8

CARBOHYDRATES

Some fundamental modifications that occur in carbohydrate metabolism are outlined, then specific examples of monosaccharides, oligosaccharides, and polysaccharides are described. The formation of amino sugars follows, leading to a discussion of aminoglycoside antibiotics. Monograph topics giving more detailed information on medicinal agents include monosaccharides and disaccharides, vitamin C, polysaccharides, aminoglycoside antibiotics based on streptamine and 2-deoxystreptamine, acarbose, lincomycin, and clindamycin.

The main pathways of carbohydrate biosynthesis and degradation comprise an important component of primary metabolism that is essential for all organisms. Carbohydrates are among the most abundant constituents of plants, animals, and microorganisms. Polymeric carbohydrates function as important food reserves, and as structural components in cell walls. Animals and most microorganisms are dependent for their very existence on the carbohydrates produced by plants. Carbohydrates are the first products formed in photosynthesis, and are the products from which plants then synthesize their own food reserves as well as other chemical constituents. These materials then become the foodstuffs of other organisms. Secondary metabolites are also ultimately derived from carbohydrate metabolism, and the relationships of the acetate, shikimate, mevalonate, and deoxyxylulose phosphate pathways to primary metabolism have already been indicated. Many of the medicinally important secondary metabolites described in the earlier chapters have been seen to contain clearly recognizable carbohydrate portions in their structures, e.g. note the frequent occurrence of glycosides. In this chapter, some of the important natural materials which can be grouped together because they are composed entirely or predominantly of basic carbohydrate units are discussed. Because of their widespread use in medicinal preparations, some materials with no inherent biological activity, and which are clearly of primary metabolic status, e.g. sucrose and starch, are also included.

MONOSACCHARIDES

Six-carbon sugars (hexoses) and five-carbon sugars (pentoses) are the most frequently encountered carbohydrate units (monosaccharides) in nature. Photosynthesis produces initially the three-carbon sugar 3-phosphoglyceraldehyde, two molecules of which are used to synthesize glucose 6-phosphate by a sequence which effectively achieves the reverse of the glycolytic reactions (Figure 8.1). Alternatively, by the complex reactions of the Calvin cycle, 3-phosphoglyceraldehyde may be used in the construction of the pentoses ribose 5-phosphate, ribulose 5-phosphate, and xylulose 5-phosphate. These sequences incorporate some of the fundamental reactions which are used in the biochemical manipulation of monosaccharide structures:

- Mutation, where repositioning of a phosphate group in the monosaccharide phosphate molecule, e.g. the isomerization of glucose 6-phosphate and glucose 1-phosphate (Figure 8.2), is achieved via an intermediate diphosphate.

- Epimerization changes the stereochemistry at one of the chiral centres, e.g. the interconversion of ribulose 5-phosphate and xylulose 5-phosphate (Figure 8.3). This reaction involves epimerization adjacent to a carbonyl group and probably proceeds through a common enol tautomer, but some other epimerizations are
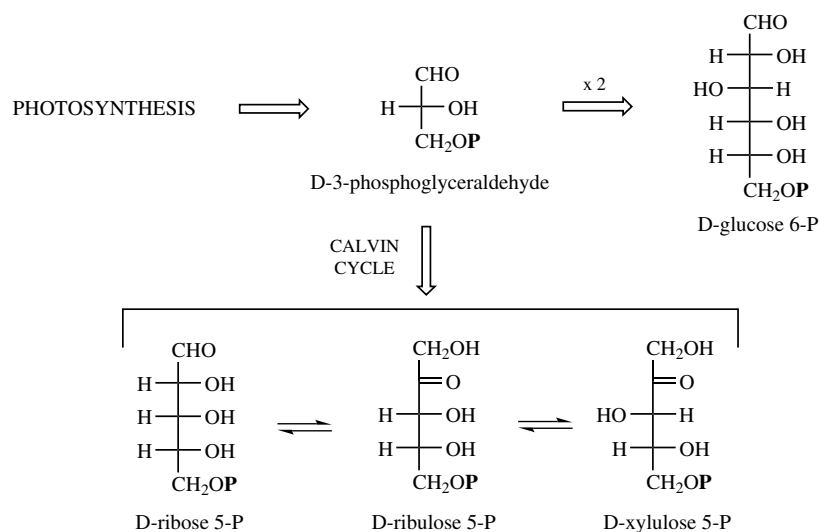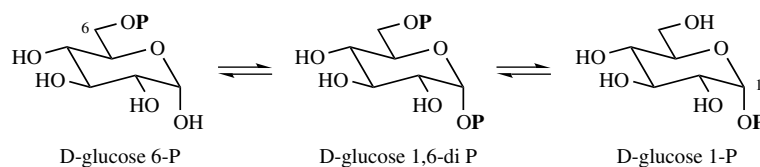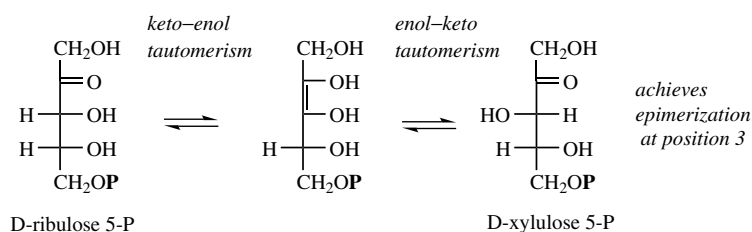
Figure 8.1

Figure 8.2

Figure 8.3

known proceed through oxidation to an inter-mediate carbonyl, followed by reduction to give the opposite configuration. The substrate for epimerization is often the UDPsugar rather than the monosaccharide phosphate.

- Aldose–ketose interconversions, e.g. glucose 6-phosphate to fructose 6-phosphate (Figure 8.4), also proceed through a common enol intermediate.
- Transfer of C_2 and C_3 units in reactions catalysed by transketolase and transaldolase respectively modify the chain length of the sugar.

Transketolase removes a two-carbon fragment from ketols such as fructose 6-phosphate (alternatively xylulose 5-phosphate or sedoheptulose 7-phosphate) through the participation of thiamine diphosphate. Nucleophilic attack of the thiamine diphosphate anion on to the carbonyl results in an addition product which then fragments by a reverse aldol reaction, generating the chain-shortened aldose erythrose 4-phosphate, and the two-carbon carbanion unit attached to TPP (Figure 8.5) (compare the role of TPP in the decarboxylation of α-keto
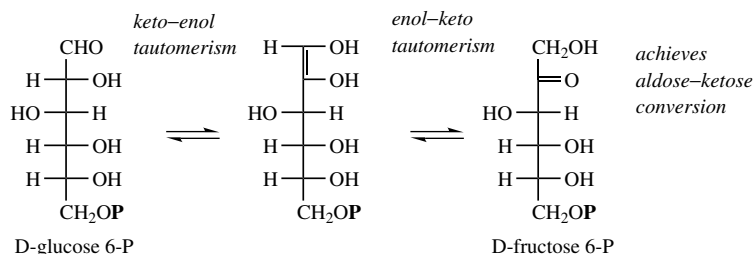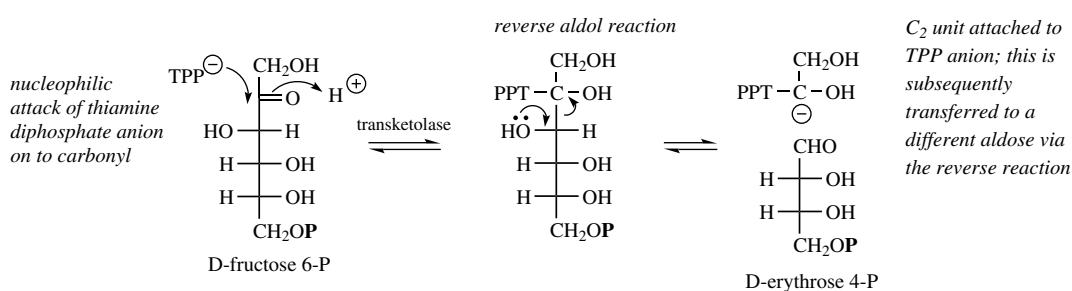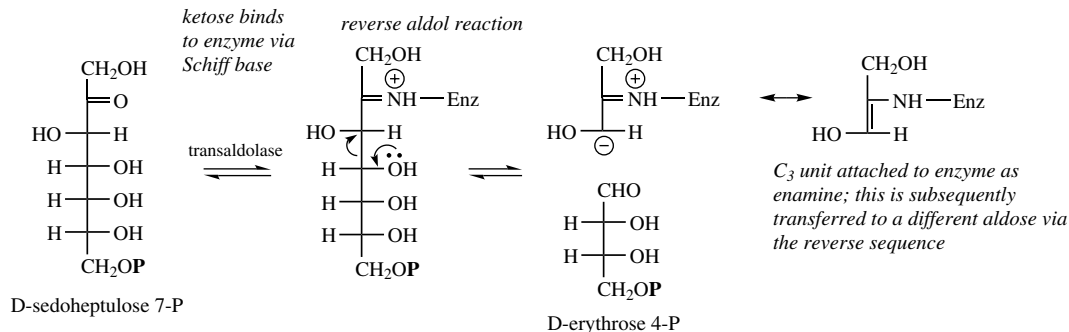
Figure 8.4

Figure 8.5

Figure 8.6

acids, page 21). Then, in what is formally the reverse of this reaction, this carbanion can attack another aldose such as ribose 5-phosphate (alternatively erythrose 4-phosphate or glyceraldehyde 3-phosphate), thus extending its chain length by two carbons. Transaldolase removes a three-carbon fragment from a ketose such as sedoheptulose 7-phosphate (alternatively fructose 6-phosphate) in a reverse aldol reaction, though this requires formation of a Schiff base between the carbonyl group and an active site lysine of the enzyme (Figure 8.6). Again, the reaction is completed by a reversal

of this process, but transferring the C_3 carbanion to another aldose such as glyceraldehyde 3-phosphate (alternatively erythrose 4-phosphate or ribose 5-phosphate) and thus increasing its length.

