Nucleotides

Outline

- 27.1 Nucleotide Biosynthesis
- 27.2 The Biosynthesis of Purines
- 27.3 Purine Salvage
- 27.4 Purine Degradation
- 27.5 Biosynthesis of Pyrimidines
- 27.6 Pyrimidine Degradation
- 27.7 Deoxyribonucleotide Biosynthesis
- 27.8 Synthesis of Thymine Nucleotides

Nucleotide Biosynthesis

- Nearly all organisms synthesize purines and pyrimidines "de novo"
- Many organisms also "salvage" purines and pyrimidines from diet and degradative pathways
- Ribose generates energy, but purine and pyrimidine rings do not
- Nucleotide synthesis pathways are good targets for anti-cancer/antibacterial strategies

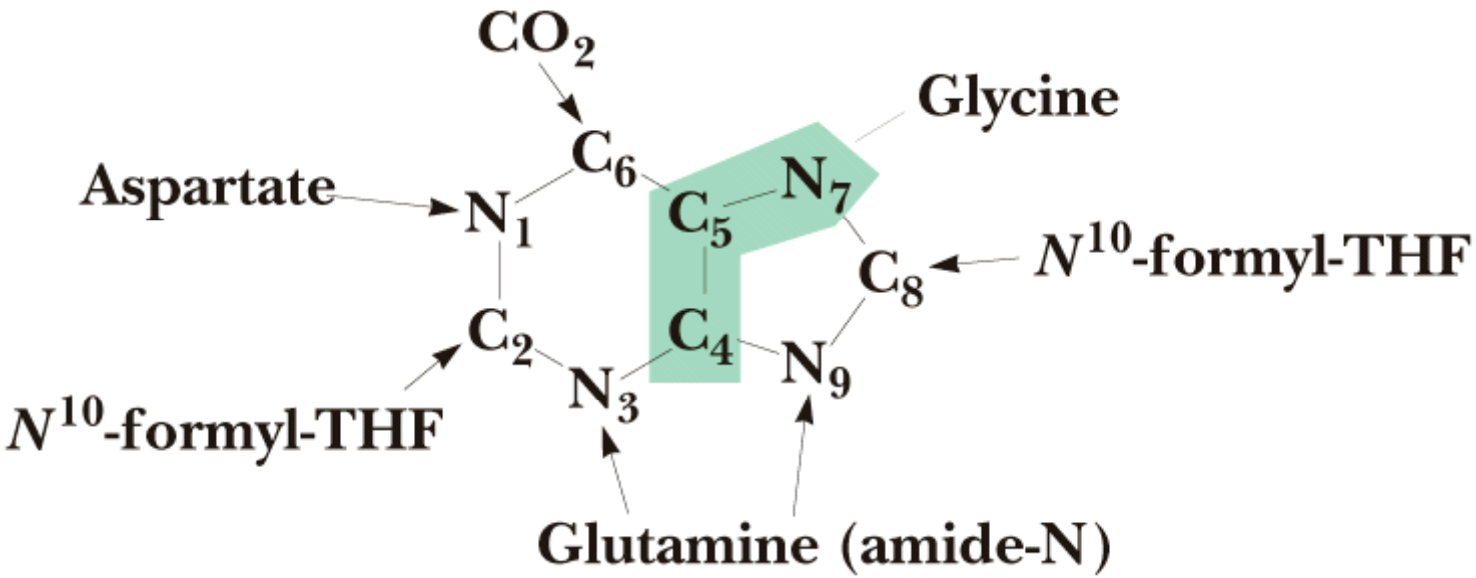
27.2 Biosynthesis of Purines

John Buchanan (1948) "traced" the sources of all nine atoms of purine ring

- N-1: aspartic acid
- N-3, N-9: glutamine
- C-4, C-5, N-7: glycine
- C-6: CO_2
- C-2, C-8: THF - one carbon units

Biochemistry 2/e - Garrett & Grisham

Garrett & Grisham: Biochemistry, 2/e
Figure 27.2



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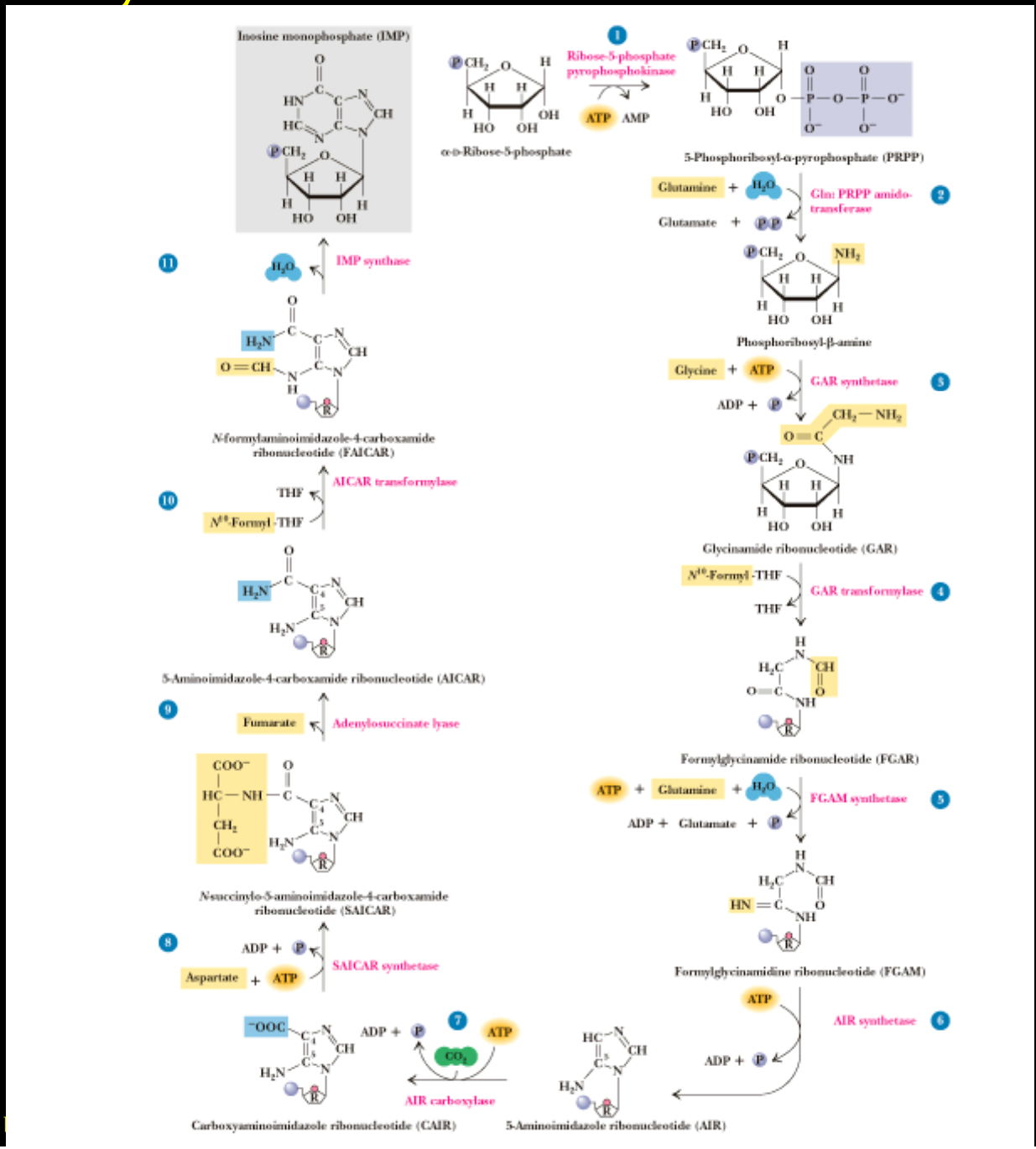
Inosine-5'-P Biosynthesis

The purine ring is built on a ribose-5-P foundation

- First step: ribose-5-P must be activated - by PP_i
- PRPP is limiting substance for purine synthesis
- But PRPP is a branch point so next step is the committed step - Gln PRPP amidotransferase
- Note that second step changes C-1 configuration
- G- and A-nucleotides inhibit this step - but at distinct sites!
- Azaserine - Gln analog - inhibitor/anti-tumor

