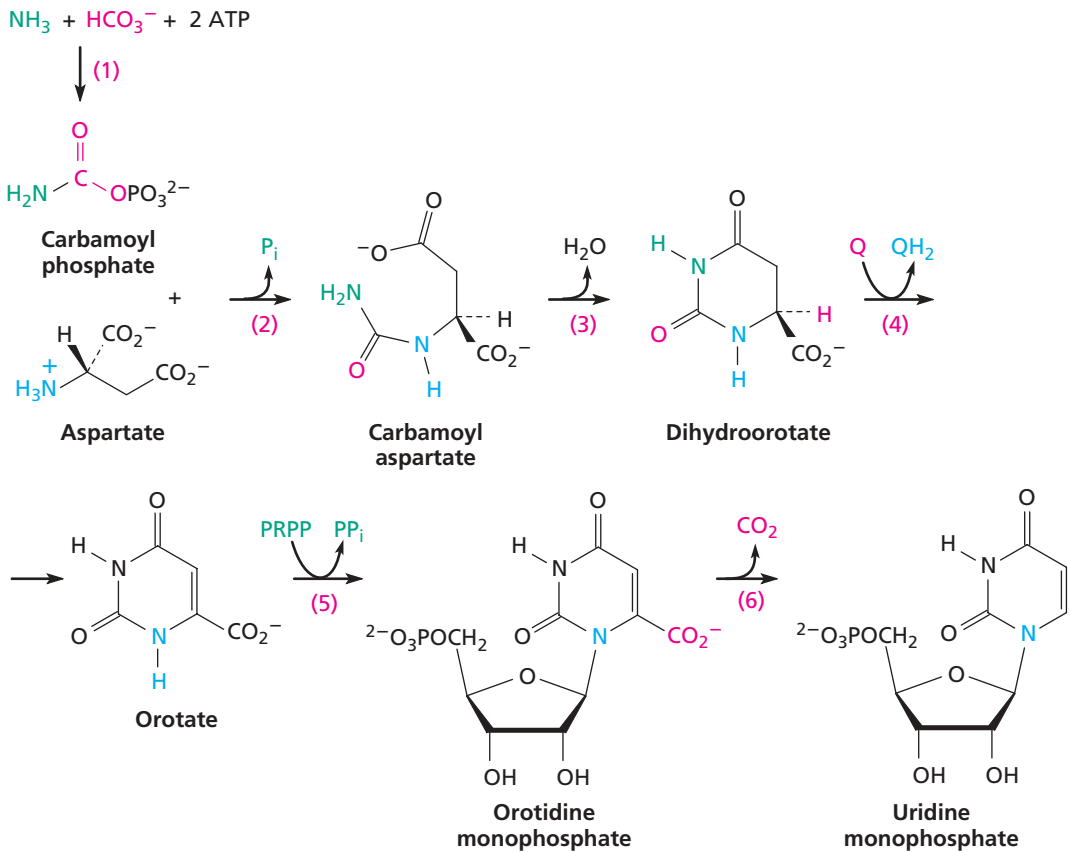


## 6.2 Biosynthesis of Pyrimidine Ribonucleotides

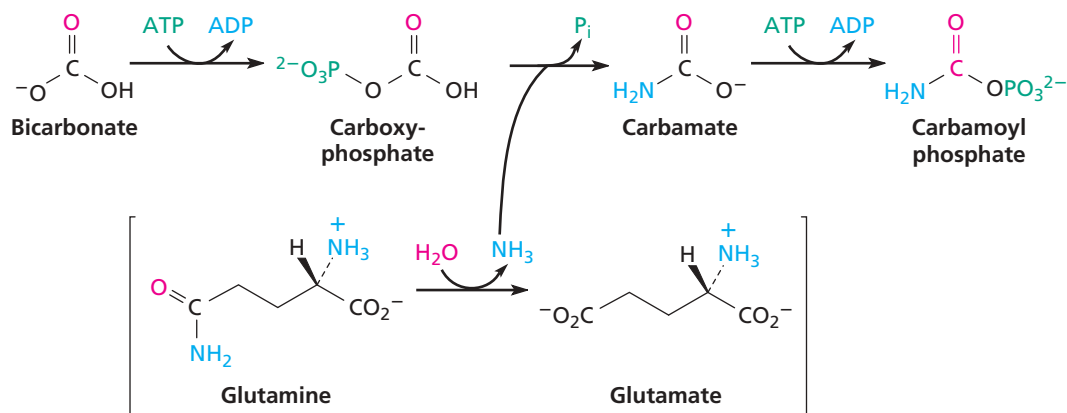
### Uridine Monophosphate

**Uridine monophosphate (UMP)** is biosynthesized in a six-step pathway from aspartate, bicarbonate, and ammonia, which itself comes from the amide nitrogen of glutamine (Figure 6.6).