- Oxidation and reduction reactions, typically employing the NAD/NADP nucleotides, alter the oxidation state of the substrate. Oxidation at C-1 converts an aldose into an aldonic acid, e.g. glucose 6-phosphate gives gluconolactone 6-phosphate and then the open-chain gluconic acid 6-phosphate (Figure 8.7). Oxidation at C-6 yields the corresponding uronic acids,
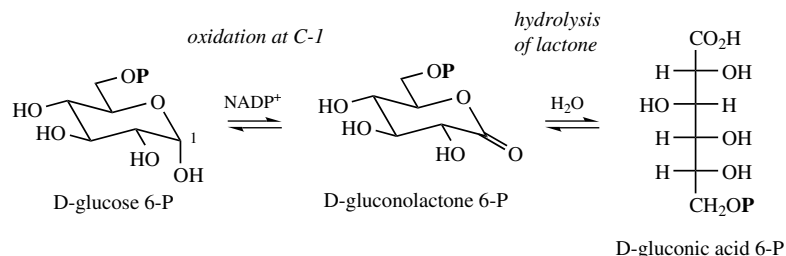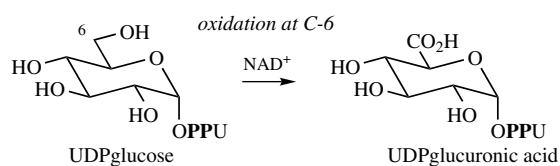
Figure 8.7

Figure 8.8

but this takes place on UDPsugar derivatives, e.g. UDPglucose to UDPglucuronic acid (Figure 8.8). Reduction is exemplified by the conversion of both glucose and fructose into the sugar alcohol sorbitol (glucitol), and of mannose into mannitol (Figure 8.9).

- Transamination reactions on keto sugars allow the introduction of amino groups as seen in the amino sugars glucosamine and galactosamine (Figure 8.10). These compounds, as their *N*-acetyl derivatives, are part of the structures of several natural polysaccharides, and

other uncommon amino sugars are components of the aminoglycoside antibiotics (see page 478).

Monosaccharide structures may be depicted in open-chain forms showing their carbonyl character, or in cyclic hemiacetal or hemiketal forms. The compounds exist predominantly in the cyclic forms, which result from nucleophilic attack of an appropriate hydroxyl on to the carbonyl (Figure 8.11). Both six-membered pyranose and five-membered furanose structures are encountered, a particular ring size usually being characteristic for any one sugar. Since the carbonyl group may be attacked from either side, two epimeric structures (anomers) are possible in each case, and in solution, the two forms are frequently in equilibrium. In natural product structures, sugar units are most likely (but not always) to be encountered in just one of the epimeric forms. The two forms are designated α or β on the basis of the
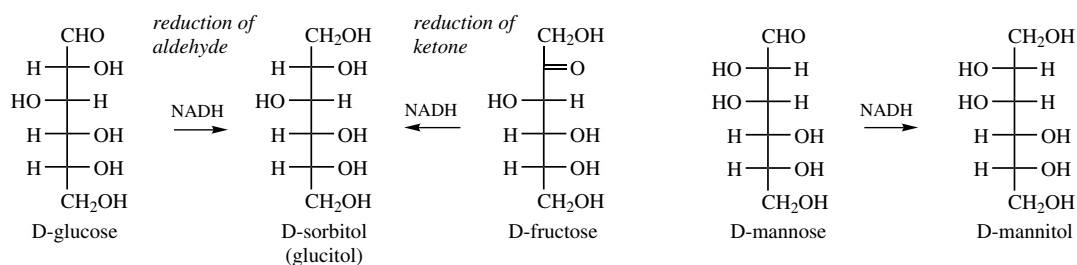

Figure 8.9

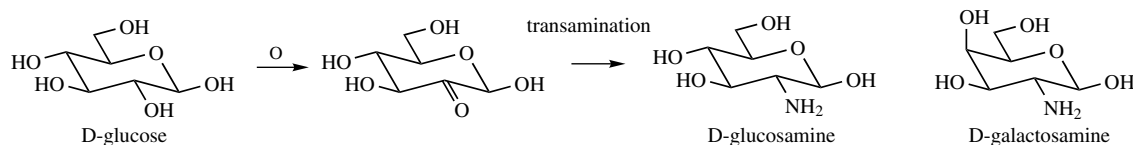**Figure 8.10**

Figure 8.11

β-D-glucose
(β-D-Glc)

β-D-galactose
(β-D-Gal)

β-D-mannose
(β-D-Mann)

α-L-rhamnose
(α-L-Rha)
[preferred conformation]

β-D-xylose
(β-D-Xyl)

α-L-arabinose
(α-L-Ara)

β-D-ribose
(β-D-Rib)

β-D-fructose
(β-D-Fru)

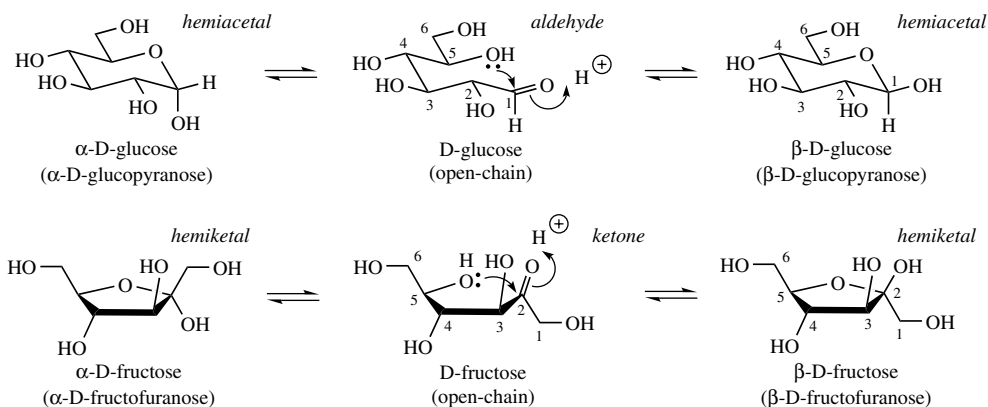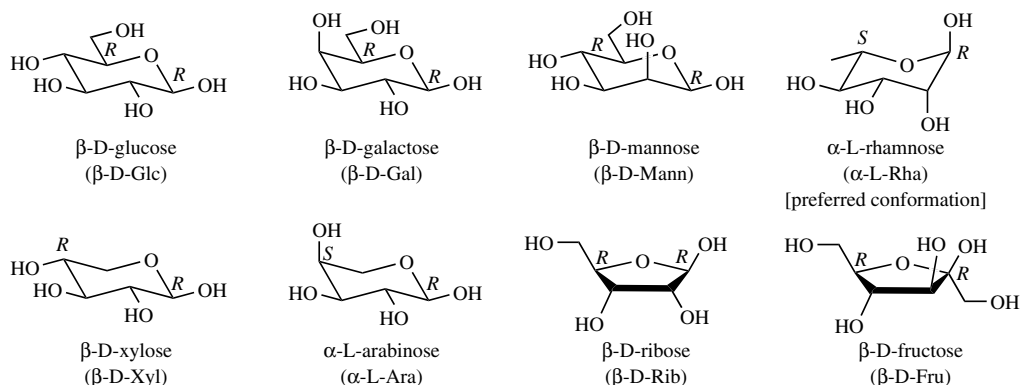**Figure 8.12**

chiralities at the anomeric centre and at the highest numbered chiral centre. If these are the same (*RS* convention) the anomer is termed β, or α if they are different. The most commonly encountered monosaccharides, and their usual anomers are shown in Figure 8.12. Note that the D- and L- prefixes are assigned on the basis of the chirality (as depicted in Fischer projection) at the highest numbered chiral centre and its relationship to D-(*R*)-(+)-glyceraldehyde or L-(*S*)-(−)-glyceraldehyde (Figure 8.13).

CHO
H—*R*—OH
CH₂OH
D-(+)-glyceraldehyde

CHO
HO—*S*—H
CH₂OH
L-(−)-glyceraldehyde

CHO
H—OH
HO—H
H—OH
H—OH
CH₂OH
D-glucose

CHO
H—OH
HO—H
HO—H
CH₂OH
L-arabinose
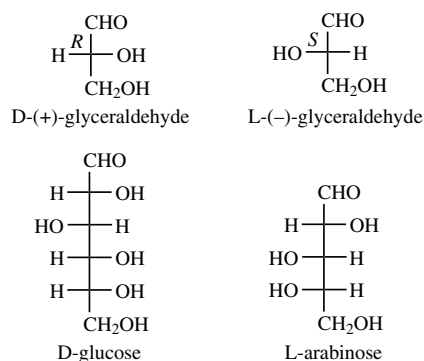
Figure 8.13

OLIGOSACCHARIDES

The formation of oligosaccharides (typically two to five monomers) and polysaccharides is dependent on the generation of an activated sugar bound to a nucleoside diphosphate. The nucleoside diphosphate most often employed is UDP, but

ADP and GDP are sometimes involved. As outlined in Chapter 2 (see page 29), a UDPsugar is formed by the reaction of a sugar 1-phosphate with UTP, and then nucleophilic displacement of the UDP leaving group by a suitable nucleophile

generates the new sugar derivative. This will be a glycoside if the nucleophile is a suitable aglycone molecule, or an oligosaccharide if the nucleophile is another sugar molecule (Figure 8.14). This reaction, if mechanistically of S_N2 type, should give an inversion of configuration at C-1 in the electrophile, generating a product with the β-configuration in the case of UDPglucose as shown. Many of the linkages formed between glucose monomers actually have the α-configuration, and it is believed that a double S_N2 mechanism operates, which also involves a nucleophilic group on the enzyme (Figure 8.14). Linkages are usually represented by a shorthand version, which indicates the atoms bonded and the configuration at the appropriate centre(s). Thus

maltose (Figure 8.15), a hydrolysis product from starch, contains two glucoses linked α1→4, whilst **lactose**, the main sugar component of cow's milk, has galactose linked β1→4 to glucose. In the systematic names, the ring size (pyranose or furanose) is also indicated. **Sucrose** ('sugar') (Figure 8.15) is composed of glucose and fructose, but these are both linked through their anomeric centres, so the shorthand representation becomes α1→β2. This means that both the hemiacetal structures are prevented from opening, and, in contrast to maltose and lactose, there can be no open-chain form in equilibrium with the cyclic form. Therefore sucrose does not display any of the properties usually associated with the masked carbonyl group, e.g. it is not a reducing sugar.