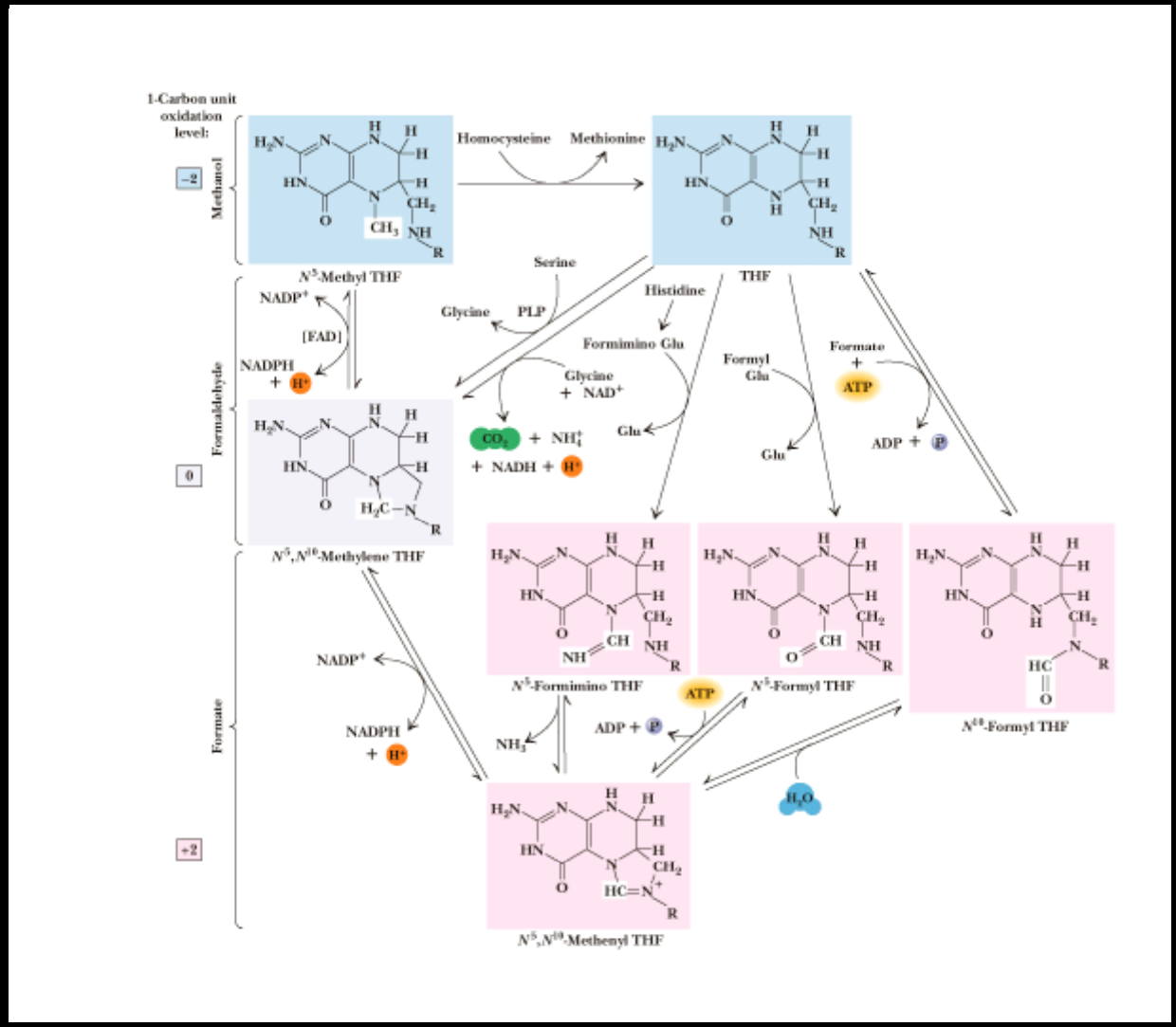
Biochemistry 2/e - Garrett & Grisham



Steps 3-5

- Step 3: Glycine carboxyl condenses with amine
  - Glycine carboxyl activated by -P from ATP
  - Amine attacks glycine carboxyl
- Step 4: Formyl group of N^{10}-formyl-THF is transferred to free amino group of GAR
- Step 5: C-4 carbonyl forms a P-ester from ATP and active NH_3 attacks C-4 to form imine

Biochemistry 2/e - Garrett & Grisham



Steps 6-8

Closure of the first ring, carboxylation and attack by aspartate

- Step 6: Similar in some ways to step 5. ATP activates the formyl group by phosphorylation, facilitating attack by N.
- Step 7: Carboxylation probably involves electron "push" from the amino group
- Step 8: Attack by the amino group of aspartate links this amino acid with the carboxyl group

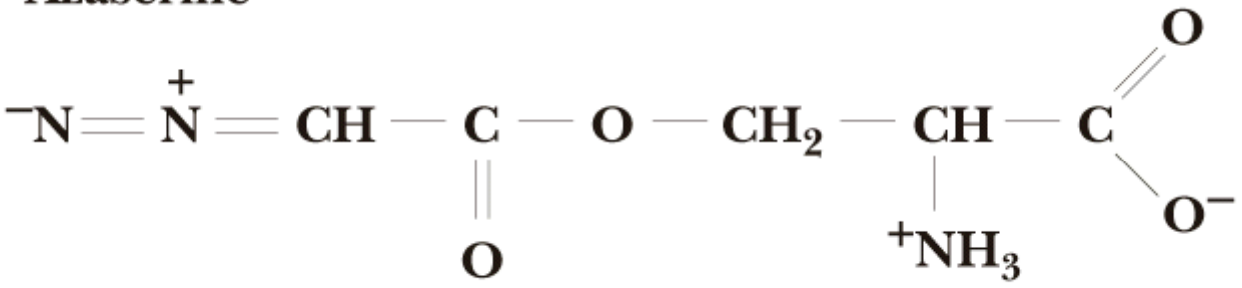
Steps 9-11

Loss of fumarate, another 1-C unit and the second ring closure

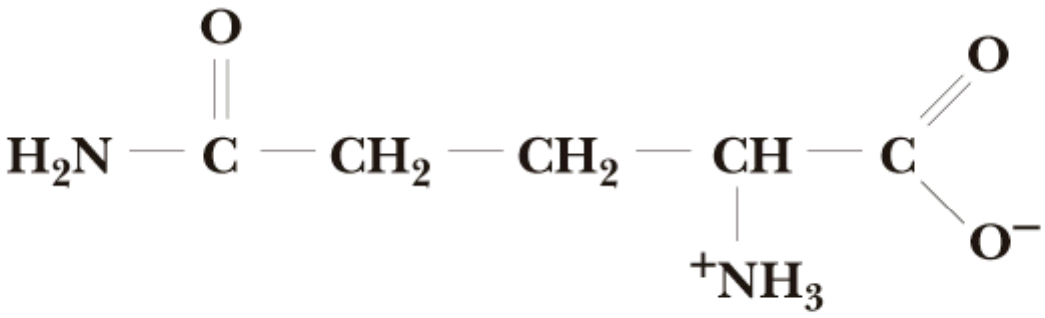
- Step 9: Deprotonation of Asp-CH_2 leads to cleavage to form fumarate
- Step 10: Another 1-C addition catalyzed by THF
- Step 11: Amino group attacks formyl group to close the second ring
- Note that 5 steps use ATP, but that this is really six ATP equivalents!
- Dependence on THF in two steps means that methotrexate and sulfonamides block purine synthesis

