19 Antibacterial agents

The fight against bacterial infections over the last 70 years has been one of the great success stories of medicinal chemistry, yet it remains to be seen whether it will last. Bacteria such as *Staphylococcus aureus* have the worrying ability to gain resistance to known drugs and so the search for new drugs is never ending. Although deaths from bacterial infection have dropped in the developed world, bacterial infection is still a major cause of death in the developing world. For example, the World Health Organisation estimated that tuberculosis was responsible for about 2 million deaths in 2002 and that one in three of the world's population was infected. The same organization estimated that in the year 2000, 1.9 million children died worldwide of respiratory infections with 70% of these deaths occurring in Africa and Asia. They also estimated that each year 1.4 million children died from gut infections and the diarrhoea resulting from these infections. In the developed world, deaths from food poisoning due to virulent strains of *Escherichia coli* have attracted widespread publicity, while tuberculosis has returned as a result of the AIDS epidemic.

The topic of antibacterial agents is a large one and terms are used in this chapter which are unique to this particular field. Rather than clutter the text with explanations and definitions, Appendix 5 contains explanations of such terms as aerobic and anaerobic organisms, antibacterial and antibiotic substances, cocci, bacilli, streptococci, and staphylococci. Appendix 5 also explains briefly the difference between bacteria, algae, protozoa, and fungi. The emphasis in this chapter is on agents that act against bacteria, but some of those described also act against protozoal infections and this may be mentioned in the text.

19.1 History of antibacterial agents

There is evidence of antibacterial herbs or potions being used for many centuries. For example, the Chinese used mouldy soybean curd to treat carbuncles, boils, and other infections. Greek physicians used wine, myrrh, and inorganic salts. In the middle ages, certain types of honey were used to prevent infections following arrow wounds. Of course in those days, there was no way of knowing that bacteria were the cause of these infections.

Bacteria are single-cell microorganisms which were first identified in the 1670s by van Leeuwenhoek, following his invention of the microscope. It was not until the nineteenth century, however, that their link with disease was appreciated. This followed the elegant experiments carried out by the French scientist Pasteur, who demonstrated that specific bacterial strains were crucial to fermentation, and that these and other microorganisms were more widespread than was previously thought. The possibility that these microorganisms might be responsible for disease began to take hold.

An early advocate of a 'germ theory of disease' was the Edinburgh surgeon Lister. Despite the protests of several colleagues who took offence at the suggestion that they might be infecting their own patients, Lister introduced **carbolic acid** as an antiseptic and sterilizing agent for operating theatres and wards. The improvement in surgical survival rates was significant.

During the latter half of the nineteenth century, scientists such as Koch were able to identify the microorganisms responsible for diseases such as tuberculosis, cholera, and typhoid. Methods of vaccination were studied, and research was carried out to try and find effective antibacterial agents or antibiotics. The scientist who can lay claim to be the father of chemotherapy—the use of chemicals against infection—was Paul Ehrlich. Ehrlich spent much of his career studying histology, then immunochemistry, and won a Nobel prize for his contributions to immunology. In 1904, however, he switched direction and entered a field which he defined as chemotherapy. Ehrlich's '**principle of chemotherapy**' was that a chemical could directly interfere with the proliferation of microorganisms, at concentrations tolerated by the host. This concept was popularly known as the '**magic bullet**', where the chemical was seen as a bullet which could search

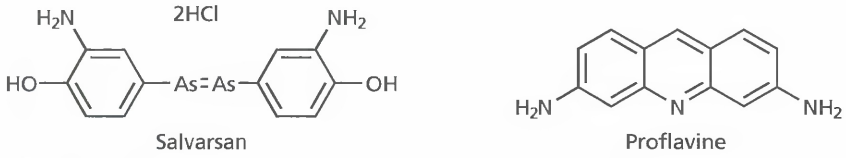


FIGURE 19.1 Salvarsan and proflavine. (The structure of salvarsan shown here is a simplification; it is in fact a cyclic trimer with no As=As bonds.)

out and destroy the invading microorganism without adversely affecting the host. The process is one of **selective toxicity**, where the chemical shows greater toxicity to the target microorganism than to the host cells. Such selectivity can be represented by a '**chemotherapeutic index**', which compares the minimum effective dose of a drug versus the maximum dose that can be tolerated by the host. This measure of selectivity was eventually replaced by the currently used **therapeutic index.**

By 1910, Ehrlich had successfully developed the first example of a purely synthetic antimicrobial drug. This was the arsenic-containing compound **salvarsan** (Fig. 19.1). Although it was not effective against a wide range of bacterial infections, it did prove effective against the protozoal disease of sleeping sickness (trypanosomiasis), and the spirochete disease of syphilis. The drug was used until 1945 when it was replaced by penicillin (see also Box 19.20).

Over the next 20 years, progress was made against a variety of protozoal diseases, but little progress was made in finding antibacterial agents, until the introduction of **proflavine** in 1934 (Fig. 19.1), a drug which was used during the Second World War against bacterial infections in deep surface wounds. Unfortunately, it was too toxic to be used against systemic bacterial infections (i.e. those carried in the bloodstream) and there was still an urgent need for agents which would fight these infections.

This need was answered in 1935 when it was discovered that a red dye called **prontosil** was effective against streptococcal infections *in vivo*. As discussed later, prontosil was eventually recognized as a prodrug for a new class of antibacterial agents—the **sulfa drugs** or **sulfonamides**. The discovery of these drugs was a real breakthrough, as they represented the first drugs to be effective against systemic bacterial infections. In fact, they were the only effective drugs until penicillin became available in the early 1940s.

Although **penicillin** was discovered in 1928, it was not until 1940 that effective means of isolating it were developed by Florey and Chain. Society was then rewarded with a drug which revolutionized the fight against bacterial infection and proved even more effective than the sulfonamides. Despite penicillin's success, it was not effective against all types of infection, and the need for new antibacterial agents still remained. Penicillin is an example of a toxic fungal metabolite that kills bacteria and allows the fungus to compete for nutrients. The realization that fungi might be a source for novel antibiotics spurred scientists into a huge investigation of microbial cultures from all round the globe.

In 1944, the antibiotic **streptomycin** was discovered from a systematic search of soil organisms. It extended the range of chemotherapy to the tubercle bacillus and a variety of Gram-negative bacteria. This compound was the first example of a series of antibiotics known as the **aminoglycoside** antibiotics. After the Second World War, the search continued leading to the discovery of **chloramphenicol** (1947), the peptide antibiotics (e.g. **bacitracin**; 1945), the tetracycline antibiotics (e.g. **chlortetracycline**; 1948), the macrolide antibiotics (e.g. **erythromycin**; 1952), the cyclic peptide antibiotics (e.g. **valinomycin**), and in 1955 the first example of a second major group of β-lactam antibiotics, **cephalosporin C.**

As far as synthetic agents were concerned, **isoniazid** was found to be effective against human tuberculosis in 1952, and in 1962 **nalidixic acid** (the first of the quinolone antibacterial agents) was discovered. A second-generation of this class of drugs was introduced in 1987 with **ciprofloxacin.**

Many antibacterial agents are now available and the vast majority of bacterial diseases have been brought under control (e.g. syphilis, tuberculosis, typhoid, bubonic plague, leprosy, diphtheria, gas gangrene, tetanus, and gonorrhoea). This represents a great achievement for medicinal chemistry and it is perhaps sobering to consider the hazards society faced in the days before penicillin. Septicaemia was a risk faced by mothers during childbirth and could lead to death. Ear infections were common, especially in children, and could lead to deafness. Pneumonia was a frequent cause of death in hospital wards. Tuberculosis was a major problem, requiring special isolation hospitals built away from populated centres. A simple cut or a wound could lead to severe infection requiring the amputation of a limb, and the threat of peritonitis lowered the success rates of surgical operations. This was in the 1930s—still within living memory for many. Perhaps those of us born since the Second World War take the success of antibacterial agents too much for granted.

19.2 The bacterial cell

The success of antibacterial agents owes much to the fact that they can act selectively against bacterial cells rather than animal cells. This is largely because bacterial and animal cells differ both in their structure and in their biosynthetic pathways. Let us consider some of the differences between the bacterial cell (defined as prokaryotic) (Fig. 19.2) and the animal cell (defined as eukaryotic).

- The bacterial cell does not have a defined nucleus, whereas the animal cell does.
- Animal cells contain a variety of structures called organelles (mitochondria, endoplasmic reticulum, etc.), whereas the bacterial cell is relatively simple.
- The biochemistry of a bacterial cell differs significantly from that of an animal cell. For example, bacteria may have to synthesize essential vitamins which animal cells can acquire intact from food. The bacterial cells must have the enzymes to catalyse these reactions. Animal cells do not, because the reactions are not required.
- The bacterial cell has a cell membrane and a cell wall, whereas the animal cell has only a cell membrane. The cell wall is crucial to the bacterial cell's survival. Bacteria have to survive a wide range of environments and osmotic pressures, whereas animal cells do not. If a bacterial cell lacking a cell wall was placed in an aqueous environment containing a low concentration of salts, water would freely enter the cell due to osmotic pressure. This would cause the cell to swell and eventually burst. The scientific term for this is **lysis**. The cell wall does not stop water flowing into the cell directly, but it does prevent the cell from swelling and so indirectly prevents water entering the cell. Bacteria can be characterized by a staining technique that allows them to be defined as Gram-positive or Gram-negative (Appendix 5). The staining technique involves the addition of a purple dye followed by washing with acetone. Bacteria with a thick cell wall (20–40 nm) absorb the dye and are stained purple, and are defined as Gram-positive. Bacteria with a thin cell wall (2–7 nm) absorb only a small amount of dye, which is washed out by acetone. These bacteria are stained pink with a second dye and are said to be Gram-negative. Although Gram-negative bacteria have a thin cell wall, they have an additional outer membrane not present in Gram-positive bacteria. This outer membrane is made up of lipopolysaccharides—similar in character to the cell membrane. These differences in cell walls and membranes have important consequences for the different vulnerabilities of Gram-positive and Gram-negative bacteria to antibacterial drugs.

19.3 Mechanisms of antibacterial action

There are five main mechanisms by which antibacterial agents act (Fig. 19.2).

- **Inhibition of cell metabolism**: antibacterial agents that inhibit cell metabolism are called **antimetabolites**. These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host. They can do this by inhibiting an enzyme-catalysed reaction which is present in the bacterial cell, but not in animal cells. The best-known examples of antibacterial agents acting in this way are the sulfonamides. It is also possible for antibacterial agents to show selectivity against enzymes that are present in both the bacterial and mammalian cell, as long as there are significant differences in structure between the two.

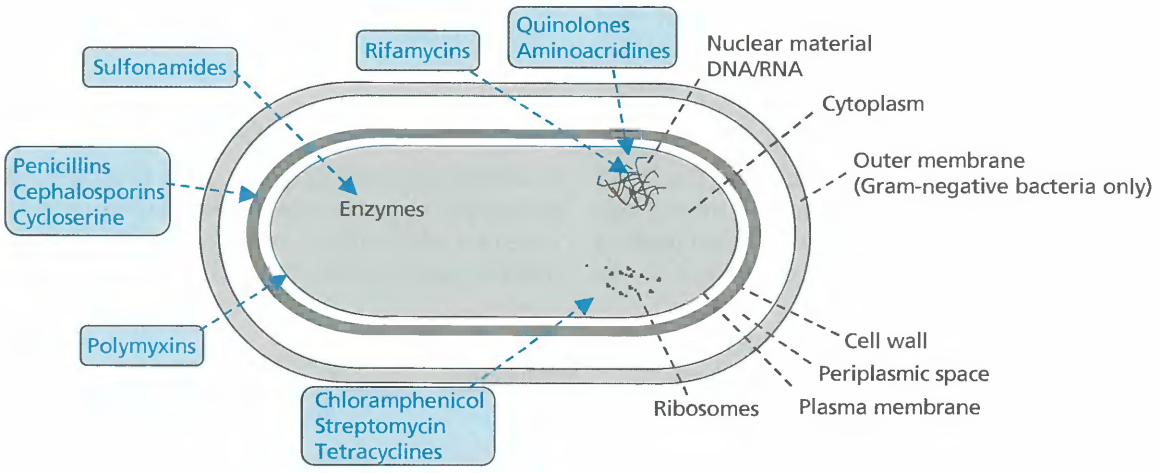


FIGURE 19.2 The bacterial cell and drug targets.

- **Inhibition of bacterial cell wall synthesis** leads to bacterial cell lysis and death. Agents operating in this way include penicillins, cephalosporins and vancomycin. As animal cells do not have a cell wall, they are unaffected by such agents.
- **Interactions with the plasma membrane:** some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell. Polymyxins and tyrothricin operate in this way.
- **Disruption of protein synthesis** means that essential proteins and enzymes required for the cell's survival can no longer be made. Agents that disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines, and chloramphenicol.
- **Inhibition of nucleic acid transcription and replication** prevents cell division and/or the synthesis of essential proteins. Agents acting in this way include nalidixic acid and proflavine.

We now consider these mechanisms in more detail.

19.4 Antibacterial agents that act against cell metabolism (antimetabolites)

19.4.1 Sulfonamides

19.4.1.1 History of sulfonamides

The best example of antibacterial agents acting as antimetabolites are the sulfonamides (sometimes called the sulfa drugs). The sulfonamide story began in 1935 when it was discovered that a red dye called **prontosil** (Fig. 19.3) had antibacterial properties *in vivo* (i.e. when given to laboratory animals). Strangely enough, no antibacterial effect was observed *in vitro*. In other words, prontosil could not kill bacteria grown in the test tube. This remained a mystery until it was discovered that prontosil was metabolized by bacteria present in the small intestine of the test animal to give a product called **sulfanilamide** (Fig. 19.3). It was this compound which was the true antibacterial agent. Thus, prontosil was an early example of a **prodrug** (section 14.6). Sulfanilamide was synthesized in the laboratory and became the first synthetic antibacterial agent found to be active against a wide range of infections. Further developments led to a range of sulfonamides which proved effective against Gram-positive organisms, especially pneumococci and meningococci.

Despite their undoubted benefits, sulfa drugs have proved ineffective against infections such as *Salmonella*—the organism responsible for typhoid. Other problems have resulted from the way these drugs are metabolized, as toxic products are frequently obtained. This led to the sulfonamides being superseded by penicillin.

19.4.1.2 Structure–activity relationships

The synthesis of a large number of sulfonamide analogues (Fig. 19.4) led to the following conclusions:

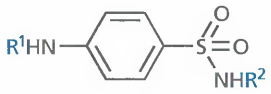


FIGURE 19.4 Sulfonamide analogues used in structure–activity relationship studies.

- The *para*-amino group is essential for activity and must be unsubstituted (i.e. R^1 = H). The only exception is when R^1 = acyl (i.e. amides). The amides themselves are inactive but can be metabolized in the body to regenerate the active compound (Fig. 19.5). Thus amides can be used as sulfonamide prodrugs.
- The aromatic ring and the sulfonamide functional group are both required.
- Both the sulfonamide and amino group must be directly attached to the aromatic ring.
- The aromatic ring must be *para*-substituted only. Extra substitution eliminates activity for steric reasons.
- The sulfonamide nitrogen must be primary or secondary.
- R^2 is the only possible site that can be varied in sulfonamides.

19.4.1.3 Sulfanilamide analogues

In sulfanilamide analogues (Fig. 19.4), R^2 is often varied by incorporating a large range of heterocyclic or aromatic structures which affects the extent to which the drug binds to plasma protein. This in turn controls the blood

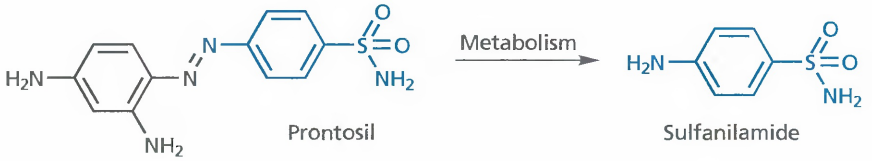


FIGURE 19.3 Metabolism of prontosil.

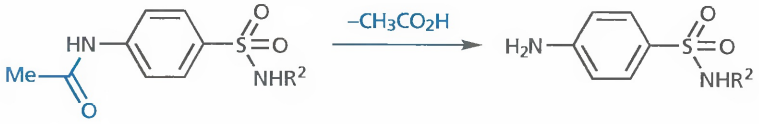


FIGURE 19.5 Metabolism of an *N*-acyl group to regenerate an active sulfonamide.

levels and lifetime of the drug. Thus, a drug that binds strongly to plasma protein will be slowly released into the blood circulation and will be longer lasting. Varying R^2 can also affect the solubility of sulfonamides. Therefore, variations of R^2 affect the pharmacokinetics of the drug, rather than its mechanism of action (Box 19.1).

19.4.1.4 Applications of sulfonamides

Before the appearance of penicillin, the sulfa drugs were the drugs of choice in the treatment of infectious diseases. Indeed, they played a significant part in world history by saving Winston Churchill's life during the Second World War. While visiting North Africa, Churchill became ill with a serious infection and was bedridden for several weeks. At one point, his condition was deemed so serious that his daughter was flown out from Britain to be at his side. Fortunately, he responded to the novel sulfonamide drugs of the day.

Penicillins largely superseded sulfonamides, and for a long time sulfonamides took a back seat. There has been a revival of interest, however, with the discovery of a new

BOX 19.1 Sulfonamide analogues with reduced toxicity

The primary amino group of sulfonamides is acetylated in the body, and the resulting amides have reduced solubility which can lead to toxic effects. For example, the metabolite formed from **sulfathiazole** (an early sulfonamide) is poorly soluble and can prove fatal if it blocks the kidney tubules (Fig. 1). It is interesting to note that certain populations are more susceptible to this than others. For example, Japanese and Chinese people metabolize sulfathiazole more quickly than the average American, and are therefore more susceptible to its toxic effects.

It was discovered that the solubility problem could be overcome by replacing the thiazole ring in sulfathiazole with a pyrimidine ring to give **sulfadiazine** (Fig. 2). The reason for the improved solubility lies in the acidity of the sulfonamide NH proton. In sulfathiazole, this proton is not very acidic (high pK_a). Therefore, sulfathiazole and its metabolite are mostly un-ionized at blood pH. Replacing the thiazole ring with a more electron-withdrawing pyrimidine ring increases the acidity of the NH proton by stabilizing the resulting anion. Therefore, sulfadiazine and its metabolite are significantly ionized at blood pH. As a consequence, they are more soluble and less toxic. Sulfadiazine was also found to be more active than sulfathiazole and soon replaced it in therapy. Silver sulfadiazine cream is still used topically to prevent infection of burns, although it is really the silver ions that provide the antibacterial effect.



FIGURE 1 Metabolism of sulfathiazole.

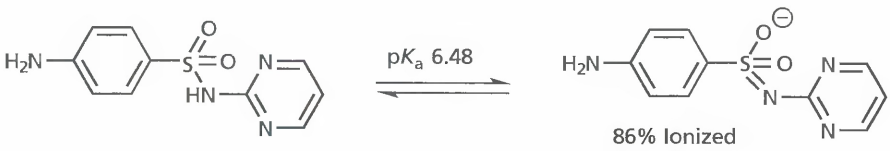


FIGURE 2 Sulfadiazine.

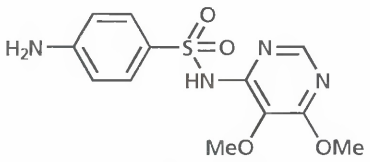


FIGURE 19.6 Sulfadoxine.

'breed' of longer lasting sulfonamides. One example of this new generation is **sulfadoxine** (Fig. 19.6), which is so stable in the body that it need only be taken once a week. The combination of sulfadoxine and **pyrimethamine** is called **Fanisdar** and has been used for the treatment of malaria.

The sulfa drugs presently have the following applications in medicine:

- treatment of urinary tract infections
- eye lotions
- treatment of infections of mucous membranes
- treatment of gut infections (Box 19.2).

It is also worth noting that sulfonamides have occasionally found uses in other areas of medicine (section 12.4.4.2).

19.4.1.5 Mechanism of action

The sulfonamides act as competitive enzyme inhibitors of dihydropteroate synthetase and block the biosynthesis of **tetrahydrofolate** in bacterial cells (Fig. 19.7). Tetrahydrofolate is important in both human and bacterial cells, as it is an enzyme cofactor that provides one carbon units for the synthesis of the pyrimidine nucleic acid bases required for DNA synthesis (section 21.3.1). If pyrimidine and DNA synthesis is blocked, then the cell can no longer grow and divide.

Note that sulfonamides do not actively kill bacterial cells. They do, however, prevent the cells growing and multiplying. This gives the body's own defence systems enough time to gather their resources and wipe out the invader. Antibacterial agents which inhibit cell growth are classed as **bacteriostatic**, whereas agents such as penicillin which actively kill bacterial cells are classed as **bactericidal**. Because sulfonamides rely on a healthy

BOX 19.2 Treatment of intestinal infections

Sulfonamides have been particularly useful against intestinal infections, and can be targeted against these by the use of prodrugs. For example, **succinyl sulfathiazole** is a prodrug of sulfathiazole (Fig. 1). The succinyl moiety contains an acidic group which means that the prodrug is ionized in the slightly alkaline conditions of the intestine. As a result, it is not absorbed into the bloodstream and is retained in the intestine. Slow enzymatic hydrolysis of the succinyl group then releases the active sulfathiazole where it is needed.

Benzoyl substitution (Fig. 2) on the aniline nitrogen has also given useful prodrugs, which are poorly absorbed through the gut wall since they are too hydrophobic (section 11.2). They can be used in the same way.

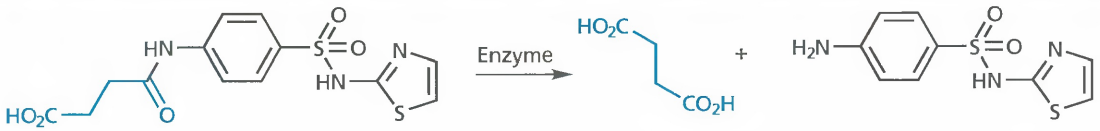


FIGURE 1 Succinyl sulfathiazole is a prodrug of sulfathiazole.

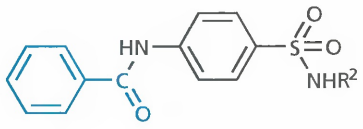


FIGURE 2 Substitution on the aniline nitrogen with benzoyl groups.

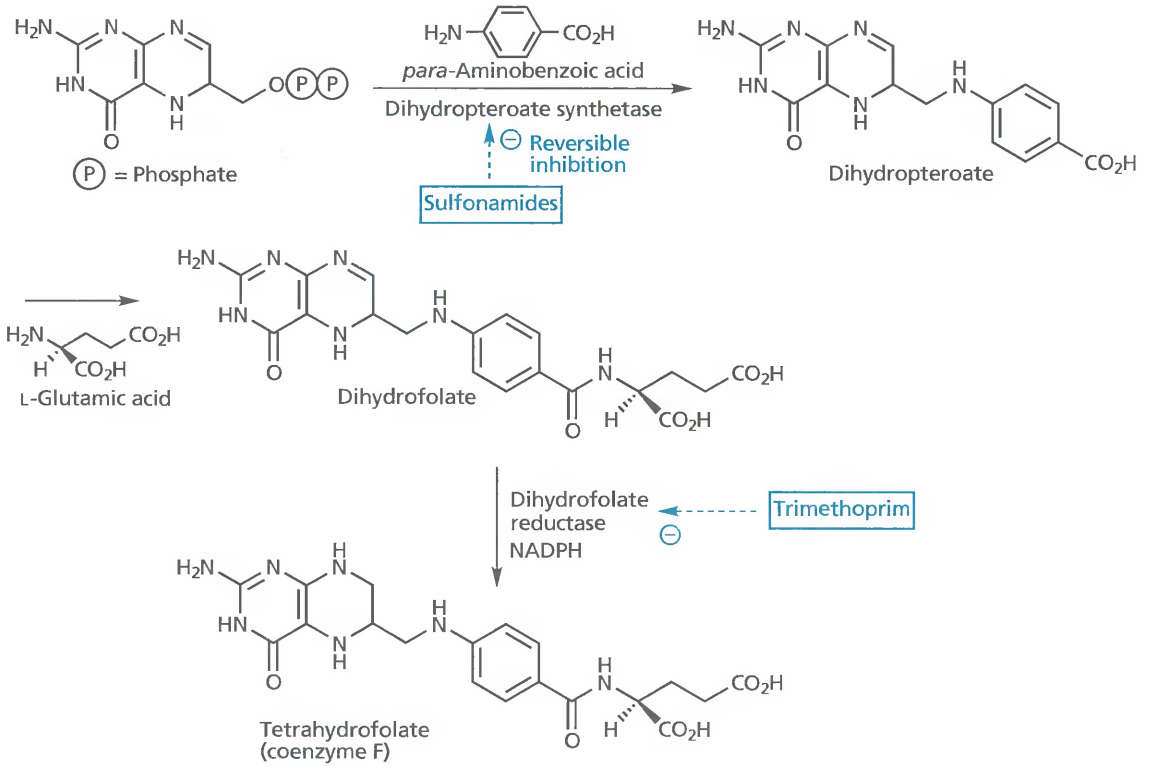


FIGURE 19.7 Mechanism of action of sulfonamides.

immune system to complete the job they have started, they are not recommended for patients with a weakened immune system. This includes people with AIDS, and patients who are undergoing cancer chemotherapy or have had an organ transplant and are taking immunosuppressant drugs.

Sulfonamides act as inhibitors by mimicking ***p*-aminobenzoic acid** (PABA)—one of the normal substrates for dihydropteroate synthetase. The sulfonamide molecule is similar enough in structure to PABA that the enzyme is fooled into accepting it into its active site (Fig. 19.8). Once it is bound, the sulfonamide prevents PABA from binding. As a result, dihydropteroate is no longer synthesized. One might ask why the enzyme does not join the sulfonamide to the other component of dihydropteroate to give a dihydropteroate analogue containing the sulfonamide skeleton. This can in fact occur, but it does the cell no good at all because the analogue is not accepted by the next enzyme in the biosynthetic pathway.

Sulfonamides are competitive enzyme inhibitors, so inhibition is reversible. This is demonstrated by certain organisms such as staphylococci, pneumococci, and gonococci which can acquire resistance by synthesizing more PABA. The more PABA there is in the cell, the more effectively it can compete with the sulfonamide inhibitor to reach the enzyme's active site. In such cases, the dose levels

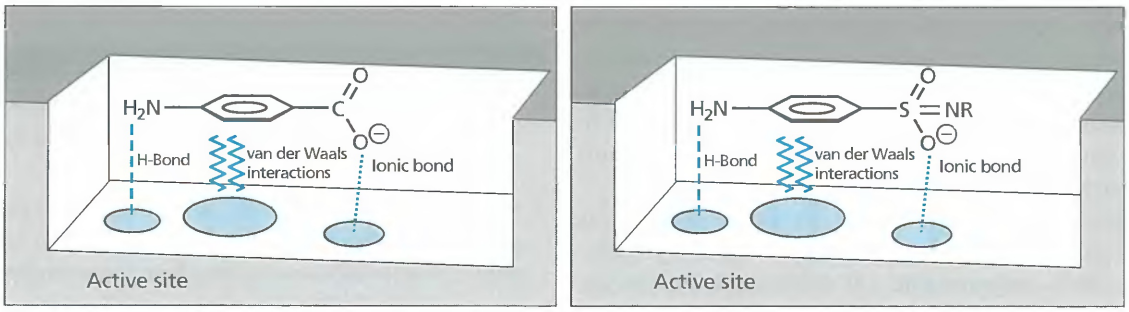


FIGURE 19.8 Sulfonamide prevents PABA from binding by mimicking PABA.

of sulfonamide have to be increased to bring back the same level of inhibition. Resistance to sulfonamides can also arise by mutations which modify the target enzyme such that it has less affinity for sulfonamides, or by decreased permeability of the cell membrane to the sulfonamide.

Tetrahydrofolate is clearly necessary for the survival of bacterial cells, but it is also vital for the survival of human cells, so why are the sulfa drugs not toxic to humans? The answer lies in the fact that human cells synthesize tetrahydrofolate in a different manner and do not contain the enzyme dihydropteroate synthetase. In human cells, tetrahydrofolate is synthesized from folic acid which is obtained from the diet as a vitamin. Folic acid is brought across cell membranes by a transport protein.

We could now ask, 'If human cells can acquire folic acid from the diet, why can't bacterial cells infecting the human body do the same, then convert it to tetrahydrofolate?' In fact, bacterial cells are unable to acquire folic acid since they lack the necessary transport protein required to carry it across the cell membrane.

To sum up, the success of sulfonamides is due to two metabolic differences between mammalian and bacterial cells.