Figure 8.14

maltose
D-Glc(α1→4)D-Glc
4-*O*-(α-D-glucopyranosyl)-D-glucopyranose

lactose
D-Gal(β1→4)D-Glc
4-*O*-(β-D-galactopyranosyl)-D-glucopyranose

sucrose
D-Glc(α1→β2)D-Fru
α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside

lactulose
D-Gal(β1→4)D-Fru
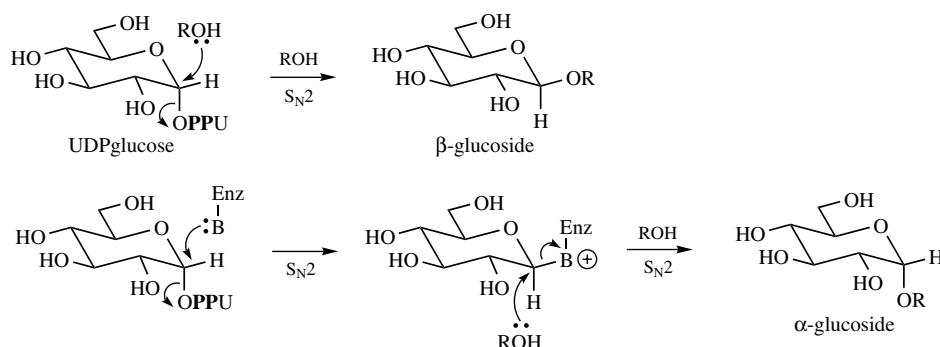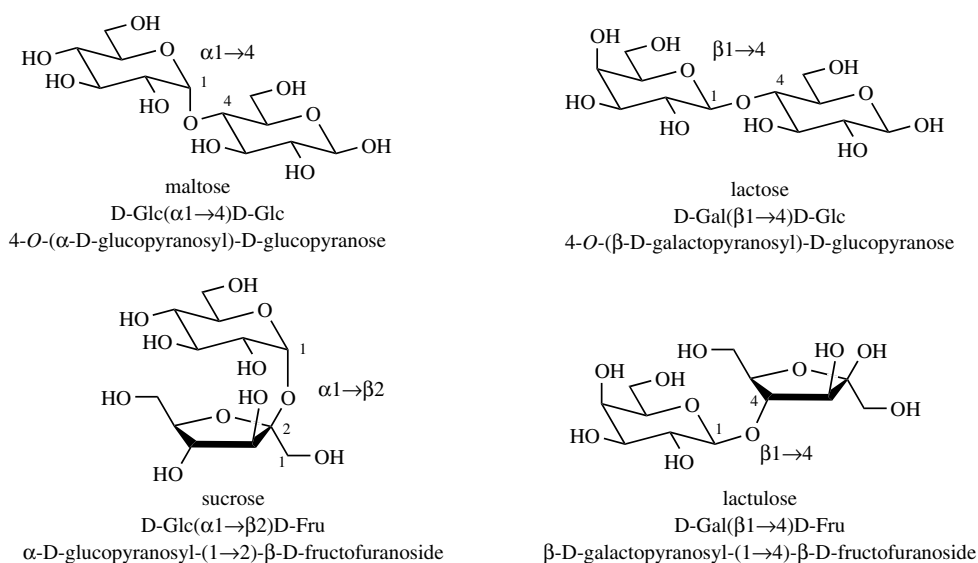β-D-galactopyranosyl-(1→4)-β-D-fructofuranoside
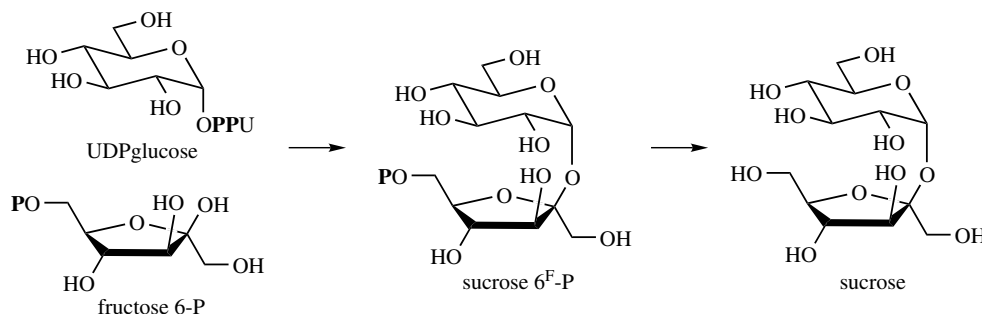
Figure 8.15

Figure 8.16

Sucrose is known to be formed predominantly by a slightly modified form of the sequence shown in Figure 8.16, in that UDPglucose is attacked by fructose 6-phosphate, and that the first formed product is sucrose 6^F-phosphate (F indicating the numbering refers to the fructose ring). Hydrolysis of the phosphate then generates sucrose (Figure 8.16).

Monosaccharides and Disaccharides

D-**Glucose** (**dextrose**) (Figure 8.9) occurs naturally in grapes and other fruits. It is usually obtained by enzymic hydrolysis of starch, and is used as a nutrient, particularly in the form of an intravenous infusion. Chemical oxidation of glucose produces gluconic acid. The soluble calcium salt **calcium gluconate** is used as an intravenous calcium supplement. D-**Fructose** (Figure 8.13) is usually obtained from invert sugar (see below) separating it from glucose, and is of benefit as a food and sweetener for patients who cannot tolerate glucose, e.g. diabetics. Fructose has the sweetness of sucrose, and about twice that of glucose. High fructose corn syrup for use as a food sweetener is a mixture of fructose and glucose containing up to 90% fructose and is produced by enzymic hydrolysis/isomerization of starch. The sugar alcohol D-**sorbitol** (Figure 8.9) is found naturally in the ripe berries of the mountain ash (*Sorbus aucuparia*; Rosaceae) but is prepared semi-synthetically from glucose. It is half as sweet as sucrose, is not absorbed orally, and is not readily metabolized in the body. It finds particular use as a sweetener for diabetic products. D-**Mannitol** (Figure 8.9) is also produced from glucose, but occurs naturally in manna, the exudate of the manna ash *Fraxinus ornus* (Oleaceae). This material has similar characteristics to sorbitol, but is used principally as a diuretic. It is injected intravenously, is eliminated rapidly into the urine, and removes fluid by an osmotic effect.

Sucrose (Figure 8.15) is obtained from a variety of sources, including sugar cane (*Saccharum officinarum*; Graminae/Poaceae), sugar beet (*Beta vulgaris*; Chenopodiaceae), and sugar maple (*Acer saccharum*; Aceraceae). It is a standard sweetening agent for foods, syrups, and drug preparations. **Invert sugar** is an equimolar mixture of glucose and fructose, obtained from sucrose by hydrolysis with acid or the enzyme invertase. During this process, the optical activity changes from $+$ to $-$, hence the reference to inversion. The high sweetness of fructose combined with that of glucose means invert sugar provides a cheaper, less calorific food sweetener than sucrose. Honey is also mainly composed of invert sugar. **Lactose** (Figure 8.15) can comprise up to 8% of mammalian milk, and is extracted from cow's milk, often as a by-product from cheese manufacture. It is only faintly sweet, and its principal use is as a diluent in tablet formulations. **Lactulose** (Figure 8.15)

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is a semi-synthetic disaccharide prepared from lactose, and composed of galactose linked β1 → 4 to fructose. It is not absorbed from the gastrointestinal tract, and is predominantly excreted unchanged. It helps to retain fluid in the bowel by osmosis, and is thus used as a laxative.

Vitamin C

Vitamin C (**ascorbic acid**) (Figure 8.17) can be synthesized by most animals except humans, other primates, guinea pigs, bats and some birds, and for these it is obtained via the diet. Citrus fruits, peppers, guavas, rose hips, and blackcurrants are especially rich sources, but it is present in most fresh fruit and vegetables. Raw citrus fruits provide a good daily source. It is a water-soluble acidic compound (an enol; see Figure 8.18) and is rapidly degraded during cooking in the presence of air. Vitamin C deficiency leads to scurvy, characterized by muscular pain, skin lesions, fragile blood vessels, bleeding gums, and tooth loss. The vitamin is essential for the formation of collagen, the principal structural protein in skin, bone, tendons, and ligaments, being a cofactor in the hydroxylation of proline to 4-hydroxyproline, and of lysine to 5-hydroxylysine (see page 409), which account for up to 25% of the collagen structure. These reactions are catalysed by 2-oxoglutarate dioxygenases (see page 27), and the ascorbic acid requirement is to reduce an enzyme-bound iron–oxygen complex. Skin lesions characteristic of scurvy are a direct result of low levels of hydroxylation in the collagen structure synthesized in the absence of ascorbic acid. Ascorbic acid is also associated with the hydroxylation of tyrosine in the pathway to catecholamines (see page 316), and in the biosynthesis of homogentisic acid, the precursor of tocopherols and plastoquinones (see page 159). Ascorbic acid is usually prepared synthetically, and is used to treat or prevent deficiency. Natural ascorbic acid is extracted from rose hips, persimmons, and citrus fruits. Large doses have been given after surgery or burns to promote healing by increasing collagen synthesis. The benefits of consuming large doses of vitamin C to alleviate the common cold and other viral infections are not proven. Some sufferers believe it to be beneficial in the
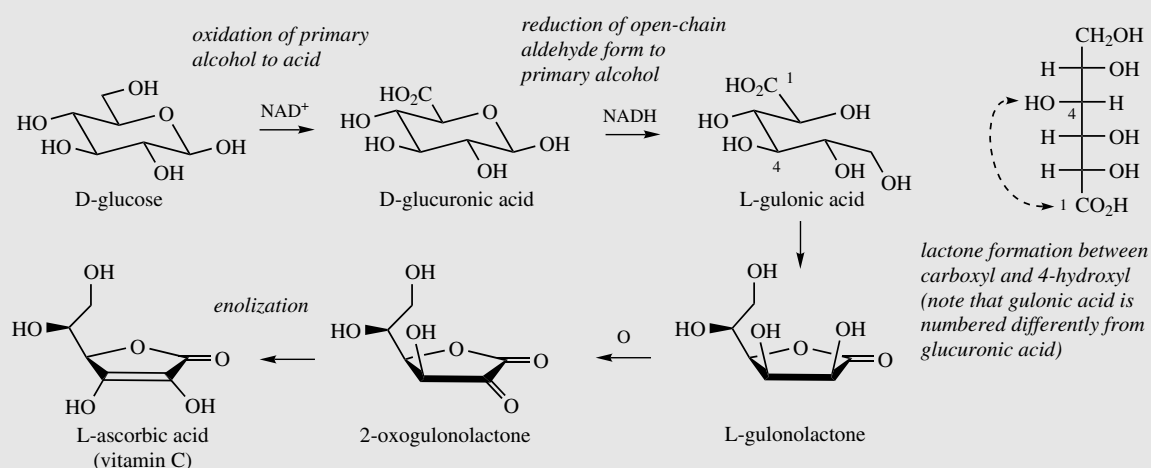

Figure 8.17

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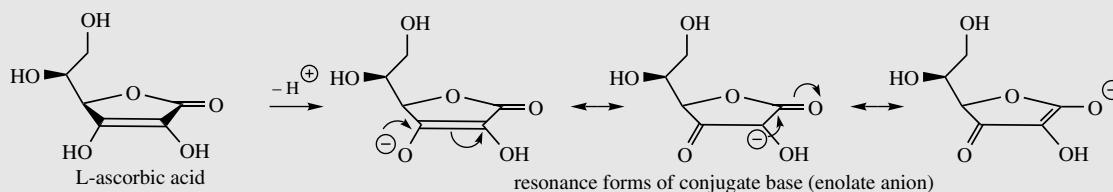L-ascorbic acid   resonance forms of conjugate base (enolate anion)

Figure 8.18

prevention and therapy of cancer. Vitamin C does have valuable antioxidant properties, and these are exploited commercially in the food industries.