Garrett & Grisham: Biochemistry, 2/e
Figure 27.4

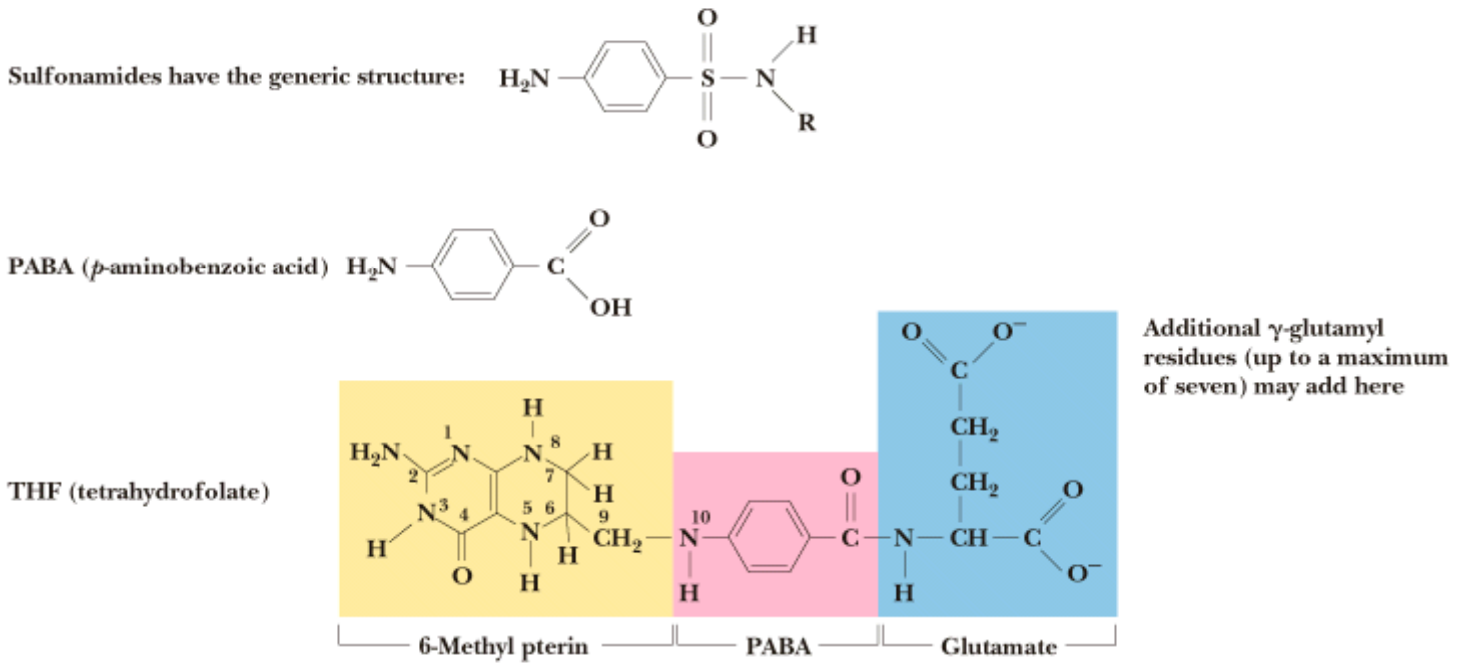
Azaserine



Glutamine



Garrett & Grisham: Biochemistry, 2/e
Figure 27.5

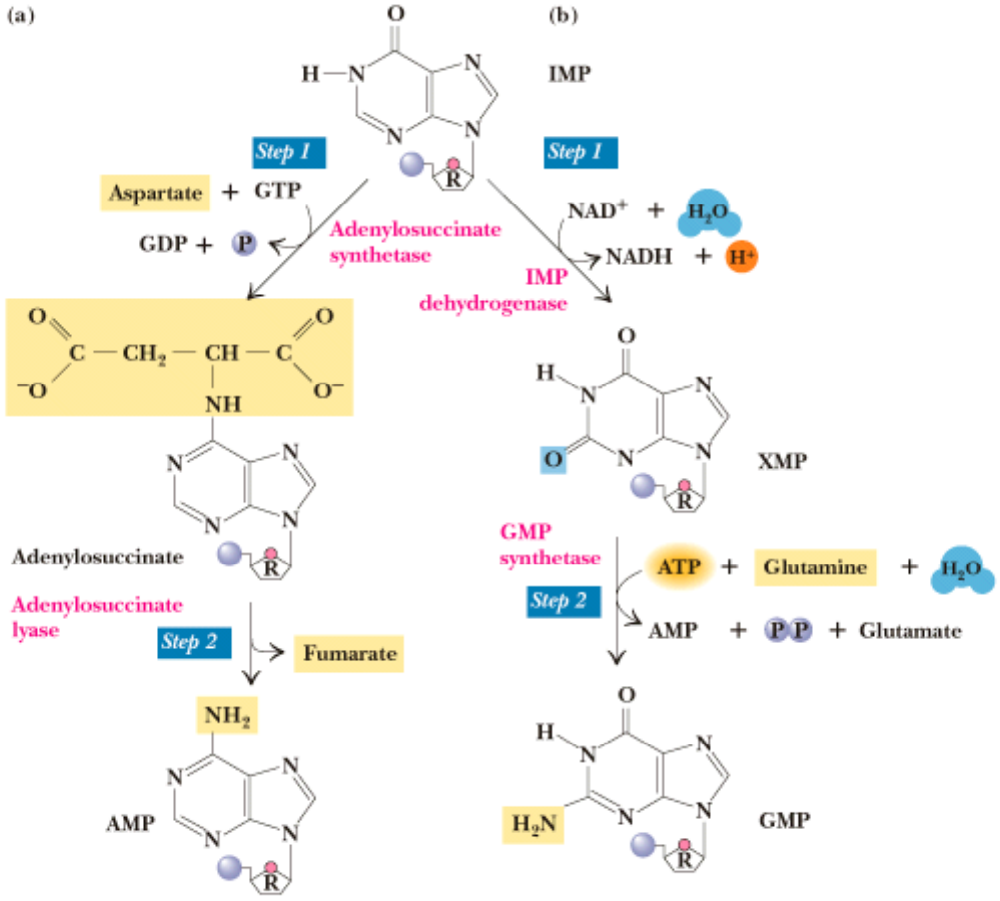


Making AMP and GMP

Reciprocal control occurs in two ways - see Figures 27.6 and 27.7

- GTP is the energy input for AMP synthesis, whereas ATP is energy input for GMP
- AMP is made by N addition from aspartate (in the familiar way - see Figure 27.6)
- GMP is made by oxidation at C-2, followed by replacement of the O by N (from Gln)
- Last step of GMP synthesis is identical to the first two steps of IMP synthesis

Garrett & Grisham: Biochemistry, 2/e
Figure 27.6



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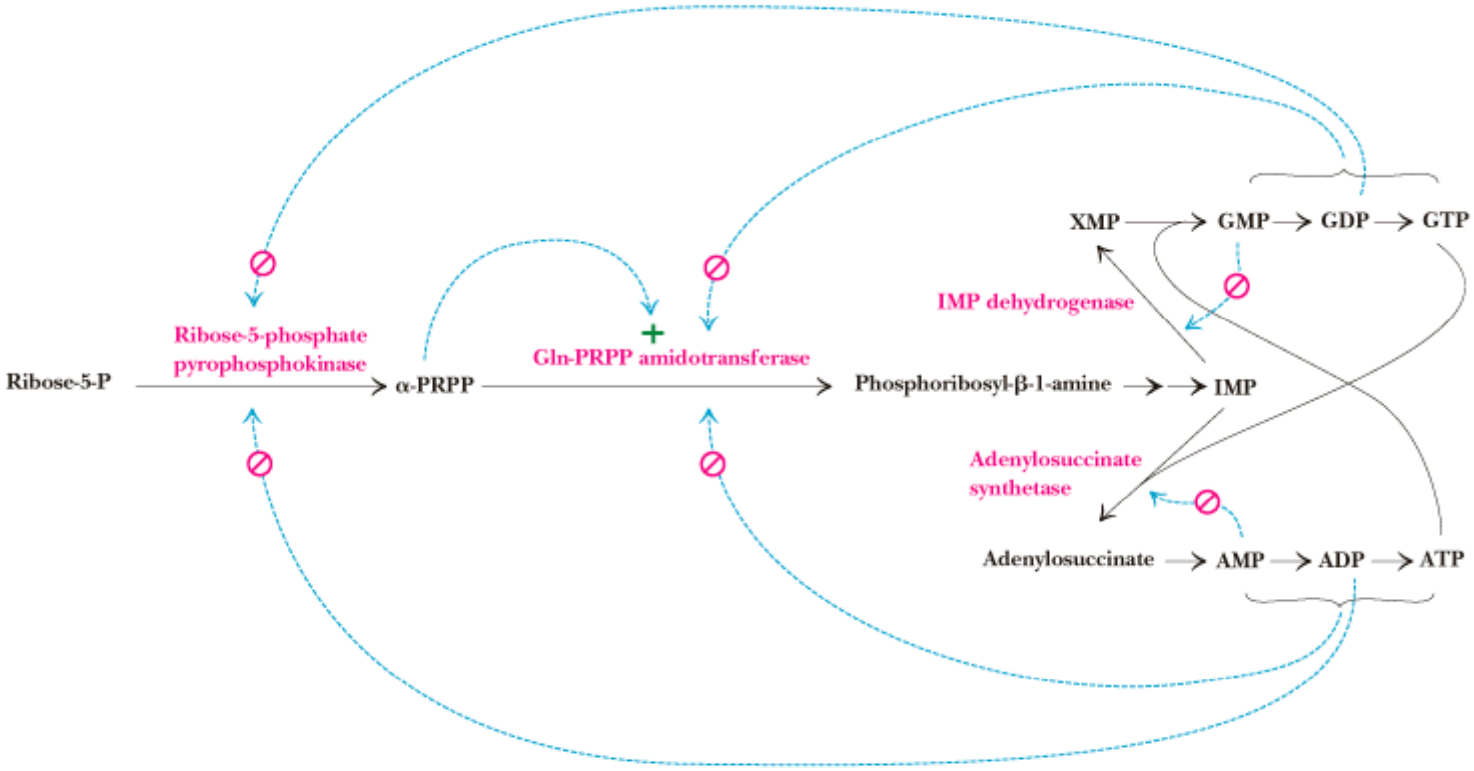
Garrett & Grisham: Biochemistry, 2/e
Figure 27.7