- Bacteria have a susceptible enzyme that is not present in mammalian cells.
- Bacteria lack the transport protein that would allow them to acquire folic acid from outside the cell.

19.4.2 Examples of other antimetabolites

Other antimetabolites in medical use include **trimethoprim** and a group of compounds known as **sulfones** (Fig. 19.9).

19.4.2.1 Trimethoprim

Trimethoprim is an orally active diaminopyrimidine structure which has proved to be a highly selective antibacterial, and antimalarial agent. It acts against **dihydrofolate reductase**—the enzyme which carries out the conversion of dihydrofolate to tetrahydrofolate—leading to the inhibition of DNA synthesis and cell growth.

Dihydrofolate reductase is present in mammalian cells as well as bacterial cells, but mutations over millions of years have resulted in a significant difference in structure between the two enzymes such that trimethoprim recognizes and inhibits the bacterial enzyme more strongly. In fact, trimethoprim is 100 000 times more active against the bacterial enzyme.

Trimethoprim is often given in conjunction with the sulfonamide **sulfamethoxazole** (Fig. 19.9) in a preparation called **cotrimoxazole**. The sulfonamide inhibits the incorporation of PABA into dihydropteroate, while trimethoprim inhibits dihydrofolate reductase. Therefore,

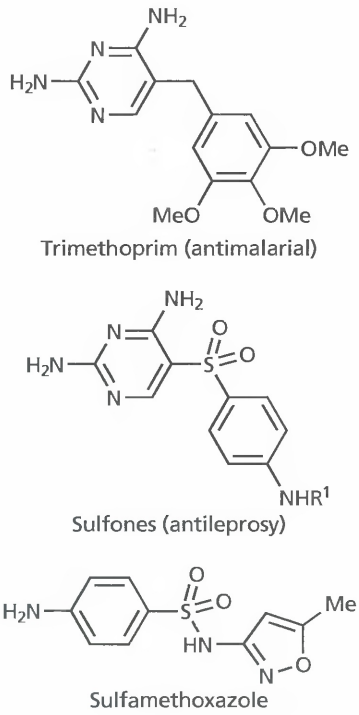


FIGURE 19.9 Examples of antimetabolites in medical use.

two enzymes in the one biosynthetic route are inhibited (Fig. 19.7). This is a very effective method of inhibiting a biosynthetic route and has the advantage that the doses of both drugs can be kept down to a safe level. To get the same level of inhibition using a single drug, the dose level would have to be much higher, leading to possible side effects. This approach has been described as '**sequential blocking**'.

Resistance to trimethoprim has been observed in strains of *E.coli* which produce a new form of the target enzyme that has less affinity for the drug.

19.4.2.2 Sulfones

The sulfones (Fig. 19.9) are the most important drugs used in the treatment of leprosy. It is believed that they inhibit the same bacterial enzyme inhibited by the sulfonamides (i.e. dihydropteroate synthetase).

KEY POINTS

- The principle of chemotherapy or the magic bullet involves the design of chemicals that show selective toxicity against bacterial cells rather than mammalian cells.
- Early antibacterial agents were salvarsan, prontosil and the sulfonamides. Following the discovery of penicillin, several classes of antibiotics were isolated from fungal strains.
- The bacterial cell differs in various respects from mammalian cells allowing the identification of drug targets which

are unique to bacterial cells, or which differ significantly from equivalent targets in mammalian cells.

- Antibacterial agents act on five main targets—cell metabolism, the cell wall, the plasma membrane, protein synthesis, and nucleic acid function.
- Sulfonamides require a primary aromatic amine group and a secondary sulfonamide group for good activity.
- Adding an aromatic or heteroaromatic group to the sulfonamide nitrogen provides a variety of sulfonamides with different pharmacokinetic properties.
- *N*-Acetylation of sulfonamides is a common metabolic reaction.
- Sulfonamides are used to treat infections of the urinary tract, gastrointestinal tract, and mucous membranes. They are also used in eye lotions.
- Sulfonamides are similar in structure to *para*-aminobenzoic acid—a component of dihydropteroate. As a result, they can bind to the bacterial enzyme responsible for dihydropteroate synthesis and act as an inhibitor.
- Mammals synthesize tetrahydrofolate from folic acid acquired from the diet. They lack the enzyme targeted by sulfonamides. Bacteria lack the transport mechanisms required to transport folic acid into the cell.
- Trimethoprim inhibits dihydrofolate reductase—an enzyme that converts folic acid to tetrahydrofolate. It has been used in combination with sulfamethoxazole in a strategy known as sequential blocking.
- Sulfones are used in the treatment of leprosy.

19.5 Antibacterial agents that inhibit cell wall synthesis

19.5.1 Penicillins

19.5.1.1 History of penicillins

In 1877, Pasteur and Joubert discovered that certain moulds could produce toxic substances which killed bacteria. Unfortunately, these substances were also toxic to humans and were of no clinical value. They did demonstrate, however, that moulds could be a potential source of antibacterial agents.

In 1928, Fleming noted that a bacterial culture that had been left several weeks open to the air had become infected by a fungal colony. Of more interest was the fact that there was an area surrounding the fungal colony where the bacterial colonies were dying. He correctly concluded that the fungal colony was producing an antibacterial agent which was spreading into the surrounding area. Recognizing the significance of this, he set out to culture and identify the fungus and showed it to be a relatively rare species of *Penicillium*. It has since been suggested that the *Penicillium* spore responsible for the fungal colony originated from another laboratory in the building, and that the spore was carried by air currents to be blown through the window of Fleming's laboratory. This in itself appears to be a remarkable stroke of good fortune. However, a series of other chance events were involved in the story—not least the weather! A period of early cold weather had encouraged the fungus to grow while the bacterial colonies had remained static. A period of warm weather then followed which encouraged the bacteria to grow. These weather conditions were the ideal experimental conditions required for:

- the fungus to produce penicillin during the cold spell
- the antibacterial properties of penicillin to be revealed during the hot spell.

If the weather had been consistently cold, the bacteria would not have grown significantly and the death of cell colonies close to the fungus would not have been seen. Alternatively, if the weather had been consistently warm, the bacteria would have outgrown the fungus and little penicillin would have been produced. As a final twist to the story, the crucial agar plate had been stacked in a bowl of disinfectant ready for washing up, but was actually placed above the surface of the disinfectant. It says much for Fleming's observational powers that he bothered to take any notice of a discarded culture plate, and that he spotted the crucial area of inhibition.

Fleming spent several years investigating the novel antibacterial substance and showed it to have significant antibacterial properties and to be remarkably non-toxic to mammals. Unfortunately, the substance was also unstable and Fleming was unable to isolate and purify the compound. He therefore came to the conclusion that penicillin was too unstable to be used clinically.

The problem of isolating penicillin was eventually solved in 1938 by Florey and Chain by using processes such as freeze-drying and chromatography, which allowed isolation of the antibiotic under much milder conditions than had previously been available. By 1941, Florey and Chain were able to carry out the first clinical trials on crude extracts of penicillin and achieved spectacular success. Further developments aimed at producing the new agent in large quantities were developed in the USA, and by 1944, there was enough penicillin to treat casualties arising from the D-Day landings.

Although the use of penicillin was now widespread, the structure of the compound was still not settled and the unusual structures being proposed proved a source of furious debate. The issue was finally settled in 1945 when Dorothy Hodgkins established the structure by X-ray crystallographic analysis. The structure was quite

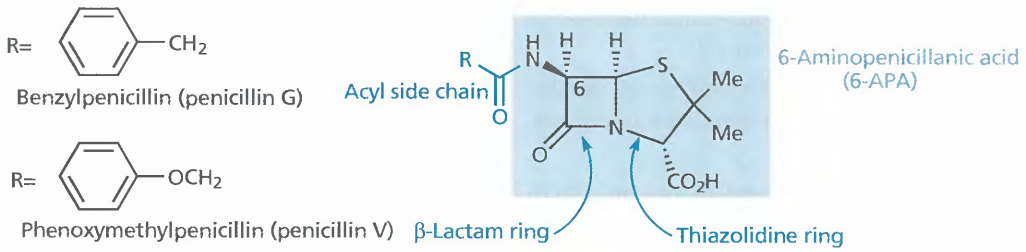


FIGURE 19.10 The structure of penicillin.

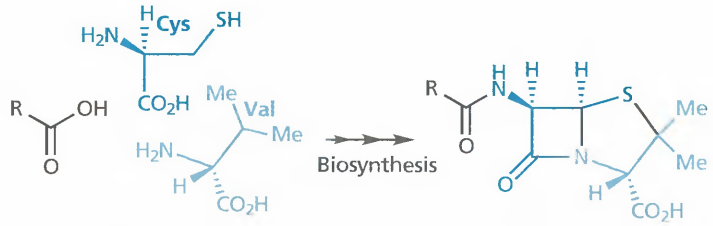


FIGURE 19.11 The biosynthetic precursors of penicillin.

surprising at the time, as penicillin was clearly a highly strained molecule—which explained why Fleming had been unsuccessful in purifying it.

The full synthesis of such a highly strained molecule presented a huge challenge—one that was met successfully by Sheehan in 1957. Unfortunately, the full synthesis was too involved to be of commercial use, but in the following year, Beechams isolated a biosynthetic intermediate of penicillin called **6-aminopenicillanic acid (6-APA)**. This revolutionized the field of penicillins by providing the starting material for a huge range of semi-synthetic penicillins.

Penicillins have been used widely, and often carelessly. As a result, penicillin-resistant bacteria have evolved and have become an increasing problem. The fight against penicillin-resistant bacteria was helped in 1976, when Beechams discovered a natural product called **clavulanic acid** which proved highly effective in protecting penicillins from the bacterial enzymes which attack them (section 19.5.4.1).

19.5.1.2 Structure of benzylpenicillin and phenoxymethylpenicillin

Penicillin (Fig. 19.10) contains a highly unstable-looking bicyclic system consisting of a four-membered β-lactam ring fused to a five-membered thiazolidine ring. The skeleton of the molecule suggests that it is derived from the amino acids cysteine and valine, and this has been established (Fig. 19.11). The overall shape of the molecule is like a half-open book, as shown in Fig. 19.12.

The acyl side chain (R) varies, depending on the components of the fermentation medium. For example, corn steep liquor (the fermentation medium first used for mass production of penicillin) contains high levels of phenylacetic acid ($PhCH_2CO_2H$) and gives **benzylpenicillin (penicillin G**; R = benzyl). A fermentation medium containing phenoxyacetic acid ($PhOCH_2CO_2H$) gives **phenoxymethylpenicillin (penicillin V**; R = $PhOCH_2$) (Fig. 19.10).

19.5.1.3 Properties of benzylpenicillin

Benzylpenicillin (penicillin G) is active against a range of bacterial infections (Box 19.3) and lacks serious side effects for most patients. However, there are various drawbacks. It cannot be taken orally since it is broken down by stomach acids, it has a narrow spectrum of activity and there are many bacterial infections against which has no effect—particularly those where the microorganism produces an enzyme called **β-lactamase**. This is an enzyme which hydrolyses the β-lactam ring of benzylpenicillin and makes it inactive. Therefore, there is scope for producing analogues with improved properties. Before looking at penicillin analogues, we shall look at penicillin's mechanism of action.

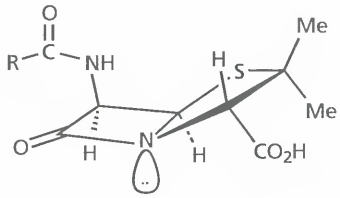


FIGURE 19.12 The three-dimensional shape of penicillin.

BOX 19.3 Clinical properties of benzylpenicillin and phenoxymethylpenicillin

Benzylpenicillin is active against non β-lactamase producing Gram-positive bacilli (e.g. *Meningitis*, *Gonorrhoea*, and early strains of staphylococci) and several Gram-negative cocci (e.g. *Neisseria*). It is effective for many streptococcal, pneumococcal, gonococcal and meningococcal infections. It is also used to treat anthrax, diphtheria, gas-gangrene, leptospirosis and Lyme disease in children. It can be effective against tetanus, although **metronidazole** is preferred. Since penicillin is bactericidal, it is most active against rapidly dividing bacteria. There are many bacterial species against which benzyl penicillin shows no activity, in particular Gram-negative bacteria and those producing β-lactamase enzymes. It is ineffective when taken orally and should be administered by intravenous or intramuscular injection.

Phenoxymethylpenicillin is recommended for the treatment of various problems such as tonsillitis, rheumatic fever, otitis media and oral infections.

Allergic reactions are suffered by some individuals when they take penicillins, varying from a rash to immediate anaphylactic shock. Anaphylactic reactions occur in 0.2% of patients with a fatality rate of 0.001%. Less serious allergic reactions are more common (1–4%).

19.5.1.4 Mechanism of action for penicillin

Structure of the cell wall

In order to understand penicillin's mechanism of action, we have to first look at the structure of the bacterial cell wall and the mechanism by which it is formed. Bacteria have cell walls in order to survive a large range of environmental conditions such as varying pH, temperature, and osmotic pressure. Without a cell wall, water would continually enter the cell as a result of osmotic pressure, causing the cell to swell and burst (lysis). The cell wall is very porous and does not block the entry of water, but it does prevent the cell swelling. Animal cells do not have a cell wall, making it the perfect target for antibacterial agents such as penicillins.

The wall is a peptidoglycan structure (Fig. 19.13). In other words, it is made up of peptide and sugar units. The structure of the wall consists of a parallel series of sugar backbones containing two types of sugar (***N*-acetylmuramic acid** (NAM) and ***N*-acetylglucosamine** (NAG) (Fig. 19.14). Peptide chains are bound to the NAM sugars, and it is interesting to note the presence of D-amino acids in these chains. In human biochemistry there are only L-amino acids, whereas bacteria have racemase enzymes that can convert L-amino acids into D-amino acids. In the final stage of cell wall biosynthesis, the peptide chains are linked together by the displacement of D-alanine from one chain by glycine in another.

About 30 enzymes are involved in the overall biosynthesis of the cell wall, but it is the final cross-linking reaction which is inhibited by penicillin. This leads to a cell wall framework that is no longer interlinked (Fig. 19.15). As a

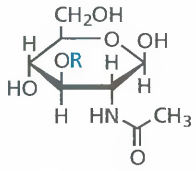


FIGURE 19.14 Sugars contained in the cell wall structure of bacteria. R = H, *N*-acetylglucosamine (NAG); R = $CHMeCO_2H$, *N*-acetylmuramic acid (NAM).

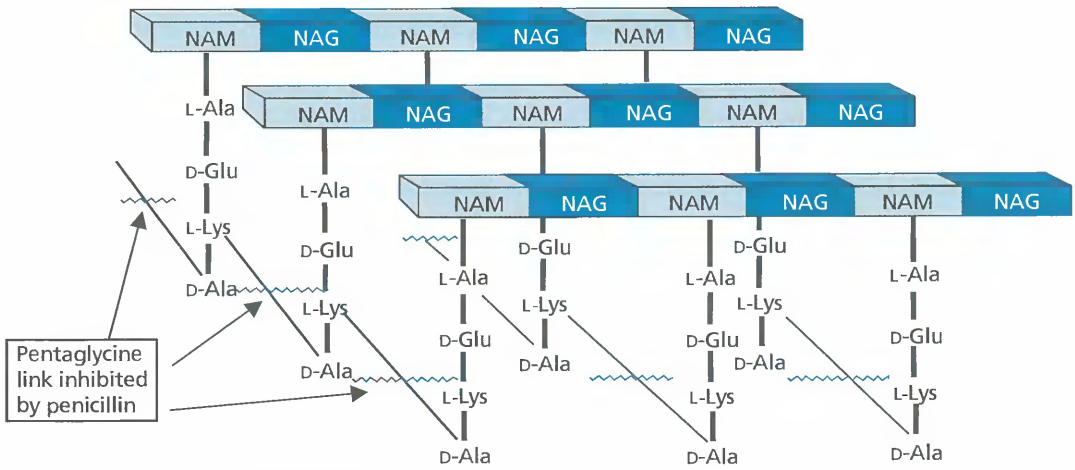


FIGURE 19.13 Peptidoglycan structure of bacterial cell walls.

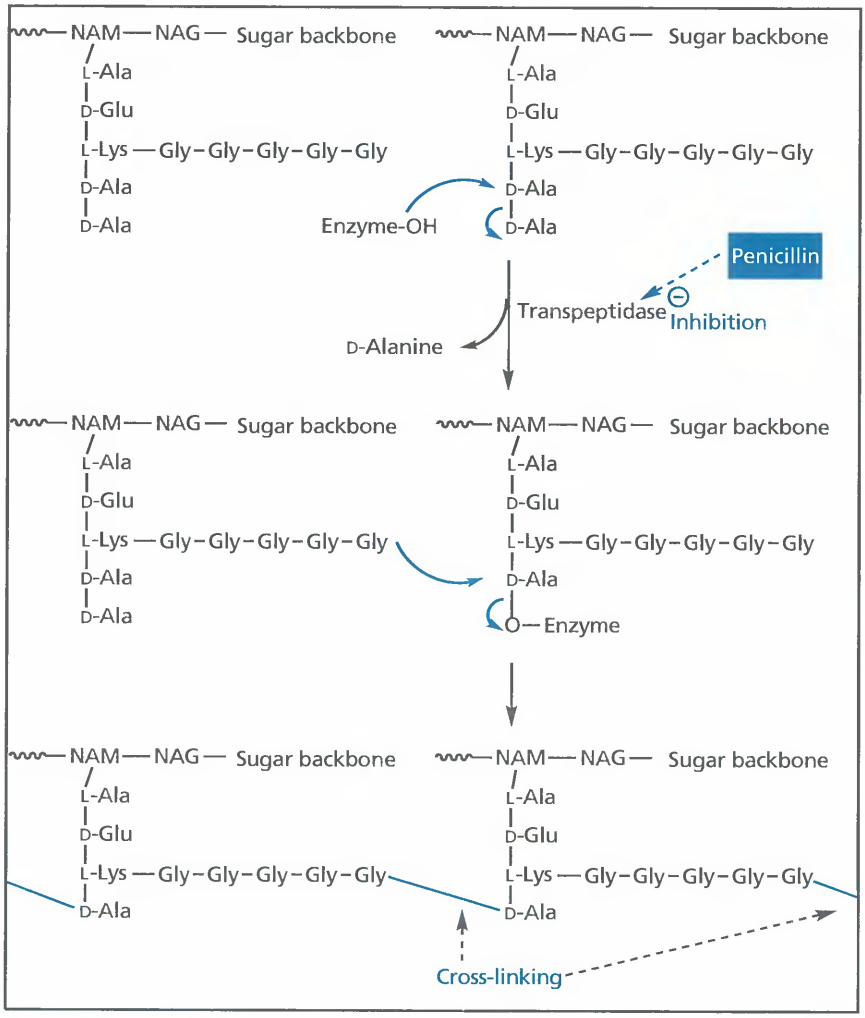


FIGURE 19.15 Cross-linking of bacterial cell walls inhibited by penicillin.

result, the wall becomes fragile and can no longer prevent the cell from swelling and bursting. The enzyme responsible for the cross-linking reaction is known as the **transpeptidase enzyme**. There are several types of the enzyme which vary in character from one bacterial species to another, but they are all inhibited to various degrees by penicillins.

There are significant differences in the thickness of the cell wall between Gram-positive and Gram-negative bacteria. The cell wall in Gram-positive bacteria consists of 50–100 peptidoglycan layers, whereas in Gram-negative bacteria it consists of only two layers.

The transpeptidase enzyme and its inhibition

The transpeptidase enzyme is bound to the outer surface of the cell membrane and is similar to a class of enzymes called the serine proteases, so called because they contain a serine residue in the active site and catalyse the hydrolysis of peptide bonds. In the normal mechanism (Fig. 19.16a), serine acts as a nucleophile to split the peptide bond between the two unusual D-alanine units on a peptide chain. The terminal alanine departs the active site, leaving the peptide chain bound to the active site. The pentaglycyl moiety of another peptide chain now enters the active site and the terminal glycine forms a peptide bond to the alanine group, displacing it from serine and linking the two chains together.

It has been proposed that penicillin has a conformation which is similar to the transition-state conformation taken up by the D-Ala-D-Ala moiety during the cross-linking reaction, and that the enzyme mistakes penicillin for D-Ala-D-Ala and binds it to the active site. Once bound, penicillin is subjected to nucleophilic attack by serine (Fig. 19.16).

The enzyme can attack the β-lactam ring of penicillin and open it in the same way as it did with the peptide bond. However, penicillin is cyclic and as a result the molecule is not split in two and nothing leaves the active site. Subsequent hydrolysis of the ester group linking the penicillin to the active site does not take place either, since the penicillin structure blocks access to the pentaglycine chain or water.

If penicillin *is* acting as a mimic for a D-Ala-D-Ala moiety, this provides another explanation for its lack of

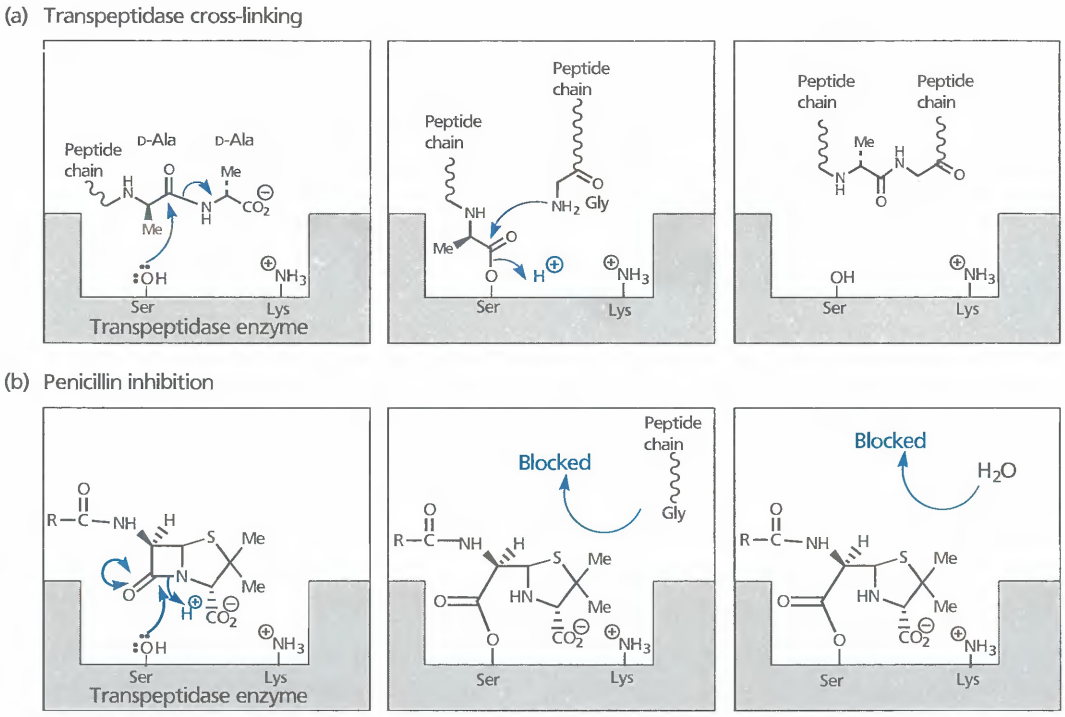


FIGURE 19.16 Mechanisms of transpeptidase crosslinking and penicillin inhibition.

toxicity, as there are no D-amino acids or D-Ala-D-Ala segments in any human protein. It is unlikely that any of the body's serine protease enzymes would recognize either the segment or penicillin itself. As a result, penicillin is selective for the bacterial transpeptidase enzyme and is ignored by the body's own serine proteases.

This theory has one or two anomalies, though. For example, **6-methylpenicillin** (Fig. 19.17) was thought to be a closer analogue to D-Ala-D-Ala. On that basis, it should fit the active site better and have higher activity. However, when this structure was synthesized, it was found to be inactive. It is now proposed that 6-methoxypenicillin is a closer analogue to acyl-D-Ala-D-Ala than 6-methylpenicillin, and penicillin structures containing a 6-methoxy substituent are indeed active. The similarity of 6-methoxypenicillin to acyl-D-Ala-D-Ala can be demonstrated using molecular modelling experiments to overlay the two molecules (Fig. 19.18). This demonstrates a slightly better match between the relevant methyl groups, in comparison to the overlay of 6-methylpenicillin with D-Ala-D-Ala (Fig. 19.18).

19.5.1.5 Resistance to penicillin

Bacterial strains vary in their susceptibility to penicillin. Some species, such as streptococci, are quite vulnerable, whereas a bacterium like *Pseudomonas aeruginosa* is particularly resistant (see Box 19.4). Other species, such as *Staphylococcus aureus*, are initially vulnerable, but acquire resistance when they are exposed to penicillin over a period of time. There are several reasons for this varied susceptibility.

Physical barriers

If penicillin is to inhibit the transpeptidase enzyme, it has to reach the outer surface of the bacterial cell membrane where the enzyme is located. Thus, penicillin has to pass through the cell walls of both Gram-positive and Gram-negative bacteria. The cell wall is much thicker in Gram-positive bacteria than in Gram-negative bacteria, so one might think that penicillin would be more effective against Gram-negative bacteria. However, this is not the case.

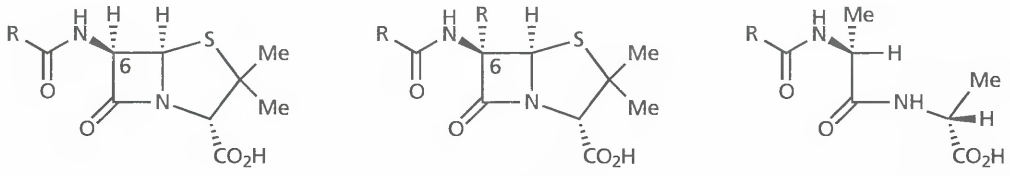


FIGURE 19.17 Comparison of penicillin, 6-substituted penicillins and acyl-D-Ala-D-Ala.

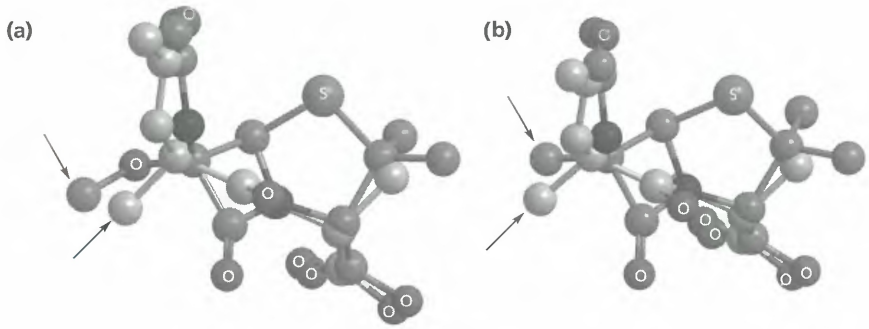


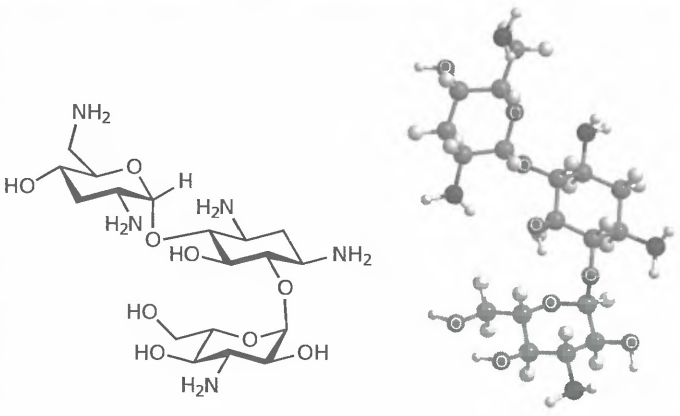
FIGURE 19.18 (**a**) Overlay of 6-methoxypenicillin (dark grey) with D-Ala-D-Ala (light grey). The important methyl groups are indicated. (**b**) Overlay of 6-methylpenicillin (dark grey) with D-Ala-D-Ala (light grey). (The side chain of penicillin has been removed for clarity in both overlays.)

BOX 19.4 Pseudomonas aeruginosa

Pseudomonas aeruginosa is an example of an **opportunistic pathogen**. Such organisms are not normally harmful to healthy individuals. Many people carry the organism without being aware of it, as their immune system keeps the organism under control. Once that immune system is weakened though, the organism can start multiplying and lead to serious illness. Hospital bound patients are particularly at risk, especially those suffering from shock or AIDS, or those undergoing cancer chemotherapy. Burn victims are particularly prone to *P. aeruginosa* skin infections and this can lead to septicaemia which can prove fatal. The organism is also responsible for serious lung infections amongst patients undergoing mechanical ventilation.