In animals, ascorbic acid is synthesized in the liver from glucose, by a pathway which initially involves oxidation to glucuronic acid. This is followed by reduction of the carbonyl function, lactone formation, oxidation of the secondary alcohol to a carbonyl, with subsequent enolization (Figure 8.17). In plants, glucose or galactose can be converted into ascorbic acid by an analogous pathway, though other sequences from glucose have also been observed to operate. Man and other primates appear to be deficient in the enzyme oxidizing gulonolactone to the ketolactone, and are thus dependent on a dietary source of vitamin C.

POLYSACCHARIDES

Polysaccharides fulfil two main functions in living organisms, as food reserves, and as structural elements. Plants accumulate starch as their main food reserve, a material that is composed entirely of glucopyranose units but in two types of molecule. **Amylose** (Figure 8.19) is a linear polymer containing some 1000–2000 glucopyranose units linked α1→4. **Amylopectin** (Figure 8.19) is a much larger molecule than amylose (the number of glucose residues varies widely but may be as high as 10^6), and it is a branched-chain molecule. In addition to α1→4 linkages, amylopectin has branches at about every 20 units through α1→6 linkages. These branches continue with α1→4 linkages, but then may have subsidiary branching, giving a treelike structure. The mammalian carbohydrate storage molecule is **glycogen**, which is analogous to amylopectin in structure, but is larger and contains more frequent branching, about every ten residues. The branching in amylopectin and glycogen is achieved by the enzymic removal of a portion of the α1→4 linked straight chain consisting of several glucose residues, then transferring this short chain to a suitable 6-hydroxyl group. A less common storage polysaccharide found in certain plants of

the Compositae/Asteraceae and Campanulaceae is **inulin** (Figure 8.19), which is a relatively small polymer of fructofuranose, linked through β2→1 bonds.

Cellulose is reputedly the most abundant organic material on earth, being the main constituent in plant cell walls. It is composed of glucopyranose units linked β1→4 in a linear chain. Alternate residues are 'rotated' in the structure (Figure 8.19), allowing hydrogen bonding between adjacent molecules, and construction of the strong fibres characteristic of cellulose, as for example in cotton. The structure of **chitin** (Figure 8.19) is rather similar to cellulose, though it is composed of β1→4 linked N-acetylglucosamine residues. Chitin is a major constituent in the shells of crustaceans, e.g. crabs and lobsters, and insect skeletons, and its strength again depends on hydrogen bonding between adjacent molecules, producing rigid sheets. Chemical deacetylation of chitin provides chitosan, a valuable industrial material used for water purification because of its chelating properties, and in wound-healing preparations. Bacterial cell walls contain **peptidoglycan** structures in which carbohydrate chains composed of alternating β1→4 linked N-acetylglucosamine and N-acetylmuramic acid (O-lactyl-N-acetylglucosamine) residues are

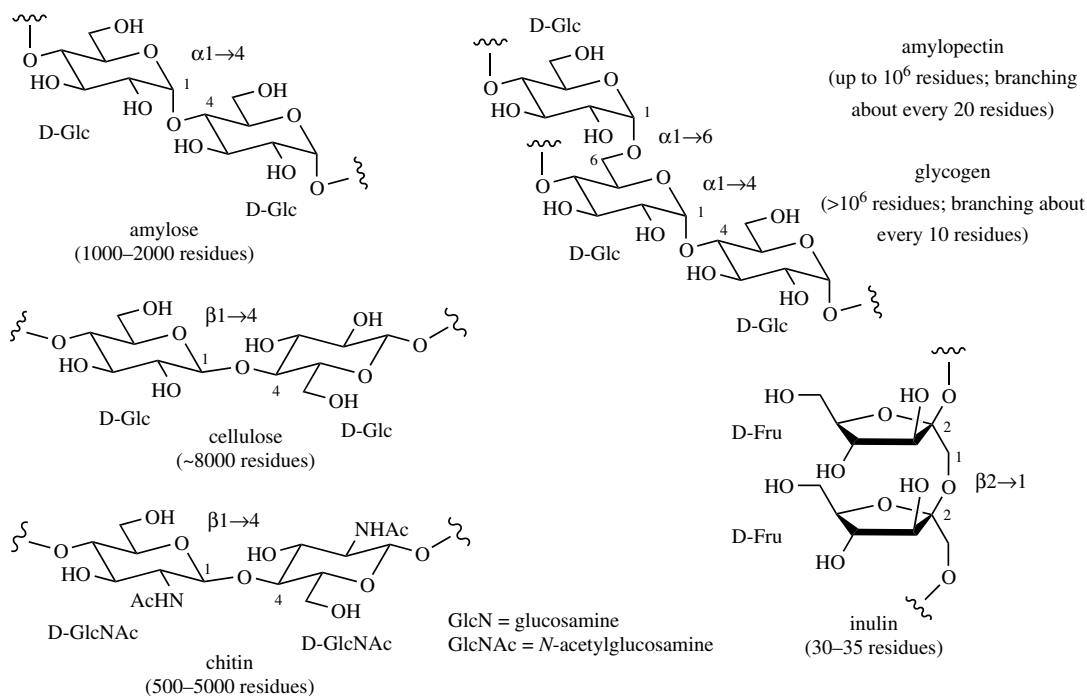**Figure 8.19**

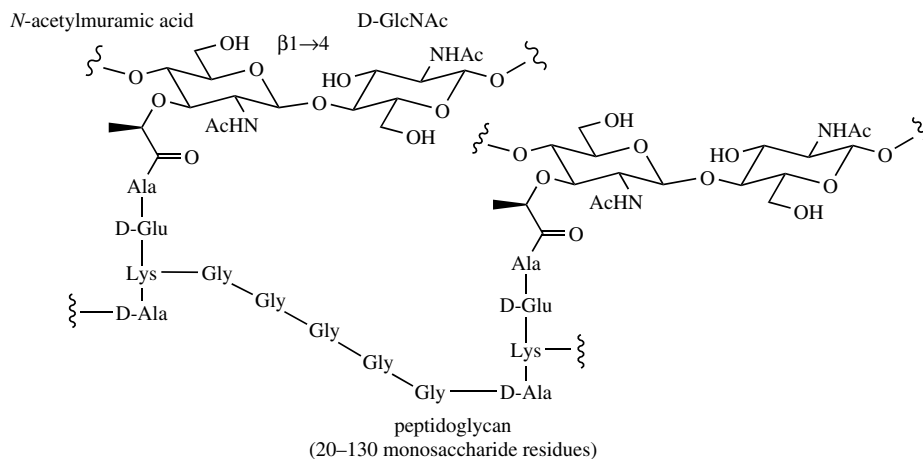peptidoglycan
(20–130 monosaccharide residues)

Figure 8.20

cross-linked via peptide structures. The peptidoglycan of *Staphylococcus aureus* is illustrated in Figure 8.20, showing the involvement of the lactyl group of the *N*-acetylmuramic acid to link the peptide with the carbohydrate via an amide/peptide bond. During the cross-linking process, the peptide chains from the *N*-acetylmuramic acid residues have a terminal –Lys–D-Ala–D-Ala

sequence, and the lysine from one chain is bonded to the penultimate D-alanine of another chain through five glycine residues, at the same time displacing the terminal D-alanine (see Figure 7.36, page 444). The biological activities of the β-lactam antibiotics, e.g. penicillins and cephalosporins (see page 444) and of the last-resort antibiotic vancomycin (see page 426)
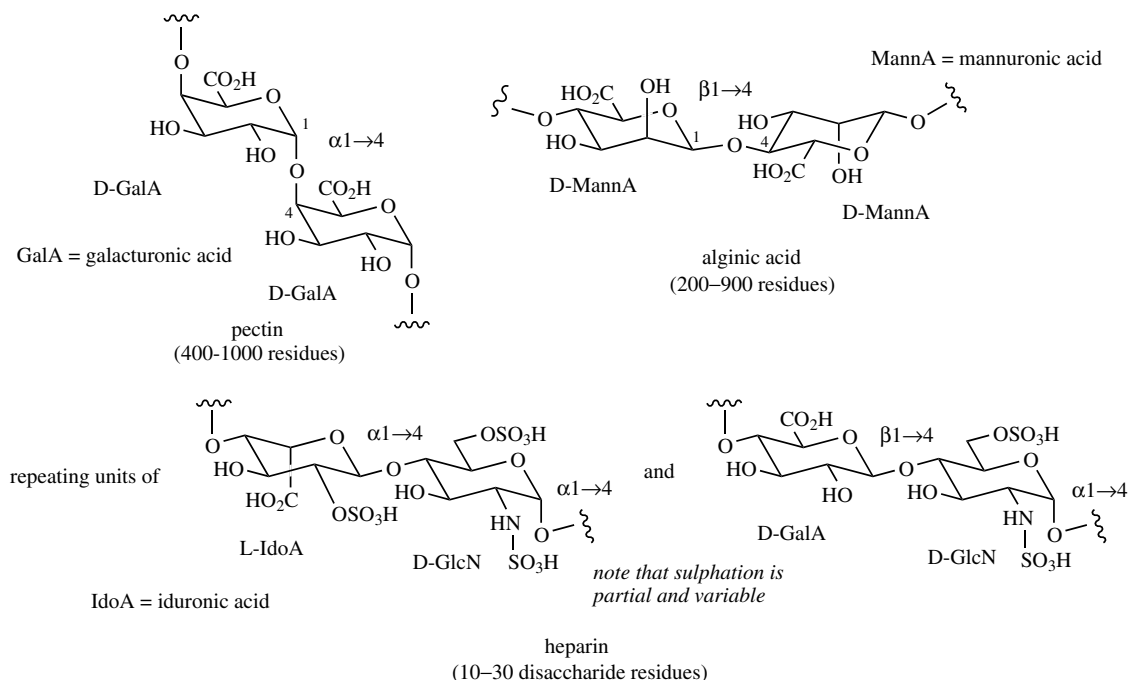
Figure 8.21

stem from an inhibition of the cross-linking mechanism during the biosynthesis of the bacterial cell wall, and relate to this terminal −D-Ala−D-Ala sequence during biosynthesis. The subdivision of bacteria into Gram-positive or Gram-negative reflects the ability of the peptidoglycan cell wall to take up Gram's dye stain. In Gram-negative organisms, an additional lipopolysaccharide cell membrane surrounding the peptidoglycan prevents attack of the dye.