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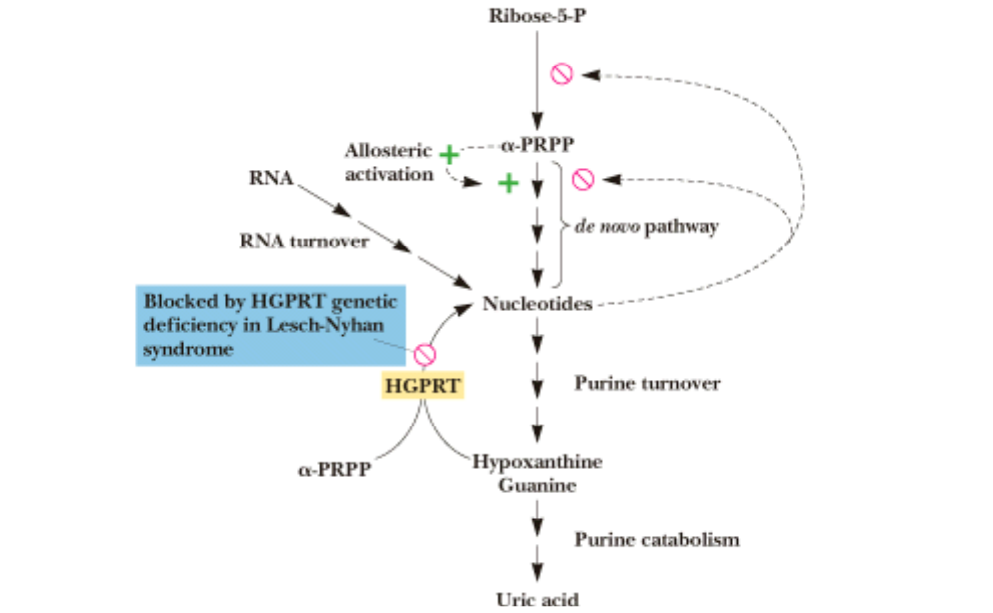
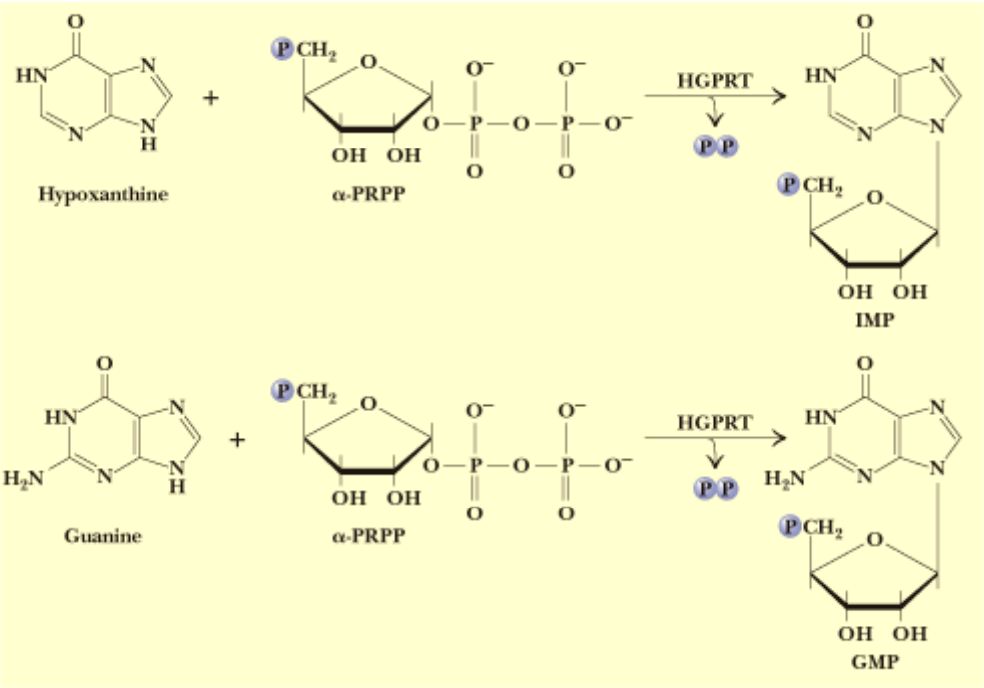
Purine Salvage

and Lesch-Nyhan syndrome

- Salvage pathways collect hypoxanthine and guanine and recombine them with PRPP to form nucleotides in the HGPRT reaction
- Absence of HGPRT is cause of Lesch-Nyhan syndrome
- In L-N, purine synthesis is increased 200-fold and uric acid is elevated in blood
- This increase may be due to PRPP feed-forward activation of *de novo* pathways

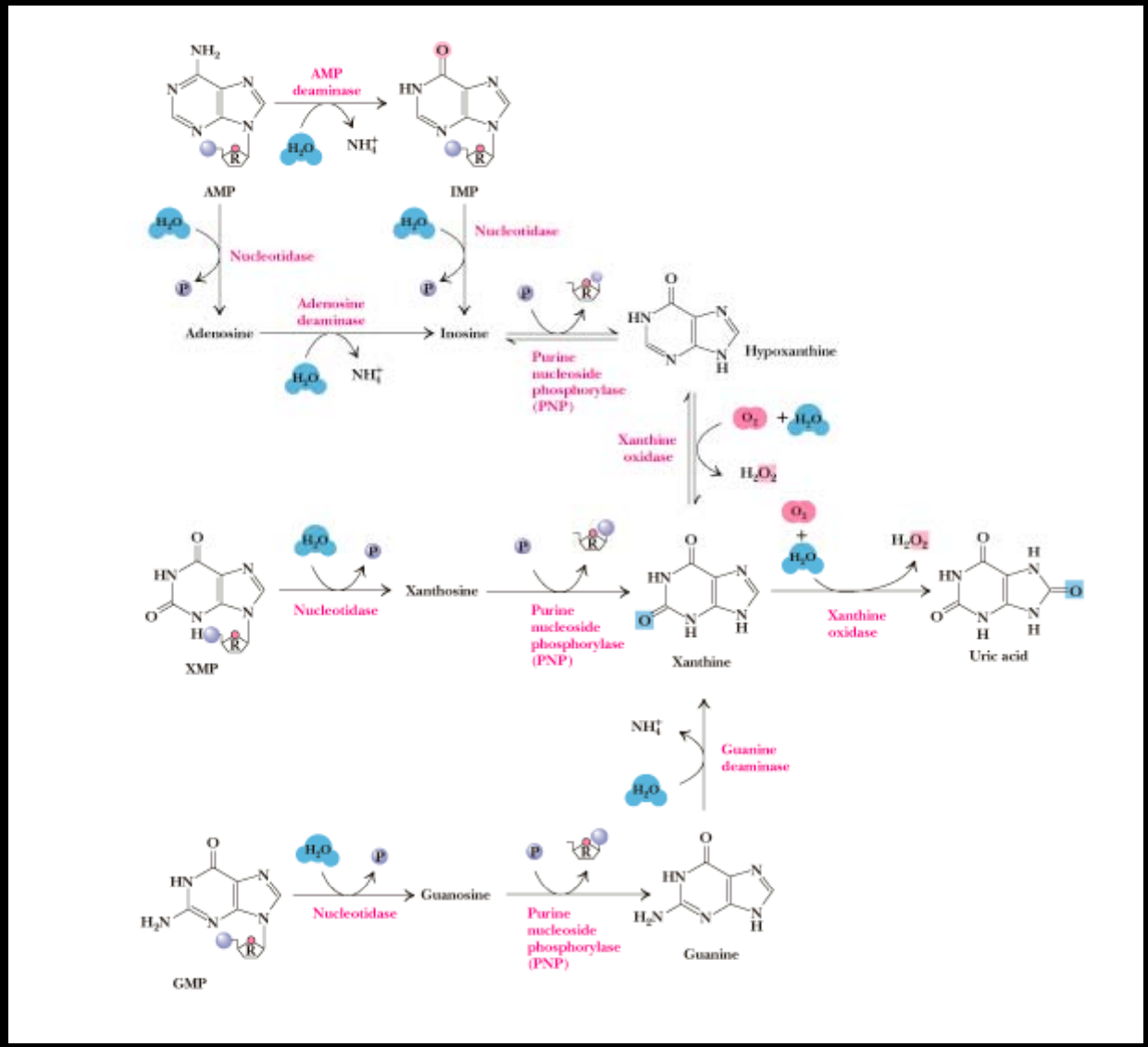

Ribose-5-P
α-PRPP
Allosteric activation
RNA
RNA turnover
de novo pathway
Nucleotides
Blocked by HGPRT genetic deficiency in Lesch-Nyhan syndrome
HGPRT
Purine turnover
α-PRPP
Hypoxanthine
Guanine
Purine catabolism
Uric acid


Hypoxanthine
α-PRPP
HGPRT
IMP
Guanine
α-PRPP
HGPRT
GMP

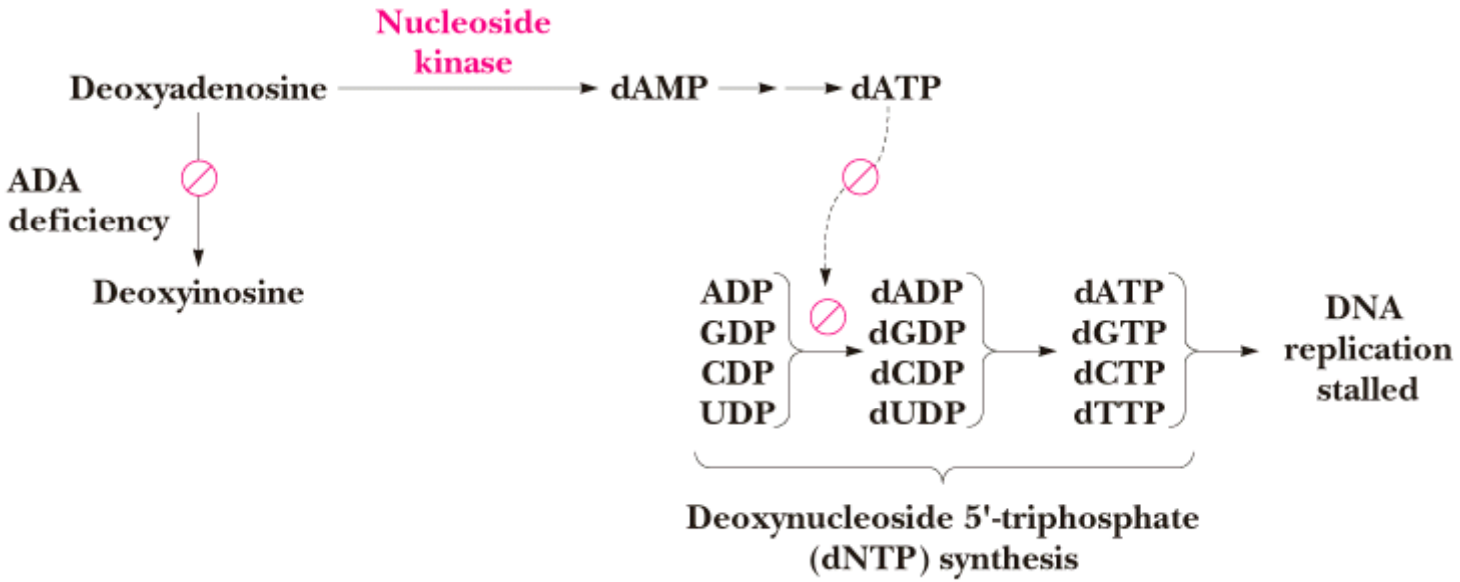
Purine Degradation

Purine catabolism leads to uric acid (see Figure 27.9)

