleave the acetyl group at C3, leaving an unreactive primary alcohol. Replacing the ester with an esterase resistant pyridinium group gives cephaloridine. The pyridine is an excellent leaving group, but as it contains a positive charge on the nitrogen (making the molecule a zwitterion) it is very poorly absorbed through the GI tract, and must therefore be injected.

The Medicinal Chemistry of Antiviral agents

Introduction
Viruses are non-cellular infectious agents that are incapable of self-replication, rather they must take over a suitable host cell and use its genetic machinery in order to multiply. Such host cells include bacterial, plant and animal cells, and there are over 400 viruses that are known to infect humans.

Viral infections can be transmitted in 4 distinct ways. They may be transmitted by aerial contact, as a result of an infected host coughing or sneezing. Examples include the viruses responsible for colds, chickenpox, flu, measles, mumps and viral pneumonia. Secondly, they may be transferable by close physical contact (intimacy or transfusion of blood). Examples include the viruses responsible for genital herpes, AIDS and rabies. Thirdly, they can be transmitted by parasitic arthropods, in particular ticks. These viruses give rise to diseases like yellow fever and tick fever.

Finally, they may be transmitted via food and water, leading to diseases like hepatitis A, polio and viral gastroenteritis.

Viral infections have led to serious 'plagues' throughout human history. The Roman Empire suffered two bouts of smallpox infections during the periods 165-180AD and 251-266AD. This disease was also responsible for the decimation of Native American tribes in North and South America during the period of European colonization. 90 years ago a flu pandemic, known as Spanish flu, killed over 20 million globally from 1918-1919, superseding the death toll resulting from the First World War.

More recently, in 2003, the outbreak of severe acute respiratory syndrome (SARS) could have had a similar effect to that of Spanish flu had the world health community not responded quickly. Viruses such as Ebola and Lassa fever have very high mortality rates, but fortunately the latency period between infection and display of symptoms is short and so sufferers can usually be isolated quickly. However, in the modern day, with frequent air travel between the Western countries and remote areas of the world does indeed increase the risk of Ebola, or indeed new viral diseases, spreading rapidly around the globe. Medical scientists can envision a potential 'supervirus' and what characteristics it would display. Firstly, it should be spread by airborne contact just like flu. Secondly, it would have a latency period similar to that of HIV, measured in years rather than days. Finally, it should have a mortality rate similar to that of Ebola. Such a virus really would give rise to an Armageddon syndrome which would wipe out more than 90% of the infected population, and the survivors would inherit a terrifying and chaotic aftermath, just like in the very recent BBC1 series *Survivors.*

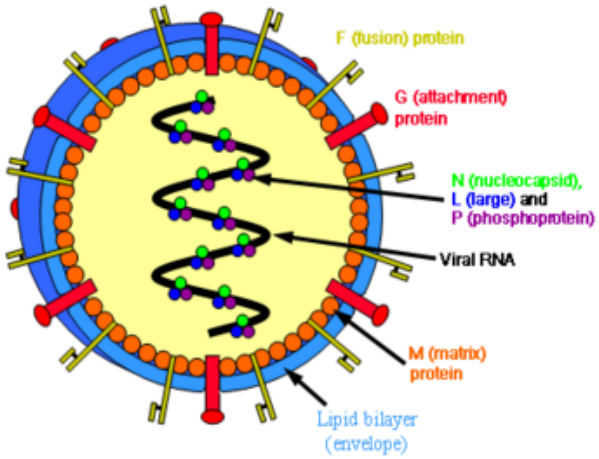


Fig 22 Schematic representation of a virus

Structure of viruses

Essentially, a virus particle consists of nucleic acid packaged in a protein/lipid case. An example is shown in detail for an RNA virus in Fig 22 above. They are extremely small, from 10-400nm and are generally only visible under an electron microscope. Viruses contain either DNA or RNA as their genetic material, and so we refer to either DNA viruses or RNA viruses. Most DNA viruses contain double stranded (ds) DNA, in which 2 complementary strands of DNA are held together via Watson-Crick base pairing. In contrast, most RNA viruses contain single stranded (ss) RNA. If the base sequence of the latter is complementary to viral messenger RNA, then it is known as the negative (-) strand, but if the base sequence of the viral RNA strand is identical to viral messenger RNA, then it is referred to as the positive (+) strand.