Polymers of uronic acids are encountered in **pectins**, which are essentially chains of galacturonic acid residues linked α1→4 (Figure 8.21), though some of the carboxyl groups are present as methyl esters. These materials are present in the cell walls of fruit, and the property of aqueous solutions under acid conditions forming gels is the basis of jam making. **Alginic acid** (Figure 8.21) is formed by β1→4 linkage of mannuronic acid residues, and is the main cell wall constituent of brown algae (seaweeds). Salts of alginic acid are valuable thickening agents in the food industry, and the insoluble calcium salt is the basis of absorbable alginate surgical dressings. The mammalian blood anticoagulant **heparin** (Figure 8.21) is also a carbohydrate polymer containing uronic acid residues, but these alternate with glucosamine derivatives. Polymers of this kind are known as anionic mucopolysaccharides, or glycosaminoglycans. Heparin consists of two

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Polysaccharides

Starch for medicinal and pharmaceutical use may be obtained from a variety of plant sources, including maize (*Zea mays*; Gramineae), wheat (*Triticum aestivum*; Gramineae), potato (*Solanum tuberosum*; Solanaceae), rice (*Oryza sativa*; Gramineae/Poaceae), and arrowroot (*Maranta arudinacea*; Marantaceae). Most contain about 25% amylose and 75% amylopectin (Figure 8.19), but these proportions can vary according to the plant tissue.

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Starch is widely used in the food industry, and finds considerable applications in medicine. Its absorbent properties make it ideal for dusting powders, and its ability to swell in water makes it a valuable formulation aid, being the basis for tablet disintegrants. **Soluble starch** is obtained by partial acid hydrolysis, and is completely soluble in hot water.

Cellulose (Figure 8.19) may be extracted from wood pulp, and is usually partially hydrolysed with acid to give microcrystalline cellulose. These materials are used as tablet diluents. Semi-synthetic derivatives of cellulose, e.g. **methylcellulose**, **hydroxymethylcellulose**, and **carboxymethylcellulose**, are used as emulsifying and suspending agents. **Cellulose acetate phthalate** is cellulose with about half the hydroxyl groups acetylated, and the remainder esterified with phthalic acid. It is used as an acid-resistant enteric coating for tablets and capsules.

Alginic acid (Figure 8.21) is obtained by alkaline (Na_2CO_3) extraction of a range of brown seaweeds, chiefly species of *Laminaria* (Laminariaceae) and *Ascophyllum* (Phaeophyceae) in Europe, and species of *Macrocystis* (Lessoniaceae) on the Pacific coast of the USA. The carbohydrate material constitutes 20–40% of the dry weight of the algae. The acid is usually converted into its soluble sodium salt or insoluble calcium salt. Sodium alginate finds many applications as a stabilizing and thickening agent in a variety of industries, particularly food manufacture, and the pharmaceutical industry, where it is of value in the formulation of creams, ointments, and tablets. Calcium alginate is the basis of many absorbable haemostatic surgical dressings. Alginic acid or alginates are incorporated into many aluminium- and magnesium-containing antacid preparations to protect against gastro-oesophageal reflux. Alginic acid released by the action of gastric acid helps to form a barrier over the gastric contents.

Agar is a carbohydrate extracted using hot dilute acid from various species of red algae (seaweeds) including *Gelidium* (Gelidiaceae) and *Gracilaria* (Gracilariaceae) from Japan, Spain, Australasia, and the USA. Agar is a heterogeneous polymer, which may be fractionated into two main components, agarose and agaropectin. Agarose yields D- and L-galactose on hydrolysis, and contains alternating $\beta1{\rightarrow}3$ linked D-galactose and $\alpha1{\rightarrow}4$ linked L-galactose, with the L-sugar in a 3,6-anhydro form. Agaropectin has a similar structure but some of the residues are methylated, sulphated, or in the form of a cyclic ketal with pyruvic acid. Agar's main application is in bacterial culture media, where its gelling properties are exploited. It is also used to some extent as a suspending agent and a bulk laxative. Agarose is now important as a support in affinity chromatography.

Tragacanth is a dried gummy exudate obtained from *Astragalus gummifer* (Leguminosae/Fabaceae) and other *Astragalus* species, small shrubs found in Iran, Syria, Greece, and Turkey. It is usually obtained by deliberate incision of the stems. This material swells in water to give a stiff mucilage with an extremely high viscosity, and provides a useful suspending and binding agent. It is chemically a complex material, and yields D-galacturonic acid, D-galactose, L-fucose, L-arabinose, and D-xylose on hydrolysis. Some of the uronic acid carboxyls are methylated.

Acacia (gum arabic) is a dried gum from the stems and branches of the tree *Acacia senegal* (Leguminosae/Fabaceae), abundant in the Sudan and Central and West Africa. Trees are tapped by removing a portion of the bark. The gum is used as a suspending agent, and adhesive and binder for tablets. The carbohydrate is a complex branched-chain material, which yields L-arabinose, D-galactose, D-glucuronic acid, and L-rhamnose on hydrolysis. Occluded enzymes (oxidases, peroxidases, and pectinases) can cause problems in some formulations, unless inactivated by heat.

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(*Continued*)

Karaya or **Sterculia Gum** is a dried gum obtained from the trunks of the tree *Sterculia urens* (Sterculiaceae) or other *Sterculia* species found in India. It exudes naturally, or may be obtained by incising through the bark. It contains a branched polysaccharide comprising L-rhamnose, D-galactose, and a high proportion of D-galacturonic acid and D-glucuronic acid residues. The molecule is partially acetylated, and the gum typically has an odour of acetic acid. It is used as a bulk laxative, and as a suspending agent. It has proved particularly effective as an adhesive for stomal appliances, rings of the purified gum being used to provide a non-irritant seal between the stomal bag and the patient's skin.

Heparin is usually extracted from the mucosa of bovine lung or porcine intestines, where it is present in the mast cells. It is a blood anticoagulant and is used clinically to prevent or treat deep-vein thrombosis. It is administered by injection or intravenous infusion and provides rapid action. It is also active *in vitro*, and is used to prevent the clotting of blood in research preparations. Heparin acts by complexing with enzymes in the blood which are involved in the clotting process. Although not strictly an enzyme inhibitor, its presence enhances the natural inhibition process between thrombin and antithrombin III by forming a ternary complex. A specific pentasaccharide sequence containing a 3-*O*-sulphated D-glucosamine residue is essential for functional binding of antithrombin. Natural heparin is a mixture of glycosaminoglycans (Figure 8.21), with only a fraction of the molecules having the required binding sequence, and it has a relatively short duration of action. Partial hydrolysis of natural heparin by chemical or enzymic means has resulted in a range of low molecular weight heparins having similar activity but with a longer duration of action. **Certoparin, dalteparin, enoxaparin**, and **tinzaparin** are examples of these currently being used clinically.

Protamine, a basic protein from the testes of fish of the salmon family, e.g. *Salmo* and *Onchorhynchus* species (see insulin, page 417), is a heparin antagonist, which may be used to counteract haemorrhage caused by overdosage of heparin.

repeating disaccharide units, in which the amino functions and some of the hydroxyls are sulphated, producing a heterogeneous polymer. The carboxyls and sulphates together make heparin a strongly acidic water-soluble material.

AMINOSUGARS

Aminosugars are readily produced from ketosugars by transamination processes. Whilst many of the natural examples, e.g. glucosamine and galactosamine (Figure 8.10), demonstrate the results of this transamination, there are some further structures where the newly introduced amino group becomes part of a heterocyclic ring system. This arises by using the amino group as a nucleophile to generate an aminohemiacetal linkage, rather than a hydroxyl to produce a hemiacetal. This, of course, is the addition step in the formation of an imine (Schiff base) (see page 18). Should the anomeric hydroxyl then be removed in subsequent

modifications such as imine formation, the product will then be a polyhydroxy-piperidine or pyrrolidine. Any confusion with ornithine/lysine-derived alkaloids (see pages 292, 307) should be dispelled by the characteristic polyhydroxy substitution. The piperidine structures **deoxynojirimycin** and **deoxymannojirimycin** (Figure 8.22) from *Streptomyces subrutilis* are good examples.