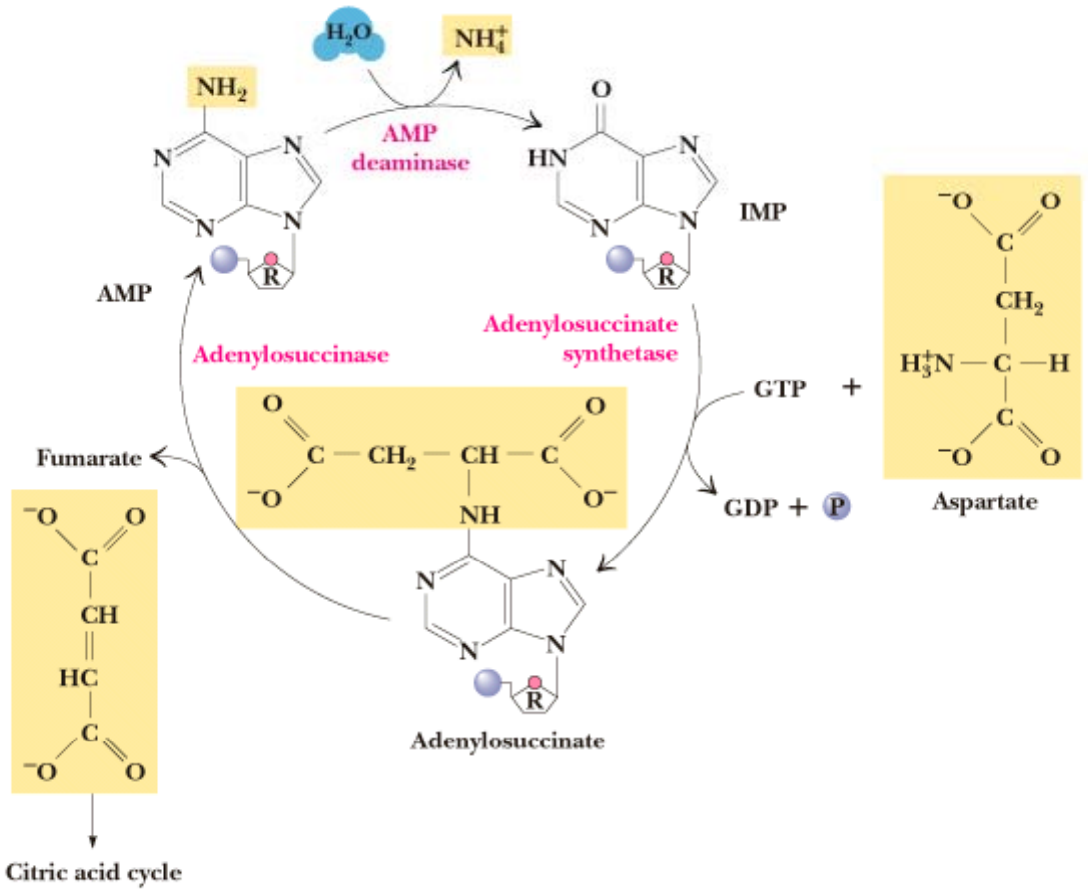
- Nucleotidases and nucleosidases release ribose and phosphates and leave free bases
- Xanthine oxidase and guanine deaminase route everything to xanthine
- Xanthine oxidase converts xanthine to uric acid
- Note that xanthine oxidase can oxidize two different sites on the purine ring system


AMP deaminase
AMP
IMP
Nucleotidase
Nucleotidase
Adenosine deaminase
Adenosine
Inosine
Purine nucleoside phosphorylase (PNP)
Hypoxanthine
Xanthine oxidase
XMP
Nucleotidase
Xanthosine
Purine nucleoside phosphorylase (PNP)
Xanthine
Xanthine oxidase
Uric acid
Guanine deaminase
GMP
Nucleotidase
Guanosine
Purine nucleoside phosphorylase (PNP)
Guanine

Garrett & Grisham: Biochemistry, 2/e
Figure 27.10



Garrett & Grisham: Biochemistry, 2/e
Figure 27.11



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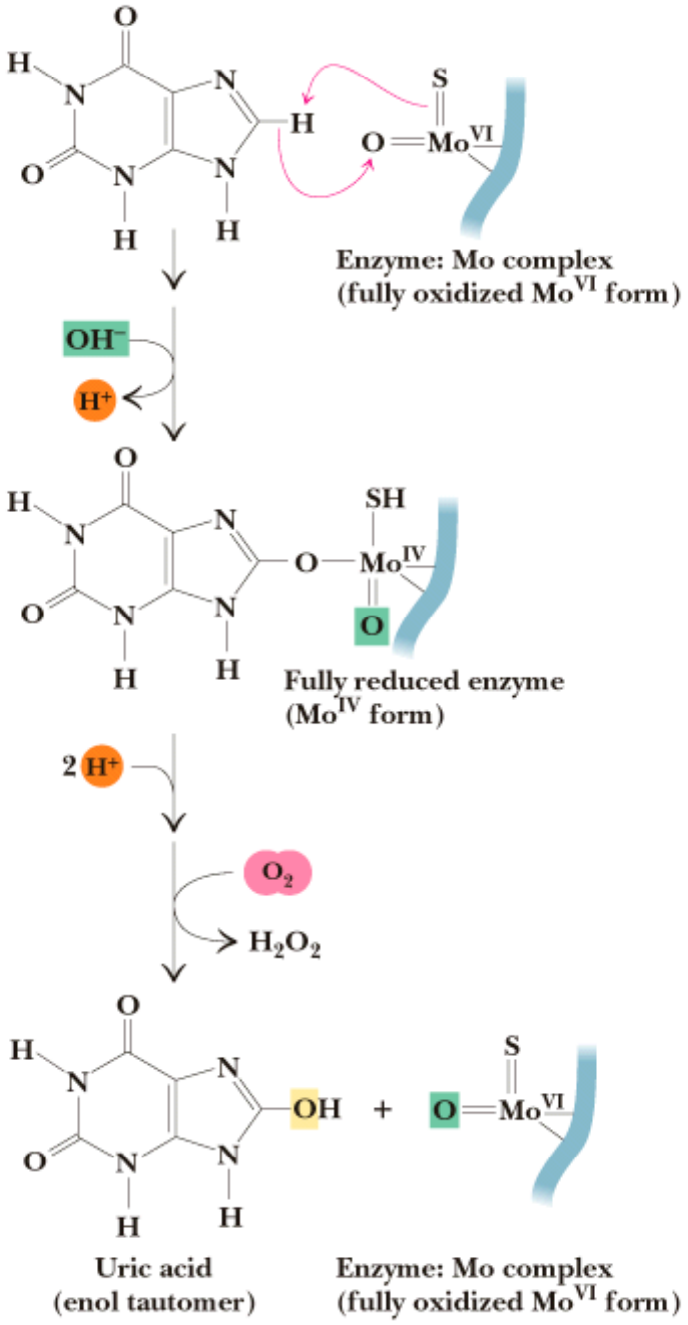
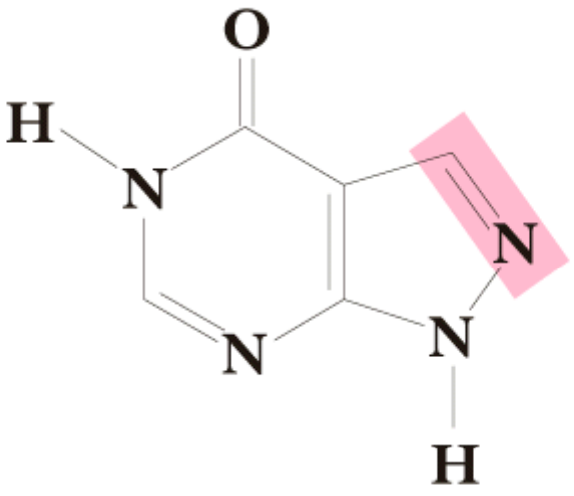
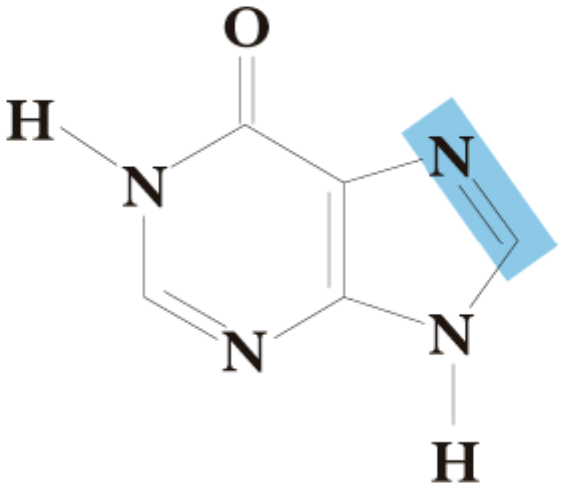
Xanthine Oxidase and Gout