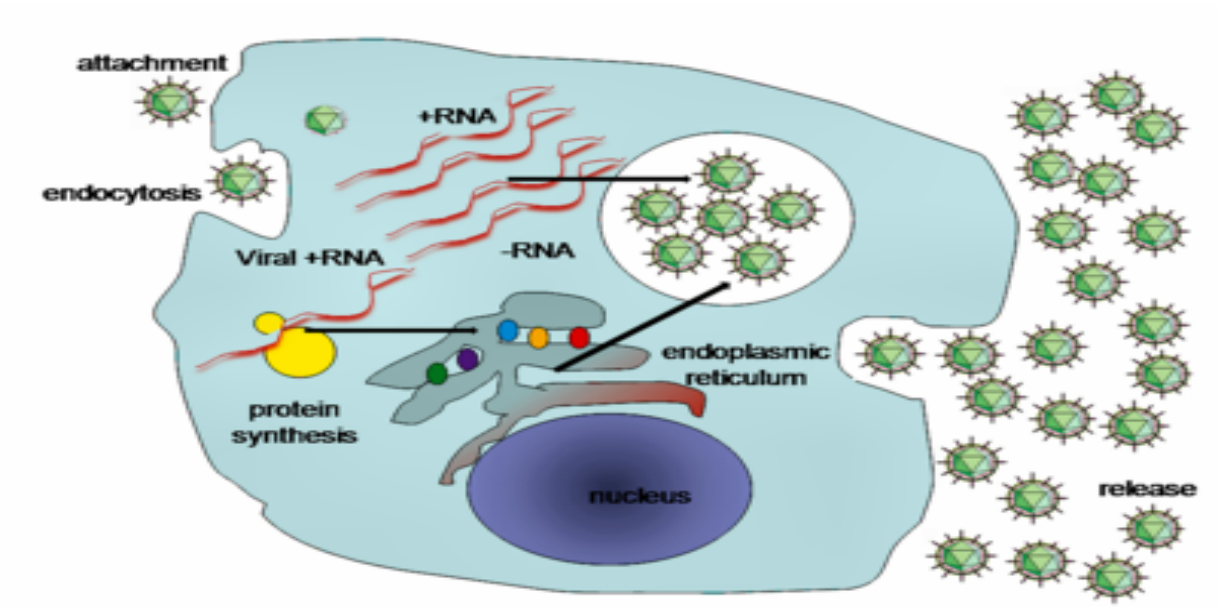


Fig 23 The five stages in the viral life cycle.

Viral Life cycle

There are 5 steps in the life cycle of a virus:

Binding The virus initially binds to a receptor on the surface of the host cell using a specific molecule on its' outer coat, which is usually a glycoprotein. The interaction is often referred to as a key-in-lock interaction. Once bound, the virus particle, or virion, can enter the next stage in the cycle, introduction of viral nucleic acid into the host cell.

Penetration Some viruses enter the cell intact and are then uncoated. Others inject their nucleic acid through the cell membrane. The net result is the release of viral nucleic acid into the cell, which is then ready to start the process of viral replication.

Replication & Transcription Viral nucleic acid gets integrated into the host genome which synthesizes viral nucleic acid, mRNA and viral proteins. The exact mechanism varies from virus to virus.

Synthesis Viral proteins and viral nucleic acid are assembled into new 'naked' virions called nucleocapsids. These are then released from the cell as fully developed virions in two possible ways.

Release Naked virions which lack any outer layer around the nucleocapsid are released by cell lysis, in which the host cell is destroyed. Viruses that contain an outer envelope are released by a process known as 'budding'. In the latter, viral outer coat proteins are first incorporated into the host cell's membrane. The nucleocapsid then binds to the inner surface of the host cell membrane and, simultaneously, viral proteins collect at the site and host cell proteins are excluded. The plasma membrane containing viral proteins then encases the nucleocapsid, and the newly formed virion is then pinched off from the cell.

Antiviral drug targets

The major challenge facing medicinal chemists attempting to treat viral infections is the fact that these pathogens reside inside host cells, utilizing their host's biochemical mechanisms to multiply. Therefore, the number of possible drug targets that are unique to the virus are fewer in comparison with microbes. The development of effective antiviral drugs has proved to be much more challenging than in the case of antibiotics. The targets must be viral proteins that are critical to the viral life cycle, especially in the early stages. These must be distinct from host proteins in order to obtain good selectivity with minimal side effects. Ideally, these viral proteins should

be common to a wide range of viruses as this increases the chances of developing a drug with a broad spectrum of antiviral activity.