The pathways to deoxynojirimycin and deoxymannojirimycin (Figure 8.22) start from the ketosugar fructose, which is aminated and then oxidized to **mannojirimycin**. This can then form a cyclic aminohemiacetal. Dehydration to the imine can follow, and reduction yields deoxymannojirimycin. **Nojirimycin** is an epimer of mannojirimycin, and analogous modifications then give deoxynojirimycin. Deoxynojirimycin is found in various strains of *Streptomyces* and *Bacillus*, as well as some plants, e.g. *Morus* spp. (Moraceae), and is attracting considerable attention in the search for anti-HIV agents. This and related structures are inhibitors of glycosidase
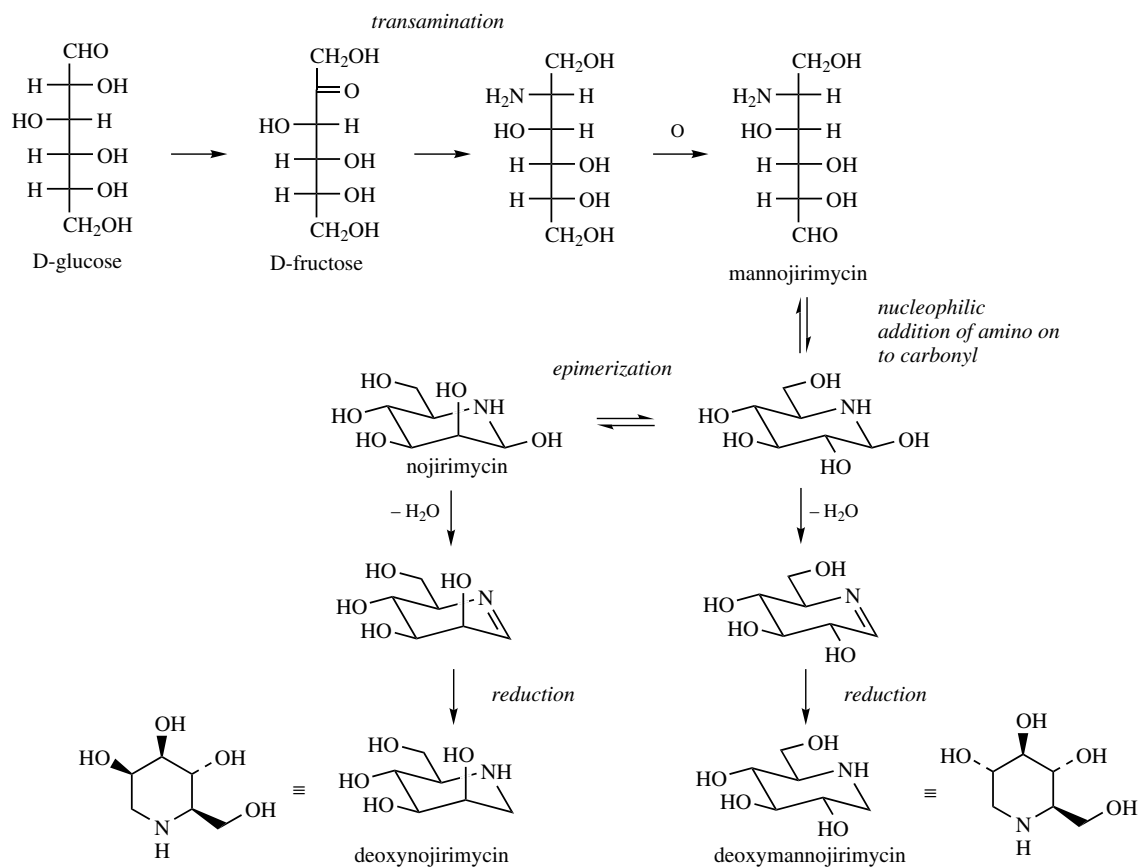
transamination

D-glucose → D-fructose → mannojirimycin

nucleophilic addition of amino on to carbonyl

epimerization

nojirimycin

−H₂O

deoxynojirimycin ≡

−H₂O

deoxymannojirimycin ≡

Figure 8.22

enzymes (compare indolizidine alkaloids such as castanospermine, page 310). By altering the constitution of glycoproteins on the surface of the virus, such compounds interfere with the binding of the HIV particle to components of the immune system.

AMINOGLYCOSIDES

The aminoglycosides* form an important group of antibiotic agents and are immediately recognizable as modified carbohydrate molecules. Typically, they have two or three uncommon sugars attached through glycoside linkages to an aminocyclitol, i.e. an amino-substituted cyclohexane system, which also has carbohydrate origins. The first of these agents to be discovered was **streptomycin** (see Figure 8.25) from *Streptomyces griseus*, whose structure contains the aminocyclitol **streptamine** (Figure 8.23), though both amino

groups are bound as guanidino substituents making **streptidine**. Other medicinally useful aminoglycoside antibiotics are based on the aminocyclitol **2-deoxystreptamine** (Figure 8.24), e.g. **gentamicin C₁** (see Figure 8.27) from *Micromonospora purpurea*. Streptamine and 2-deoxystreptamine are both derived from glucose 6-phosphate. The route to the streptamine system can be formulated to involve oxidation (in the acyclic form) of the 5-hydroxyl, allowing removal of a proton from C-6 and generation of an enolate anion (Figure 8.23). The cyclohexane ring is then formed by attack of this enolate anion on to the C-1 carbonyl. Reduction and hydrolysis of the phosphate produces *myo*-**inositol**. The amino groups as in **streptamine** are then introduced by oxidation/transamination reactions. **Streptidine** incorporates amidino groups from arginine, by nucleophilic attack of the aminocyclitol amino group on to the imino function
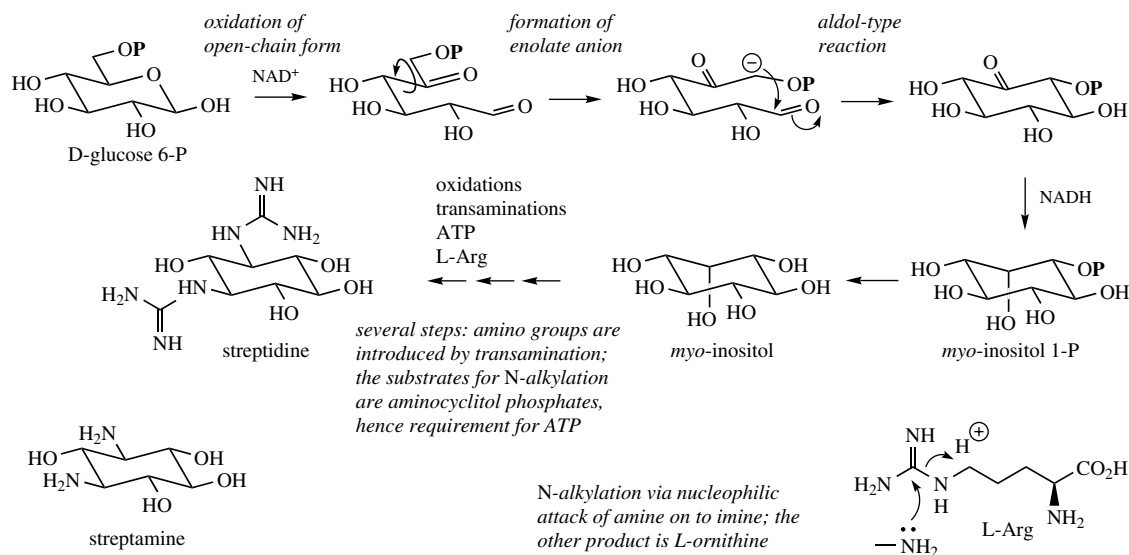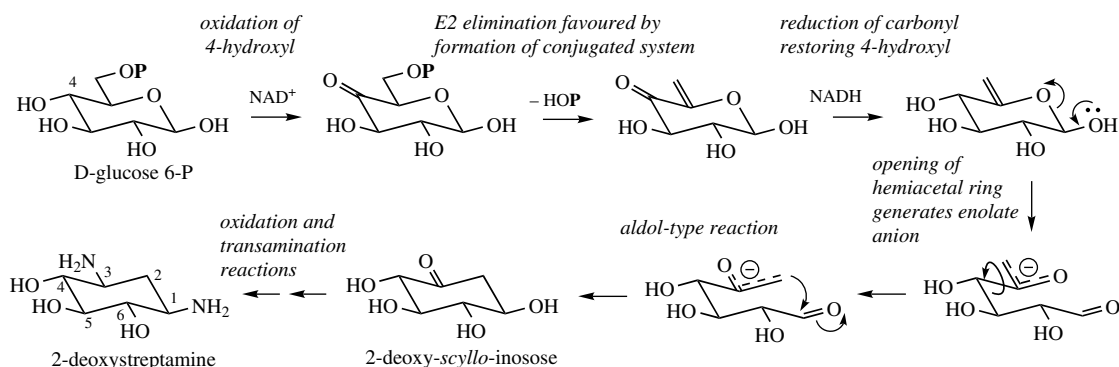
Figure 8.23

Figure 8.24

of arginine (Figure 8.23). However, streptamine itself is not a precursor, and the first guanidino side-chain is built up before the second amino group is introduced. The *N*-alkylation steps also involve aminocyclitol phosphate substrates. The biosynthesis of 2-deoxystreptamine shares similar features, but the sequence involves loss of the oxygen function from C-6 of glucose 6-phosphate in an elimination reaction (Figure 8.24). The elimination is facilitated by oxidation of the 4-hydroxyl, which thus allows a conjugated enone to develop in the elimination step, but the original hydroxyl is reformed by reduction after the elimination. The cyclohexane ring is then formed by attack of an enolate anion on to the C-1 carbonyl giving a tetrahydroxy-

cyclohexanone, and transamination reactions allow formation of **2-deoxystreptamine**. The pathway in Figure 8.24 is remarkably similar to that operating in the biosynthesis of dehydroquinic acid from the seven-carbon sugar DAHP in the early part of the shikimate pathway (see page 122).