The cells of *P. aeruginosa* are rod shaped and can appear blue or green in colour, which is why it was given the name aeruginosa. It prefers to grow in moist environments and has been isolated from soil, water, plants, animals, and humans. It can even grow in distilled water and contact lens solutions. In hospitals, there are several possible sources of infection including respiratory equipment, sinks, uncooked vegetables, and flowers brought by visitors.

P. aeruginosa is a difficult organism to treat because it has an intrinsic resistance to a wide variety of antibacterial agents including many penicillins, cephalosporins, tetracyclines, quinolones, and chloramphenicol. There are two reasons for this. The outer membrane of the cell has a low permeability to drugs, and even if a drug does enter the cell, there is an efflux system which can pump it back out again. Nevertheless, there are drugs that have proved effective against the organism, in particular aminoglycosides such as tobramycin or gentamicin, and penicillins such as ticarcillin. These are often given in combination with each other.



Tobramycin.

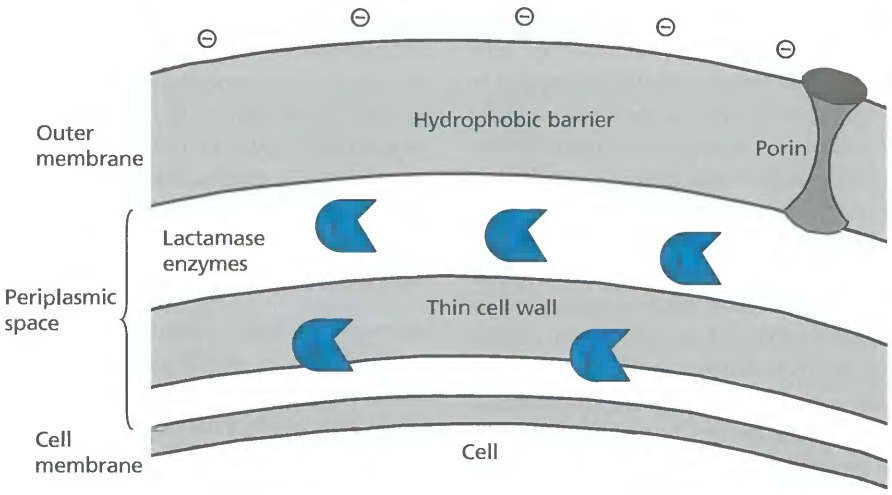


FIGURE 19.19 Outer surface of a Gram-negative bacterial cell.

Although the cell wall is a strong, rigid structure, it is also highly porous and small molecules like penicillin can move through it without difficulty. One can imagine the cell wall being like several layers of chicken wire, and the penicillin molecules as small pebbles able to pass through the gaps.

If the cell wall does not prevent penicillin reaching the cell membrane, what does? As far as Gram-positive bacteria are concerned there is no barrier, and that is why penicillin G has good activity against these organisms. As far as Gram-negative bacteria are concerned, they have an outer lipopolysaccharide membrane surrounding the cell wall which is impervious to water and polar molecules such as penicillin (Fig. 19.19). That can explain why Gram-negative bacteria are generally resistant, but not why some Gram-negative bacteria are susceptible and some are not. Should they not all be resistant?

The answer lies in protein structures called **porins**, which are located in the outer membrane. These act as pores through which water and essential nutrients can pass to reach the cell. Small drugs such as penicillin can also pass this way, but whether they do or not depends on the structure of the porin as well as the characteristics of the penicillin (i.e. its size, structure, and charge). In general, drugs have less chance of passing through the porins if they are large, have a negative charge, and are hydrophobic. In contrast, a small hydrophilic drug that can exist as a zwitterion will move through quickly. Therefore, porins play a crucial role in controlling the amount of penicillin capable of reaching the periplasmic space between the outer membrane and cell membranes. If access is slow, the concentration of penicillin at the transpeptidase enzyme may not be sufficient to inhibit it effectively.

Presence of β-lactamase enzymes

The presence of β-lactamase enzymes is the most important mechanism by which bacteria gain resistance to penicillin. β-Lactamases are enzymes which have mutated from transpeptidases and so they are quite similar in nature. For example, they have a serine residue in the active site and can open up the β-lactam ring of penicillin to form an ester link to the structure. Unlike the transpeptidase enzyme, β-lactamases are able to hydrolyse the ester link and shed the ring-opened penicillin. They do this so effectively that 1000 penicillin molecules are hydrolysed per second (Fig. 19.20).

Some Gram-positive bacterial strains are resistant to penicillin because they can release β-lactamase into the surrounding environment such that penicillin is intercepted before it reaches the cell membrane. The enzyme eventually dissipates through the cell wall and is lost, so the bacterium has to keep generating the enzyme to maintain its protection. *Staphylococcus aureus* is a Gram-positive bacterium that used to be susceptible to penicillin, but 95% of *S. aureus* strains now release a β-lactamase which hydrolyses penicillin G.

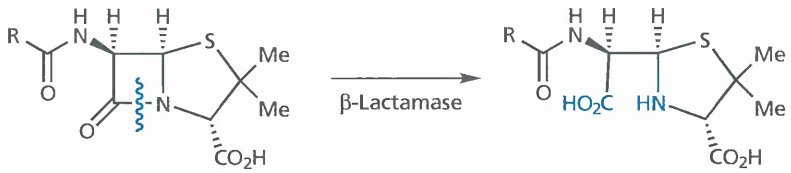


FIGURE 19.20 β-lactamase deactivation of penicillin.

Most if not all Gram-negative bacteria produce β-lactamases which makes them more resistant to penicillins. Moreover, the β-lactamase released is trapped in the periplasmic space between the cell membrane and the outer membrane because it cannot pass through the latter. As a result, any penicillin managing to penetrate the outer membrane encounters a higher concentration of β-lactamase than it would with Gram-positive bacteria. This might suggest again that all Gram-negative bacteria should be resistant to penicillin. However, there are various types of β-lactamase enzyme produced by both Gram-positive and Gram-negative bacteria which vary in their substrate selectivity. Some are selective for penicillins (penicillinases), some for cephalosporins (section 19.5.2) (cephalosporinases) and some for both penicillins and cephalosporins. The differing levels of enzyme and their differing affinities for different β-lactams account for the varying susceptibilities of Gram-negative bacteria to different β-lactams.

High levels of transpeptidase enzyme produced

In some Gram-negative bacteria, excess quantities of transpeptidase are produced, and penicillin is incapable of inactivating all the enzyme molecules present.

Affinity of the transpeptidase enzyme to penicillin

There are several forms of the transpeptidase enzyme present within any bacterial cell and these vary in their affinity for the different β-lactams. Differences in the relative proportions of these enzymes across bacterial species account in part for the variable susceptibility of these bacteria to different penicillins. For example, early strains of *Staphylococcus aureus* contained transpeptidase enzymes which had a high affinity for penicillin and were effectively inhibited. Penicillin-resistant strains of *Staphylococcus aureus* acquired a transpeptidase enzyme called penicillin binding protein (PBP) 2a, which has a much lower affinity to penicillins. The presence of low-affinity transpeptidases is also a problem with enterococci and pneumococci.

Transport back across the outer membrane of Gram-negative bacteria

There are proteins in the outer membrane of some Gram-negative bacteria which are capable of pumping penicillin out of the periplasmic space, thus lowering its concentration and effectiveness. The extent to which this happens varies from species to species and also depends on the structure of the penicillin. This is known as an **efflux** process.

Mutations and genetic transfers

Mutations can occur which will affect any or all of the above mechanisms such that they are more effective in resisting the effects of β-lactams. Small portions of DNA carrying the genes required for resistance can also be transferred from one cell to another by means of genetic vehicles called **plasmids**. These are small pieces of circular bacterial extra-chromosomal DNA. If the transferred DNA contains a gene coding for a β-lactamase enzyme or some other method of improved resistance, then the recipient cell acquires immunity. Genetic material can also be transferred between bacterial cells by viruses and by the uptake of free DNA released by dead bacteria.

19.5.1.6 Methods of synthesizing penicillin analogues

Having studied the mechanism of action of penicillin G and the various problems surrounding resistance, we now look at how analogues of penicillin G can be synthesized which might have improved stability and activity. A method of preparing analogues is required which is cheap, efficient, and flexible. Sheehan's full synthesis of penicillin is too long and low yielding (1%) to be practical, so that limits the options to fermentation methods or semi-synthetic procedures.

Fermentation

Originally, the only way to prepare different penicillins was to vary the fermentation conditions. Adding different carboxylic acids to the fermentation medium resulted in penicillins with different acyl side chains (e.g. **phenoxymethylpenicillin;** Fig. 19.10). Unfortunately, there was a limitation to the sort of carboxylic acid which was accepted by the biosynthetic route (i.e. only acids of general formula RCH_2CO_2H). This in turn restricted the variety of analogues which could be obtained. The other major disadvantage was the tedious and time-consuming nature of the method.

Semi-synthetic procedure

In 1959, Beechams isolated a biosynthetic intermediate of penicillin from *Penicillium chrysogenum* grown in a fermentation medium which was deficient in a carboxylic acid. The intermediate (**6-aminopenicillanic acid; 6-APA**) proved to be one of Sheehan's synthetic intermediates, and so it was possible to use this to synthesize a huge number of analogues by a semi-synthetic method. Thus, fermentation yielded 6-APA, which could then be treated with a range of acid chlorides (Fig. 19.21).

6-APA is now produced more efficiently by hydrolysing penicillin G or penicillin V with an enzyme (**penicillin acylase**) (Fig. 19.22) or by a chemical method that allows the hydrolysis of the side chain in the presence of the highly strained β-lactam ring. The latter procedure is described in more detail in section 19.5.2.2 where it is used to hydrolyse the side chain from cephalosporins.

We have emphasized the drive to make penicillin analogues with varying acyl side chains, but what is so special about the acyl side chain? Could changes not be made elsewhere in the molecule? In order to answer

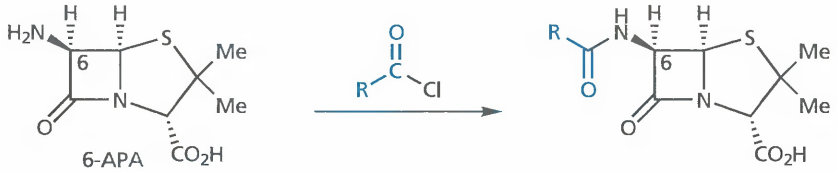


FIGURE 19.21 Penicillin analogues synthesized by acylating 6-APA.

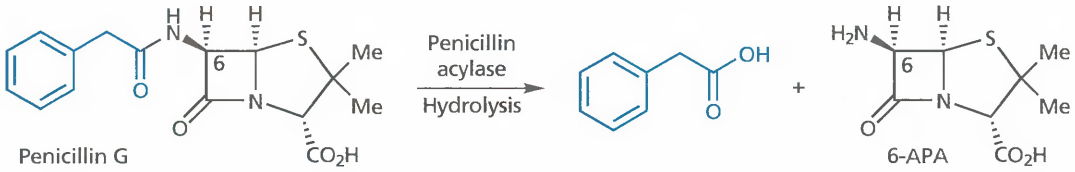


FIGURE 19.22 Synthesis of 6-APA from penicillin G.

these questions we need to look at the structure-activity relationships of penicillins.

19.5.1.7 Structure–activity relationships of penicillins

A large number of penicillin analogues have been synthesized and studied. The results of these studies led to the following SAR conclusions (Fig. 19.23).

- The strained β-lactam ring is essential.
- The free carboxylic acid is essential. This is usually ionized and penicillins are administered as sodium or potassium salts. The carboxylate ion binds to the charged nitrogen of a lysine residue in the binding site.
- The bicyclic system is important. (This confers further strain on the β-lactam ring—the greater the strain, the greater the activity, but the greater the instability of the molecule to other factors.)
- The acylamino side chain is essential.
- Sulfur is usual but not essential.
- The stereochemistry of the bicyclic ring with respect to the acylamino side chain is important.

The results of this analysis led to the inevitable conclusion that very little variation is tolerated by the penicillin nucleus and that any variations are restricted to the acylamino side chain.

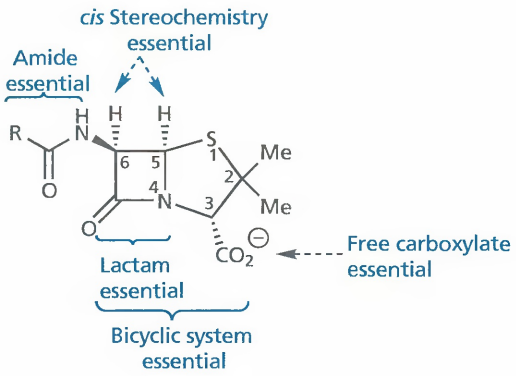


FIGURE 19.23 Structure–activity relationships of penicillins.

19.5.1.8 Penicillin analogues

In this section we consider the penicillin analogues which proved successful in tackling the problems of acid sensitivity, β-lactamase sensitivity and limited breadth of activity.

Acid sensitivity of penicillins

There are three reasons for the acid sensitivity of penicillin G.

- **Ring strain:** The bicyclic system in penicillin consists of a four-membered ring fused to a five-membered ring. As a result, penicillin suffers large angle and torsional strains. Acid-catalysed ring opening relieves these strains by breaking open the more highly strained β-lactam ring (Fig. 19.24).
- **A highly reactive β-lactam carbonyl group:** The carbonyl group in the β-lactam ring is highly susceptible to nucleophiles and does not behave like a normal tertiary amide. The latter is resistant to nucleophilic attack because the carbonyl group is stabilized by the neighbouring nitrogen atom as shown in Fig. 19.25; the nitrogen can feed its lone pair of electrons into the carbonyl group to form a dipolar resonance structure with bond angles of 120°. This resonance stabilization is impossible for the β-lactam ring, because of the increase in angle strain that would result in having a double bond within a 4-membered β-lactam ring. The preferred bond angles for a double bond are 120° but the bond angles of the β-lactam ring are constrained to 90°. As a result, the lone pair is localized on the nitrogen atom, and the carbonyl group is more electrophilic than one would expect for a tertiary amide.
- **Influence of the acyl side chain (neighbouring group participation):** Figure 19.26 demonstrates how the

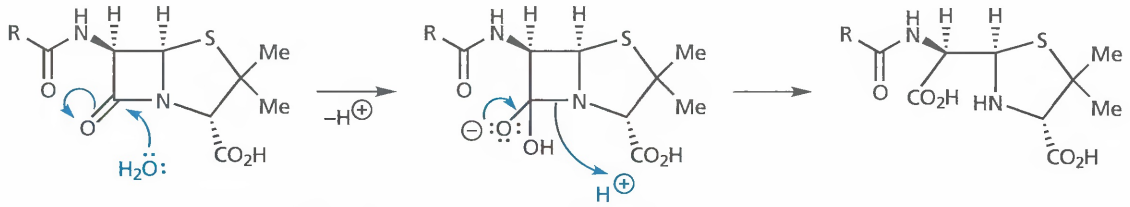


FIGURE 19.24 Ring opening of the β-lactam ring under acidic conditions.

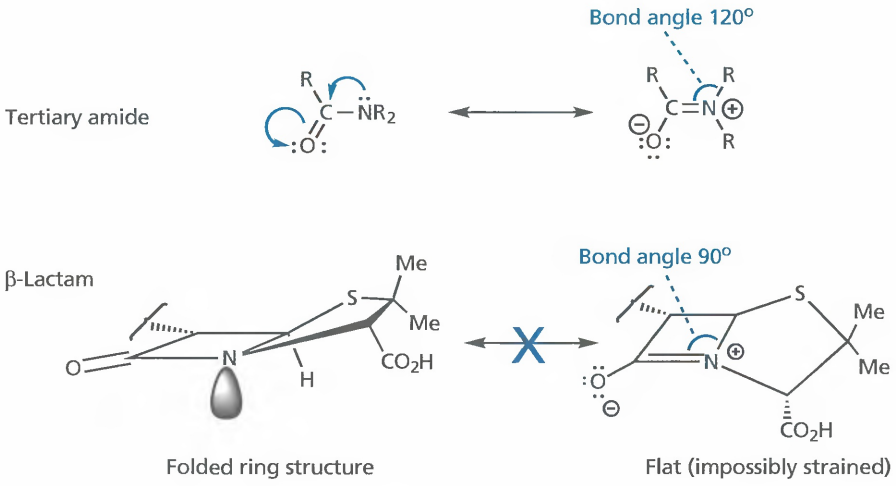


FIGURE 19.25 Comparison of *tertiary* amide and β-lactam carbonyl groups.

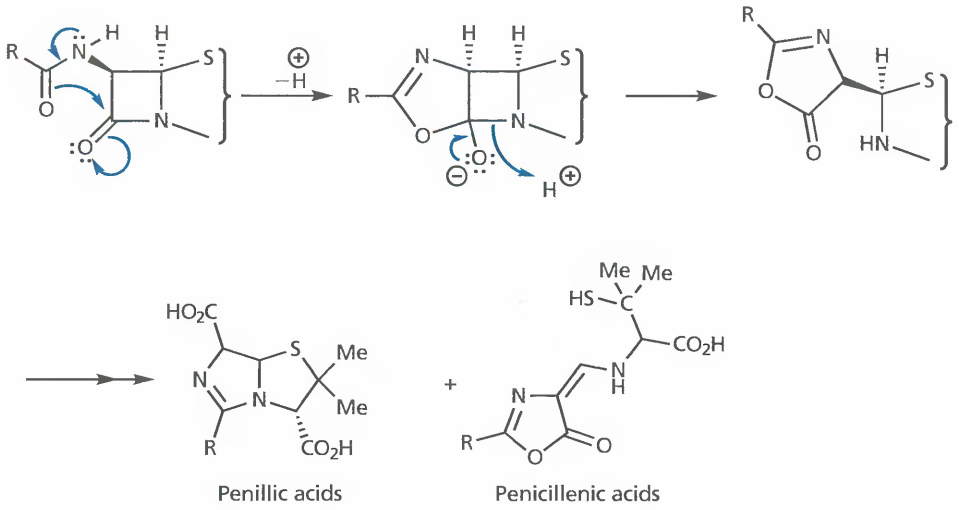


FIGURE 19.26 Influence of the acyl side chain on the acid sensitivity of penicillins.

neighbouring acyl group can actively participate in a mechanism to open up the lactam ring. Thus, penicillin G has a self-destruct mechanism built into its structure.

Acid-resistant penicillins

It can be seen that countering acid sensitivity is a difficult task. Nothing can be done about the first two factors, as the β-lactam ring is vital for antibacterial activity. Therefore, only the third factor can be tackled. The task then becomes one of reducing the amount of neighbouring group participation taking place. This was achieved by placing an electron-withdrawing group in the side chain which could draw electrons away from the carbonyl oxygen and reduce its tendency to act as a nucleophile (Fig. 19.27).

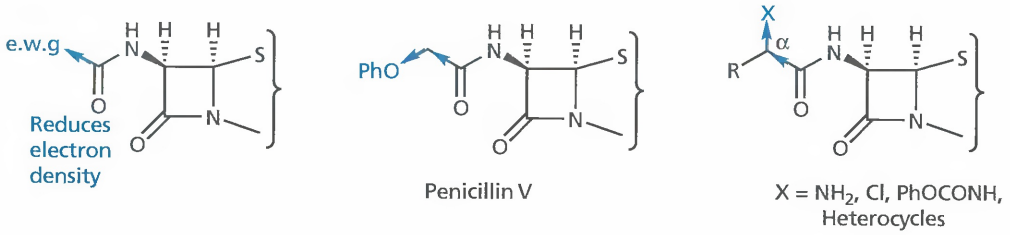


FIGURE 19.27 Reduction of neighbouring group participation with an electron-withdrawing group (e.w.g.).

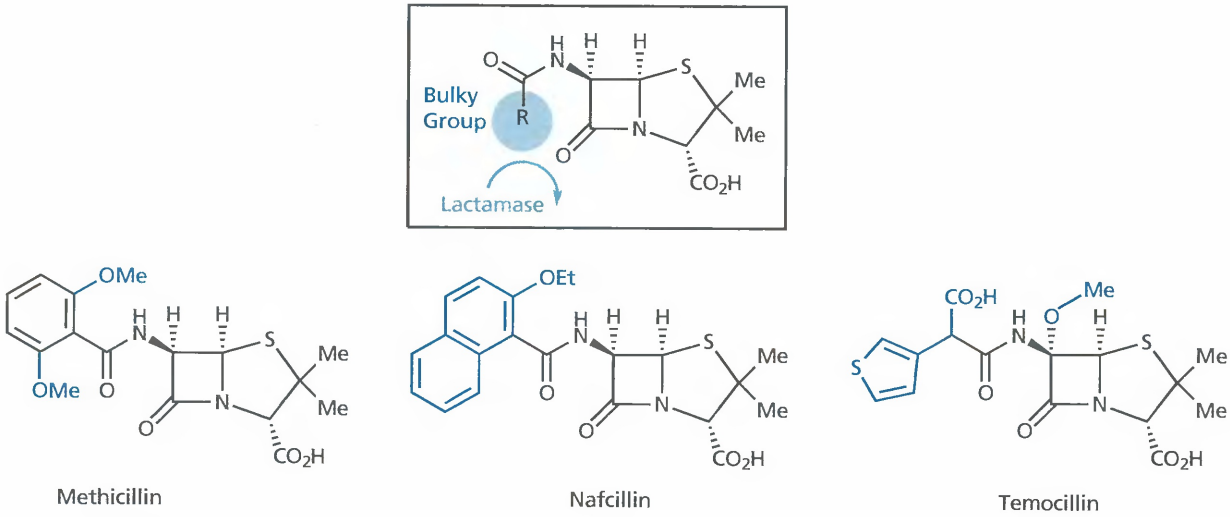


FIGURE 19.28 The use of steric shields to blocking penicillin from reaching the β-lactamase active site.

Phenoxymethylpenicillin (penicillin V) has an electronegative oxygen on the acyl side chain with the electron-withdrawing effect required. The molecule has better acid stability than penicillin G and is stable enough to survive the acid in the stomach, so it can be given orally.

A range of penicillin analogues with an electron-withdrawing substituent (X) on the α-carbon of the side chain (Fig. 19.27) has also proved resistant to acid hydrolysis and can be given orally (e.g. **ampicillin;** see Fig. 19.30).

To conclude, the problem of acid sensitivity is fairly easily solved by having an electron-withdrawing group on the acyl side chain.

Penicillinase-resistant penicillins

The problem of β-lactamases (or penicillinases) became critical in 1960, when the widespread use of penicillin G led to an alarming increase of penicillin-resistant *S. aureus* infections. At one point, 80% of all *S. aureus* infections in hospitals were due to virulent, penicillin-resistant strains. Alarmingly, these strains were also resistant to all other available antibiotics. Fortunately, a solution to the problem was just around the corner—the design of penicillinase-resistant penicillins.

The strategy of steric shields (section 14.2.1) was successfully used to block penicillin from accessing the penicillinase or β-lactamase active site by placing a bulky group on the side chain (Fig. 19.28). However, there was a problem. If the steric shield was *too* bulky, then it also prevented the penicillin from attacking the transpeptidase target enzyme. Therefore, a great deal of work had to be done to find the ideal shield which would be large enough to ward off the lactamase enzyme, but would be small enough to allow the penicillin to bind to the target enzyme. The fact that the β-lactam ring interacts with both enzymes in the same way highlights the difficulty in finding the ideal shield.

Fortunately, shields were found which could make that discrimination. **Methicillin** (Fig. 19.28) was the first effective semi-synthetic penicillin with resistance to the *S. aureus* β-lactamase enzyme and reached the clinic just

in time to treat the growing *S. aureus* problem. The steric shields are the two *ortho*-methoxy groups on the aromatic ring.

Methicillin is by no means an ideal drug, however. With no electron-withdrawing group on the side chain, it is acid sensitive, and has to be injected. It also shows poor activity against many other bacterial strains. Better penicillinase resistant agents have since been developed (Box 19.5), and methicillin is no longer used clinically. **Nafcillin** is a penicillin which is resistant to β-lactamase enzymes and contains a naphthalene ring which acts as its steric shield. **Temocillin** is another β-lactamase resistant penicillin and is interesting in that it has a 6-methoxy group present.

In general, β-lactamase-resistant penicillins are kept as 'reserve troops'. They are only introduced into the fray if an infection proves resistant to a broad-spectrum penicillin as a result of the presence of a β-lactamase enzyme (e.g. penicillin-resistant *S. aureus* and *S. epidermidis*).

Unfortunately, 95% of *S. aureus* strains detected in hospitals have become resistant to methicillin and the other penicillinase-resistant penicillins as a result of mutations to the transpeptidase enzyme. These bacteria are referred to as MRSA. The abbreviation stands for *m*ethicillin *r*esistant *S. aureus*, but the term applies to all the penicillinase-resistant penicillins and not just methicillin.

Broad-spectrum penicillins

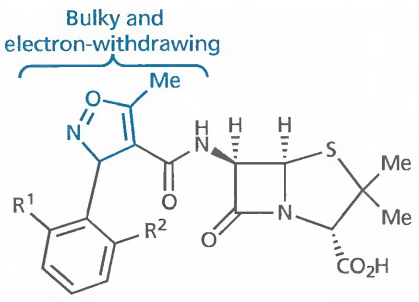
There are a variety of factors affecting whether a particular bacterial strain will be susceptible to a penicillin. The spectrum of activity shown by any penicillin depends on its structure, its ability to cross the cell membrane of Gram-negative bacteria, its susceptibility to β-lactamases,

BOX 19.5

The isoxazolyl penicillins

The incorporation of an isoxazolyl ring into the penicillin side chain led to orally active compounds which were stable to the β-lactamase enzyme of *S. aureus*. The isoxazolyl ring acts as the steric shield but it is also electron-withdrawing giving the structure acid stability.

Oxacillin, cloxacillin, flucloxacillin and dicloxacillin are all useful against *S. aureus* infections. The only difference between them is the type of halogen substitution on the aromatic ring. These substituents affect pharmacokinetic properties such as absorption and plasma protein binding.



Oxacillin	$R^1 = R^2 = H$
Cloxacillin	$R^1 = Cl$, $R^2 = H$
Flucloxacillin	$R^1 = Cl$, $R^2 = F$
Dicloxacillin	$R^1 = Cl$, $R^2 = Cl$

Incorporation of a five-membered heterocycle into a penicillin side chain.

BOX 19.6

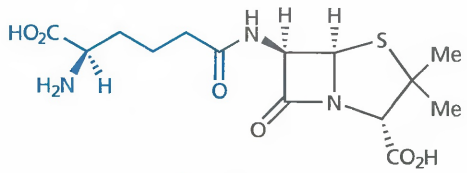
Clinical aspects of β-lactamase-resistant penicillins

Methicillin was useful in the 1960s against penicillin-resistant *S. aureus* infections. However, it is no longer used clinically. **Nafcillin** has more intrinsic activity than methicillin against staphylococci and streptococci, and is administered by injection. **Temocillin** is not active against Gram-positive bacteria or bacteria with altered penicillin-binding proteins. It should be reserved for the treatment of infections caused by β-lactamase producing strains of Gram-negative bacteria, including those resistant to third generation cephalosporins. It is used for the treatment of septicaemia, urinary tract infections and lower respiratory-tract infections caused by susceptible Gram-negative bacteria.

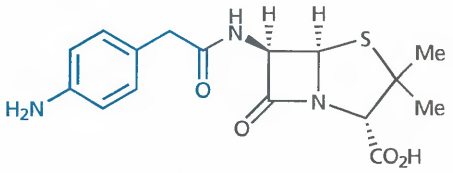
Oxacillin, cloxacillin, flucloxacillin and **dicloxacillin** are all useful agents against *S. aureus* infections. Cloxacillin is better absorbed through the gut wall than oxacillin, whereas flucloxacillin is less bound to plasma protein, resulting in higher levels of the free drug in the blood supply. They all show inferior activity to the original penicillins if they are used against bacteria that lack the β-lactamase enzyme. They are also inactive against Gram-negative bacteria. Flucloxacillin is the drug of choice for the treatment of penicillin-resistant staphylococcal infections in the ear. **Co-fluampicil** is a combination of flucloxacillin with ampicillin, which is used against streptococcal or staphylococcal infections.

its affinity for the transpeptidase target enzyme, and the rate at which it is pumped back out of cells by Gram-negative organisms. All these factors vary in importance across different bacterial species and so there are no clear cut tactics which can be used to improve the spectrum of activity. Consequently, the search for broad-spectrum antibiotics was one of trial and error which involved making a huge variety of analogues. These changes were again confined to variations in the side chain and gave the following results:

- Hydrophobic groups on the side chain (e.g. penicillin G) favour activity against Gram-positive bacteria, but result in poor activity against Gram-negative bacteria.
- If the hydrophobic character is increased, there is little effect on Gram-positive activity, but what activity there is against Gram-negative bacteria drops even more.
- Hydrophilic groups on the side chain have little effect on Gram-positive activity (e.g. **penicillin T**) or cause a reduction of activity (e.g. **penicillin N**) (Fig. 19.29). However, they lead to an increase in activity against Gram-negative bacteria.
- Enhancement of Gram-negative activity is found to be greatest if the hydrophilic group (e.g. NH_2, OH, CO_2H) is attached to the carbon, α to the carbonyl group on the side chain.