- XO in liver, intestines (and milk) can oxidize hypoxanthine (twice) to uric acid
- Humans and other primates excrete uric acid in the urine, but most N goes out as urea
- Birds, reptiles and insects excrete uric acid and for them it is the major nitrogen excretory compound
- Gout occurs from accumulation of uric acid crystals in the extremities
- Allopurinol, which inhibits XO, is a treatment

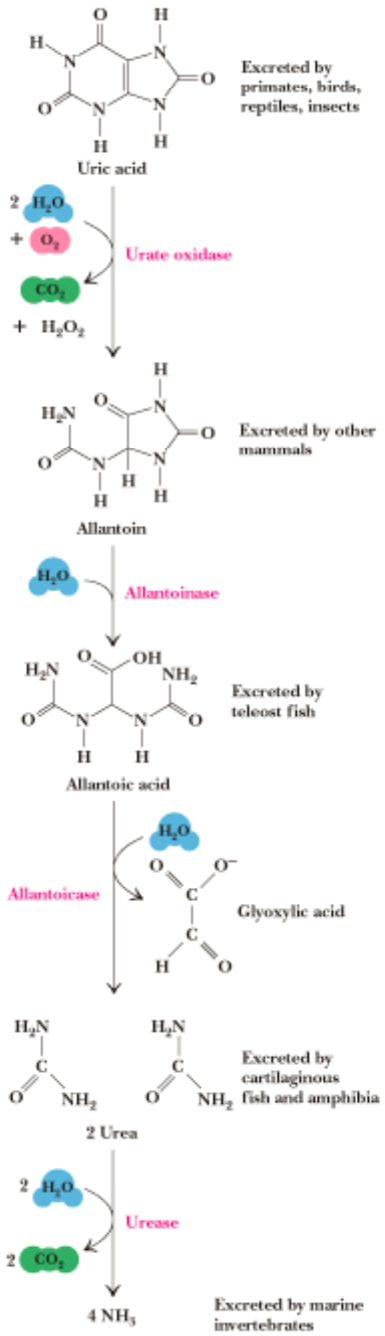

Enzyme: Mo complex
(fully oxidized Mo^VI form)
OH⁻
H⁺
Fully reduced enzyme
(Mo^IV form)
2 H⁺
O₂
H₂O₂
Uric acid
(enol tautomer)
Enzyme: Mo complex
(fully oxidized Mo^VI form)



Allopurinol



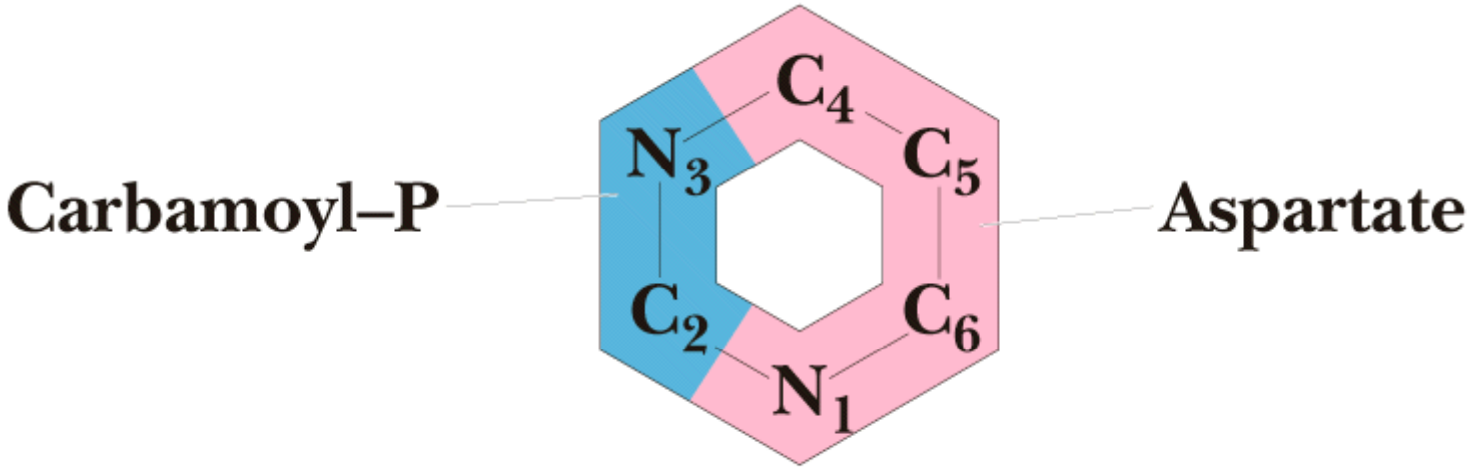
Hypoxanthine


Excreted by primates, birds, reptiles, insects
Uric acid
2 H_2O
+ O_2
Urate oxidase
CO_2
+ H_2O_2
Excreted by other mammals
Allantoin
H_2O
Allantoinase
Excreted by teleost fish
Allantoic acid
Allantoicase
H_2O
Glyoxylic acid
Excreted by cartilaginous fish and amphibia
2 Urea
2 H_2O
Urease
2 CO_2
4 NH_3
Excreted by marine invertebrates

Pyrimidine Biosynthesis

- In contrast to purines, pyrimidines are not synthesized as nucleotides
- Rather, the pyrimidine ring is completed before a ribose-5-P is added
- Carbamoyl-P and aspartate are the precursors of the six atoms of the pyrimidine ring

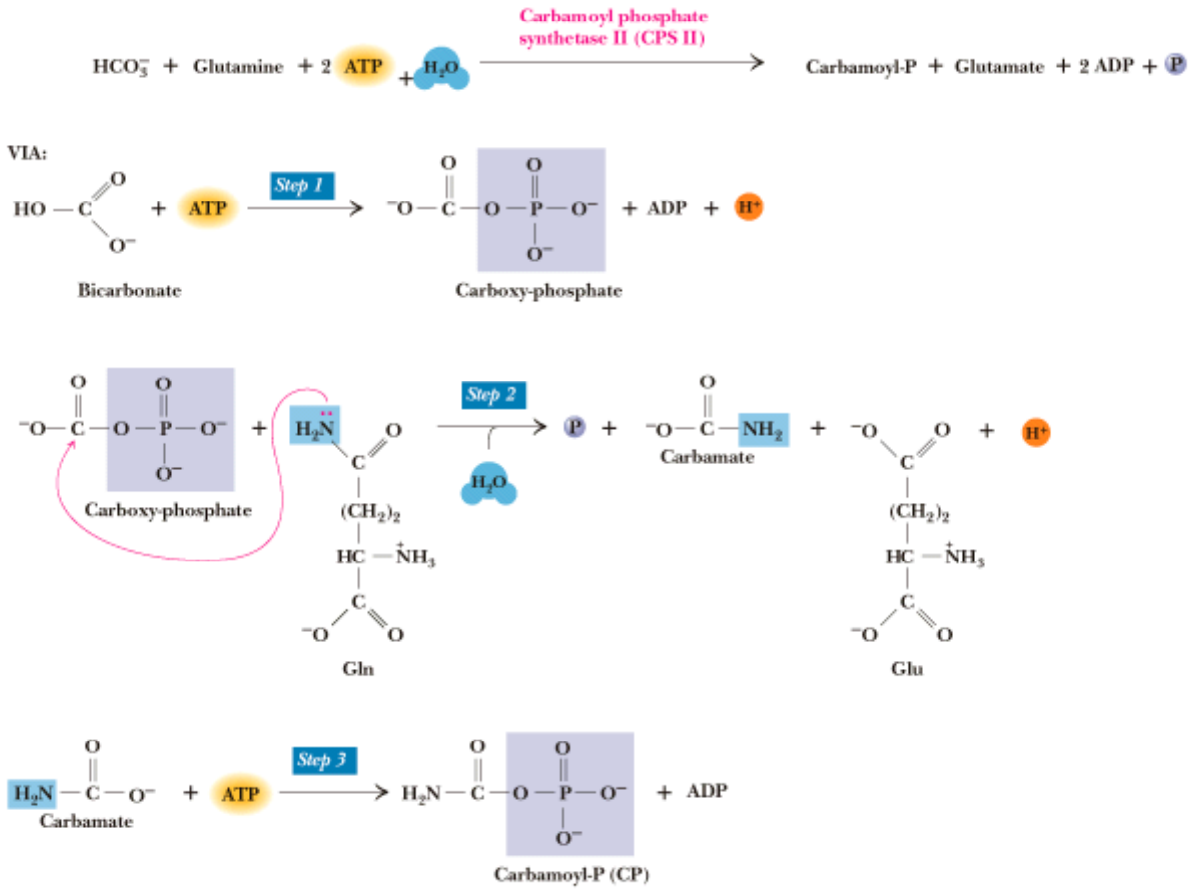
Garrett & Grisham: Biochemistry, 2/e
Figure 27.15



CPS II

- Carbamoyl phosphate for pyrimidine synthesis is made by carbamoyl phosphate synthetase II (CPS II)
- This is a cytosolic enzyme (whereas CPS I is mitochondrial and used for the urea cycle)
- Substrates are HCO_3^-, glutamine, 2 ATP
- See Figure 27.16