DNA viruses

DNA viruses are responsible for many of the well known viral infections. These include chickenpox, shingles, glandular fever, cold sores, genital warts and eye infections. These viruses are known as the *herpes viruses*. Nucleoside analogues have been particularly effective in treating them.

Acyclovir

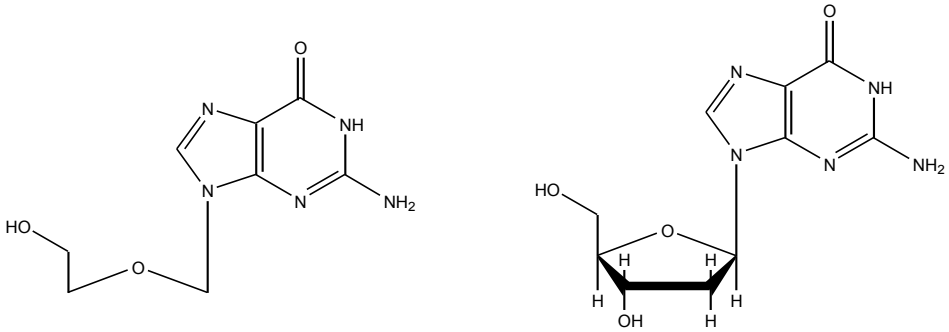


Fig 24 Acyclovir, an analogue of 2'-deoxyguanosine, is the world's most prescribed antiviral drug.

Acyclovir is an analogue of the naturally occurring nucleoside 2'-deoxyguanosine, a key component of DNA. Its' antiviral potency was discovered by compound screening and it was first prescribed in 1981. It is highly active against α-type herpes viruses, including HSV-1 and HSV-2 (which cause cold sores and genital warts respectively) and varicella zoster virus (VZV) which causes chickenpox and shingles. It interferes with viral proliferation by inhibiting the viral DNA polymerase enzyme, and is highly selective and non-toxic.

How it works

Look carefully at the structure of acyclovir in Fig 24 above. Acyclovir has the same nucleobase as deoxyguanosine but instead contains an incomplete sugar ring. This latter feature is of profound importance to its' antiviral efficacy. But let us first consider the important topic of DNA replication in general. Recall the nature of DNA itself. It is a polymer in which nucleosides are linked together via phosphate groups that append the 3'-O of one with the 5'-O of another. Two such strands interact with each other via Watson-Crick base pairings between an adenine residue on one strand with a thymine base on the other, and likewise between a guanine base on one strand with a cytidine residue on the other. These pairings arise as a result of hydrogen bonding between proton donors on one base (N-Hs) with proton acceptors (lone pairs of electrons on either O or N) on the other. The two strands intertwine to form a double helix structure in which the hydrogen-bonded base pairs form the steps, and the sugar-phosphate backbone the rungs.

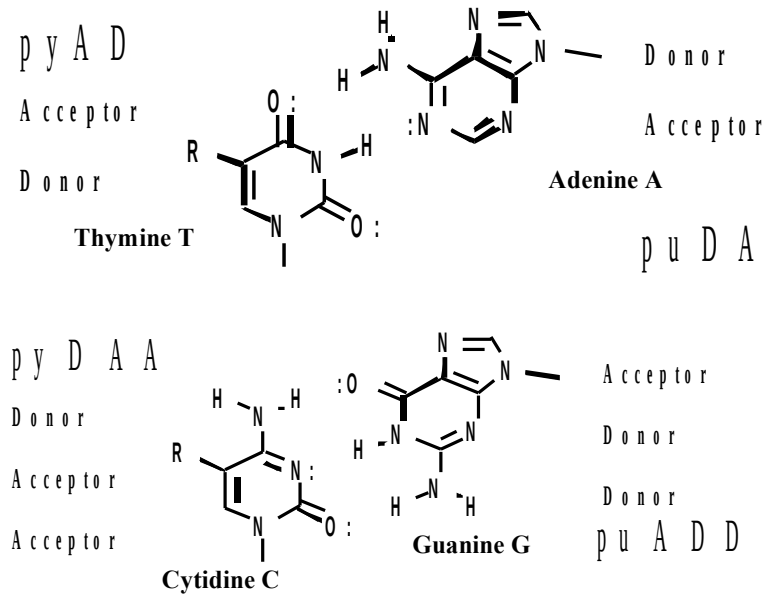


Fig 26 DNA strands are held together via Watson-Crick pairings between adenine and thymine, and cytidine and guanine.