Penicillin N



Penicillin T

FIGURE 19.29 Effect of side chain hydrophilic groups on antibacterial activity.

Those penicillins having useful activity against both Gram-positive and Gram-negative bacteria are known as **broad-spectrum antibiotics** (Box 19.8). There are three classes of broad-spectrum antibiotics. All have an α-hydrophilic group which aids the passage of these penicillins through the porins of the Gram-negative bacterial outer cell membrane.

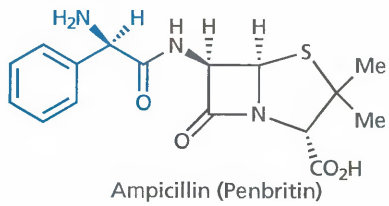
Broad-spectrum penicillins: the aminopenicillins

Ampicillin (Fig. 19.30; Beechams 1964) and **amoxicillin** are orally active compounds having a very similar structure, and are commonly used as a first line of defence against infection. Both compounds are acid resistant because of the presence of the electron-withdrawing amino group. There are no steric shields present, and so these agents are sensitive to β-lactamase enzymes. Both structures are poorly absorbed through the gut wall as both the amino group and the carboxylic group are ionized. This problem can be alleviated by using a prodrug where one of the polar groups is masked with a protecting group which can be removed metabolically once the prodrug has been absorbed (Box 19.7).

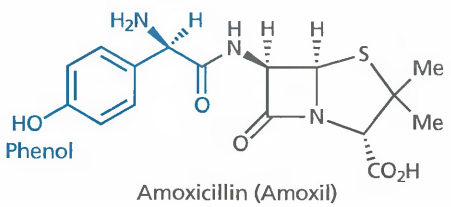
Broad-spectrum penicillins: the carboxypenicillins

Carbenicillin (Fig. 19.31) was the first example of this class of compounds. It shows a broad-spectrum of activity due to the hydrophilic carboxylic acid group (ionized at pH 7) on the side chain. It is particularly interesting to note that the stereochemistry of this group is important. The α-carbon is asymmetric and only one of the two enantiomers is active.

Carfecillin and indanyl carbenicillin (Fig. 19.31) are prodrugs for carbenicillin and show an improved absorption through the gut wall. Aryl esters are better than alkyl esters since the former are chemically more susceptible to hydrolysis because of the electron-withdrawing inductive effect of the aryl ring. An extended ester is not required in this case since the aryl ester is further from the β-lactam ring and is not shielded (cf. Box 19.7). **Ticarcillin** is similar in structure to carbenicillin but has a thiophene ring in place of the phenyl group.



Ampicillin (Penbritin)



Amoxicillin (Amoxil)

FIGURE 19.30 Broad-spectrum penicillins—the aminopenicillins.

BOX 19.7 Ampicillin prodrugs

Pivampicillin, talampicillin, and bacampicillin are prodrugs of ampicillin (Fig. 1). In all three cases, the esters used to mask the carboxylic acid group seem rather elaborate and one may ask why a simple methyl ester is not used. The answer is that methyl esters of penicillins are not metabolized in humans. The bulky penicillin skeleton is so close to the ester that it acts as a steric shield, and prevents the esterase enzymes, which catalyse this reaction from accepting the penicillin ester as a substrate.

Fortunately, acyloxymethyl esters *are* susceptible to esterases. These 'extended' esters contain a second ester group further away from the penicillin nucleus, which is more exposed to attack. The hydrolysis products are inherently unstable and decompose spontaneously to release formaldehyde and reveal the free carboxylic acid (Fig. 2). The release of formaldehyde is not ideal, as it is a toxic chemical. However, it is formed naturally in the body through enzymatic demethylation of various compounds found in the diet, and the levels produced from the prodrugs described above cause little problem because the drugs are only taken for a short duration of time.

Such extended esters can be used to prepare prodrugs of other penicillins, but one has to be careful that one does not go to the other extreme and make the penicillin too lipophilic. For example, the 1-acyloxyalkyl ester of penicillin G is too lipophilic and has poor solubility in water. Fortunately, the problem can easily be avoided by making the extended ester more polar (e.g. by attaching valine as in Fig. 3).

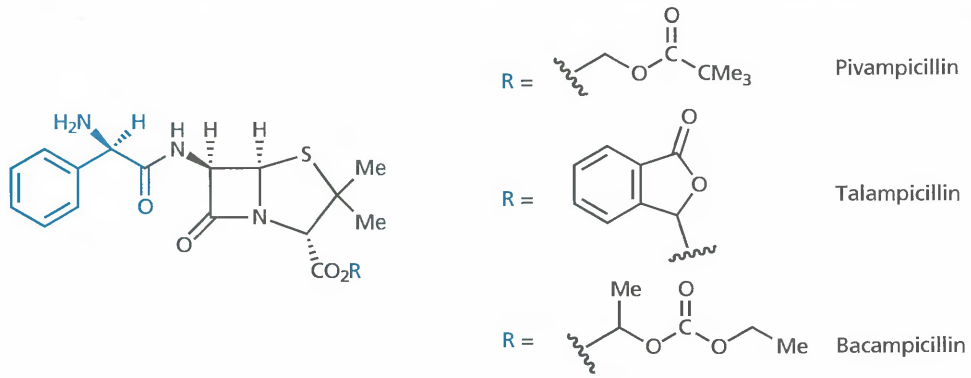


FIGURE 1 Prodrugs used to aid absorption of antibiotic through gut wall.

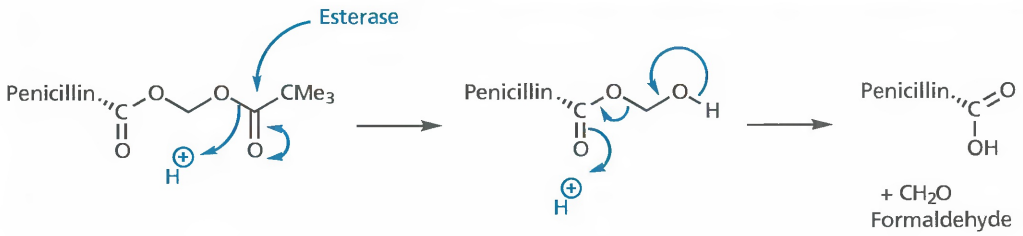


FIGURE 2 Mechanism by which acyloxymethyl esters are hydrolysed.

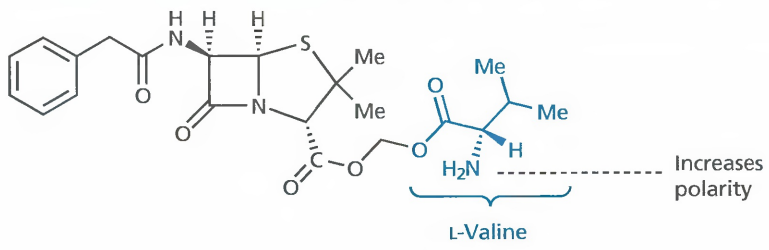


FIGURE 3 Polar extended ester for Penicillin G.

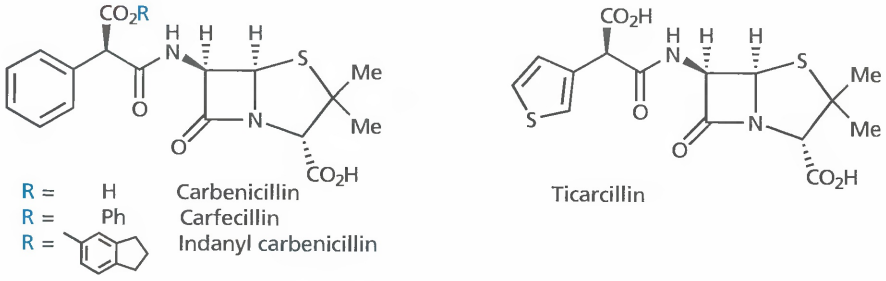


FIGURE 19.31 Carboxypenicillins.

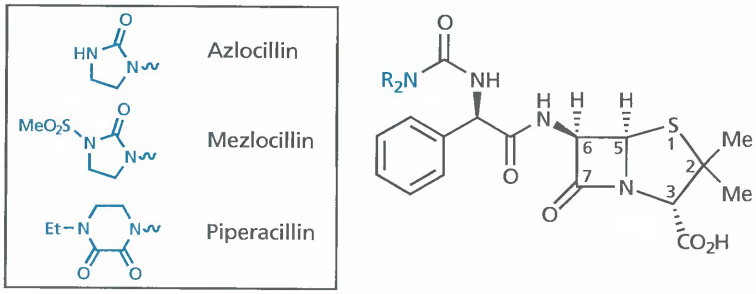


FIGURE 19.32 Ureidopenicillins.

Broad-spectrum penicillins: the ureidopenicillins

Ureidopenicillins (Fig. 19.32) are the newest class of broad-spectrum penicillins and have a urea functional group at the α-position. They generally have better properties than the carboxypenicillins and have largely replaced them in the clinic.

19.5.1.9 Synergism of penicillins with other drugs

There are several examples in medicinal chemistry where the presence of one drug enhances the activity of another. In many cases this can be dangerous, leading to an effective overdose of the enhanced drug. In some cases, though, it can be useful. There are two interesting examples where the activity of penicillin has been enhanced by the presence of another drug.

One of these is the effect of clavulanic acid, described in Section 19.5.4.1. The other is the administration of penicillins with a compound called **probenecid** (Fig. 19.33). Probenecid is a moderately lipophilic carboxylic acid and as such is similar to penicillin. It is found that probenecid can block facilitated transport of penicillin through the kidney tubules. In other words, probenecid slows down the rate at which penicillin is excreted by competing with it in the excretion mechanism. Probenecid also competes with penicillin for binding sites on albumin. As a result, penicillin levels in the bloodstream are enhanced and the antibacterial activity increases—a useful tactic if faced with a particularly resistant bacterium.

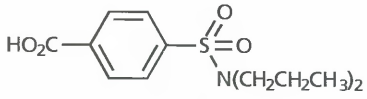


FIGURE 19.33 Probenecid.

KEY POINTS

- Penicillins have a bicyclic structure consisting of a β-lactam ring fused to a thiazolidine ring. The strained β-lactam ring reacts irreversibly with the transpeptidase enzyme responsible for the final cross-linking of the bacterial cell wall.
- Penicillin analogues can be prepared by fermentation or by a semi-synthetic synthesis from 6-aminopenicillanic acid. Variation of the penicillin structure is limited to the acyl side chain.
- Penicillins can be made more resistant to acid conditions by incorporating an electron-withdrawing group into the acyl side chain.
- Steric shields can be added to penicillins to protect them from bacterial β-lactamase enzymes.
- Broad-spectrum activity is associated with the presence of an α-hydrophilic group on the acyl side chain of penicillin.

BOX 19.8 Clinical aspects of broad-spectrum penicillins

Ampicillin and **amoxicillin** have a similar spectrum of activity to penicillin G but are more active against Gram-negative cocci and enterobacteria. They are non-toxic and can be taken orally, but they are sensitive to β-lactamases and are inactive against *Pseudomonas aeruginosa*. Some patients get diarrhoea when they take these penicillins. This is a result of poor absorption from the gut, with ampicillin being more poorly absorbed than amoxicillin. If penicillins are used at high doses for prolonged periods, they abolish the normal gut microflora, and this allows the colonization of resistant Gram-negative bacilli or fungi, which cause the intestinal problems. Ampicillin is currently used to treat sinusitis, bronchitis and a variety of other infections including oral, ear and urinary tract infections. Amoxicillin has been used in the treatment of bronchitis, pneumonia, typhoid, gonorrhoea, Lyme disease and urinary tract infections. Its spectrum of activity is increased when administered with **clavulanic acid** (section 19.5.4.1).

Carbenicillin was the first penicillin to show activity against *Pseudomonas aeruginosa*. Compared to ampicillin, it is active against a wider range of Gram-negative bacteria, and was used particularly against penicillin-resistant strains. However, it is less active than ampicillin against various other bacterial strains, and requires high dose levels. Toxic side effects are observed and the drug shows a marked reduction in activity against Gram-positive bacteria. It is also acid sensitive and has to be injected. Better penicillins such as the ureidopenicillins have since been developed and so the use of carbenicillin is now discouraged.

Carfecillin and indanyl carbenicillin proved useful for the treatment of urinary tract infections, but have generally been superseded by fluoroquinolone antibacterial agents (section 19.8.1).

Ticarcillin is administered by injection, and has an identical antibacterial spectrum to carbenicillin. However, it has the advantage that smaller doses can be used. It is also 2–4 times more effective against *P. aeruginosa*, and has fewer side effects. The drug is mainly used against infections due to *Pseudomonas* and *Proteus* species, and is currently administered with clavulanic acid to broaden its spectrum of activity (section 19.5.4.1).

Ureidopenicillins are generally more active than the carboxypenicillins against streptococci and *Haemophilus* species. They show similar activity against Gram-negative aerobic rods such as *P. aeruginosa*, but are generally more active against other Gram-negative bacteria. Unfortunately, they have to be injected. Examples include **azlocillin**, which is 8–16 times more active than carbenicillin against *P. aeruginosa*, and is used primarily for the treatment of infections caused by that organism. It is susceptible to β-lactamases. **Mezlocillin** has a similar spectrum of activity to carbenicillin but is more active since it has a higher affinity for transpeptidases, and can cross the outer membrane of Gram-negative bacteria more effectively. **Piperacillin** is similar to ampicillin in its activity against Gram-positive species. It also has good activity against anaerobic species of both cocci and bacilli, and can be used against a variety of infections. It is more active than ticarcillin against *Pseudomonas aeruginosa*. Piperacillin can be administered alongside **tazobactam** to widen its spectrum of activity (section 19.5.4.2).

- Prodrugs of penicillins are useful in masking polar groups and improving absorption from the gastrointestinal tract. Extended esters are used which undergo enzyme catalysed hydrolysis to produce a product which spontaneously degrades to release the penicillin.
- Probenecid can be administered with penicillins to hinder the excretion of penicillins.

19.5.2 Cephalosporins

19.5.2.1 Cephalosporin C

Discovery and structure of cephalosporin C

The second major group of β-lactam antibiotics to be discovered were the cephalosporins. The first cephalosporin (**cephalosporin C**) was derived from a fungus obtained in the mid 1940s from sewer waters on the island of Sardinia. This was the work of an Italian professor who noted that the waters surrounding the sewage outlet periodically cleared of microorganisms. He reasoned that an organism might be producing an antibacterial substance and so he collected samples and managed to isolate a fungus called *Cephalosporium acremonium* (now called *Acremonium chrysogenum*). The crude extract from this organism was shown to have antibacterial properties, and in 1948, workers at Oxford University isolated cephalosporin C, but it was not until 1961 that the structure was established by X-ray crystallography.

The structure of cephalosporin C (Fig. 19.34) has similarities to that of penicillin in that it has a bicyclic system

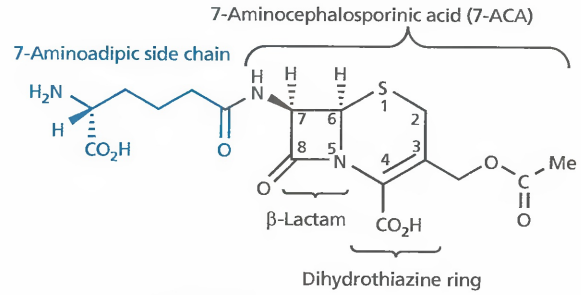


FIGURE 19.34 Cephalosporin C.

containing a four-membered β-lactam ring. This time the β-lactam ring is fused to a six-membered dihydrothiazine ring. The larger ring relieves the strain in the bicyclic system to some extent, but it is still a reactive system. A study of the cephalosporin skeleton reveals that cephalosporins can be derived from the same biosynthetic precursors as penicillin (i.e. cysteine and valine) (Fig. 19.35).

Properties of cephalosporin C

Cephalosporin C is not particularly potent compared to penicillins (1/1000 the activity of penicillin G), but the antibacterial activity it *does* have is more evenly directed against Gram-negative and Gram-positive bacteria. Another in-built advantage of cephalosporin C is its greater resistance to acid hydrolysis and to β-lactamase enzymes. It is also less likely to cause allergic reactions. Therefore, cephalosporin C became a useful lead compound for the development of broad-spectrum antibiotics. The aim was to produce analogues with increased potency, while retaining the breadth of activity.

Structure–activity relationships of cephalosporin C

Many analogues of cephalosporin C have been made which demonstrate the importance of the β-lactam ring within the bicyclic system, the ionized carboxylate group at position 4 and the acylamino side chain at position 7. These results tally closely with those obtained for the penicillins. The strain effect of a six-membered ring fused to a four-membered ring is less than for penicillin, but this is partially offset by the effect of the acetyloxy group at position 3. This can act as a good leaving group in the inhibition mechanism (Fig. 19.36).

There is a limited number of places where modifications can be made (Fig. 19.37), but there are more possibilities than with penicillins:

- variations of the 7-acylamino side chain
- variations of the 3-acetoxymethyl side chain
- extra substitution at carbon 7.

19.5.2.2 Synthesis of cephalosporin analogues at position 7

Access to analogues with varied side chains at position 7 initially posed a problem. Unlike penicillins, it proved impossible to obtain cephalosporin analogues by fermentation. Similarly, it was not possible to obtain **7-ACA (7-aminocephalosporinic acid)** either by

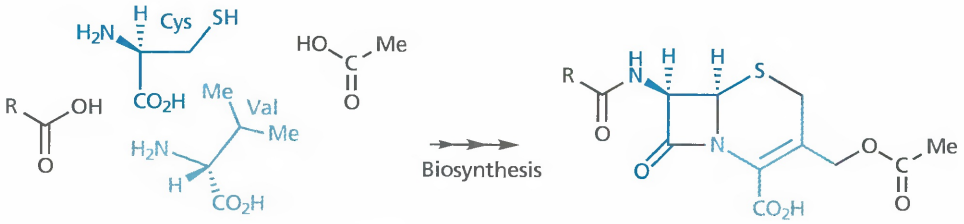


FIGURE 19.35 Biosynthetic precursors of cephalosporin C.

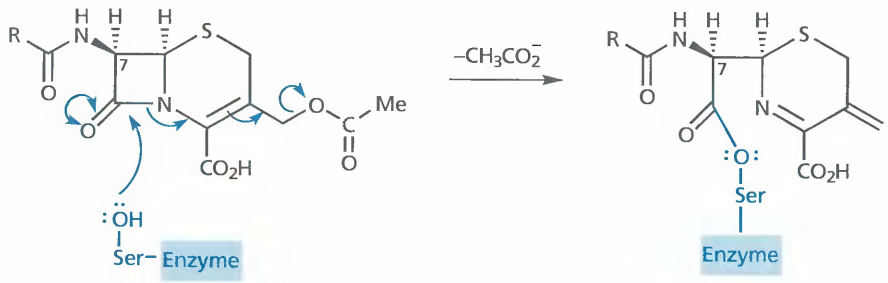


FIGURE 19.36 Mechanism by which cephalosporins inhibit the transpeptidase enzyme.

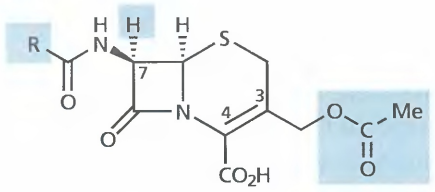


FIGURE 19.37 Positions for possible modification of cephalosporin C. The shading indicates positions which can be varied.

fermentation or by enzymatic hydrolysis of cephalosporin C, thus preventing the semi-synthetic approach analogous to the preparation of penicillins from 6-APA (section 19.5.1.6).

Therefore, a way had to be found of obtaining 7-ACA from cephalosporin C by chemical hydrolysis. This is no easy task, as a secondary amide has to be hydrolysed in the presence of a highly reactive β-lactam ring. Normal hydrolytic procedures are not suitable and so a special method had to be worked out (Fig. 19.38).

The strategy used takes advantage of the fact that the β-lactam nitrogen is unable to share its lone pair of electrons with its neighbouring carbonyl group. The first step of the procedure requires the formation of a double bond between the nitrogen on the side chain and its neighbouring carbonyl group (Fig. 19.39). This is only possible for the secondary amide group, since ring constraints prevent the β-lactam nitrogen forming a double bond within the β-lactam ring. A chlorine atom is now introduced to form an imino chloride which can then be treated with an alcohol to give an imino ether. This functional group is now more susceptible to hydrolysis than the β-lactam ring and so treatment with aqueous acid successfully gives the desired 7-ACA which can then be acylated to give a range of analogues.

19.5.2.3 First-generation cephalosporins

Examples of first-generation cephalosporins include **cephalothin, cephaloridine, cefalexin** and **cefazolin** (Figs. 19.40–19.43). In general, they have a lower activity than comparable penicillins, but a better range. Most

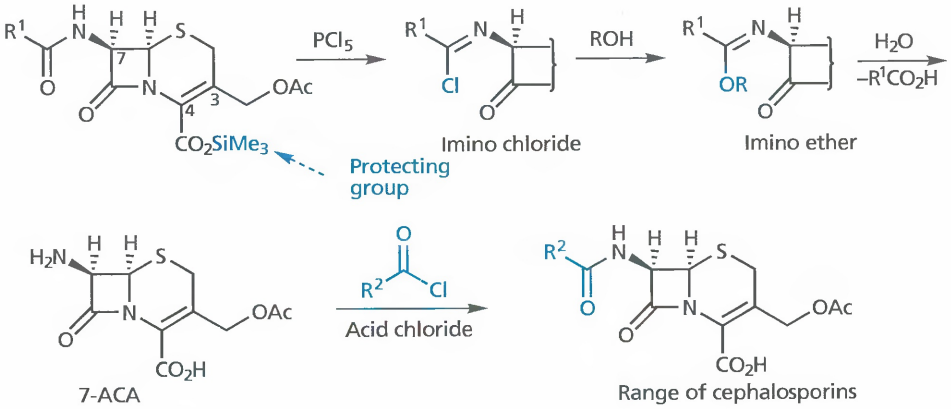


FIGURE 19.38 Synthesis of 7-ACA and cephalosporin analogues.

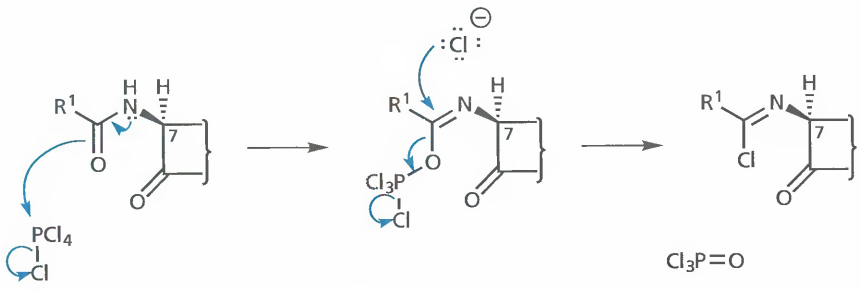


FIGURE 19.39 Mechanism for imino chloride formation.

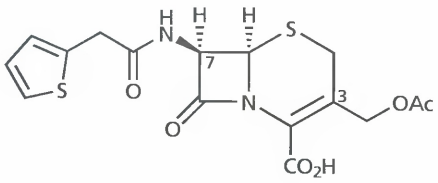


FIGURE 19.40 Cephalothin.

are poorly absorbed through the gut wall and have to be injected. As with penicillins, the appearance of resistant organisms has posed a problem, particularly with Gram-negative organisms. These contain β-lactamases which are more effective than the β-lactamases of Gram-positive organisms. Steric shields are successful in protecting cephalosporins from these β-lactamases, but also prevent them from inhibiting the transpeptidase target enzymes.

One of the most commonly used first-generation cephalosporins was **cephalothin** (Fig. 19.40). A disadvantage with cephalothin is the fact that the acetyloxy group at position 3 is readily hydrolysed by esterase enzymes to give the less active alcohol (Fig. 19.41). The acetyloxy group is important to the mechanism of inhibition and acts as a good leaving group, whereas the alcohol is a much poorer leaving group. Therefore, it would be useful if this metabolism could be blocked to prolong activity. Replacing the ester with a metabolically stable pyridinium group gives **cephaloridine** (Fig. 19.42). The pyridine can still act as a good leaving group for the inhibition mechanism, but is not cleaved by esterases. Cephaloridine exists as a zwitterion and is soluble in water, but like most first-generation cephalosporins, it is poorly absorbed through the gut wall and has to be injected.

Cefalexin (Fig. 19.42) has a methyl substituent at position 3 (Box 19.9). This is bad for activity, since it is not a good leaving group, but it appears to help oral absorption, and cefalexin is one of the few cephalosporins which can be taken orally. The mechanism of absorption through the gut wall is poorly understood and it is not clear why the 3-methyl group is so advantageous. Although the methyl group at position 3 is bad for activity, the presence of a hydrophilic amino group at the α-carbon of the 7-acylamino side chain helps to compensate. **Cefazolin** (Fig. 19.43) is another example of a first-generation cephalosporin.

19.5.2.4 Second-generation cephalosporins

Cephamycins

A methoxy substituent at position 7 of the cephalosporin skeleton has proved advantageous. Compounds such as these are known as cephamycins. The parent compound **cephamycin C** (Fig. 19.44) was isolated from a culture of *Streptomyces clavuligerus* and was the first β-lactam to be isolated from a bacterial source. Modification of the side chain gave **cefoxitin** (Fig. 19.44) which showed a broader spectrum of activity than most first-generation cephalosporins. This is due to greater resistance to β-lactamase enzymes which may be due to the steric hindrance provided by the extra methoxy group. Cefoxitin shows good metabolic stability to esterases due to the presence of a urethane group rather than an ester at position 3 (section 14.2.2).

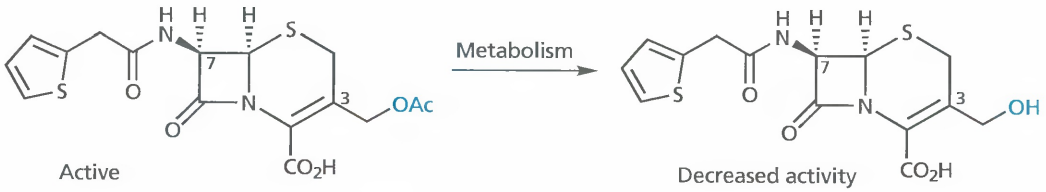


FIGURE 19.41 Metabolic hydrolysis of cephalothin.

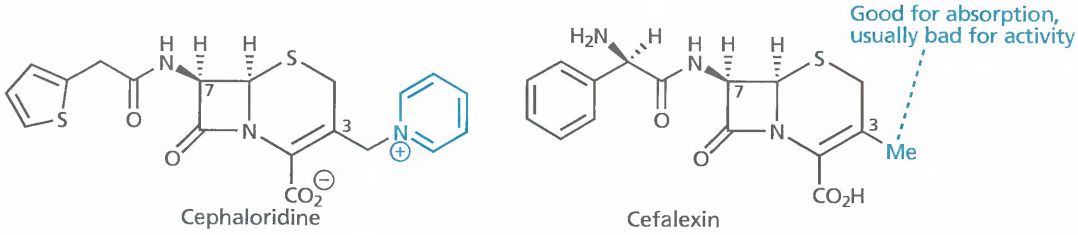
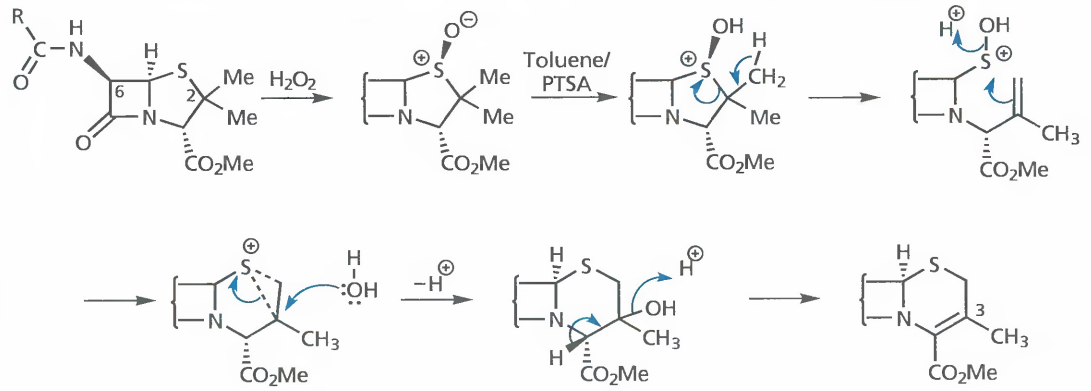


FIGURE 19.42 Cephaloridine and cefalexin.