Garrett & Grisham: Biochemistry, 2/e
Figure 27.16



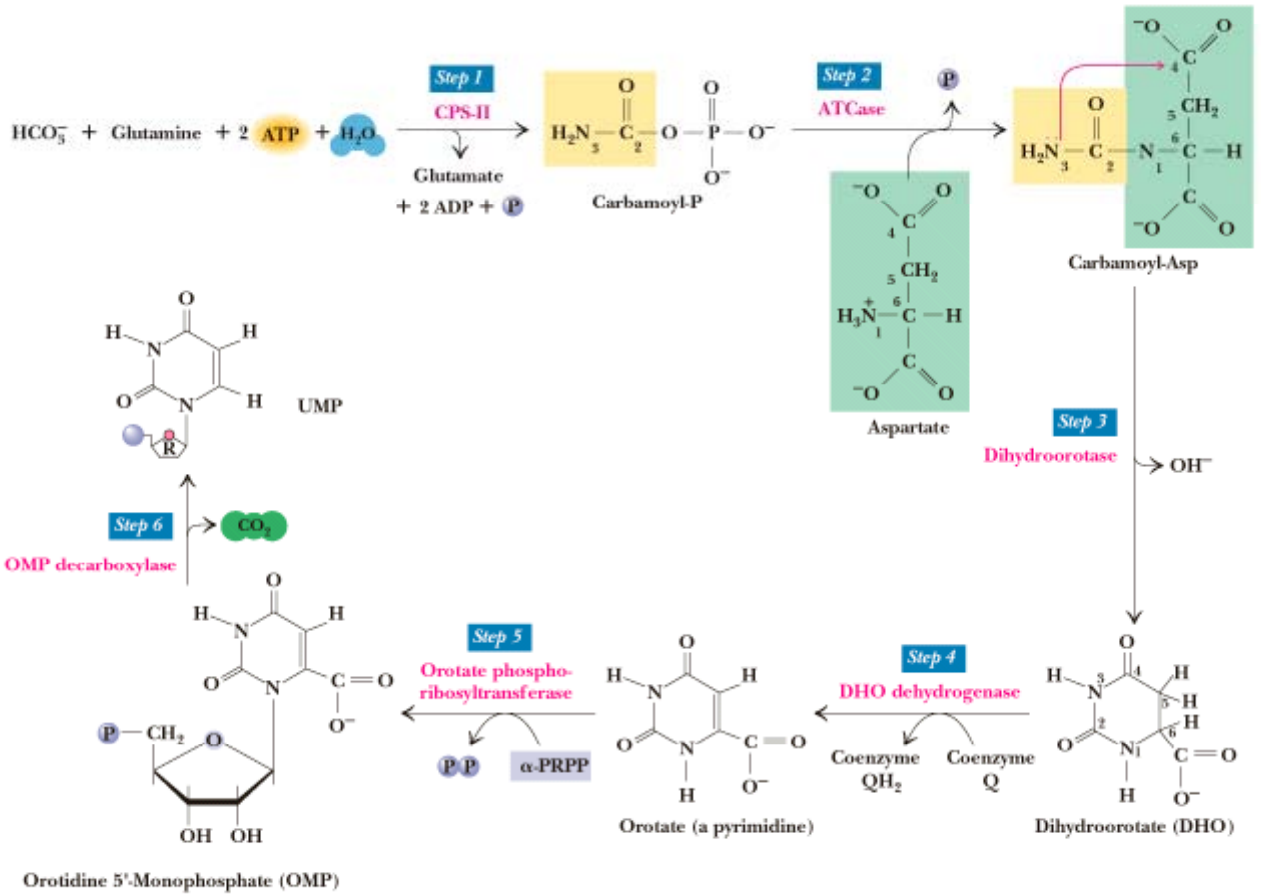
de novo Pyrimidine Synthesis

- Aspartate transcarbamoylase (ATCase) catalyzes the condensation of carbamoyl phosphate with aspartate to form carbamoyl-aspartate
- Note that carbamoyl phosphate represents an ‘activated’ carbamoyl group

More Pyrimidine Synthesis

- Step 3: ring closure and dehydration - catalyzed by dihydroorotase
- Step 4: Synthesis of a true pyrimidine (orotate) by DHO dehydrogenase
- Step 5: Orotate is joined with a ribose-P to form orotidine-5'-phosphate
- The ribose-P donor is PRPP
- Step 6: OMP decarboxylase makes UMP

Garrett & Grisham: Biochemistry, 2/e
Figure 27.17



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Metabolic channeling

- Eukaryotic pyrimidine synthesis involves channeling and multifunctional polypeptides
- UDP is made from UMP, and UTP is made for UDP
- CTP sythetase forms CTP from UTP and ATP