Note that the cytidine-guanine (C-G) base pair is held together by three hydrogen bonds, while the adenine-thymine (A-T) base pair is held together by two hydrogen bonds. They are referred to as Watson-Crick pairs after James Watson and Francis Crick, who first elucidated the double helical nature of DNA in 1953 based on the X-ray crystallographic images obtained by Rosalind Franklin.

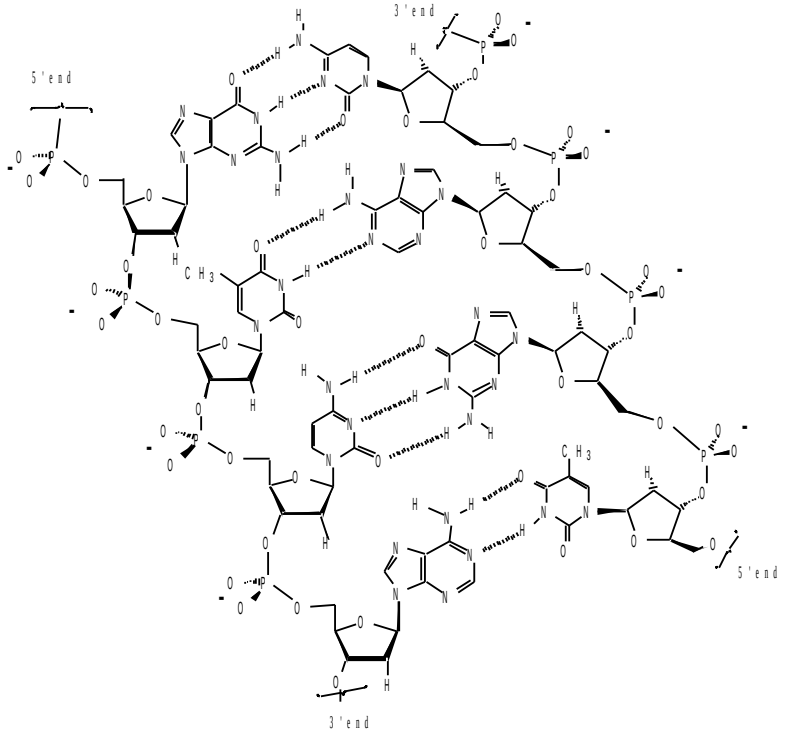


Fig 27 The DNA double helix is held together via hydrogen bonded interactions between base-pairs (A-T and C-G) on opposite strands. Each strand contains a sugar-phosphate backbone in which a phosphate group links the 3'-O of one sugar with the 5'-O of another.

DNA replication occurs using a DNA strand as a template for the construction of a new strand that is its' Watson-Crick complement. Thus for example, if we have a template strand that has the base sequence A-T-G-C-C-T-T-A-T-C, then the newly synthesized strand will have the base sequence T-A-C-G-G-A-A-T-A-G. The process is catalyzed by an enzyme called DNA polymerase. The new strand is synthesized using nucleoside 5'-O- triphosphates as building blocks. The four nucleoside 5'-O-triphosphates are shown in Fig 28 below. Nucleoside 5'-O-triphosphates are biosynthesized by three successive phosphorylations of the relevant nucleosides by enzymes known as *kinases.*