BOX 19.9 Synthesis of 3-methylated cephalosporins

The synthesis of 3-methylated cephalosporins involves the use of a penicillin starting material as shown below. The synthesis, which was first demonstrated by Eli Lilly Pharmaceuticals, involves oxidation of sulfur followed by an acid catalysed ring expansion, where the five-membered thiazolidine ring in penicillin is converted to the six-membered dihydrothiazine ring in cephalosporin.



Synthesis of 3-methylated cephalosporins from a penicillin.

Oximinocephalosporins

A major advance in cephalosporin research has been the development of the oximinocephalosporins. These contain an iminomethoxy group at the α-position of the acyl side chain, which significantly increases the stability of cephalosporins against the β-lactamases produced by some organisms (e.g. *Haemophilus influenza*). The first useful agent in this class of compounds was **cefuroxime** (Fig. 19.45) which, like cefoxitin, has an increased resistance to β-lactamases and mammalian esterases, but unlike cefoxitin, retains activity against streptococci and to a lesser extent staphylococci.

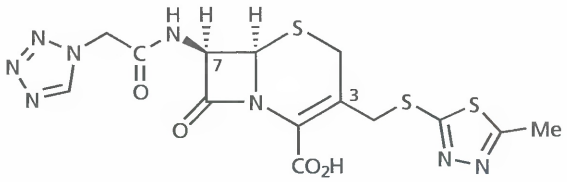


FIGURE 19.43 Cefazolin.

19.5.2.5 Third-generation cephalosporins

Replacing the furan ring of the aforesaid oximinocephalosporins with an aminothiazole ring enhances the penetration of cephalosporins through the outer membrane of Gram-negative bacteria and may also increase affinity for the transpeptidase enzyme. As a result, third-generation cephalosporins containing this ring have a marked increase in activity against these bacteria. A variety of such structures have been prepared such as **ceftazidime, cefotaxime, ceftizoxime** and **ceftriaxone**

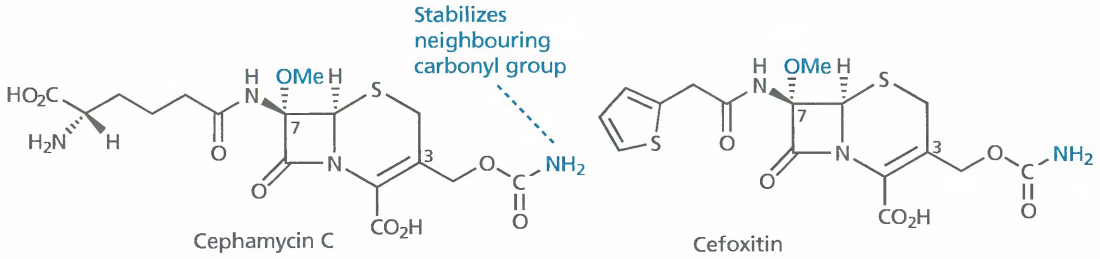


FIGURE 19.44 Cephamycin C and cefoxitin.

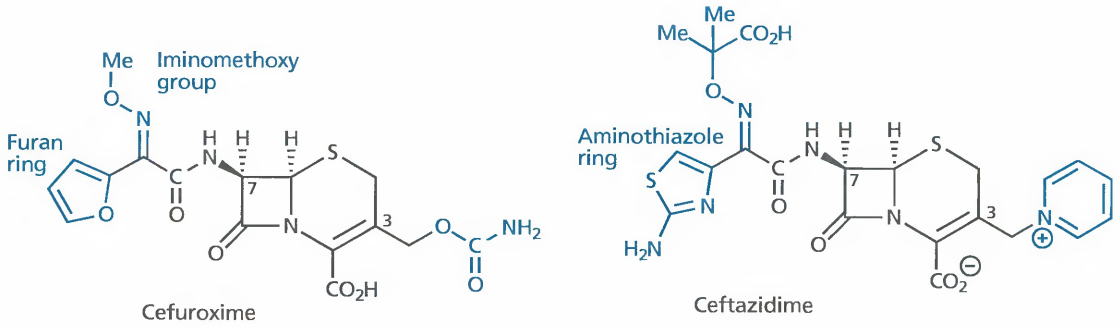


FIGURE 19.45 Cefuroxime and ceftazidime.

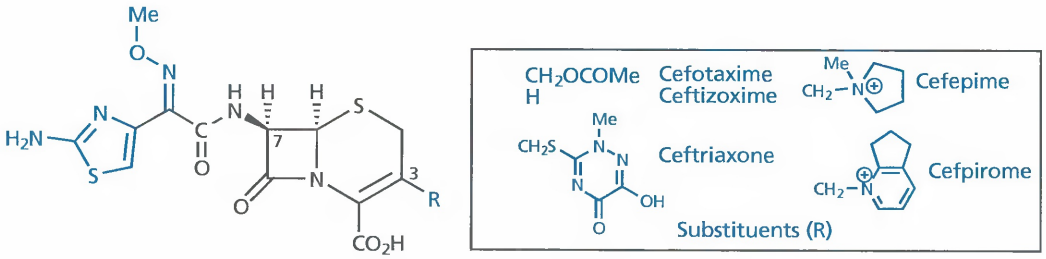


FIGURE 19.46 Third-generation and fourth-generation oximinocephalosporins.

(Figs. 19.45–46) with different substituents at position 3 to vary the pharmacokinetic properties of the analogues. They play a major role in antimicrobial therapy due to their activity against Gram-negative bacteria, many of which are resistant to other β-lactams. Because such infections are uncommon outside hospitals, physicians are discouraged from prescribing these drugs routinely, and they are viewed as 'reserve troops' to be used for troublesome infections which do not respond to the more commonly prescribed β-lactams.

19.5.2.6 Fourth-generation cephalosporins

Cefepime and **cefpirome** (Fig. 19.46) are oximinocephalosporins which have been classed as fourth-generation cephalosporins. They are zwitterionic compounds having a positively charged substituent at position 3 and a negatively charged carboxylate group at position 4. This property appears to radically enhance the ability of these compounds to penetrate the outer membrane of Gram-negative bacteria. They are also found to have a good affinity for the transpeptidase enzyme and a low affinity for a variety of β-lactamases.

19.5.2.7 Resistance to cephalosporins

The activity of a specific cephalosporin against a particular bacterial cell is dependent on the same factors as those for penicillins; that is, ability to reach the transpeptidase enzyme, stability to any β-lactamases which might be present, and the affinity of the antibiotic for the target. For example, most cephalosporins (with the exception of cephaloridine) are stable to the β-lactamase produced by *S. aureus*, and as this is a Gram-positive organism, the β-lactams can reach the transpeptidase enzyme without difficulty. Therefore, the relative ability of cephalosporins to inhibit *S. aureus* comes down to their affinity for the target transpeptidase enzyme. Agents such as the cephamycins and ceftazidime have poor affinity whereas other cephalosporins have a higher affinity. The MRSA organism contains a modified transpeptidase enzyme (PBP2a) for which both penicillins and cephalosporins have a poor affinity.

KEY POINTS

- Cephalosporins contain a strained β-lactam ring fused to a dihydrothiazine ring.
- In general, first-generation cephalosporins offer advantages over penicillins in that they have greater stability to acid and β-lactamases, and have a good ratio of activity against Gram-positive and Gram-negative bacteria. However, they have poor oral availability and are generally lower in activity.
- Variation of the 7-acylamino side chain alters antimicrobial activity whereas variation of the side chain at position

BOX 19.10 Clinical aspects of cephalosporins

In general, cephalosporins are useful broad-spectrum antibacterial agents for the treatment of septicaemia, pneumonia, meningitis, biliary tract infections, peritonitis and urinary tract infections. **Cephalosporin C** itself has been used in the treatment of urinary tract infections, since it is found to concentrate in the urine and survive the body's hydrolytic enzymes.

First-generation cephalosporins

First-generation cephalosporins have good activity against Gram-positive cocci, and they can be used to treat some community-derived Gram-negative infections (i.e. infections not caught in a hospital). They can also be used against *S. aureus* and streptococcal infections when penicillins have to be avoided. **Cephalothin** is more active than penicillin G against some Gram-negative bacteria, and is less likely to cause allergic reactions. It can also be used against β-lactamase producing *S. aureus* strains.

Cefalexin is useful for the treatment of urinary tract infections which do not respond to other drugs or which occur in pregnancy. It is also useful in treating infections of the respiratory tract, ear, skin and mouth. **Cefazolin** is recommended for use as a prophylactic to prevent infection when surgical procedures are used to implant foreign bodies.

Second-generation cephalosporins

In general, the second-generation cephalosporins have variable activity against Gram-positive cocci, but increased activity against Gram-negative bacteria. **Cefoxitin** is active against bowel flora including *Bacteroides fragilis,* and was once recommended for peritonitis. **Cefuroxime** has a wide spectrum of activity and is useful against organisms that have become resistant to penicillin. However, it is not active against 'difficult' bacteria such as *Pseudomonas aeruginosa.* It is used clinically against *Neisseria gonorrhoeae* and respiratory infections caused by *H. influenza, Moraxella catarrhalis,* and susceptible strains of *S. pneumoniae.* It is also used for surgical prophylaxis as well as for the treatment of Lyme disease. **Cefotaxime** is used in surgical prophylaxis and for the treatment of gonorrhoea, meningitis, and infections due to *Haemophilus epiglottis.*

Third-generation cephalosporins

Third-generation cephalosporins have good activity against Gram-negative bacteria, but vary in their activity against Gram-positive cocci. The ability to attack *P. aeruginosa* also varies from structure to structure, and they lack activity against the MRSA organisms and *Enterobacter* species. **Ceftazidime** is an injectable cephalosporin that has excellent activity against *P. aeruginosa,* as well as other Gram-negative bacteria. As the drug can cross the blood–brain barrier, it can be used to treat meningitis. Compared with the other aminothiazole structures, ceftazidime has good activity against streptococci, but loses activity against strains of methicillin-susceptible *S. aureus.* This is due to a decreased binding affinity for the transpeptidase enzyme present in *S. aureus.* **Ceftriaxone** is used for surgical prophylaxis and as a prophylactic for meningococcal meningitis.

Fourth-generation cephalosporins

Fourth-generation cephalosporins have activity against Gram-positive cocci and a broad array of Gram-negative bacteria including *P. aeruginosa* and many of the enterobacterial species. **Cefpirome** is administered as an intravenous injection or infusion and has been used against a variety of sensitive Gram-positive and Gram-negative bacteria.

3 predominantly alters the metabolic and pharmacokinetic properties of the compound. Introduction of a methoxy substitution at C-7 is possible.

- Semisynthetic cephalosporins can be prepared from 7-aminocephalosporanic acid (7-ACA).
- 7-ACA is obtained from the chemical hydrolysis of cephalosporins. This requires prior activation of the side chain to make it more reactive than the β-lactam ring.
- Deacetylation of cephalosporins occurs metabolically to produce inactive metabolites. Metabolism can be blocked by replacing the susceptible acetoxy group with metabolically stable groups.
- Methyl substitution at position 3 of cephalosporins is good for oral absorption but is bad for activity unless a hydrophilic group is present at the α-position of the acyl side chain.
- 3-Methylated cephalosporins can be synthesized from penicillins.
- Cephamycins are cephalosporins containing a methoxy group at position 7.
- Oximinocephalosporins have resulted in second, third, and fourth generations of cephalosporins with increased potency and a broader spectrum of activity, particularly against Gram-negative bacteria.

19.5.3 Other β-lactam antibiotics

Although penicillins and cephalosporins are the best known and most researched β-lactams, there are other β-lactam structures which are of great interest in the antibacterial field.

19.5.3.1 Carbapenems

Thienamycin (Fig. 19.47) was the first example of this class of compounds, and was isolated from *Streptomyces cattleya* in 1976. It is potent with an extraordinarily broad range of activity against Gram-positive and Gram-negative bacteria, including *P. aeruginosa*. It has low toxicity and shows a high resistance to β-lactamases. This resistance has been ascribed to the presence of the hydroxyethyl side chain. Unfortunately, it shows poor metabolic and chemical stability, and is not absorbed from the gastrointestinal tract. The unexpected features of the structure of thienamycin are the missing sulfur atom and acylamino side chain, both of which were thought to be essential to antibacterial activity. Furthermore, the stereochemistry of the side chain at substituent 6 is opposite from the usual stereochemistry in penicillins, another factor in the resistance of this agent to β-lactamases. **Imipenem** and **meropenem** are clinically useful analogues of thienamycin (Box 19.11). Imipenem is susceptible to metabolism by a dehydropeptidase enzyme, whereas meropenem is more resistant due to the different substituent at position 2. **Ertapenem** was approved in 2002 and is similar in structure to meropenem. It has an extra substituent on the carbapenem ring (R^1 = Me) which provides further stability against dehydropeptidases, while the ionized benzoic acid contributes to high protein binding and prolongs the half-life of the drug such that once daily dosing is allowed. In general, the carbapenems have the broadest spectrum of activity of all the β-lactam antibiotics.

19.5.3.2 Monobactams

Monocyclic β-lactams such as the nocardicins (Fig. 19.48) have been isolated from natural sources. At least seven nocardicins have been isolated by the Japanese company Fujisawa. They show moderate activity *in vitro* against a narrow group of Gram-negative bacteria including *P. aeruginosa*. However, it is surprising that they should show any activity at all since they contain a single β-lactam ring unfused to any other ring system. The presence of a fused second ring has always been thought to be essential in order to strain the β-lactam ring sufficiently for antibacterial activity.

One explanation for the surprising activity of the nocardicins is that they operate via a different mechanism from penicillins and cephalosporins. There is some evidence supporting this in that the nocardicins are inactive against Gram-positive bacteria and generally show a different spectrum of activity from the other β-lactam antibiotics. It is possible that these compounds act on cell wall synthesis by inhibiting a different enzyme. They also show low levels of toxicity. **Aztreonam** (Fig. 19.48) is an example of a monobactam which has reached the clinic, and was developed from a naturally occurring monobactam isolated from *Chromobacterium violaceum*,

19.5.4 β-Lactamase inhibitors

19.5.4.1 Clavulanic acid

Clavulanic acid (Fig. 19.49) was isolated from *Streptomyces clavuligerus* by Beechams in 1976. It has weak and unimportant antibiotic activity, but it is a powerful and irreversible inhibitor of most β-lactamases and is now used as such in combination with traditional penicillins such as amoxicillin (**Augmentin**). This allows the dose levels of amoxicillin to be decreased and also increases the spectrum of activity. However, it should be noted that there are various types of β-lactamases. Although

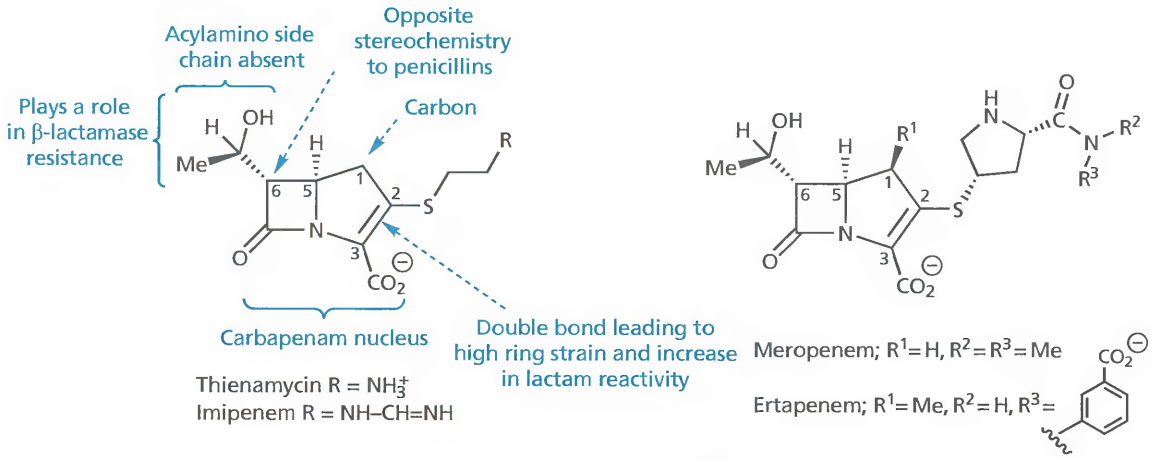


FIGURE 19.47 Carbapenems.

BOX 19.11 Clinical aspects of miscellaneous β-lactam antibiotics

Imipenem is active against a variety of aerobic, anaerobic, Gram-positive and Gram-negative infections, and can be effective against some infections that do not respond to cephalosporins, or infections which have become resistant to more conventional β-lactams. It can be used against hospital-acquired septicaemia, and for surgical prophylaxis. The structure is metabolized by a dehydropeptidase enzyme to produce metabolites that are toxic to the kidney, but this can be alleviated by administrating the drug alongside **cilastatin**—a dehydropeptidase inhibitor which protects imipenem from metabolism. Administration is by intramuscular injection or by intravenous infusion. **Meropenem** is also effective against a variety of aerobic, anaerobic, Gram-positive and Gram-negative infections, and is administered by intravenous injection or infusion. Meropenem is slightly less active than imipenem against Gram-positive bacteria, but is more active against Gram-negative bacteria. Unlike imipenem, meropenem is active against *P. aeruginosa*, and can be administered on its own since it is more resistant to dehydropeptidases. Both meropenem and imipenem penetrate the outer membrane of Gram-negative bacteria through porins, but meropenem enters more efficiently, so it has a higher activity against these bacteria. The drug has been used to treat pneumonia, meningitis, abdominal infections, and urinary tract infections. **Ertapenem** is administered by intravenous infusion and is used for the treatment of abdominal infections, acute gynaecological infections, community-acquired pneumonia, and diabetic foot infections of the skin and soft tissue. It is also used as a prophylactic for colorectal surgery. **Aztreonam** is used against Gram-negative infections including *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Neisseria meningitidis*. It is administered by intravenous injection and can be safely used for patients with allergies to penicillin or cephalosporins. It has no activity against Gram-positive organisms or anaerobic bacteria, as it does not bind to the transpeptidases produced by these organisms. However, it can bind to and inhibit the transpeptidases produced by Gram-negative aerobic organisms.

clavulanic acid is effective against most of these, it is not effective against all. Clavulanic acid is also administered intravenously with ticarcillin as **Timentin**.

The structure of clavulanic acid was the first example of a naturally occurring compound where the β-lactam ring was not fused to a sulfur-containing ring—it is fused instead to an oxazolidine ring. The structure is also unusual in that it does not have an acylamino side chain.

Many analogues have now been made and the essential requirements for β-lactamase inhibition are:

- a strained β-lactam ring
- the enol ether

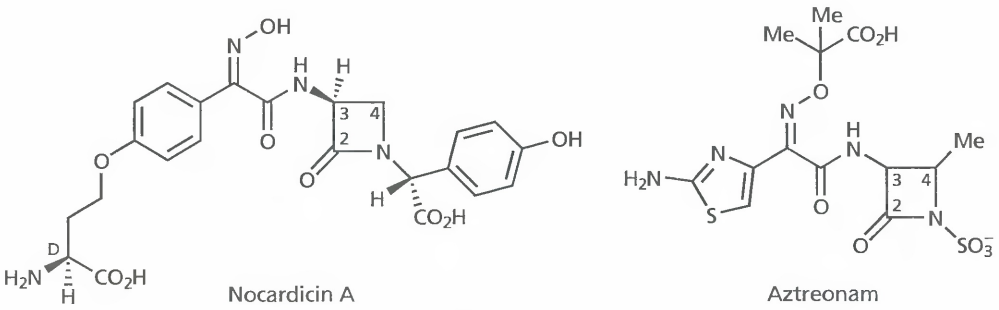


FIGURE 19.48 Monobactams.

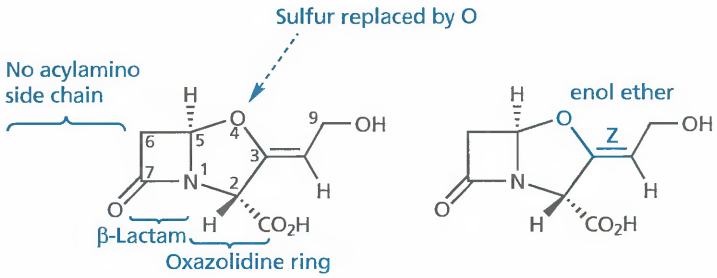


FIGURE 19.49 Clavulanic acid.

- the *Z* configuration for the double bond of the enol ether (activity is reduced but not eliminated if the double bond is *E*)
- no substitution at C-6
- (*R*)-stereochemistry at positions 2 and 5
- the carboxylic acid group.

It is also thought that the 9-hydroxyl group is involved in a hydrogen bonding interaction with the active site of the β-lactamase enzyme.

Clavulanic acid is a mechanism-based irreversible inhibitor and could be classed as a suicide substrate (section 7.5). The drug fits the active site of β-lactamase, and the β-lactam ring is opened by a serine residue in the same manner as penicillin. However, the acyl-enzyme intermediate then reacts further with another enzymatic nucleophilic group (possibly NH_2) to bind the drug irreversibly to the enzyme (Fig. 19.50). The mechanism requires the loss or gain of protons at various stages, and an amino acid such as histidine in the active site would be capable of acting as a proton donor/acceptor (compare sections 3.5.2 and 22.14.3.2).

19.5.4.2 Penicillanic acid sulfone derivatives

The agents **sulbactam** and **tazobactam** have also been developed as β-lactamase inhibitors and are used clinically (Fig. 19.51). They too act as suicide substrates for β-lactamase enzymes and have similar properties. Sulbactam has a broader spectrum of activity against β-lactamases than clavulanic acid, but is less potent. It is combined with ampicillin for intravenous administration in a preparation called **Unasyn.** Tazobactam is similar to sulbactam and has a similar spectrum of activity against β-lactamases. However, its potency is more like clavulanic acid. It is administered intravenously with piperacillin in a preparation called **Tazocin or Zosyn.** This combination has the broadest spectrum of activity of the various combinations described so far.

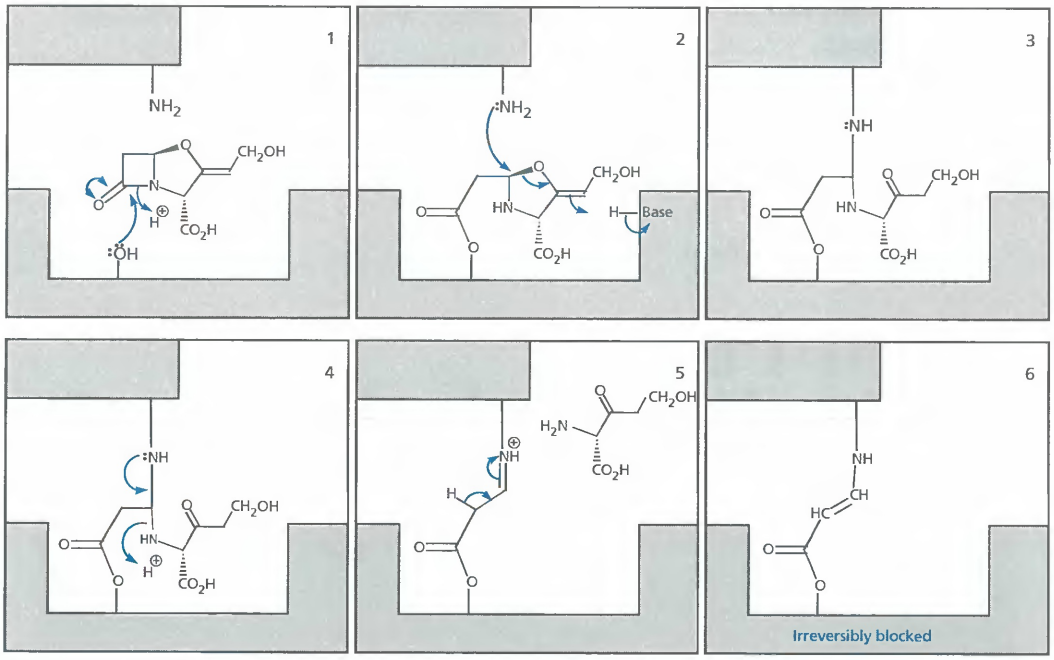


FIGURE 19.50 Clavulanic acid acting as an irreversible mechanism-based inhibitor.

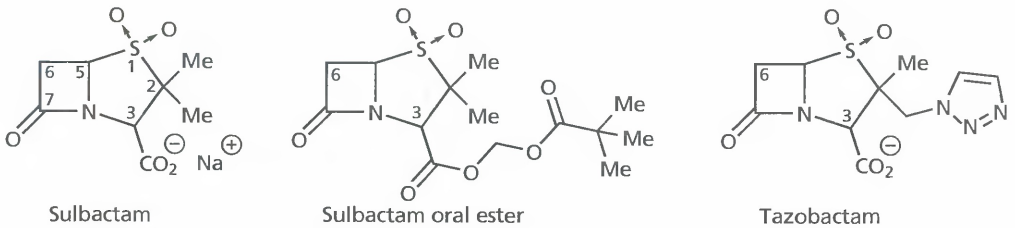


FIGURE 19.51 Penicillanic acid sulfones.

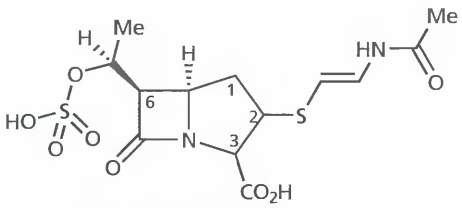


FIGURE 19.52 MM 13902.

19.5.4.3 Olivanic acids

The olivanic acids (e.g. **MM 13902**) (Fig. 19.52) were isolated from strains of *Streptomyces olivaceus* and are carbapenem structures like thienamycin. They are very strong inhibitors of β-lactamase, in some cases 1000 times more potent than clavulanic acid. They are also effective against the β-lactamases which can break down cephalosporins and which are unaffected by clavulanic acid. Unfortunately, olivanic acids lack chemical stability.

19.5.5 Other drugs that act on bacterial cell wall biosynthesis

β-Lactams are not the only antibacterial agents which inhibit cell wall biosynthesis. The antibacterial agents **vancomycin**, **D-cycloserine,** and **bacitracin** also inhibit biosynthesis, though at different stages. In order to synthesize the cell wall, *N*-acetylmuramic acid (NAM) is linked to three amino acids, then to the dipeptide D-Ala-D-Ala (Fig. 19.53). The D-Ala-D-Ala dipeptide is derived from two L-alanine units which are first racemized then linked together.

NAM with its pentapeptide chain is then linked to a C55 carrier lipid with the aid of a translocase enzyme and carried to the outer surface of the cell membrane, where the lipid carrier acts as an anchor holding the glycopeptide in place for the subsequent steps. These steps involve the addition of *N*-acetylglucosamine (NAG) and a pentaglycine chain to give the complete 'building block'. A transglycosidase enzyme catalyses the attachment of the disaccharide building block to the growing cell wall and at the same time a carrier lipid is released to pick up another molecule of NAM/pentapeptide. Crosslinking between the various chains of the cell wall finally takes place catalysed by the transpeptidase enzyme as described previously (section 19.5.1.4).