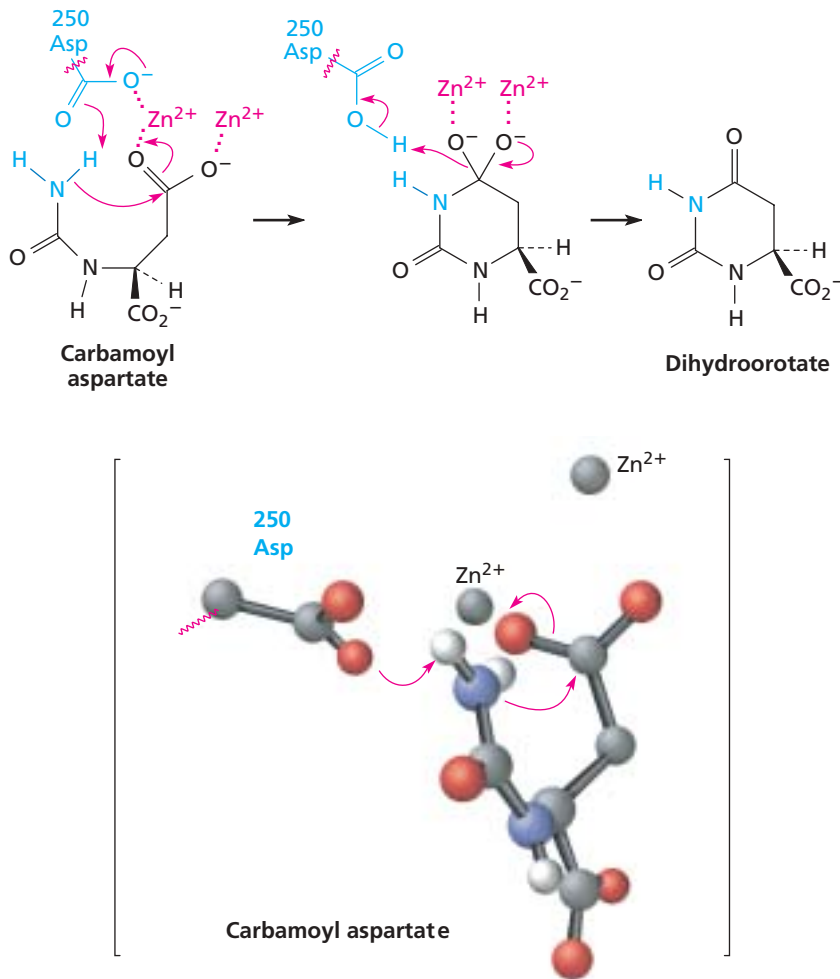


**FIGURE 6.6** Pathway for the biosynthesis of uridine monophosphate (UMP) from carbamoyl phosphate and aspartate.

**Step 1. Carbamoyl phosphate synthesis** UMP biosynthesis begins with formation of carbamoyl phosphate, catalyzed by carbamoyl phosphate synthetase II.<sup>5</sup> The reaction is identical to that occurring in the urea cycle (Section 5.2) except that the ammonia used for pyrimidine synthesis comes from hydrolysis of glutamine within the synthetase enzyme rather than from free ammonia as in the urea cycle.



**Steps 2–3. Reaction with aspartate and cyclization** Carbamoyl phosphate reacts with aspartate in a nucleophilic acyl substitution reaction with phosphate as the leaving group to give carbamoyl aspartate. Cyclization then forms dihydroorotate. The cyclization is catalyzed by dihydroorotase<sup>6</sup> and is mechanistically interesting because it accomplishes the formation of an amide bond between a poor nucleophile (a urea-like nitrogen) and a poor electrophile (a carboxylate). What evidently happens is that the carboxylate is activated by coordination to two Lewis-acidic  $\text{Zn}^{2+}$  ions, and both reacting centers are surrounded by various charged groups within the enzyme that electrostatically stabilize the reaction intermediates. Deprotonation of the urea  $\text{—NH}_2$  by an aspartate residue and concurrent addition to the carboxylate carbonyl group in a nucleophilic acyl substitution reaction gives the product. Figure 6.7 shows both the mechanism and an X-ray crystal structure of the substrate bound in the active site.



**FIGURE 6.7** Mechanism of the cyclization of carbamoyl aspartate to dihydroorotate, along with an X-ray crystal structure of the substrate bound in the active site.