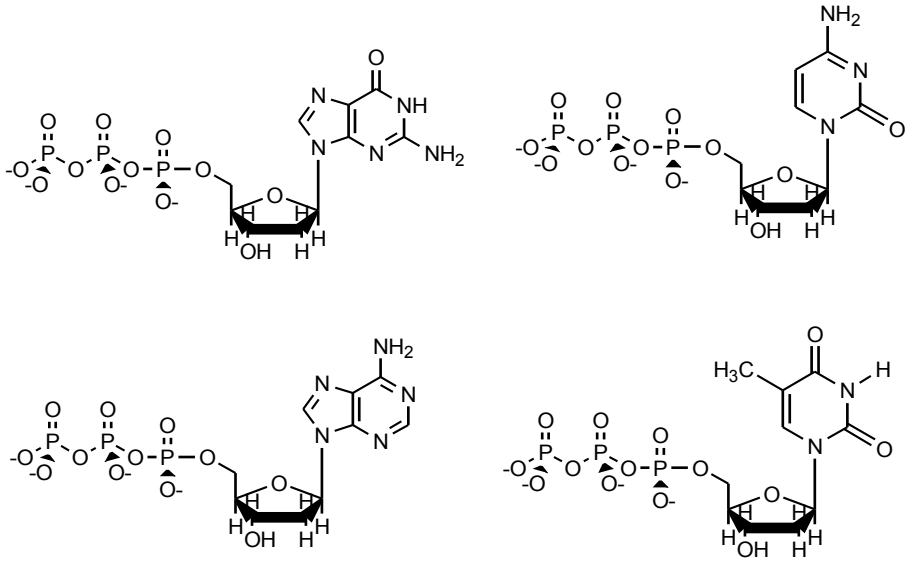
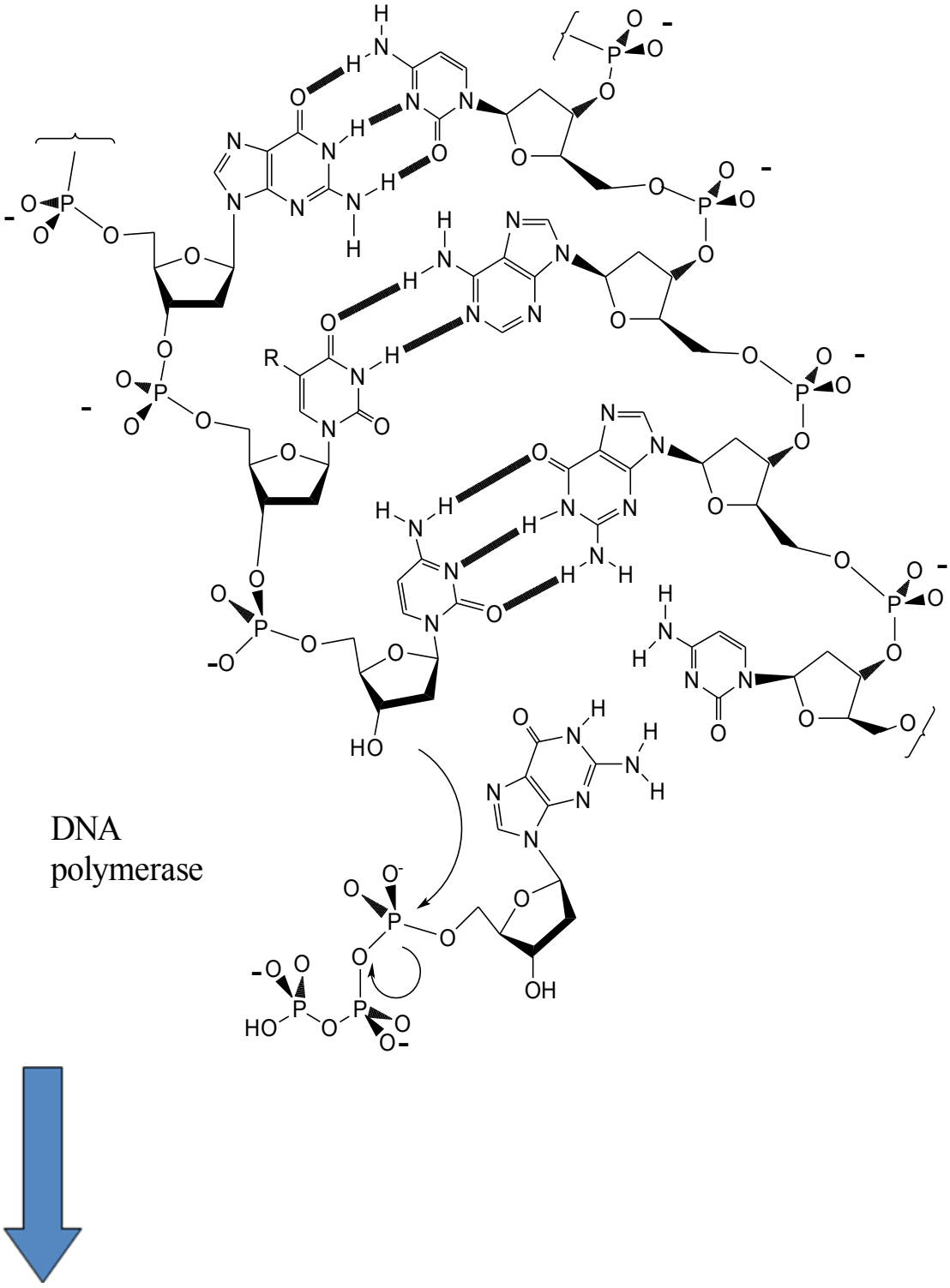


Fig 28 Nucleoside 5'-O-triphosphates are used as the building blocks in DNA synthesis which is catalyzed by DNA polymerase.