19.5.5.1 D-Cycloserine and bacitracin

D-Cycloserine (Fig. 19.54) is a simple molecule produced by *Streptomyces garyphalus* which has broad-spectrum activity and acts within the cytoplasm to prevent the formation of D-Ala-D-Ala. It does this by mimicking

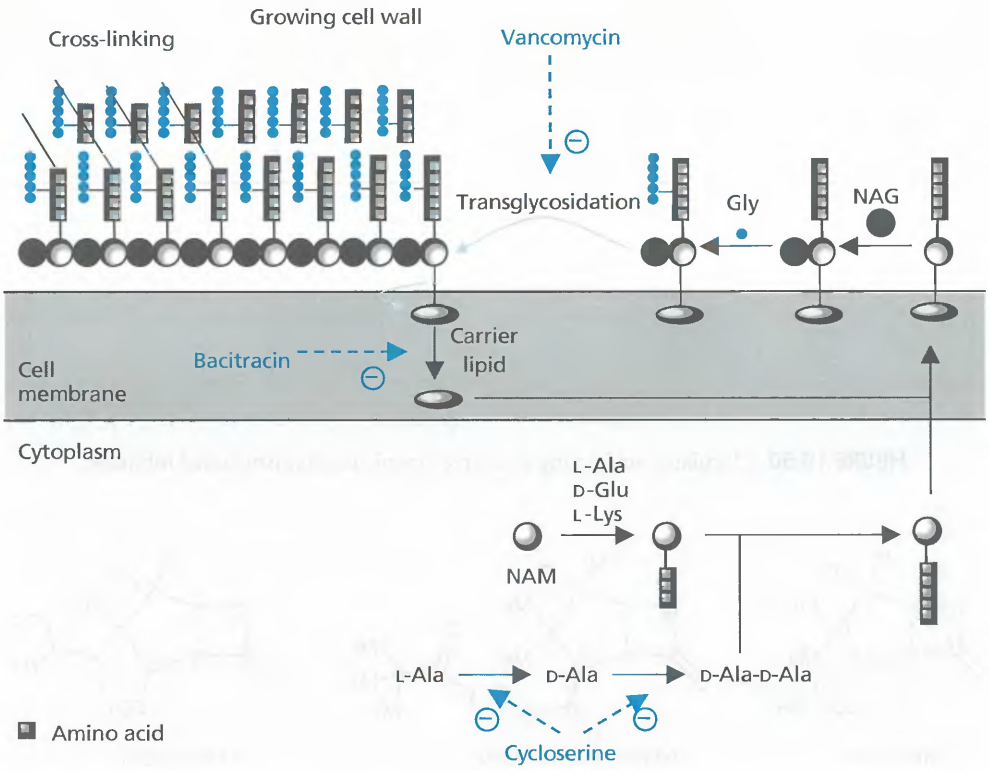


FIGURE 19.53 Cell wall biosynthesis.



FIGURE 19.54 D-Cycloserine as a mimic for D-alanine.

the structure of D-alanine and inhibiting the enzymes L-alanine racemase (responsible for racemizing L-Ala to L-Ala) and D-Ala-D-Ala ligase (responsible for linking the two D-alanine units together).

Bacitracin is a polypeptide complex produced by *Bacillus subtilis*, which binds to the lipid carrier responsible for transporting the NAM/pentapeptide unit across the cell membrane and prevents it from carrying out that role.

19.5.5.2 The glycopeptides: vancomycin and vancomycin analogues

Vancomycin (Fig. 19.55) is a narrow-spectrum bactericidal glycopeptide produced by a microorganism called *Streptomyces orientalis*, which was found in Borneo and India. Its name is aptly derived from the verb 'to vanquish'. Vancomycin was introduced in 1956 for the treatment of infections due to penicillin-resistant *S. aureus*, but was discontinued when methicillin became available. Since then, it has been reintroduced and it is now the main stand-by drug for MRSA. Vancomycin and related glycopeptides are often the last resort in treating patients with drug-resistant infections. As such, they have become extremely important and a great deal of research is currently being carried out in this area.

Vancomycin is derived biosynthetically from a linear heptapeptide containing five aromatic residues. These undergo oxidative coupling with each other to produce three cyclic moieties within the structure. Chlorination, hydroxylation and the final addition of two sugar units then complete the structure (Fig. 19.56).

The cyclizations described transform a highly flexible heptapeptide molecule into a rigid structure which holds the peptide backbone in a fixed conformation. Moreover, there is an extra element of rigidity to the structure which may not be apparent at first sight. The aromatic rings (A–E) cannot rotate and are fixed in space because of hindered single bond rotation. For example, the aromatic rings C and E have a chloro substituent which prevents these rings becoming coplanar with ring D. Similarly, rings A and B have phenol substituents which prevent them becoming coplanar.

The fixed conformation of the hexapeptide chain is important to vancomycin's unique mechanism of action, which involves targeting the cell wall's building blocks rather than a protein or a nucleic acid. To be specific, there is a pocket in the vancomycin structure into which the tail of the building block's pentapeptide moiety can fit. The

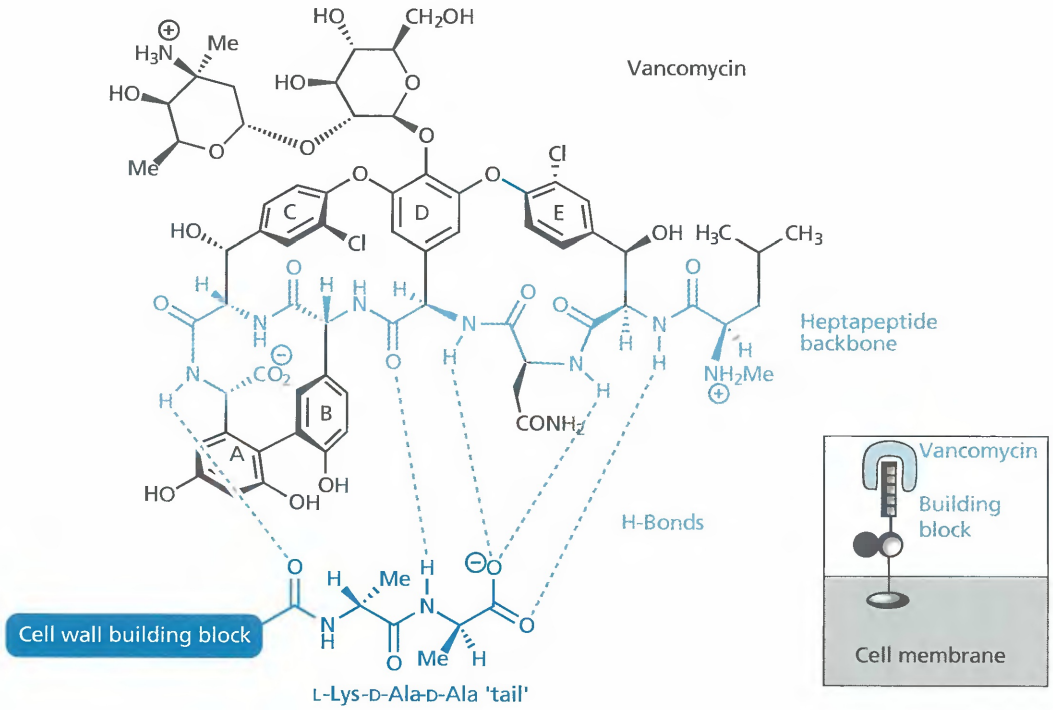


FIGURE 19.55 Vancomycin and its binding interactions to the L-Lys-D-Ala-D-Ala moiety.

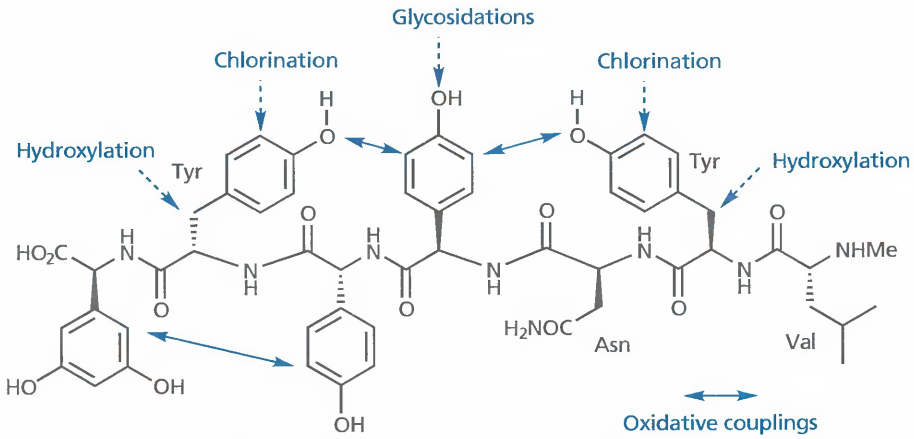


FIGURE 19.56 Reactions involved in the biosynthesis of vancomycin.

pentapeptide is then held there by the formation of five hydrogen bonds between it and the hexapeptide chain of vancomycin (Fig. 19.55). Dimerization can now occur where a highly stable vancomycin dimer is bound to two tails. Because vancomycin is a large molecule, it caps the tails and acts as a steric shield, blocking access to the transglycosidase and transpeptidase enzymes (Fig. 19.57).

Dimerization occurs head to tail such that the heptapeptide chains of each vancomycin molecule interact through four hydrogen bonds (Fig. 19.58). The sugar and chloro-groups also play an important role in this

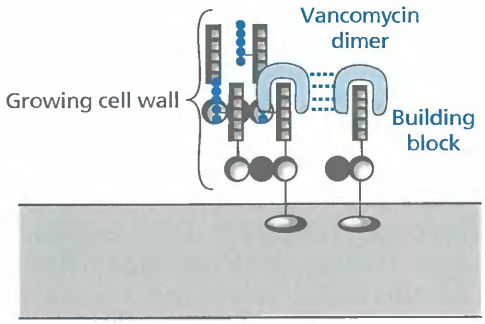


FIGURE 19.57 'Capping' of pentapeptide 'tails' by vancomycin.

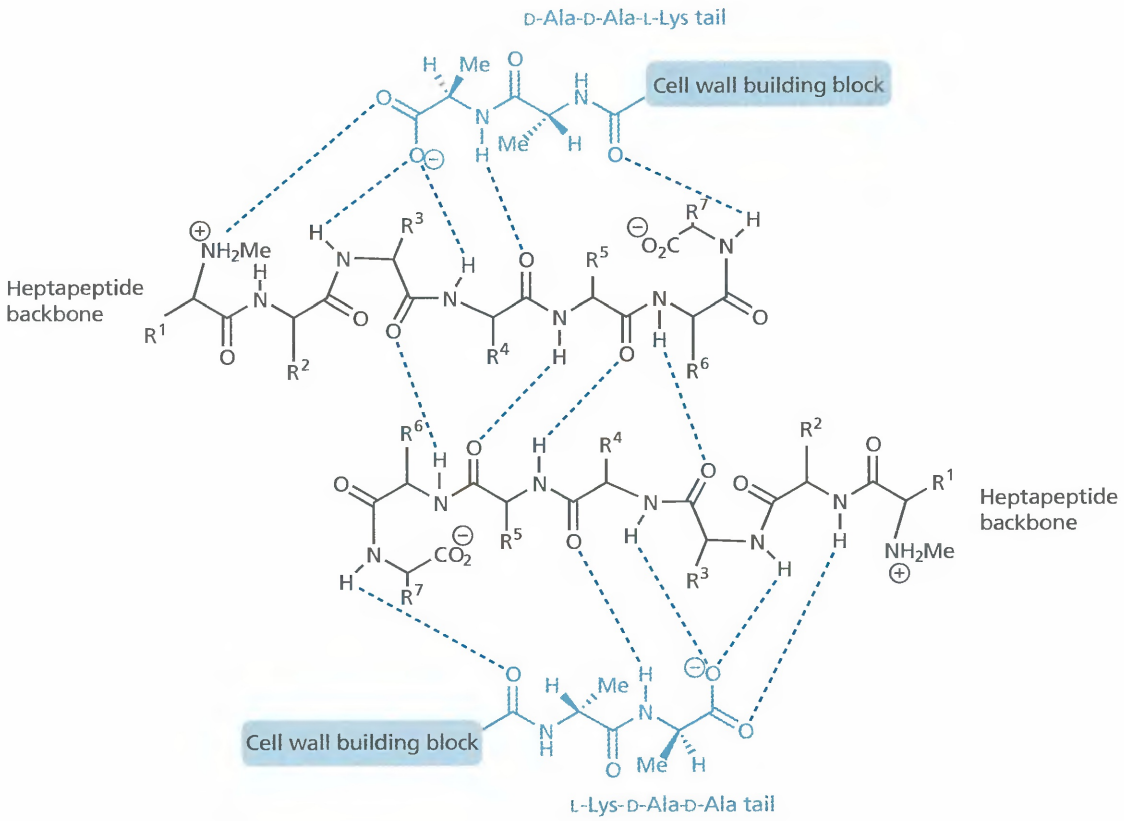


FIGURE 19.58 Dimerization of vancomycin. The dashed lines represent hydrogen bonds.

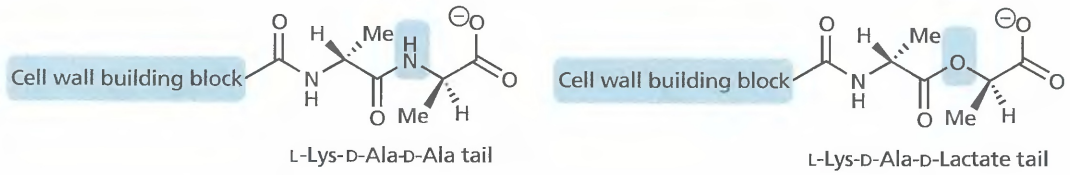


FIGURE 19.59 Modification of the pentapeptide chain leading to resistance.

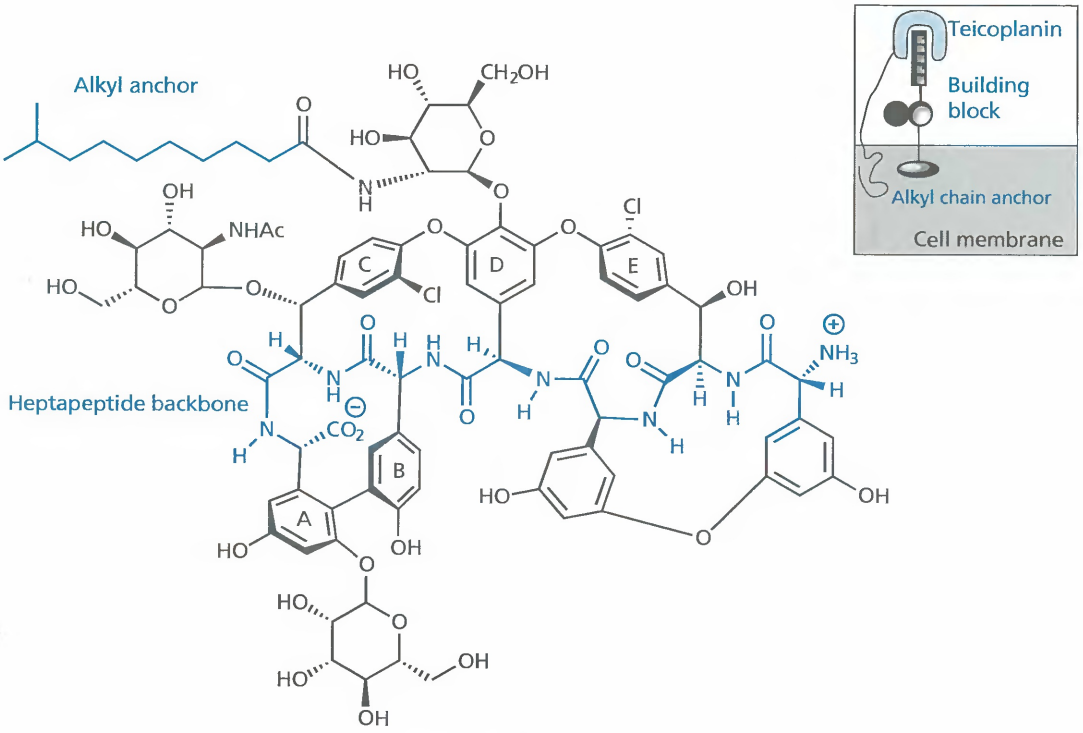


FIGURE 19.60 Teicoplanin.

dimerization and activity drops if either of these groups is absent.

Because vancomycin is such a large molecule, it is unable to cross the outer cell membrane of Gram-negative bacteria and consequently lacks activity against those organisms. It is also unable to cross the inner cell membrane of Gram-positive bacteria, but this is not required since the construction of the cell wall takes place outside the cell membrane.

Bacterial resistance to vancomycin has been slow to develop, although some hospital strains of *S. aureus* were identified in 1996 which *do* show resistance (**VRSA**). Of particular concern is the appearance of **vancomycin-resistant enterococci** (VRE) in 1989. These are organisms that can cause life-threatening gut infections in patients whose immune system is weakened. Resistance in the latter organisms has arisen from a modification of the cell wall precursors where the terminal D-alanine group in the pentapeptide chain has been replaced by D-lactic acid, resulting in a terminal ester link rather than an amide link (Fig. 19.59). This removes one of the NH groups involved in the hydrogen bonding interaction with vancomycin. It may not sound like much, but it is sufficient to weaken the binding affinity and make the antibiotic ineffective. The building block is still acceptable to the transglycosylase and transpeptidase enzyme. In the latter case, lactate acts as the leaving group rather than D-alanine.

Teicoplanin (Fig. 19.60) is another member of the vancomycin family which has been isolated from a soil microorganism called *Actinoplanes teichomyceticus*. Unlike vancomycin, it fails to dimerize, but the long alkyl chain anchors the antibiotic to the outer surface of the cell membrane where it is perfectly placed to interact with the building blocks of the cell wall synthesis (Fig. 19.54). It is used clinically for the treatment of Gram-positive infections and is less toxic than vancomycin.

Another naturally occurring member of the vancomycin family is **eremomycin** (Fig. 19.61). A biphenyl

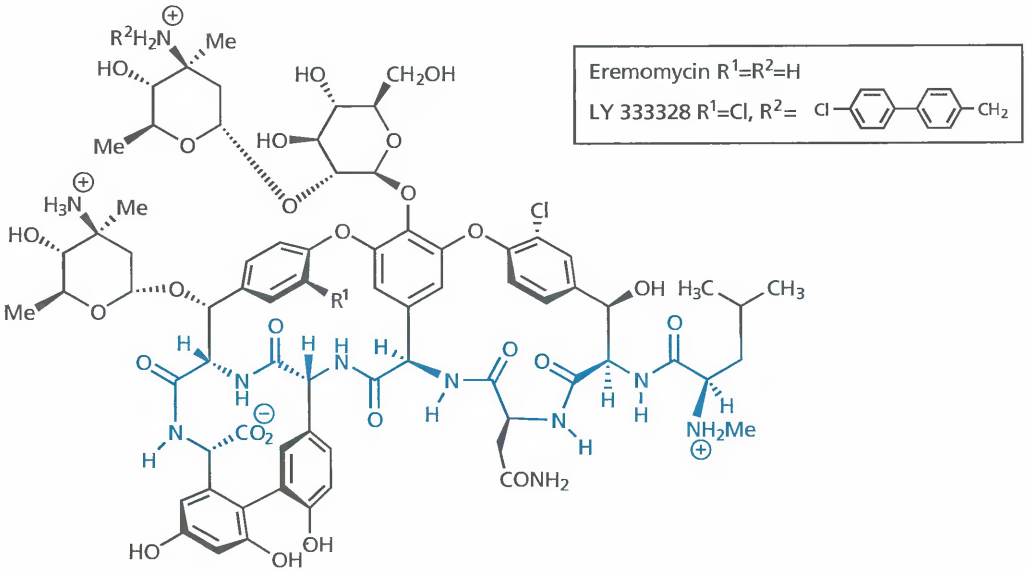


FIGURE 19.61 Eremomycin and LY 333328.

hydrophobic 'tail' was added to act as an anchor, resulting in a compound (**LY 333328**) which is 1000 times more active than vancomycin.

Although the complexity of the glycopeptides is an advantage in their targeting and selectivity, it is a problem when it comes to synthesizing analogues. Therefore, work has been carried out to try and prepare simplified analogues of vancomycin which are easier to synthesize, yet retain the desired selectivity. Structures such as those shown in Fig. 19.62 have been prepared which are capable of binding to D-Ala-D-Ala and D-Ala-D-Lac. These now represent lead compounds for the development of effective antibacterial agents.

There are another two mechanisms by which glycopeptide antibiotics may have an antibacterial activity. First, it is possible that glycopeptide dimers disrupt the cell membrane structure. This is supported by the fact that glycopeptide antibiotics enhance the activity of aminoglycosides by increasing their absorption through the cell membrane. Second, RNA synthesis is

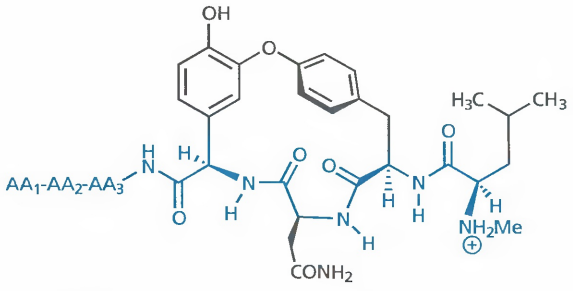


FIGURE 19.62 Simplified analogues of the glycopeptides.

BOX 19.12

Clinical aspects of cycloserine, bacitracin and vancomycin

D-Cycloserine is administered orally in combination with other drugs to treat tuberculosis that is resistant to first-line drugs. Bacitracin is used alongside polymyxin B sulfate for the topical treatment of skin infections. The same preparation is also used for the topical treatment of eye infections caused by *Pseudomonas aeruginosa*. Neomycin sulfate and bacitracin are used together for the topical treatment of skin infections as a cream or dusting powder.

Vancomycin and **teicoplanin** are bactericidal, and are active against aerobic and anaerobic Gram-positive bacteria including MRSA. Vancomycin is not absorbed orally and is administered by intravenous injection every 12 hours for the prophylaxis and treatment of endocarditis and other serious infections caused by Gram-positive cocci. Vancomycin is also given orally to treat gut infections caused by *Clostridium difficile*. This organism may appear following the use of broad-spectrum antibiotics, and produces harmful toxins. Vancomycin has also been used in eye drops. Teicoplanin can be administered once daily and is used for potentially serious Gram-positive infections including endocarditis, dialysis-associated peritonitis and serious infections due to *Staphylococcus aureus*. It is also used as a prophylactic in endocarditis and orthopaedic surgery.

known to be disrupted in the presence of glycopeptides. The possibility of three different mechanisms of action explains why bacteria are slow to acquire resistance to the glycopeptides.

KEY POINTS

- β-Lactamase inhibitors are β-lactam structures that have negligible antibacterial activity but inhibit β-lactamases. They can be administered alongside penicillins to protect them from β-lactamases and to broaden their spectrum of activity.
- Carbapenems and monobactams are examples of other β-lactam structures with clinically useful antibacterial activity.
- Glycopeptides such as vancomycin bind to the building blocks for cell wall synthesis, preventing their incorporation into the cell wall. They also block the cross-linking reaction for those units already incorporated in the wall. The glycopeptides are the drugs of last resort against drug-resistant strains of bacteria.
- Bacitracin binds to and inhibits the carrier lipid responsible for carrying the cell wall components across the cell membrane.
- Cycloserine inhibits the synthesis of D-Ala-D-Ala.

19.6 Antibacterial agents that act on the plasma membrane structure

19.6.1 Valinomycin and gramicidin A

The peptides **valinomycin** and **gramicidin A** both act as ion-conducting antibiotics (ionophores) and allow the uncontrolled movement of ions across the cell membrane. These agents are described in section 10.6.

19.6.2 Polymyxin B

The polypeptide antibiotic **polymyxin B** (Fig. 19.63) derives from a soil bacterium called *Bacillus polymyxa*. It also operates within the cell membrane and shows a selective toxicity for bacterial cells over animal cells. This appears to be related to the ability of the compound to bind selectively to the different plasma membranes. The mechanism of this selectivity is not fully understood. Polymyxin B acts like valinomycin (section 10.6.2), but it causes the leakage of small molecules such as nucleosides from the cell.

19.6.3 Killer nanotubes

Work is currently in progress to design cyclic peptides that will self-assemble in the cell membranes of bacteria to form tubules that have been labelled as 'killer nanotubes' (section 10.6.1).

19.6.4 Cyclic lipopeptides

Daptomycin (Fig. 19.64) is a member of a new class of antibacterial agents called the cyclic lipopeptides. It is a natural product derived from a bacterial strain *Streptomyces roseosporus*, and works by disrupting multiple functions of the bacterial cell membrane. The lipid portion of the molecule is derived from decanoic acid and the yield of product obtained is increased if decanoic acid is added to the fermentation medium.

KEY POINTS

- Ionophores act on the plasma membrane and result in the uncontrolled movement of ions across the cell membrane leading to cell death.
- Polymyxin B operates selectively on the plasma membrane of bacteria and causes the uncontrolled movement of small molecules across the membrane.
- Cyclic peptides are being designed which will self-assemble to form nanotubes in the cell membranes of bacteria.
- Cyclic lipopeptides are a new class of antibiotic.

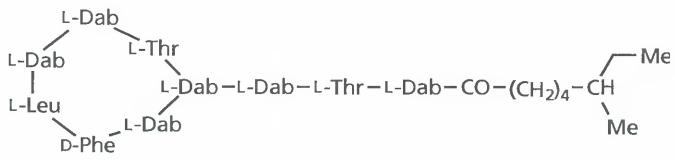


FIGURE 19.63 Polymyxin B (Dab= α,γ-Diaminobutyric acid with peptide link through the α-amino group).

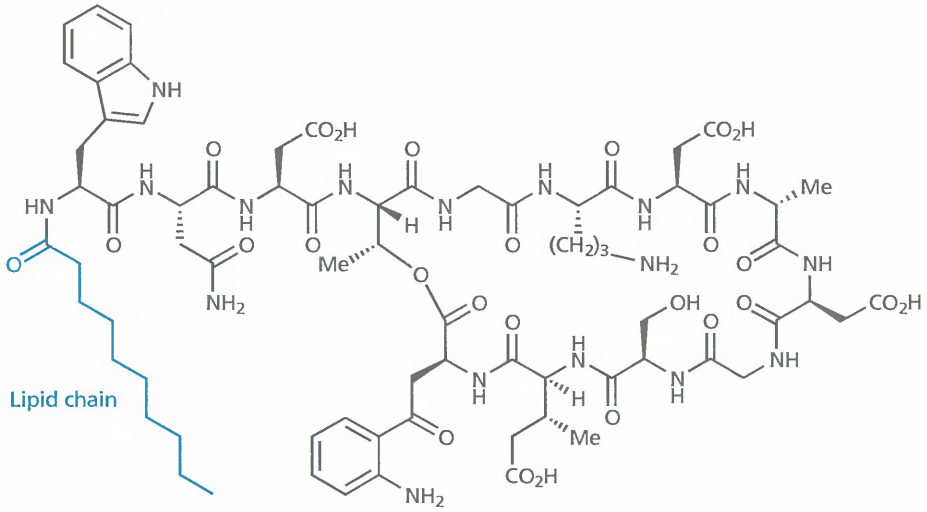


FIGURE 19.64 Daptomycin.

BOX 19.13

Clinical aspects of drugs acting on the plasma membrane

Valinomycin and gramicidin A show no selective toxicity for bacterial cells over mammalian cells, and are therefore useless as systemic therapeutic agents. However, gramicidin is present as a minor constituent in some topical applications.

Polymyxin B is injected intramuscularly and is useful against *Pseudomonas* strains which are resistant to other antibacterial agents. It can be used topically for the treatment of minor skin infections, and has good activity against Gram-negative bacteria. It is less effective against Gram-positive bacteria, as it is difficult for such a big molecule to pass through the thicker cell wall. It is used in combination with **bacitracin** for the treatment of eye and skin infections, or with **dexamethasone** and **neomycin** for the treatment of eye infections. The ear-drop preparation **Otosprin** contains **hydrocortisone** and polymyxin B to treat ear infections and inflammation.

Daptomycin was approved in 2003 for the treatment of Gram-positive infections. It is administered by intravenous infusion and has a spectrum of activity similar to valinomycin. In order to guard against resistance developing against it, the drug is held in reserve for skin and soft tissue infections caused by drug-resistant Gram-positive bacteria such as MRSA. It can be administered alongside other antibacterial agents for mixed infections involving Gram-positive bacteria, Gram-negative bacteria and some anaerobes.

19.7 Antibacterial agents that impair protein synthesis: translation

The agents described in this section all inhibit protein synthesis by binding to ribosomes and inhibiting different stages of the translation process (Fig. 19.65). Selective toxicity is due to either different diffusion rates through the cell barriers of bacterial versus mammalian cells or to a difference between the ribosomal target structures. The bacterial ribosome is a 70S particle (see section 6.2.2) made up of a 30S subunit and a 50S subunit. The 30S subunit binds messenger RNA (mRNA) and initiates protein synthesis. The 50S subunit combines with the 30S subunit–mRNA complex to form a ribosome, then binds aminoacyl transfer RNA (tRNA) and catalyses the building of the protein chain. There are two main binding sites for the tRNA molecules. The peptidyl site (P-site) binds the tRNA bearing the peptide chain. The acceptor aminoacyl site (A-site) binds the tRNA bearing the next amino acid, to which the peptide chain will be transferred (section 6.2.2). The ribosomes of eukaryotic cells are bigger (80S), consisting of a 60S large subunit and a 40S small subunit. They are sufficiently different in structure from prokaryotic ribosomes that it is possible for some drugs to distinguish between them.