**Step 4. Dehydrogenation** Introduction of a double bond into dihydroorotate to give orotate is catalyzed by dihydroorotate dehydrogenase<sup>7, 8</sup> a flavin-dependent enzyme that, in humans, uses coenzyme Q, also called ubiquinone, as the ultimate electron acceptor. The reaction occurs by base abstraction of the *pro-S* hydrogen at C5 and donation of hydride ion from C6 to FMN. The FMNH<sub>2</sub> is then reoxidized

by coenzyme Q. As shown in Figure 6.8, CoQ is a benzoquinone with a long hydrocarbon tail that allows it to dissolve readily in lipid membranes. Its function is to act as a redox agent in the transport of electrons between enzymes embedded in the inner mitochondrial membrane.

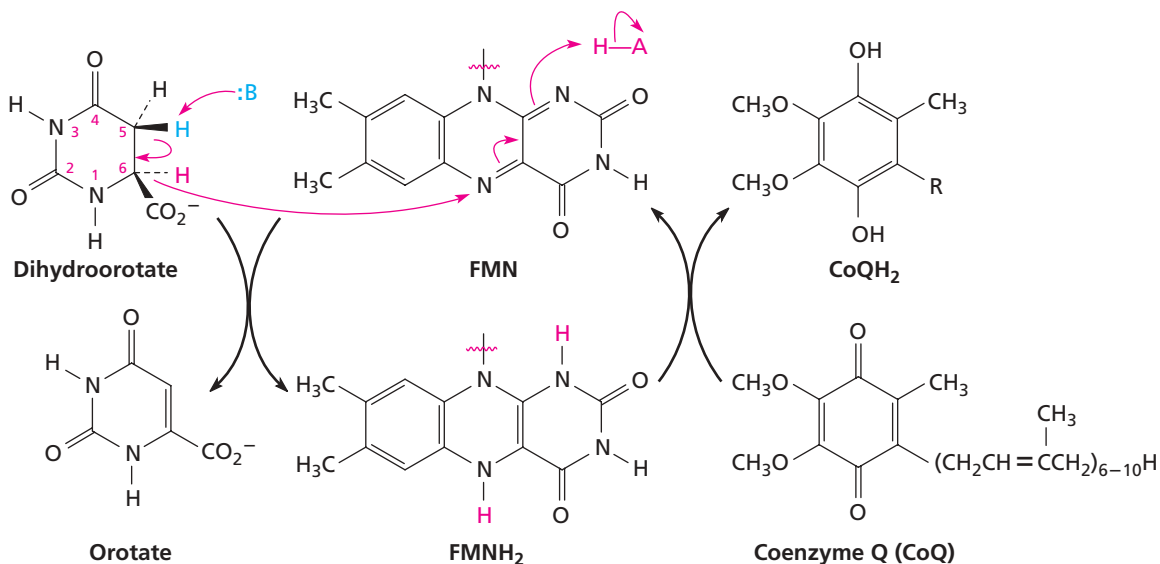


FIGURE 6.8 Mechanism of the dehydrogenation of dihydroorotate to orotate.

**Step 5. Ribonucleotide formation** Orotate reacts with 5-phosphoribosyl  $\alpha$ -diphosphate (PRPP) to give the ribonucleotide orotidine monophosphate (OMP). This ribonucleotide formation takes place by a nucleophilic substitution reaction, catalyzed by orotate phosphoribosyltransferase.<sup>9</sup> Although the reaction occurs with an inversion of stereochemistry, the likely mechanism involves spontaneous, S<sub>N</sub>1-like loss of diphosphate ion to give an oxonium-ion intermediate, much like what occurs in the hydrolysis of a polysaccharide catalyzed by an inverting glycosidase (Section 4.1, Figure 4.2). The 5-phosphoribosyl  $\alpha$ -diphosphate precursor is formed from  $\alpha$ -D-ribose 5-phosphate by reaction with ATP in the presence of PRPP synthetase (Figure 6.9).

**Step 6. Decarboxylation** The final step in UMP biosynthesis is the decarboxylation of OMP, catalyzed by orotidine monophosphate decarboxylase.<sup>10</sup> This enzyme contains no cofactors and holds the distinction of having the greatest