The other component parts of streptomycin, namely L-streptose and 2-deoxy-2-methylamino-L-glucose (*N*-methyl-L-glucosamine) (Figure 8.25), are also derived from D-glucose 6-phosphate, though the detailed features of these pathways will not be considered further. Undoubtedly, these materials are linked to streptidine through stepwise glycosylation reactions via their nucleoside sugars (Figure 8.25).
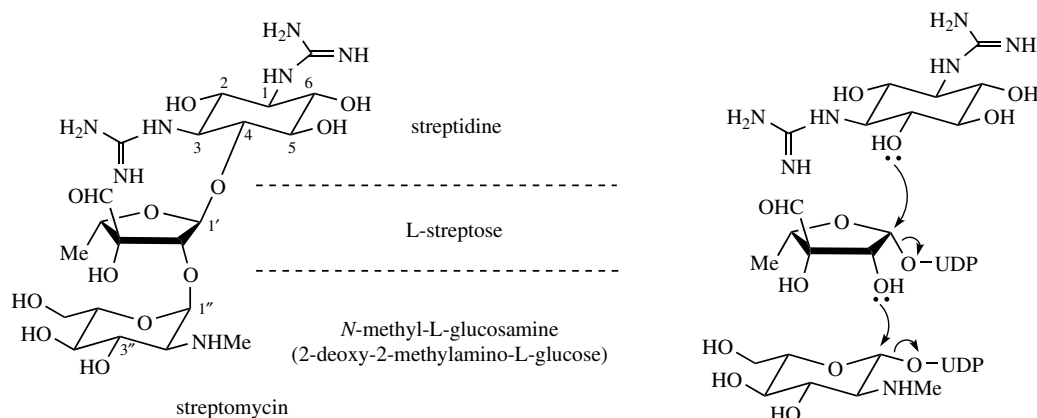
Figure 8.25

Aminoglycoside Antibiotics

The aminoglycoside antibiotics have a wide spectrum of activity, including activity against some Gram-positive and many Gram-negative bacteria. They are not absorbed from the gut, and for systemic infections must be administered by injection. However, they can be administered orally to control intestinal flora. The widespread use of aminoglycoside antibiotics is limited by their nephrotoxicity, which results in impaired kidney function, and by their ototoxicity, which is a serious side-effect and can lead to irreversible loss of hearing. They are thus reserved for treatment of serious infections where less toxic antibiotics have proved ineffective. The aminoglycoside antibiotics interfere with protein biosynthesis by acting on the smaller 30S subunit of the bacterial ribosome. Streptomycin is known to interfere with the initiation complex, but most agents block the translocation step as the major mechanism of action. Some antibiotics can also induce a misreading of the genetic code to yield membrane proteins with an incorrect amino acid sequence leading to altered membrane permeability. This actually increases aminoglycoside uptake and leads to rapid cell death.

Bacterial resistance to the aminoglycoside antibiotics has proved to be a problem, and this has also contributed to their decreasing use. Several mechanisms of resistance have been identified. These include changes in the bacterial ribosome so that the affinity for the antibiotic is significantly decreased, reduction in the rate at which the antibiotic passes into the bacterial cell, and plasmid transfer of extrachromosomal R-factors. This latter mechanism is the most common and causes major clinical problems. Bacteria are capable of acquiring genetic material from other bacteria, and in the case of the aminoglycosides this has led to the organisms becoming capable of producing enzymes that inactivate the antibiotic. The modifications encountered are acetylation, adenylylation, and phosphorylation. (Note adenylic acid = adenosine 5′-phosphate). The enzymes are referred to as AAC (aminoglycoside acetyltransferase), ANT (aminoglycoside nucleotidyltransferase) (sometimes AAD (aminoglycoside adenylyltransferase)), and APH (aminoglycoside phosphotransferase). They differ with respect to the reaction catalysed, the position of derivatization (see numbering scheme in gentamicin, Figure 8.26), and the range of substrates attacked. Thus, some clinically significant inactivating enzymes are
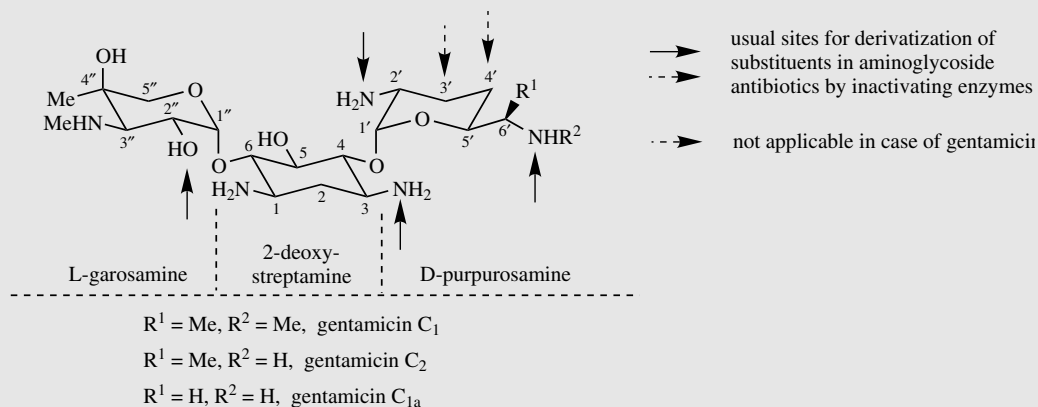
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R^1 = Me, R^2 = Me, gentamicin C$_1$

R^1 = Me, R^2 = H, gentamicin C$_2$

R^1 = H, R^2 = H, gentamicin C$_{1a}$

Figure 8.26

- AAC(3) and AAC(6′), which acetylate the 3- and 6′-amino functions respectively in gentamicin, tobramycin, kanamycin, neomycin, amikacin, and netilmicin,
- ANT(2″), which adenylylates the 2″-hydroxy group in gentamicin, tobramycin, and kanamycin,
- APH(3′), which phosphorylates the 3′-hydroxyl in neomycin and kanamycin, and
- APH(3″), which phosphorylates the 3″-hydroxyl of streptomycin.

Other changes which may be imparted include acetylation of groups at position 2′, adenylylation of position 4′ substituents, and phosphorylation of the position 2″ substituent. Position 6 in the streptamine portion of streptomycin is also susceptible to adenylylation and phosphorylation.

Aminoglycoside antibiotics are produced in culture by strains of *Streptomyces* and *Micromonsopora*. Compounds obtained from *Streptomyces* have been given names ending in -*mycin*, whilst those from *Micromonospora* have names ending in -*micin*.

Streptamine-containing Antibiotics

Streptomycin (Figure 8.25) is produced by cultures of a strain of *Streptomyces griseus*, and is mainly active against Gram-negative organisms. Because of its toxic properties it is rarely used in modern medicine except against resistant strains of *Mycobacterium tuberculosis* in the treatment of tuberculosis.

Spectinomycin (Figure 8.27) is not strictly an aminoglycoside, but its structure does contain a modified streptamine portion linked by a glycoside bond to a deoxy sugar. It is sometimes written as a ketone at position 4, though this exists as a hydrate as shown in Figure 8.27. Spectinomycin is produced by cultures of *Streptomyces spectabilis*, and although it displays a broad antibacterial spectrum, it is only used against *Neisseria gonorrhoea* for the treatment of gonorrhoea where the organism has proved resistant to other antibiotics. It is known to inhibit protein biosynthesis on the 30S ribosomal subunit, but does not appear to cause any misreading of the genetic code.

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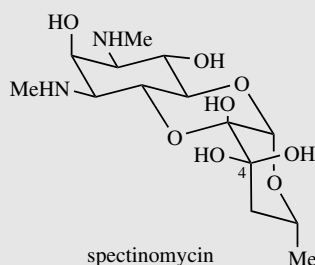
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spectinomycin

Figure 8.27

2-Deoxystreptamine-containing Antibiotics

Gentamicin is a mixture of antibiotics obtained from *Micromonospora purpurea*. Fermentation yields a mixture of gentamicins A, B, and C, from which gentamicin C is separated for medicinal use. This is also a mixture, the main component being gentamicin C_1 (Figure 8.26) (50–60%), with smaller amounts of gentamicin C_{1a} and gentamicin C_2. These three components differ in respect to the side-chain in the purpurosamine sugar. Gentamicin is clinically the most important of the aminoglycoside antibiotics, and is widely used for the treatment of serious infections, often in combination with a penicillin when the infectious organism is unknown. It has a broad spectrum of activity, but is inactive against anaerobes. It is active against pathogenic enterobacteria such as *Enterobacter, Escherichia*, and *Klebsiella*, and also against *Pseudomonas aeruginosa*. Compared with other compounds in this group, its component structures contain fewer functionalities that may be attacked by inactivating enzymes, and this means gentamicin may be more effective than some other agents.

  Sisomicin (Figure 8.28) is a dehydro analogue of gentamicin C_{1a}, and is produced by cultures of *Micromonospora inyoensis*. It is used medicinally in the form of the semi-synthetic *N*-ethyl derivative **netilmicin** (Figure 8.28), which has a similar activity to gentamicin, but causes less ototoxicity.

  The **kanamycins** (Figure 8.29) are a mixture of aminoglycosides produced by *Streptomyces kanamyceticus*, but have been superseded by other drugs. **Amikacin** (Figure 8.29) is a semi-synthetic acyl derivative of kanamycin A, the introduction of the 4-amino-2-hydroxybutyryl group helping to protect the antibiotic against enzymic deactivation at several positions, whilst
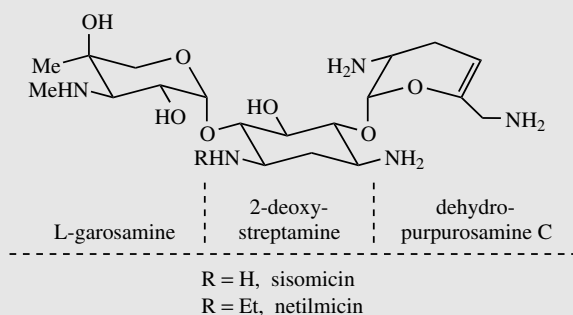

L-garosamine   2-deoxy-   dehydro-
                  streptamine   purpurosamine C

R = H, sisomicin
R = Et, netilmicin

Figure 8.28
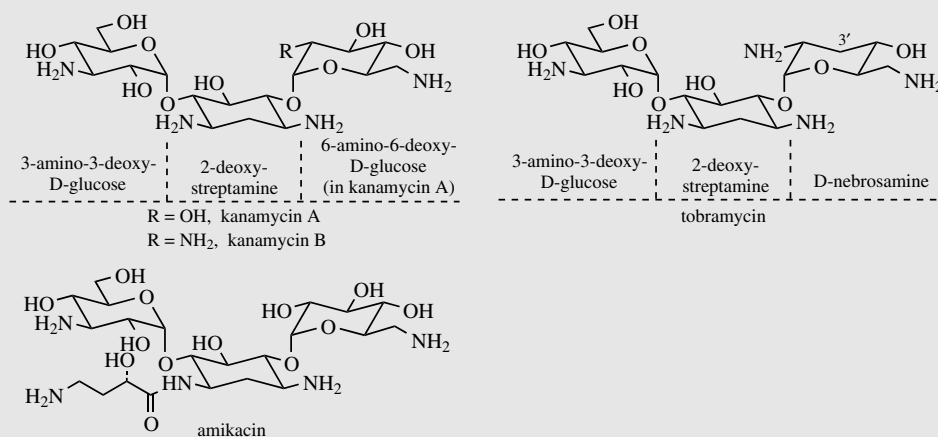
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Figure 8.29

still maintaining the activity of the parent molecule. It is stable to many of the aminoglycoside inactivating enzymes, and is valuable for the treatment of serious infections caused by Gram-negative bacteria which are resistant to gentamicin. **Tobramycin** (Figure 8.29) (also called nebramycin factor 6) is an analogue of kanamycin B isolated from *Streptomyces tenebrarius*, and is also less prone to deactivation in that it lacks the susceptible 3′-hydroxyl group. It is slightly more active towards *Pseudomonas aeruginosa* than gentamicin, but shows less activity against other Gram-negative bacteria.