When a cytidine base (C) is present in the template strand, the DNA polymerase enzyme brings a deoxyguanosine-5'-O-triphosphate molecule into the active site, where it is positioned opposite its Watson-Crick partner. The 3'-OH of the growing synthesized strand is now primed by the enzyme for a nucleophilic attack on the first phosphorus atom of the triphosphate group; this results in a loss of diphosphate and the incorporation of deoxyguanosine-5'-O-phosphate into the growing strand. The presence of a free 3'-OH group enables further chain extension to occur using the requisite nucleoside-5'-O-triphosphate.


DNA
polymerase

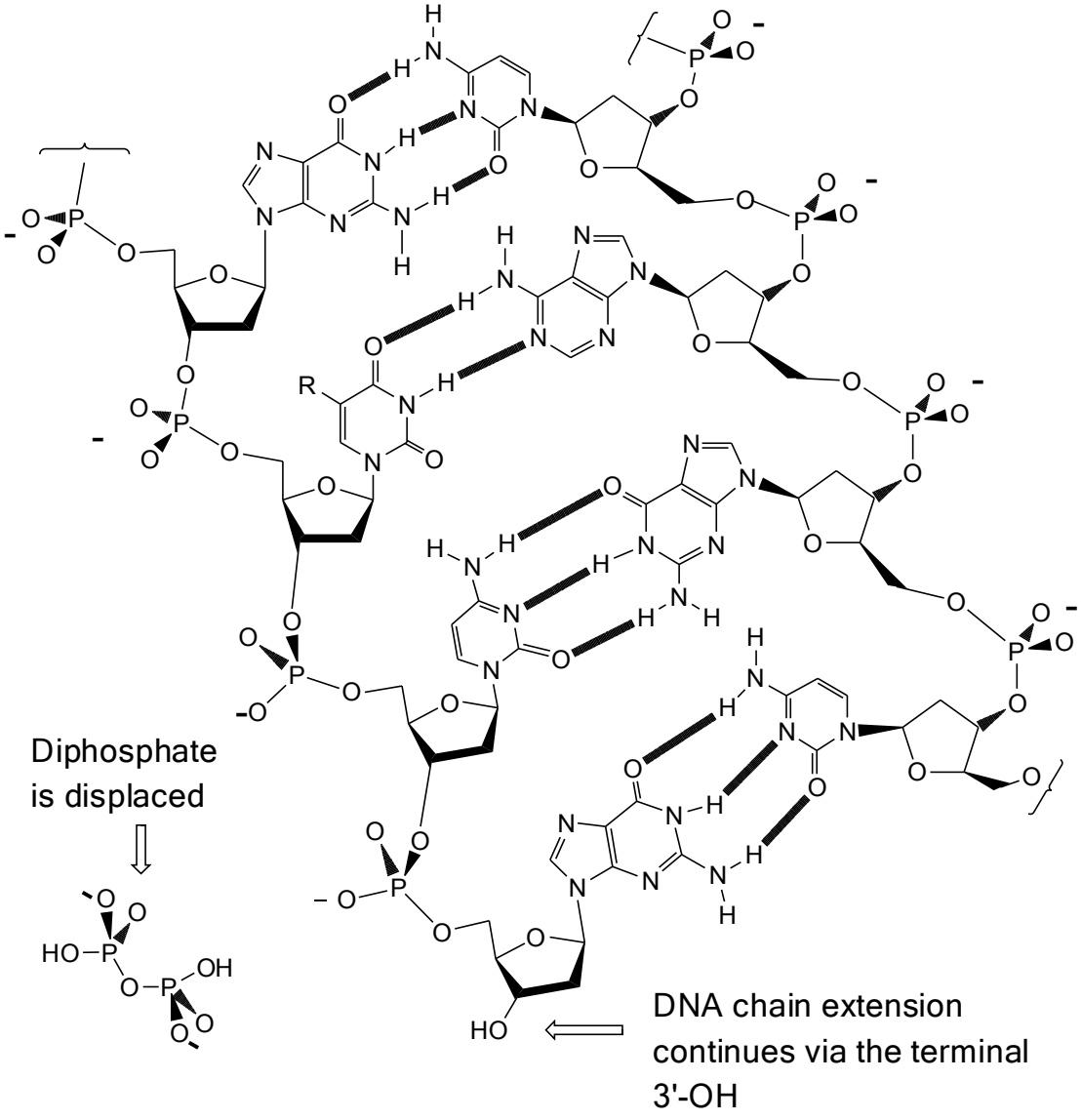


Fig 29 DNA replication is catalyzed by the enzyme DNA polymerase, and uses the relevant nucleoside-5'-O-triphosphate, which undergoes a nucleophilic attack on the first phosphorus atom by the 3'-OH group of the terminal nucleoside in the growing DNA chain.