19.7.1 Aminoglycosides

Streptomycin (Fig. 19.66) was isolated from the soil microorganism *Streptomyces griseus* in 1944, and is an

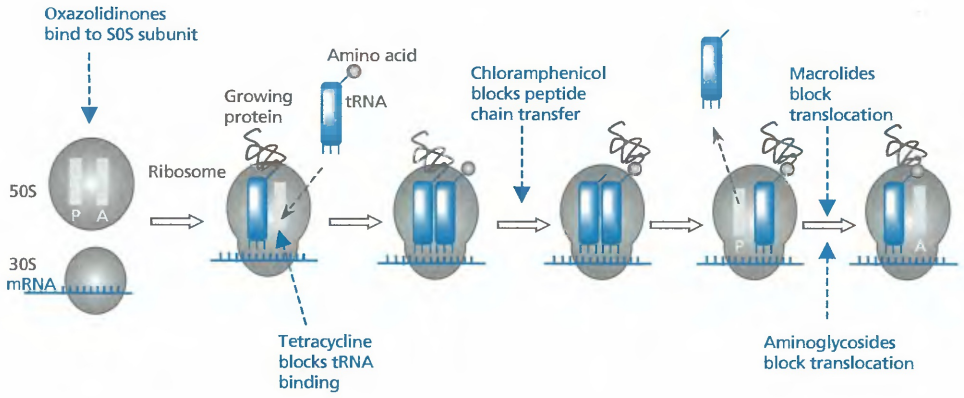
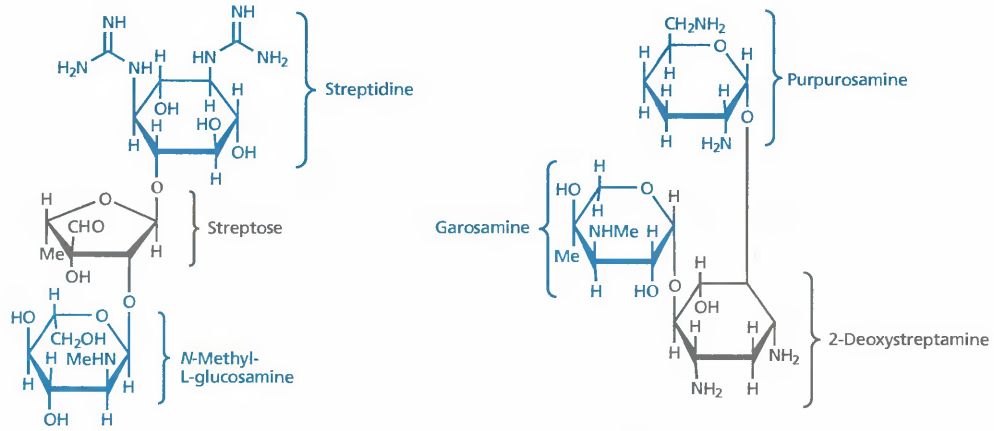


FIGURE 19.65 Stages at which antibacterial agents inhibit translation.



Streptomycin (from *Streptomyces griseus*) Gentamicin C1a

FIGURE 19.66 Aminoglycosides.

example of an aminoglycoside—a carbohydrate structure which includes basic amine groups. Streptomycin was the next most important antibiotic to be discovered after penicillin. A variety of other aminoglycosides such as **gentamicin C1a** (Fig. 19.66) have been isolated from various organisms. At pH 7.4, they are positively charged, which is beneficial to activity and also means that they work best in slightly alkaline conditions. The ionic charge is important to how these agents are absorbed through the outer membrane of Gram-negative bacteria. An ionic interaction takes place with various negatively charged groups in the membrane (lipopolysaccharides, phospholipids and proteins) which displaces magnesium and calcium ions. These ions normally act as bridges between adjacent lipopolysaccharide molecules, and their displacement results in rearrangement of these molecules to produce pores through which the drug can pass. The drug still has to cross the cell membrane to enter the cell and this is an energy-dependent process. Once across the membrane, it is trapped inside the cell and so it is possible for high concentrations of the drug to build up within.

Binding to bacterial ribosomes now takes place to inhibit protein synthesis. The binding is specifically to the 30S ribosomal subunit and prevents the movement of the ribosome along mRNA so that the triplet code on mRNA can no longer be read. In some cases, protein synthesis is terminated and the shortened proteins end up in the cell membrane. This can lead to a further increase in cell permeability, resulting in an even greater uptake of the drug. Aminoglycosides are bactericidal rather than bacteriostatic and it is thought that their activity may be due to their effects, both on the ribosomes and the outer cell membrane.

Because the ribosomes in human cells are different in structure from those in bacterial cells, they have a much lower binding affinity for the aminoglycosides, which explains their selectivity.

Box 19.14 Clinical aspects of aminoglycosides

Aminoglycosides are fast acting, but they can also cause ear and kidney problems if the dose levels are not carefully controlled. They are effective in the treatment of infections caused by aerobic Gram-negative bacteria, including *P. aeruginosa*. Indeed, they used to be the only compounds effective against that organism. Some Gram-negative bacteria are resistant to aminoglycosides due mainly to enzymes that catalyse reactions such as *O*-phosphorylations, *O*-adenylations (addition of an adenine group) and *N*-acylations. Resistance can also occur from alterations of the ribosomes such that they bind aminoglycosides less strongly, or by less efficient uptake mechanisms. Because the aminoglycosides are polar in nature, they have to be injected. They are also unable to cross the blood–brain barrier efficiently, and so they cannot be used for the treatment of meningitis unless they are injected directly into the central nervous system. The activity of aminoglycosides is increased if they are administered with agents that disrupt cell wall synthesis, since this increases uptake of the former. However, bacteriostatic agents should not be taken with aminoglycosides, as these inhibit the energy-dependent uptake process by which the aminoglycosides cross the cell membrane.

Streptomycin was the first effective agent used against tuberculosis. However, resistance soon developed and a multidrug therapy involving streptomycin, **isoniazid**, and ***para*-aminosalicylic acid** was used until the early 1970s. At that point, **rifampicin** became available, allowing different multidrug therapies to be used. Streptomycin is now rarely used for the treatment of tuberculosis, but it may be used if there is a known resistance to isoniazid, in which case it is administered by intramuscular injection. Streptomycin is still used to treat enterococcal endocarditis, and as an adjunct to **doxycycline** in brucellosis.

Gentamycin is administered by intramuscular or slow intravenous injection for the treatment of a number of infections including septicaemia, neonatal sepsis, CNS infections including meningitis, biliary tract infections, acute pyelonephritis or prostatis, endocarditis, and pneumonia in hospital patients. It can be used topically in drops for the treatment of eye and ear infections.

19.7.2 Tetracyclines

The tetracyclines are bacteriostatic antibiotics that have a broad spectrum of activity and are the most widely prescribed form of antibiotic after penicillins. They are also capable of attacking the malarial parasite. One of the best known tetracyclines is **chlortetracycline (aureomycin)** (Fig. 19.67), which was isolated in 1948 from a mud growing microorganism in Missouri called *Streptomyces aureofaciens*—so called because of its golden colour. Further tetracyclines such as **tetracycline** and **doxycycline** (Fig. 19.66) have been synthesized or discovered.

The tetracyclines inhibit protein synthesis by binding to the 30S subunit of ribosomes and preventing aminoacyl-tRNA from binding. This stops the further addition of amino acids to the growing protein chain. Protein release is also inhibited.

In the case of Gram-negative bacteria, tetracyclines cross the outer membrane by passive diffusion through the porins. Passage across the inner membrane is dependent on a pH gradient, which suggests that a proton-driven carrier is involved. Selectivity is due to the ability of bacterial cells to concentrate these agents faster than human cells. This is fortunate because tetracyclines are capable of inhibiting protein synthesis in mammalian cells—particularly in mitochondria.

Widespread resistance to tetracyclines has occurred, caused partly by the use of tetracyclines to cure animal infections, and as a food additive to promote the growth of new born animals.

19.7.3 Chloramphenicol

Chloramphenicol (Fig. 19.67) was originally isolated from a microorganism called *Streptomyces venezuela* found in a field near Caracas, Venezuela. It is now prepared synthetically and has two asymmetric centres. Only the *R,R*-isomer is active.

Chloramphenicol binds to the 50S subunit of ribosomes and appears to act by inhibiting the movement of ribosomes along mRNA, probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended. Since it binds to the same region as macrolides and lincosamides, these drugs cannot be used in combination. The nitro group and both alcohol groups are involved in binding interactions. The dichloroacetamide group is also important, but can be replaced by other electronegative groups. Chloramphenicol is quite toxic and the nitro-substituent is thought to be responsible for this.

19.7.4 Macrolides

Macrolides are bacteriostatic agents. The best-known example of this class of compounds is **erythromycin**—a metabolite isolated in 1952 from the soil microorganism

Box 19.15 Clinical aspects of tetracyclines and chloramphenicol

The tetracyclines are broad-spectrum antibiotics that are active against both Gram-positive and Gram-negative bacteria. Commonly used tetracyclines in the clinic are **tetracycline, demeclocycline, doxycycline, lymecycline, minocycline** and **oxytetracycline.** The use of chlortetracycline has decreased over the years because it kills the intestinal flora that produce vitamin K. However, it is still administered alongside tetracycline and demeclocycline in the preparation **Deteclo.**

In general, the tetracyclines can be divided into short-lasting compounds such as chlortetracycline, an intermediate group of compounds such as demeclocycline, and longer-acting compounds such as doxycycline. Minocycline has the broadest spectrum of activity. The tetracyclines were originally used for many types of respiratory infections, but have been largely replaced by β-lactams because of the problems of resistance. However, they are still the agents of choice for the treatment of Lyme disease, rickettsia, and infections caused by chlamydia. They are also used to treat acne and a variety of different infections including respiratory and genital infections. Doxycycline has been found to be useful for the treatment and prophylaxis of malaria, and is cheaper than other antimalarial agents. One drawback is the possibility of skin hypersensitivity to sunlight. The drug can also be used for the treatment of a variety of diseases including syphilis, sinusitis, oral herpes simplex, and acne. It is a possible agent for the treatment or prophylaxis of anthrax.

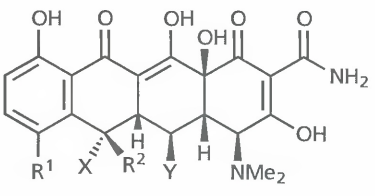
Tetracyclines should be avoided for young children and pregnant mothers since they can bind to developing teeth and bone, leading to tooth discolouration. Resistance to tetracyclines can arise through several mechanisms. Some organisms have effective efflux mechanisms that pump the drug back out of the cell. Resistance can also arise from alterations in the bacterial ribosomes, such that they have lower affinity for the agents.

Chloramphenicol is a potent broad-spectrum antibiotic and in some regions of the world, it is the drug of choice for the treatment of typhoid, when more expensive drugs cannot be afforded. It can also be used in severe bacterial infections that are insensitive to other antibacterial agents, and is widely used for eye infections. It can also be used for ear infections, but the preparation can cause hypersensitivity reactions in about 10% of patients taking it. The drug should only be used in these restricted scenarios as it is quite toxic, especially to bone marrow. The drug is inadequately metabolized in babies leading to a combination of symptoms described as the **gray baby syndrome**, which can be fatal. In adults, the drug undergoes a phase II conjugation reaction to form a glucuronic acid conjugate (section 11.4.5), which is excreted. This reaction fails to take place efficiently in newborn babies, and so the drug levels increase to toxic levels. Bacteria with resistance to the drug contain an enzyme called chloramphenicol acetyltransferase which catalyses the acylation of the hydroxyl groups.

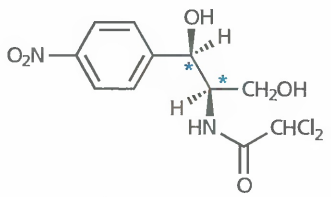
Streptomyces erythreus in the Philippines, and one of the safest antibiotics in clinical use. The structure (Fig. 19.68) consists of a 14-membered macrocyclic lactone ring with a sugar and an aminosugar attached. The sugar residues are important for activity.

Erythromycin acts by binding to the 50S subunit of bacterial ribosomes. It works by inhibiting translocation, but other mechanisms of action also appear likely. Since erythromycin and chloramphenicol bind to the same region of the ribosome, they should not be administered together as they will compete with each other and be less effective.

Erythromycin is unstable to stomach acids, but can be taken orally in a tablet form. The formulation of the tablet involves a coating that is designed to protect the tablet during its passage through the stomach, but which is soluble once it reaches the intestines (enterosoluble). The acid sensitivity of erythromycin is due to the presence



Chlortetracycline (Aureomycin) (R^1=Cl, R^2 = Me, X=OH, Y=H)
Tetracycline (R^1=H, R^2 = Me, X=OH, Y=H)
Doxycycline (Vibramycin) (R^1=H, R^2= Me, X=H, Y=OH)
Demeclocycline (R^1=Cl, R^2= H, X=OH, Y=H)



Chloramphenicol

FIGURE 19.67 Tetracyclines and chloramphenicol. The asterisks indicate asymmetric centres

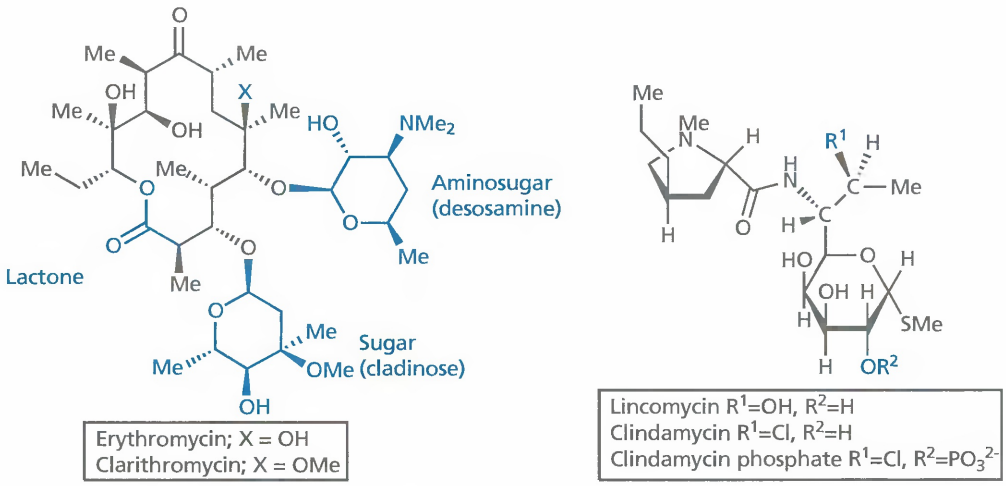


FIGURE 19.68 Macrolides and lincosamides.

of a ketone and two alcohol groups which are set up for the acid-catalysed intramolecular formation of a ketal (Fig. 19.69). One way of preventing this is to protect the hydroxy groups. For example, **clarithromycin** is a methoxy analogue of erythromycin which is more stable to gastric juices and has improved oral absorption. Another method of increasing acid stability is to increase the size of the macrocycle to a 16-membered ring.

Azithromycin (Fig. 19.70) contains a 15-membered macrocycle where an *N*-methyl group has been incorporated into the macrocycle. It is one of the world's best-selling drugs. **Telithromycin** (Fig. 19.70) is a semi-synthetic derivative of erythromycin and reached the European market in 2001. The cladinose sugar in erythromycin has been replaced with a keto-group, and a carbamate ring has been fused to the macrocyclic ring. The two hydroxyl groups that cause the intramolecular ketal formation in erythromycin have been masked, one as a methoxy group, and the other as part of the carbamate ring.

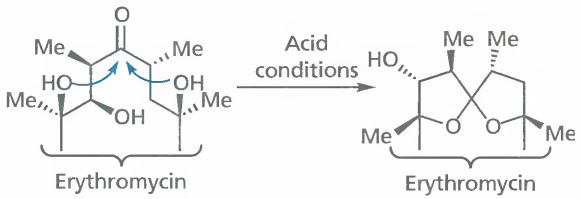


FIGURE 19.69 Intramolecular ketal formation in erythromycin.

19.7.5 Lincosamides

The lincosamide antibiotics (Fig. 19.68) have similar antibacterial properties to the macrolides and act in the same fashion. **Lincomycin** was the first of these agents and was isolated in 1962 from a soil organism called *Streptomyces lincolnensis* found near Lincoln, Nebraska. Chemical modification led to the clinically useful **clindamycin,** which has increased activity.

19.7.6 Streptogramins

Pritinamycin is a mixture of macrolactone structures obtained from *Streptomyces pristinaespiralis*. Two of the

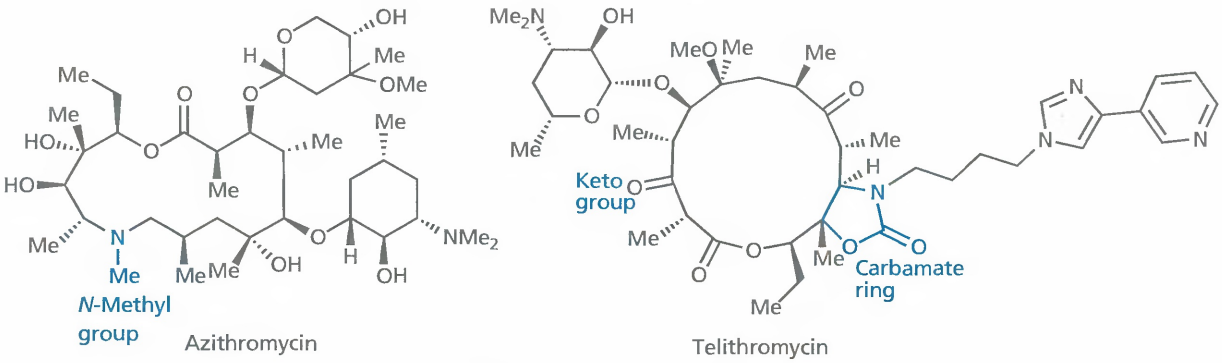


FIGURE 19.70 Azithromycin and telithromycin.

components (**quinupristin** and **dalfopristin**) have been isolated. These agents bind to different regions of the 50S subunit of the bacterial ribosome and form a complex with it. It is found that the binding of dalfopristin increases the binding affinity for quinupristin and so the two agents act in synergy with each other. Quinupristin inhibits peptide chain elongation, while dalfopristin interferes with the transfer of the peptide chain from one tRNA to the next.

19.7.7 Oxazolidinones

The oxazolidinones are a new class of clinically useful synthetic antibacterial agents which have been discovered in recent years. They inhibit protein synthesis at a much earlier stage than previous agents, and as a result do not suffer the same resistance problems.

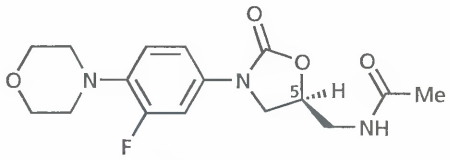


FIGURE 19.71 Linezolid.

Before protein synthesis can start, a 70S ribosome has to be formed by the combination of a 30S ribosome with a 50S ribosome. The oxazolidinones bind to the 50S ribosome and prevent this from happening. As a result, translation cannot start. Other agents that inhibit protein synthesis, do so during the translation process itself (Fig. 19.65). **Linezolid** (Fig. 19.71) was the first of this class of compounds to reach the market in 2000.

Box 19.16 Clinical aspects of macrolides, lincosamides, streptogramins and oxazolidinones

Macrolides

Erythromycin has an antibacterial spectrum that is similar to penicillins, and can be used as an alternative to penicillins for those patients having penicillin allergies. It has been used against penicillin-resistant staphylococci, but newer penicillins are now preferred for these infections due to increased resistance against erythromycin. It is very useful for the treatment of respiratory infections including whooping cough and legionnaires disease. It can also be used to treat syphilis and diphtheria, as well as oral and skin infections. Topically, it can be used for the treatment of acne. **Clarithromycin** has slightly greater activity than erythromycin, with fewer gastrointestinal side effects. Therefore, it is often prescribed instead of erythromycin. Clarithromycin is one of the drugs used in the treatment of ulcers caused by the presence of *Helicobacter pylori* (section 25.4). **Azithromycin** is slightly less active than erythromycin against Gram-positive infections, but is more active against Gram-negative infections including *H. influenza*, against which erythromycin shows poor activity. Azithromycin can also be used for the treatment of Lyme's disease. **Telithromycin** is a derivative of erythromycin and has a similar spectrum of activity to other macrolides. It should only be used for specified infections such as pneumonia, tonsillitis and sinusitis.

Resistance to macrolides is due to effective efflux mechanisms that pump the drug back out the cell. The ribosomal target site may also change in character such that binding is weakened. Enzyme-catalysed modifications can also occur. Recently there has been research into finding novel macrolides that can be effective against respiratory infections due to resistant strains of *Streptococcus pneumoniae*, as well as the organism *Haemophilus influenza*.

Lincosamides

Clindamycin can be taken orally and is active against Gram-positive cocci, including streptococci and penicillin-resistant staphylococci. It is active against peripheral infections involving the anaerobic *B. fragilis*, and is recommended for the treatment of joint and bone infections caused by staphylococci. It is also used topically for the treatment of acne.

Streptogramins

Pritinamycin has been used orally in the treatment of Gram-positive cocci infections, including MRSA. **Quinupristin** and **dalfopristin** are used intravenously in combination (**Synercid**). At present these agents are reserved for life-threatening Gram-positive infections for which there are no alternative therapies; for example hospital-acquired pneumonia, skin and soft tissue infections and infections due to vancomycin-resistant *Enterococcus faecium*.

Oxazolidinones

The oxazolidinones have a broad spectrum of activity and are active against bacterial strains which have acquired resistance to other antibacterial agents acting against protein synthesis. **Linezolid** has good activity against most clinically important Gram-positive bacteria, including MRSA. It can also be taken orally with 100% uptake from the gastrointestinal tract. Unfortunately, there is a high level of side effects related to its use and, since it is a bacteriostatic agent, there is a greater risk of bacterial resistance developing.

19.8 Agents that act on nucleic acid transcription and replication

19.8.1 Quinolones and fluoroquinolones

The quinolone and fluoroquinolone antibacterial agents are particularly useful in the treatment of urinary tract infections and also for the treatment of infections which prove resistant to the more established antibacterial agents.

Nalidixic acid (Fig. 19.72) was the first therapeutically useful agent in this class of compounds, and was synthesized in 1962. Various analogues were synthesized but offered no great advantage. However, a breakthrough was made in the 1980s, with the development of **enoxacin** (Fig. 19.72) which showed improved broad-spectrum activity. The development of enoxacin was based on the discovery that a single fluorine atom at position 6 greatly increased activity as well as increasing uptake into the bacterial cell. A basic substituent such as a piperazinyl ring at position 7 was also beneficial for a variety of pharmacokinetic reasons, due to the ability of the basic substituent to form a zwitterion with the carboxylic acid group at position 3.

The introduction of a cyclopropyl substituent at position 1 further increased broad-spectrum activity, while replacement of the nitrogen at position 8 with carbon reduced adverse reactions and increased activity against *S. aureus*. This led to **ciprofloxacin** (Fig. 19.72 and Box 19.17), the most active of the fluoroquinolones against Gram-negative bacteria.

The quinolones and fluoroquinolones inhibit the replication and transcription of bacterial DNA by stabilizing the complex formed between DNA and topoisomerases (section 9.2). In Gram-positive bacteria, the stabilized complexes are between DNA and **topoisomerase IV**, with the drugs showing a 1000-fold selectivity for the bacterial enzyme over the corresponding enzyme in human cells. In Gram-negative bacteria, the main target for fluoroquinolones is the complex between DNA and a topoisomerase II enzyme called **DNA gyrase**. It has the same role as topoisomerase IV in reverse and is required when the DNA double helix is being supercoiled after replication and transcription.

A large number of fluoroquinolones have now been synthesized. Those agents having good activity all have a similar bicyclic ring system which includes a pyridone ring and a carboxylic acid at position 3. A problem with first-generation and second-generation fluoroquinolones is that they generally show only moderate activity against *S. aureus*, with resistance being quick to arise. Furthermore, only marginal activity is shown against anaerobes and *Streptococcus pneumoniae*. Third-generation fluoroquinolones such as **ofloxacin, levofloxacin,** and **moxifloxacin** (Fig. 19.73) began to be developed in the early 1990s to tackle these issues. Ofloxacin has an asymmetric centre and is sold as a racemic mixture of both enantiomers, one of which is active and one of which is not. Levofloxacin is the active enantiomer of oflaxacin and is twice as active as one would expect.

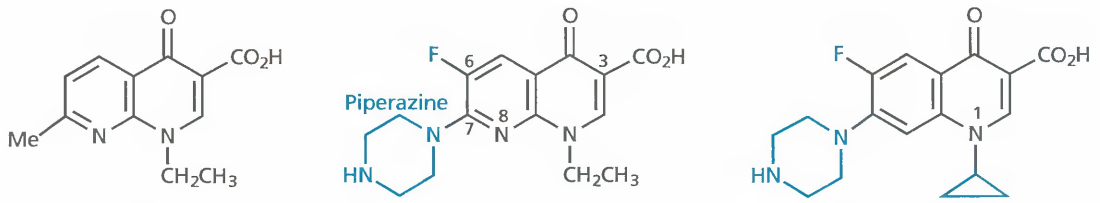


FIGURE 19.72 Quinolones and fluoroquinolones.

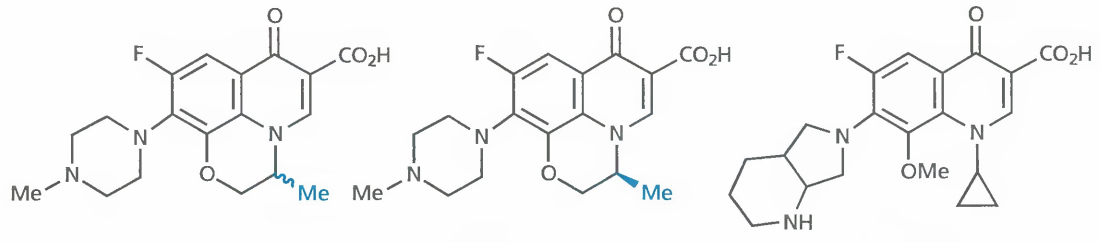
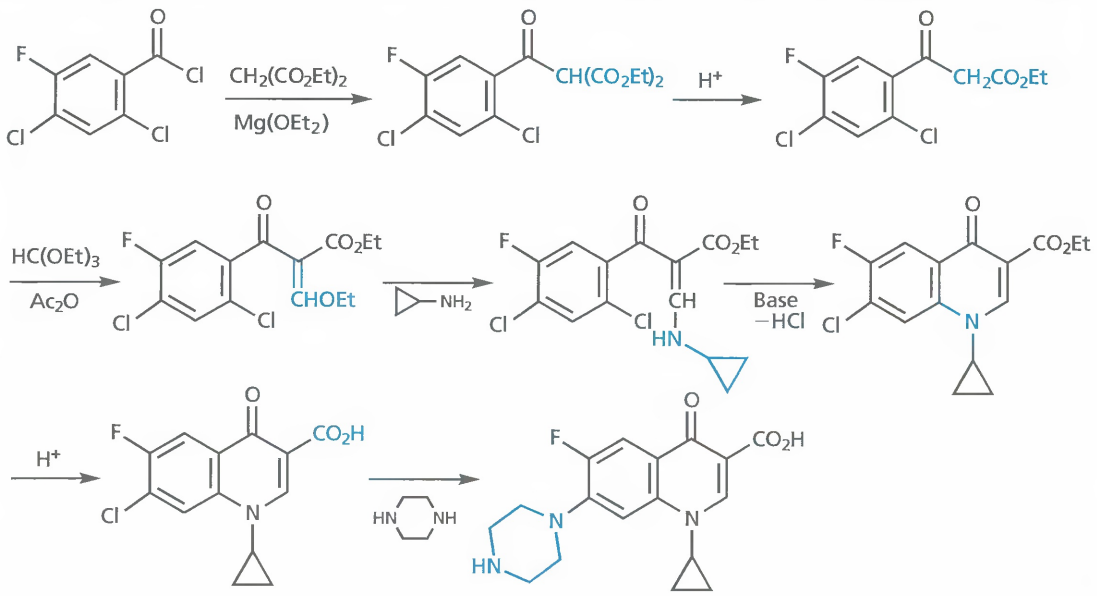


FIGURE 19.73 Third-generation fluoroquinolones.

Box 19.17 Synthesis of ciprofloxacin

The synthesis of ciprofloxacin is a seven stage route and is applicable to a wide range of fluoroquinolones. It involves the construction of the 'right-hand' pyridone ring onto an aromatic ring which already contains the fluoro-substituent. The cyclopropyl-substituent is incorporated just before ring closure and the piperazinyl-substituent is added at the final stage of the synthesis.



Synthesis of ciprofloxacin.