Neomycin is a mixture of neomycin B (**framycetin**) (Figure 8.30) and its epimer neomycin C, the latter component accounting for some 5–15% of the mixture. It is produced by cultures of *Streptomyces fradiae*, and, in contrast to the other clinically useful aminoglycosides described, contains three sugar residues linked to 2-deoxystreptamine. One of these is the common sugar D-ribose. Neomycin has good activity against Gram-positive and Gram-negative bacteria, but is very ototoxic. Its use is thus restricted to oral treatment of intestinal infections (it is poorly absorbed from the digestive tract) and topical applications in eyedrops, eardrops, and ointments.
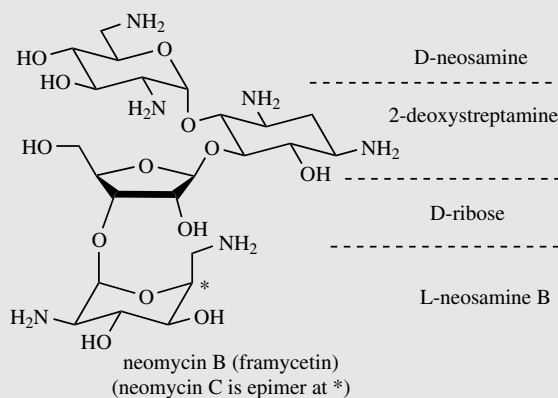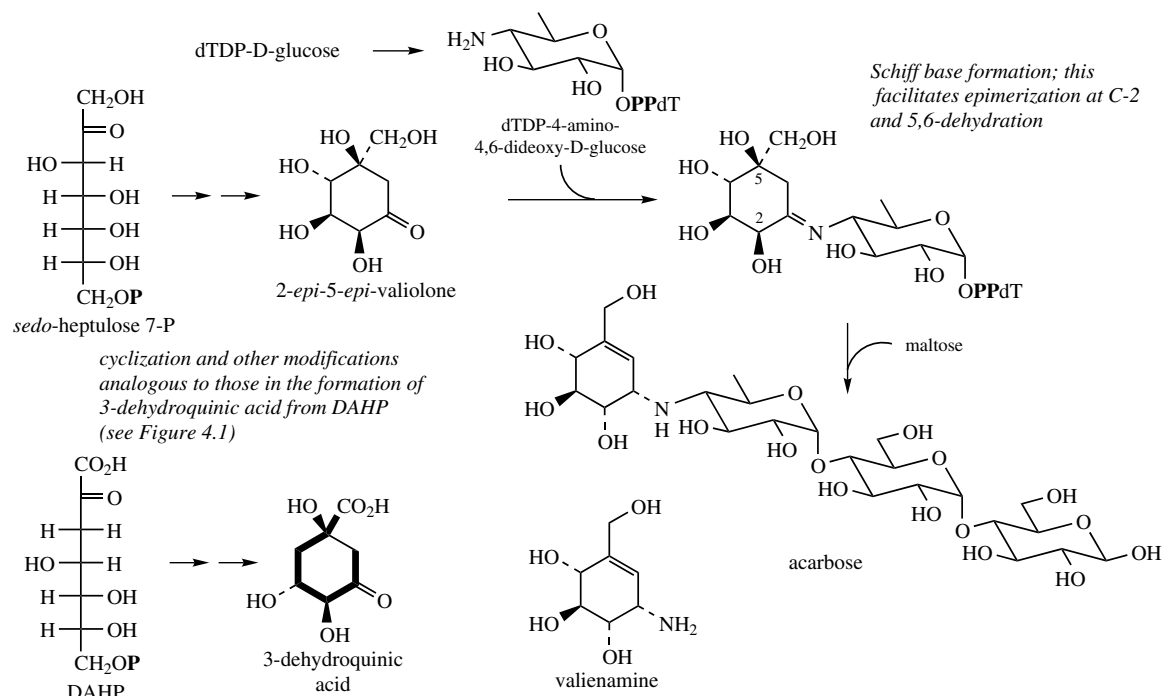

Figure 8.30

Figure 8.31

The aminocyclitol found in **acarbose*** (Figure 8.31) is based on **valienamine**, though this is not a precursor, and the nitrogen is introduced via an imine with the aminosugar, 4-amino-4,6-dideoxyglucose in the form of its deoxyTDP derivative. The cyclitol involved is 2-*epi*-5-*epi*-valiolone, and this appears to be produced from the seven-carbon sugar derivative *sedo*-heptulose 7-phosphate. The reaction sequence is exactly analogous to that seen in the transformation of DAHP into 3-dehydroquinic acid at the beginning of the shikimate pathway (page 122). The valienamine moiety requires subsequent

epimerization and dehydration steps, and these are readily seen to be facilitated by the imine function. Unusually, the two further glucose units are not added sequentially, but via the preformed dimer maltose. Acarbose is produced by strains of *Actinoplanes* sp., and is of clinical importance in the treatment of diabetes.

The antibiotic **lincomycin*** (Figure 8.32) from *Streptomyces lincolnensis* bears a superficial similarity to the aminoglycosides, but has a rather more complex origin. The sugar fragment is termed methyl α-thiolincosaminide, contains a thiomethyl group, and is known to be derived

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Acarbose

Acarbose is obtained commercially from fermentation cultures of selected strains of an undefined species of *Actinoplanes*. It is an inhibitor of α-glucosidase, the enzyme that hydrolyses starch and sucrose. It is employed in the treatment of diabetic patients, allowing better utilization of starch- or sucrose-containing diets, by delaying the digestion of such foods and thus slowing down the intestinal release of α-D-glucose. It has a small but significant effect in lowering blood glucose, and is used either on its own, or alongside oral hypoglycaemic agents, in cases where dietary control with or without drugs has proved inadequate. Flatulence is a common side-effect.
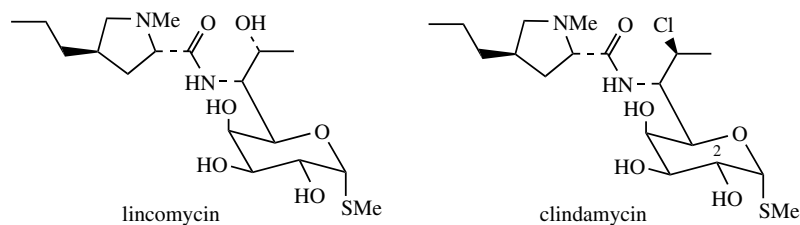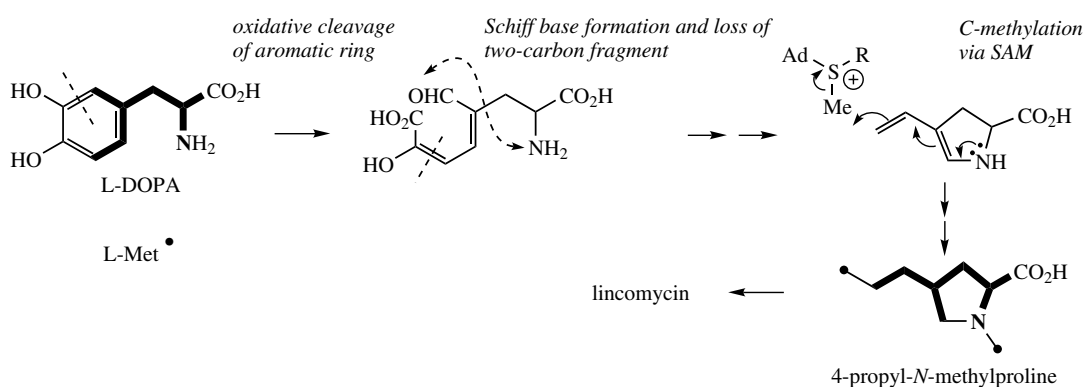
Figure 8.32

Figure 8.33

from two molecules of glucose, one of which provides a five-carbon unit, the other a three-carbon unit. The 4-propyl-*N*-methylproline fragment does not originate from proline, but is actually a metabolite from the aromatic amino acid L-DOPA (Figure 8.33). Oxidative cleavage of the aromatic ring (see page 27) provides all the carbons for the pyrrolidine ring, the carboxyl, and two carbons of the propyl side-chain. The terminal carbon of the propyl is supplied by L-methionine, as are the *N*-methyl, and the *S*-methyl in the sugar fragment. Two carbons from DOPA are lost during the biosynthesis.

Lincomycin and Clindamycin

Lincomycin (Figure 8.32) is obtained from cultures of *Streptomyces lincolnensis*. The semi-synthetic derivative **clindamycin** (Figure 8.32) obtained by chlorination of the lincomycin with resultant inversion of stereochemistry is more active and better absorbed from the gut, and has largely replaced lincomycin. Both antibiotics are active against most Gram-positive bacteria, including penicillin-resistant staphylococci. Their use is restricted by side-effects. These include diarrhoea and occasionally serious pseudomembraneous colitis, caused by overgrowth of resistant strains of *Clostridium difficile*, which can cause fatalities in elderly patients. However, this may be controlled by the additional administration of vancomycin (see page 426). Clindamycin finds particular application in the treatment of staphylococcal joint and bone infections such as osteomyelitis since it readily penetrates into bone. **Clindamycin 2-phosphate** is also of value, especially in the topical treatment of acne vulgaris and vaginal infections. Lincomycin and clindamycin inhibit protein biosynthesis by blocking the peptidyltransferase site on the 50S subunit of the bacterial ribosome. Microbial resistance may develop slowly, and in some cases has been traced to adenylylation of the antibiotic.

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