The α-herpes viruses, which include HSV-1 and HSV-2, have their own specific viral thymidine kinase enzyme. Fortunately this viral kinase enzyme is over 100 times better at phosphorylating acyclovir than is the host cell kinase. So acyclovir is effectively converted firstly to the 5'-O-monophosphate and then onto the 5'-O-triphosphate (the 2nd and 3rd phosphorylation steps are carried out by host cell kinases) *only* in a herpes virus infected cell. This makes acyclovir a highly selective and relatively non-toxic antiviral agent.

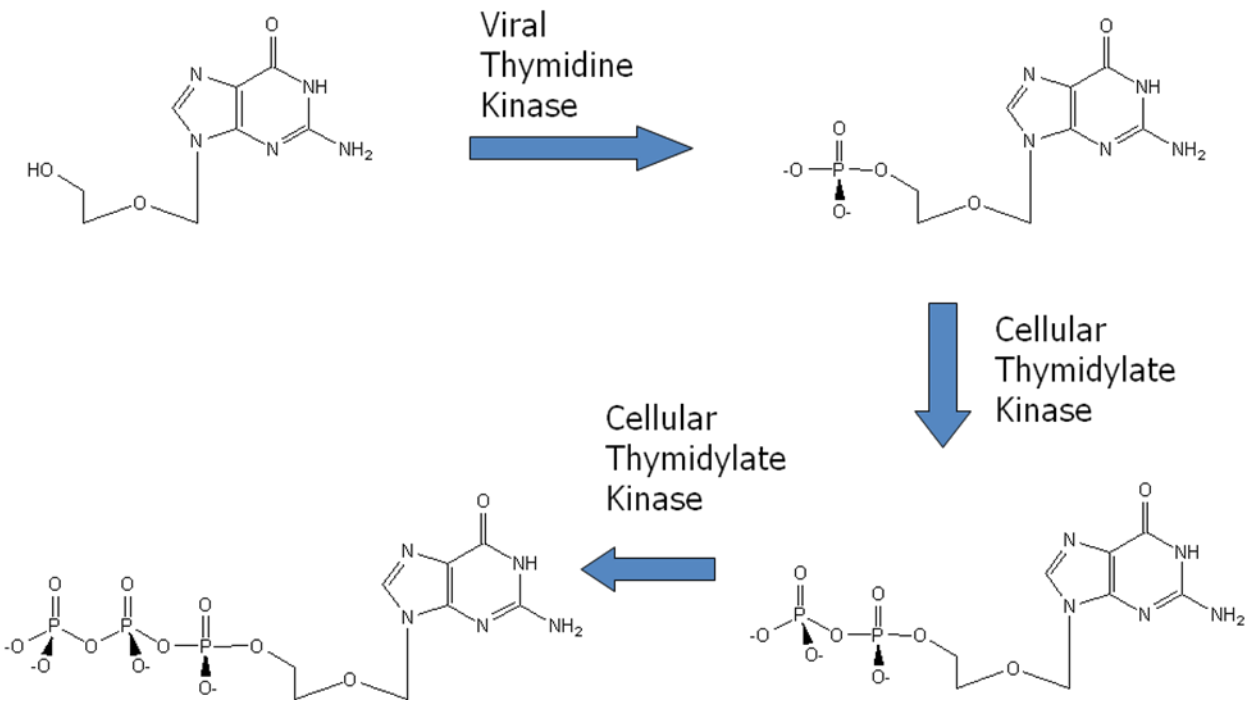


Fig 30 Acyclovir is converted into the active 5'-O-triphosphate only in an HSV-infected cell.

Acyclovir 5'-O-triphosphate is a good mimic for 2'-deoxyguanosine 5'-O-triphosphate, and is 50 times more selective a substrate for the viral DNA polymerase than it is for the host cell DNA polymerase. This results in acyclovir being incorporated in a growing viral DNA chain in place of guanosine. Because the acyclovir lacks a 3'-OH (unlike guanosine) no further DNA extension can occur and so viral DNA chain termination results, inhibiting the proliferation of the herpes viruses.