Box 19.18 Clinical aspects of quinolones and fluoroquinolones

Nalidixic acid is active against Gram-negative bacteria and is useful in the short-term therapy of uncomplicated urinary tract infections. It can be taken orally, but unfortunately, bacteria can develop a rapid resistance to it. **Enoxacin** has a greatly increased spectrum of activity against Gram-negative and Gram-positive bacteria. It also shows improved oral absorption, tissue distribution and metabolic stability, as well as an improvement in the level and spectrum of activity, particularly against Gram-negative bacteria such as *P. aeruginosa*. **Ciprofloxacillin** is used in the treatment of a large range of infections involving the urinary, respiratory, and gastrointestinal tracts (e.g. travellers' diarrhoea), as well as infections of skin, bone, and joints. It is also used for gonorrhoea and septicaemia, and as part of a cocktail of drugs for anthrax. It has been claimed that ciprofloxacin may be the most active broad-spectrum antibacterial agent on the market. In contrast to nalidixic acid, resistance to the fluoroquinolones is slow to appear, but when it does appear, it is mainly due to efflux mechanisms which pump the drug back out of the cell. Less common resistance mechanisms include mutations to the topoisomerase enzymes, which reduce their affinity to the agents, and alteration of porins in the outer membrane of Gram-negative organisms to limit access.

Third-generation fluoroquinolones show improved activity against *Streptococcus pneumoniae,* while maintaining activity against enterobacteria. **Ofloxacin** is administered orally or by intravenous infusion to treat urinary tract infections, lower respiratory tract infections, skin and soft tissue infections, septicaemia and gonorrhoea. **Levofloxacin** has a greater activity against pneumococci than ciprofloxacillin and is a second-line treatment for community-acquired pneumonia. It is also used for acute sinusitis, chronic bronchitis, urinary tract infections, skin infections and soft tissue infections. **Moxifloxacin** also has greater activity against pneumococci than ciprofloxacin. It is used to treat sinusitis and is a second line treatment of community-acquired pneumonia.

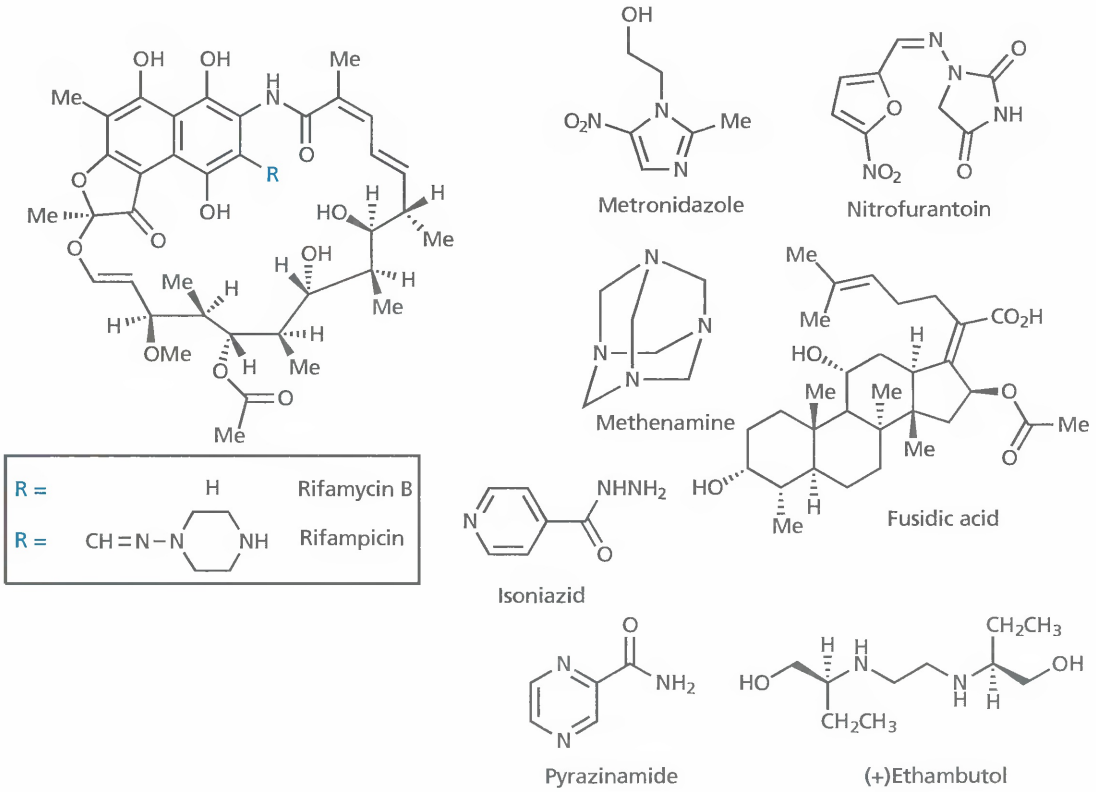


FIGURE 19.74 Miscellaneous agents.

19.8.2 Aminoacridines

Aminoacridine agents such as the yellow-coloured **proflavine** are topical antibacterial agents which were used particularly in the Second World War to treat deep surface wounds. The best agents are completely ionized at pH 7 and they interact directly with bacterial DNA by intercalation (section 9.1). Despite the success of these drugs as topical agents, they are not suitable for the treatment of systemic bacterial infections, as they are toxic to host cells.

19.8.3 Rifamycins

Rifampicin (Fig. 19.74) is a semi-synthetic rifamycin made from **rifamycin B**—an antibiotic which was isolated from *Streptomyces mediterranei* in 1957. It inhibits Gram-positive bacteria and works by binding non-covalently to DNA-dependent RNA polymerase and inhibiting the start of RNA synthesis. The DNA-dependent RNA polymerases in eukaryotic cells are unaffected, since the drug binds to a peptide chain not present in the mammalian RNA polymerase. It is therefore highly selective. The flat naphthalene ring and several of the hydroxyl groups are essential for activity, and the molecule exists as a zwitterion, giving it good solubility both in lipids and aqueous acid.

19.8.4 Nitroimidazoles and nitrofurantoin

Metronidazole (Fig. 19.74) is a nitroimidazole structure which was introduced in 1959 as an anti-protozoal agent, but began to be used as an antibacterial agent in the 1970s. The mechanism of action involves the drug entering the bacterial cell where the nitro group is reduced. This lowers the concentration of metronidazole within the cell setting up a concentration gradient down which more drug can flow. The reduction mechanism also proves toxic to the cell since free radicals are formed which act on DNA. **Nitrofurantoin** also undergoes reduction within bacterial cells to form radical species which act on DNA.

19.9 Miscellaneous agents

A variety of miscellaneous agents are shown in Fig. 19.74. **Methenamine** is used to treat urinary tract infections where it degrades in acid conditions to give formaldehyde as the active agent (section 14.6.6). **Fusidic acid** is a steroid structure derived from the fungus *Fusidium coccineum* and is used as a topical antibacterial agent. **Isoniazid** is the most widely used drug for the treatment of tuberculosis. It acts by inhibiting the synthetic pathways leading to

Box 19.19 Clinical aspects of rifamycins and miscellaneous agents

Rifampicin is bactericidal and is mainly used in the treatment of tuberculosis and staphylococci infections that resist penicillin. It is used in combination with dapsone in treating leprosy, and is also used for the treatment of brucellosis, legionnaires' disease and serious staphylococcal infection. It is a very useful antibiotic, showing a high degree of selectivity against bacterial cells over mammalian cells. Unfortunately, it is also expensive, which discourages its use against a wider range of infections. Rifampicin is a key component of any antituberculosis regimen, but it poses a special problem when treating tuberculosis in AIDS patients, since it enhances the activity of the cytochrome P450 enzyme family (CYP3A). These enzymes metabolize the HIV-protease inhibitors used in HIV therapy, thus lowering their effectiveness. Increased cytochrome P450 activity also decreases the effect of oral anticoagulants, oral contraceptives, and barbiturates.

Metronidazole has good activity in treating infections caused by anaerobic bacteria and protozoa, including difficult-to-treat organisms such as *Bacteroides fragilis* and *Clostridium difficile*. It is well distributed round the body and crosses the blood-brain barrier, so it can be used for the treatment of brain abscesses and other central nervous system infections involving anaerobic bacteria. Metronitrazole is used for the treatment of leg ulcers, bacterial vaginosis, pelvic inflammatory disease, and can also be used as an alternative to penicillins for oral infections including tooth abcesses. It is administered with amoxicillin (or with tetracycline and bismuth) in the treatment of gastric ulcers involving *H. pylori* (section 25.4). The drug is effective against *Guardia* infections derived from polluted water supplies – a common hazard when visiting the third world. Finally, nitroimidazoles are commonly combined with cephalosporins or aminoglycosides to treat infections involving both aerobic and anaerobic organisms. Resistance is rare, although it has been observed. **Nitrofurantoin** is used to treat uncomplicated urinary tract infections.

Methenamine can be used to treat urinary tract infections, but only if the urine is acidic, and the infection is in the lower urinary tract infection. It can be used as a prophylactic and as a treatment for chronic and recurrent lower urinary tract infections.

Fusidic acid is a topical antibacterial agent that is used in eye drops and skin creams. It can penetrate intact and damaged skin, so it is useful for the treatment of boils. It has also been used to eradicate MRSA colonies carried in the nasal passages of hospital patients and health workers. **Isoniazid** is the most widely used drug for the treatment of tuberculosis and is part of a four drug cocktail which is the first choice treatment for the initial phase of the disease.

mycolic acid, an important constituent of mycobacterial cell walls. It is activated in bacterial cells by a catalase-peroxidase enzyme. Resistant strains of tuberculosis (TB) block the action of this enzyme. **Ethambutol** and **pyrazinamide** are synthetic compounds which are both front-line drugs in the treatment of tuberculosis. Ethambutol inhibits arabinosyl transferase enzymes that are involved in the biosynthesis of the mycobacterial cell wall.

KEY POINTS

- Aminoglycosides, tetracyclines, chloramphenicol, streptogramins, lincosamides, and macrolides inhibit protein synthesis by binding to the bacterial ribosomes involved in the translation process.
- Resistance can arise from a variety of mechanisms such as drug efflux, altered binding affinity of the ribosome, altered membrane permeability, and metabolic reactions.
- Oxazolidinones prevent the formation of the 70S ribosome by binding to the 50S subunit.
- Quinolones and fluoroquinolones inhibit topoisomerase enzymes, resulting in inhibition of replication and transcription.
- Aminoacridines are useful topical antibacterial agents that can intercalate with bacterial DNA and hinder replication and transcription.
- Rifamycins inhibit the enzyme RNA polymerase and prevent RNA synthesis. This in turn prevents protein synthesis. Rifampicin is used to treat tuberculosis and staphylococcus infections.
- Nitroimidazoles are used against infections caused by protozoa and anaerobic bacteria.

19.10 Drug resistance

Medicinal chemists are still actively seeking new and improved antibacterial agents to combat the worrying ability of bacteria to acquire resistance to current drugs. For example, 60% of *Streptococcus pneumoniae* strains are resistant to β lactams and 60% of *Staphylococcus aureus* strains are resistant to **methicillin**. The last resort in treating *S. aureus* infections is **vancomycin**, but resistance is beginning to appear to that antibiotic as well. Some strains of *Enterococcus faecalis* appearing in urinary and wound

infections are resistant to all known antibiotics and are untreatable. If antibiotic resistance continues to grow, medicine could be plunged back to the 1930s. Indeed many of today's advanced surgical procedures would become too risky to carry out due to the risks of infection. Old diseases are already making a comeback. For example, a new antibiotic resistant strain of tuberculosis (**multidrug-resistant TB**—MDRTB) appeared in New York and took 4 years and $10 million to bring under control. These strains were resistant to two of the front-line drugs used against TB (isoniazid and rifamycin), and had various levels of resistance against another two (streptomycin and ethambutol). Other examples of bacterial strains acquiring resistance include penicillin-resistant meningococci and pneumococci in South Africa, penicillin-resistant gonococci in Asia and Africa, ampicillin-resistant *H. influenza* in the USA and Europe, and chloramphenicol-resistant meningococci in France and South East Asia. Resistance to trimethoprim in some of the developing nations has meant that the drug has become ineffective as a treatment of dysentery within 10 years.

Drug resistance can arise due to a variety of factors described in section 19.5.1.5, but the cell must have the necessary genetic information. This information can be obtained by mutation or by the transfer of genes between cells.

19.10.1 Drug resistance by mutation

Bacteria multiply at such a rapid rate that there is always a chance that a mutation will render a bacterial cell resistant to a particular agent. This feature has been known for a long time and is the reason why patients should fully complete a course of antibacterial treatment even though their symptoms may have disappeared well before the end of the course. If this rule is adhered to, the vast majority of the invading bacterial cells will be wiped out, leaving the body's own defence system to mop up any isolated survivors or resistant cells. If the treatment is stopped too soon, however, then the body's defences struggle to cope with the survivors. Any isolated resistant cell is then given the chance to multiply, resulting in a new infection which will, of course, be completely resistant to the original drug. Patients failing to complete a course of therapy was a major factor in the appearance of MDRTB.

These mutations occur naturally and randomly and do not require the presence of the drug. Indeed, it is likely that a drug-resistant cell is present in a bacterial population even before the drug is encountered. This was demonstrated with the identification of **streptomycin**-resistant cells from old cultures of *E. coli* which had been freeze-dried to prevent multiplication before the introduction of streptomycin into medicine.

19.10.2 Drug resistance by genetic transfer

A second way in which bacterial cells can acquire drug resistance is by gaining that resistance from another bacterial cell. This occurs because it is possible for genetic information to be passed on directly from one bacterial cell to another. There are two main methods by which this can take place—**transduction** and **conjugation**.

In transduction, small segments of genetic information known as **plasmids** are transferred by means of bacterial viruses (**bacteriophages**) which leave the resistant cell and infect a non-resistant cell. If the plasmid contains the gene required for drug resistance, then the recipient cell will be able to use that information and gain resistance. For example, the genetic information required to synthesize β-**lactamases** can be passed on in this way, rendering bacteria resistant to penicillins. The problem is particularly prevalent in hospitals where currently over 90% of *staphylococcal* infections are resistant to antibiotics such as penicillin, erythromycin, and tetracycline. It may seem odd that hospitals should be a source of drug-resistant strains of bacteria. In fact, they are the perfect breeding ground. Drugs commonly used in hospitals are present in the air in trace amounts. It has been shown that breathing in these trace amounts kills sensitive bacteria in the nose and allows the nostrils to act as a breeding ground for resistant strains.

In conjugation, bacterial cells pass genetic material directly to each other. This is a method used mainly by Gram-negative, rod-shaped bacteria in the colon, and involves two cells building a connecting bridge of sex pili through which the genetic information can pass.

19.10.3 Other factors affecting drug resistance

The more useful a drug is, the more it will be used and the greater the possibilities of resistant bacterial strains emerging. The original penicillins were used widely in human medicine, but were also commonly used in veterinary medicine. Antibacterial agents have also been used in animal feeding to increase animal weight and this, more than anything else, has resulted in drug-resistant bacterial strains. It is sobering to think that many of the original bacterial strains which were treated so dramatically with penicillin V or penicillin G are now resistant to those early penicillins. In contrast, these two drugs are still highly effective antibacterial agents in poorer, developing African nations, where the use (and abuse) of the drugs has been far less widespread.

The ease with which different bacteria acquire resistance varies. For example, *S. aureus* is notorious for its

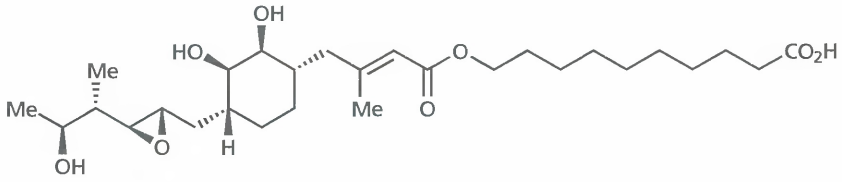


FIGURE 19.75 Mupirocin.

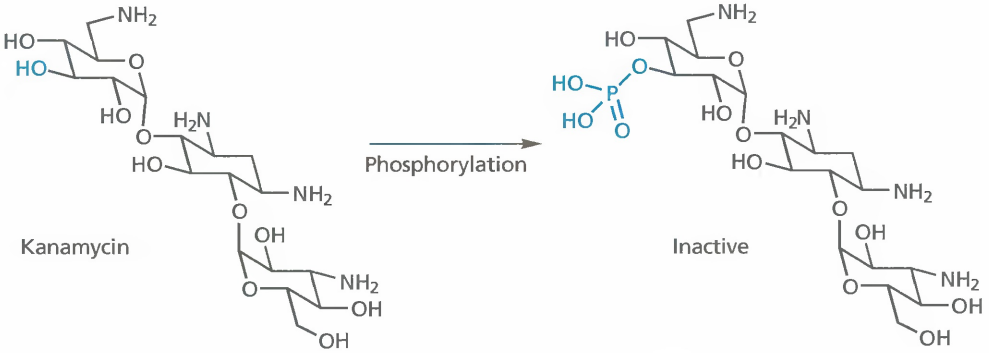


FIGURE 19.76 The phosphorylation reaction causing resistance to kanamycin.

ability to acquire drug resistance due to the ease with which it can undergo transduction. On the other hand, the microorganism responsible for syphilis seems incapable of acquiring resistance and is still susceptible to the original drugs used against it.

19.10.4 The way ahead

The ability of bacteria to gain resistance to drugs is an ever-present challenge to the medicinal chemist and it is important to continue designing new antibacterial agents. Identifying potential new targets is essential in this never-ending battle. The sequencing of genomes and a study of the proteins present in bacterial cells promises to give more detailed understanding of the molecular details of infectious agents leading to the identification of new drug targets. For example, *Mycobacterium tuberculosis*—the causative agent of TB—has a complex cell wall where three types of polymers are attached to peptidoglycan. The detailed mechanisms by which these polymers are synthesized and incorporated into the cell wall are being investigated to identify new targets for antibacterial drugs which will disrupt the cell wall structure.

It is also beginning to be appreciated that the drugs with the least susceptibility to resistance are those with several different modes of action. Therefore, designing drugs which act on a number of different targets rather than one specific target is more likely to be successful.

Examples of new targets include enzymes known as **aminoacyl tRNA synthetases**. These enzymes are an ancient group of enzymes responsible for attaching amino acids to tRNA. As they are ancient, there is a considerable sequence divergence between the bacterial and human enzymes, making selective inhibition possible. Isoleucyl tRNA synthetase is one such enzyme which is known to be inhibited by **mupirocin** (Fig. 19.75)—a clinically useful antibiotic isolated from *Pseudomonas fluorescens* with activity against MRSA. Mupirocin is used as a topical agent for skin infections, and has also been used to combat the transmission of *S. aureus* within hospitals by treating the nasal passages of patients and hospital staff. Unfortunately, the widespread use of the agent for this purpose has led to strains of *S. aureus* with increasing resistance to the drug. Research is now being carried out to find novel inhibitors for a different aminoacyl tRNA synthetase present in *S. aureus*, mainly tyrosine tRNA synthetase.

Another potential approach in countering resistance is to modify the antibiotics such that they gain resistance to the mechanisms of resistance used against them! For example, **kanamycin** is an aminoglycoside which is no longer used since resistant bacteria can phosphorylate one of the hydroxyl groups present (Fig. 19.76).

An active analogue has been synthesized which replaces the susceptible alcohol with a ketone (Fig. 19.77). This ketone is in equilibrium with the hydrated gem-diol. When phosphorylation occurs on the diol, the phosphate group thus formed acts as a good leaving group and the ketone is regenerated. *In vitro* tests showed that this agent was active against strains of bacteria which are resistant to kanamycin.

Another approach is to design molecules with an inbuilt self-destruct mechanism. One of the problems with antibiotics in medicine or veterinary practice is

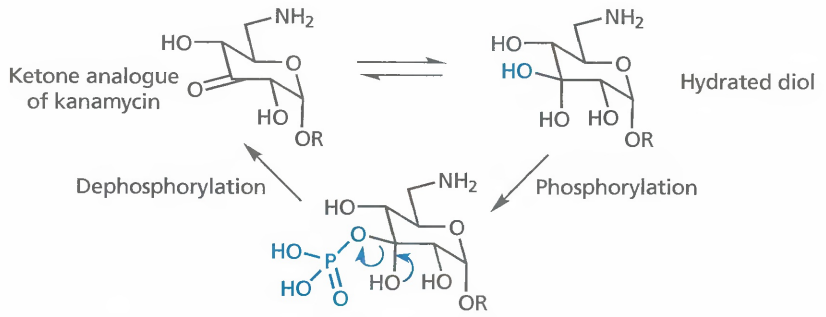


FIGURE 19.77 Analogue of kanamycin, which is resistant to phosphorylation.

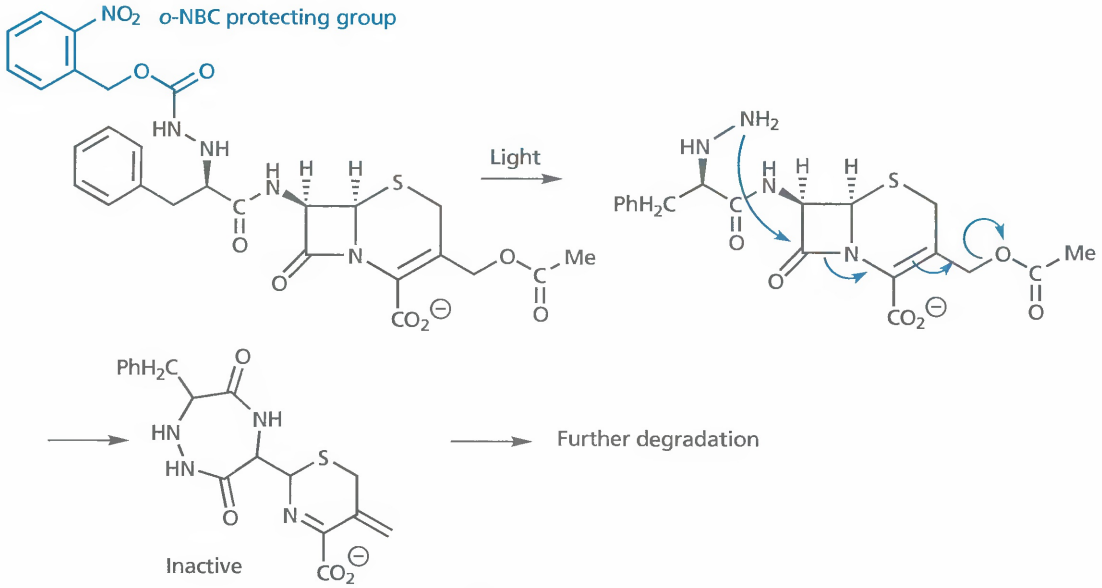


FIGURE 19.78 Self-destruct mechanism.

that much of the active antibiotic is excreted, giving bacteria in the environment the opportunity to gain resistance. This problem should be reduced by incorporating a self destruct mechanism which kicks in once the antibiotic is excreted. For example work has been carried out on a cephalosporin containing a protected hydrazine group (Fig. 19.78). The protecting group concerned is *ortho*-nitrobenzylcarbamate which is susceptible to light. Once the antibiotic is excreted and exposed to light, the protecting group is lost allowing the nucleophilic hydrazine moiety to react with the β-lactam ring and deactivate the molecule. This works *in vitro* but has still be tested *in vivo*.

KEY POINTS

- Bacteria can gain resistance to antibacterial drugs. Different strains gain resistance more easily than others. *Staphylococcus aureus* strains are quick to gain antibacterial resistance. The MRSA strain is a *S. aureus* strain that is resistant to most antibacterials, including methicillin.
- Vancomycin is the antibacterial agent of last resort in the treatment of resistant bacterial strains.
- There are many mechanisms by which bacteria can acquire resistance against antibacterial agents, but they all result from a change in the cell's genetic make-up.
- Drug resistance can result from mutation of a cell's genetic information, or from transfer of genetic information from one cell to another. Genetic information can be transferred from one cell to another by transduction or conjugation.
- Care has to be taken to use antibacterial agents in a responsible manner to reduce the chances of resistance developing.
- It is important to identify new targets that can be used for the design of novel antibacterial agents.

Box 19.20 Organoaresenicals as antiparasitic drugs

The first effective antimicrobial drug to be synthesized was the organoaresenical, salvarsan (section 19.1). In the late 1940s, another organoaresenical called **melarsoprol** was introduced into medicine and is the first-choice drug for the treatment of trypanosomiasis and sleeping sickness (Fig. 1).

One of the mechanisms by which melarsoprol might act is through a reaction with the cysteine residues of enzymes involved in glycolysis (Fig. 2). As a result, glycolysis is blocked leading to a loss of cell motility and eventual cell death. Other mechanisms of action have been proposed.

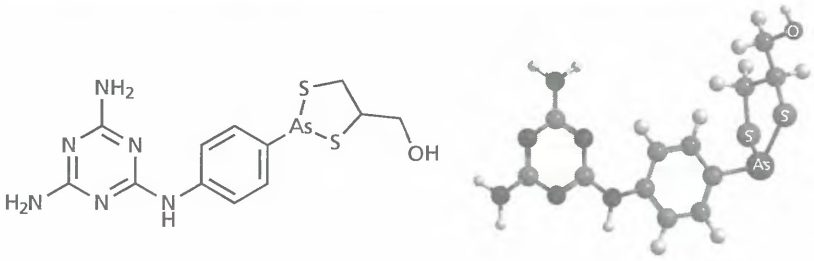


FIGURE 1 Melarsoprol.

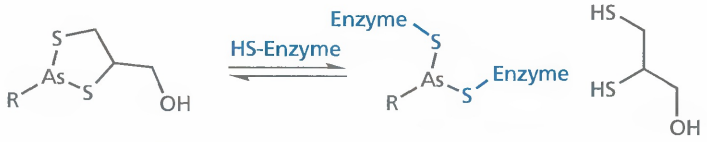


FIGURE 2 Mechanism of action of melarsoprol.

QUESTIONS

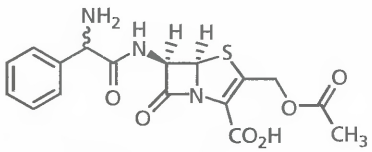
1. How would you convert penicillin G to 6-aminopenicillanic acid (6-APA) using chemical reagents? Suggest how you would make ampicillin from 6-APA.

2. Penicillin is produced biosynthetically from cysteine and valine. If the biosynthetic pathway could accept different amino acids, what sort of penicillin analogues might be formed if valine was replaced by alanine, phenylalanine, glycine, or lysine? What sort of penicillin analogue might be formed if cysteine was replaced by serine? (See Appendix 1 for amino acid structures.)

3. Referring to Question 2, why do you think penicillin analogues like this are not formed during the fermentation process?

4. The activity of sulfonamides is decreased if they are taken at the same time as procaine. Suggest why this might be the case.

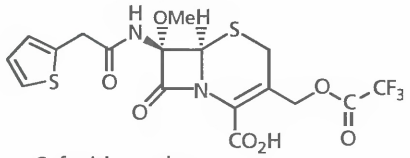
5. Discuss whether you think the following penicillin analogue would be a useful antibacterial agent.



Penicillin analogue

6. Explain what effect replacing the methoxy groups on methicillin with ethoxy groups might have on the properties of the agent.

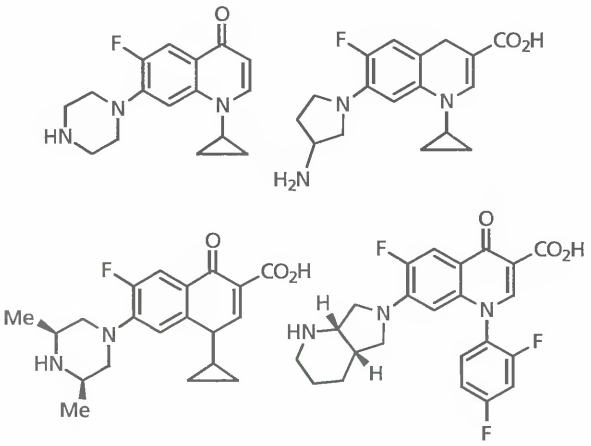
7. The following structure is an analogue of cefoxitin. What sort of properties do you think it might have compared to cefoxitin itself?



Cefoxitin analogue

8. Show the mechanism by which the prodrug bacampicillin (Box 19.7) is converted to ampicillin. What are the by-products?

9. Which of the following structures would you expect to have the best antibacterial activity?



10. Devise a synthesis for the structure chosen in Question 9.

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Saunders, J. (2000) Quinolones as anti-bacterial DNA gyrase inhibitors. Chapter 10 in: *Top drugs: top synthetic routes.* Oxford University Press, Oxford.

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Titles for general further reading are listed on p. 725.