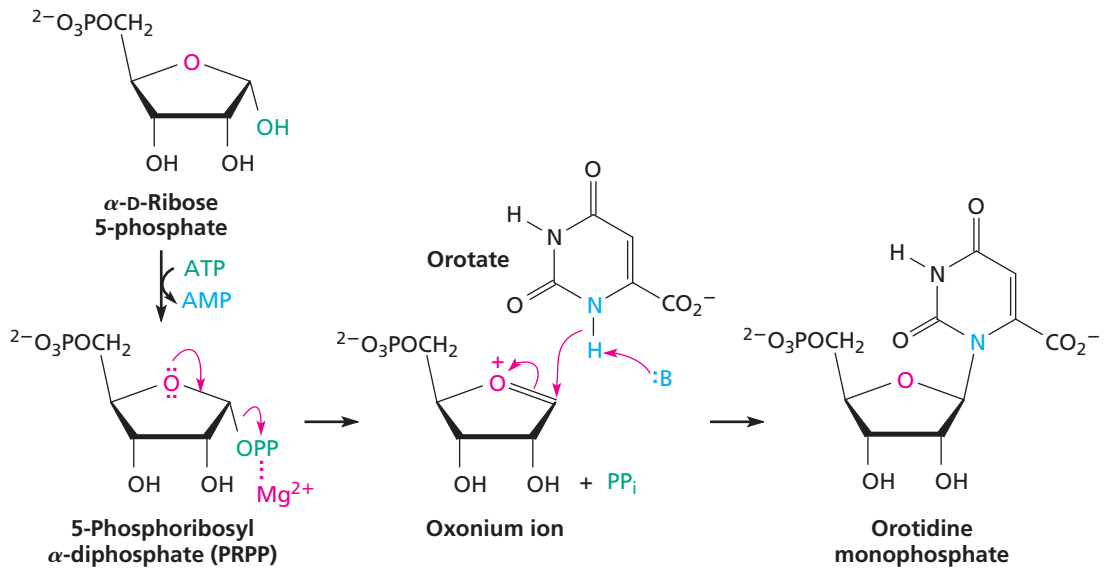
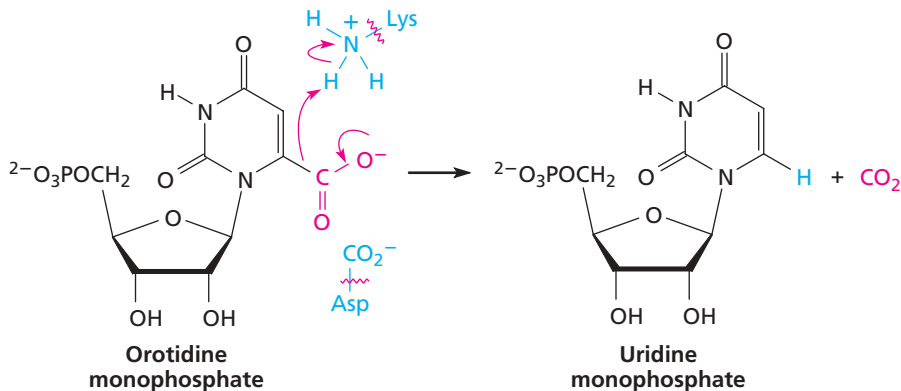


FIGURE 6.9 Mechanism of the formation of orotidine monophosphate.

experimentally determined rate acceleration known for any enzyme, a factor of  $2 \times 10^{23} \text{ M}^{-1}$  for the catalyzed versus uncatalyzed reaction! Mechanistically, the decarboxylation is unusual because the substrate is not a  $\beta$  keto acid and has no obvious electron sink nearby to accept electrons as  $\text{CO}_2$  leaves. It's thought instead that the decarboxylation occurs in a single step, driven by electrostatic interactions between the substrate and charged residues in the active site. An aspartate residue held near the carboxylate destabilizes the ground state, while a protonated lysine stabilizes the transition state and provides a proton as  $\text{CO}_2$  departs.



### Cytidine Triphosphate

Following its synthesis from orotate, uridine monophosphate is converted into the corresponding triphosphate (UTP) by two sequential reactions with ATP. Uridine triphosphate is then converted into **cytidine triphosphate** by a reaction that is essentially the reverse of the cytidine  $\rightarrow$  uridine conversion seen in cytidine catabolism (Section 6.1). The primary difference between the two processes is that the cytidine  $\rightarrow$  uridine conversion requires no ATP while the uridine  $\rightarrow$  cytidine conversion is coupled to ATP hydrolysis for energetic reasons. Catalyzed by CTP synthase,<sup>11</sup> glutamine is first hydrolyzed to glutamate plus ammonia at one site in the enzyme, a process similar to what occurs in carbamoyl phosphate synthesis (Section 6.2). The ammonia then moves through a channel in the enzyme to the next reaction site.

In the second site, uridine triphosphate is phosphorylated on the pyrimidine oxygen by ATP, and the resultant imino phosphate undergoes nucleophilic acyl substitution by addition of  $\text{NH}_3$  to the  $\text{C}=\text{N}$  double bond followed by elimination of  $\text{P}_i$ .