Acyclovir's high selectivity against α-type herpes viruses has made it the most successful and widely used antiviral agent over the past three decades. It does however have some disadvantages. The polar structure results in a relatively low bioavailability (15-30%). It is ineffective against β-type herpes viruses, and there are emerging strains of type α-herpes viruses that display acyclovir-resistance (mutated viral thymidine kinases and viral DNA polymerases for which acyclovir is not a good substrate).

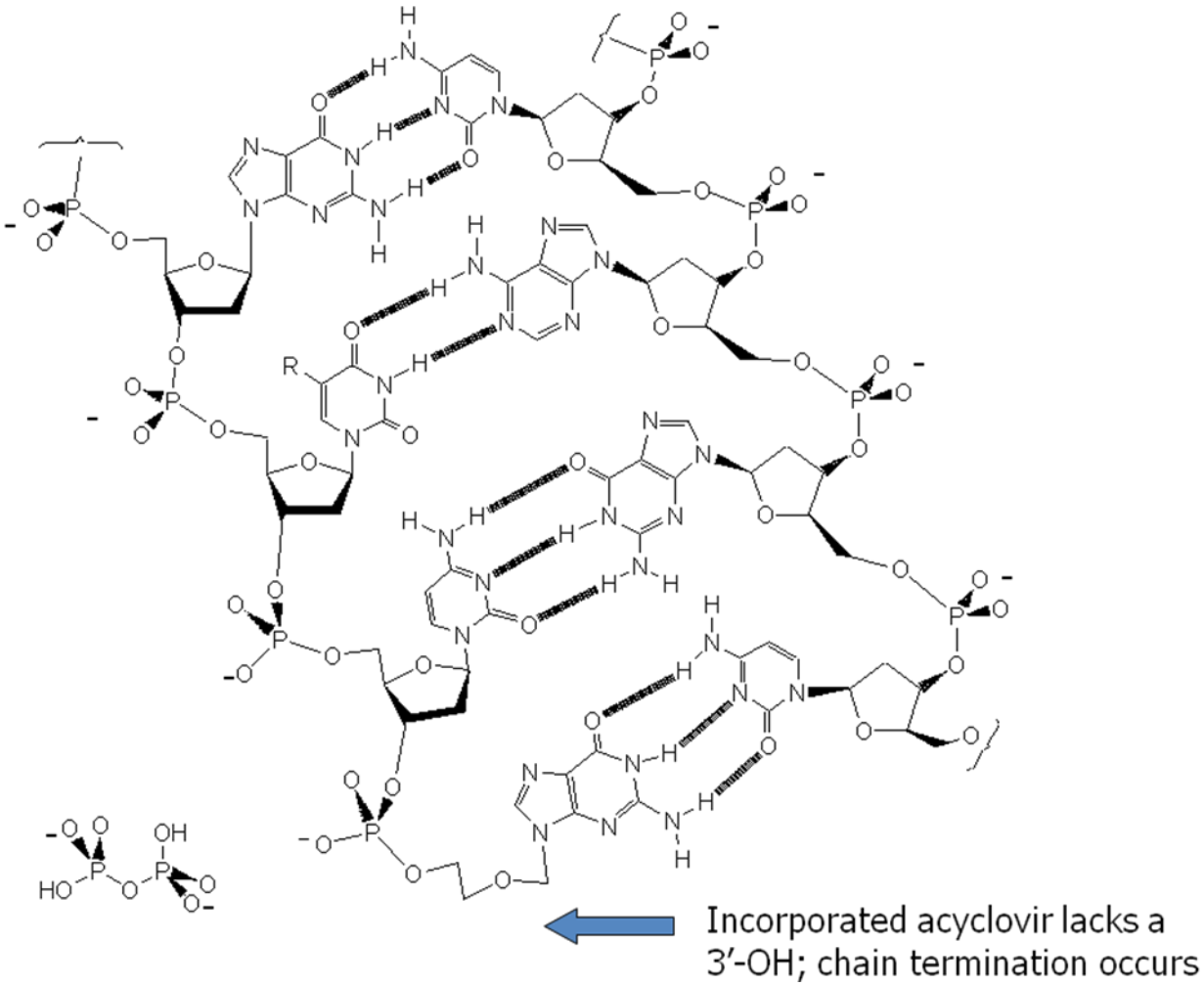


Fig 30 Acyclovir 5'-O-triphosphate is a good mimic for 2'-deoxyguanosine-5'-O-triphosphate, and is selectively incorporated by viral DNA polymerase into the growing viral DNA chain.