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Figure 27.18

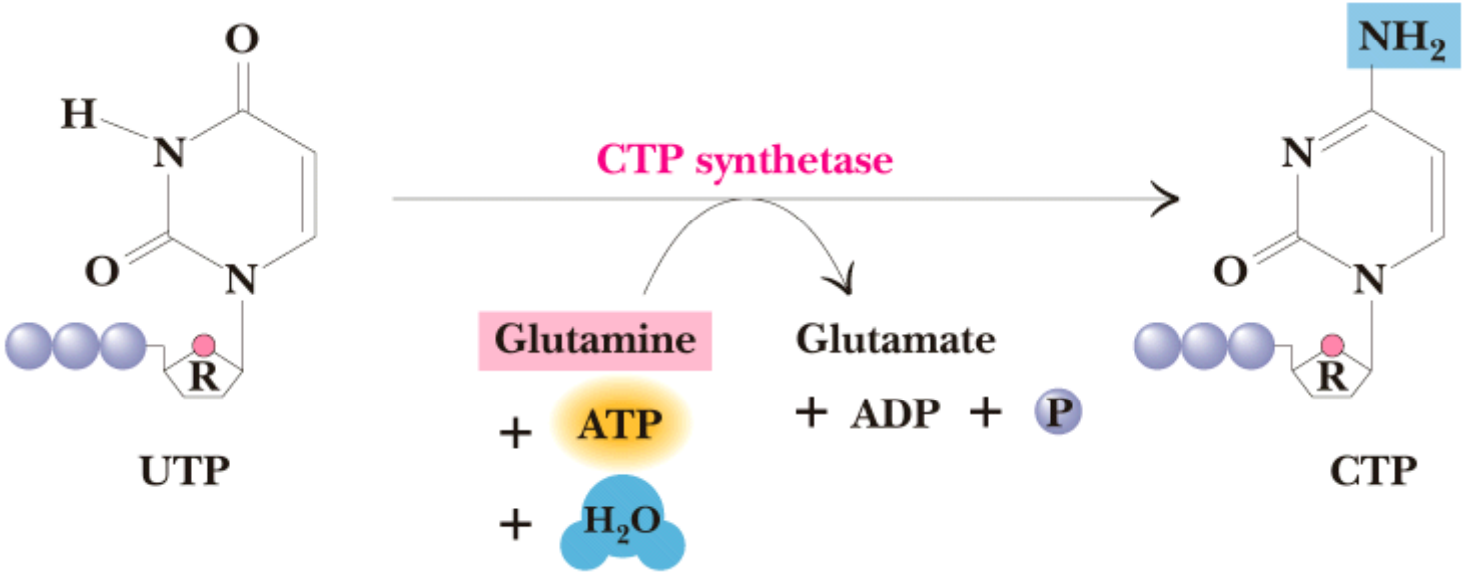

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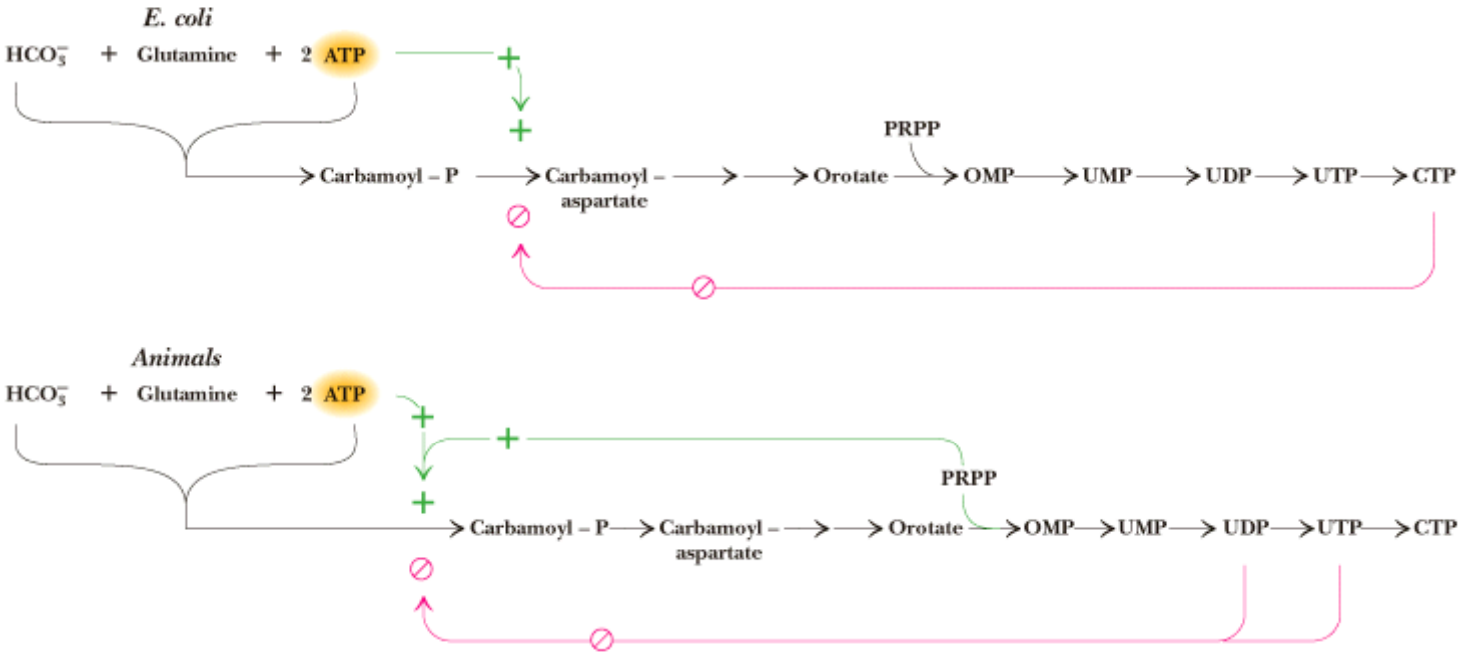
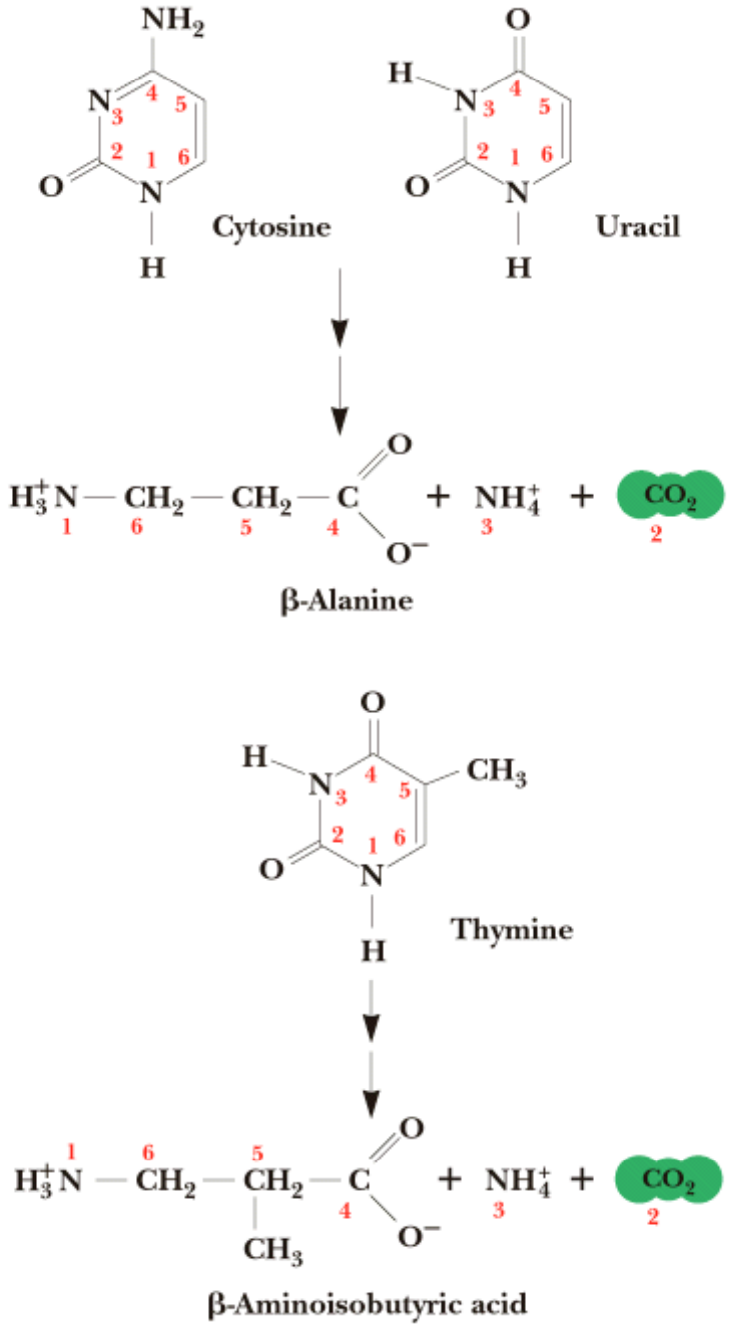
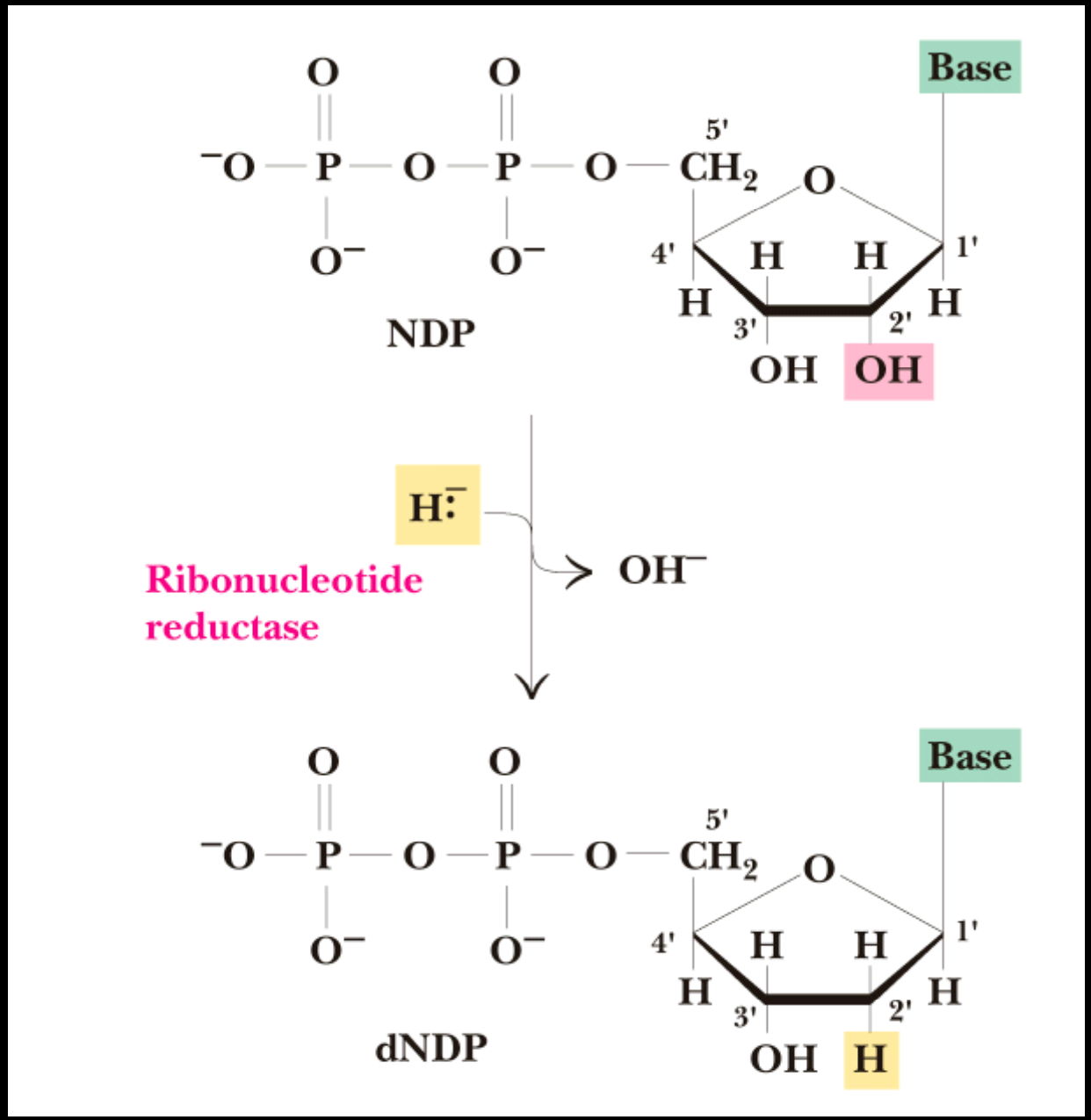
Figure 27.19




Cytosine
Uracil
β-Alanine
Thymine
β-Aminoisobutyric acid


Base
NDP
H:⁻
OH⁻
Ribonucleotide reductase
Base
dNDP

Deoxyribonucleotide Biosynthesis

- Reduction at 2'-position commits nucleotides to DNA synthesis
- Replacement of 2'-OH with hydride is catalyzed by ribonucleotide reductase
- An $\alpha_2\beta_2$-type enzyme - subunits R_1 (86 kD) and R_2 (43.5 kD)
- R_1 has two regulatory sites, a specificity site and an overall activity site

Ribonucleotide Reductase

- Activity depends on Cys^{439}, Cys^{225}, and Cys^{462} on R_1 and on Tyr^{122} on R_2
- Cys^{439} removes 3'-H, and dehydration follows, with disulfide formation between Cys^{225} and Cys^{462}
- The net result is hydride transfer to C-2'
- Thioredoxin and thioredoxin reductase deliver reducing equivalents

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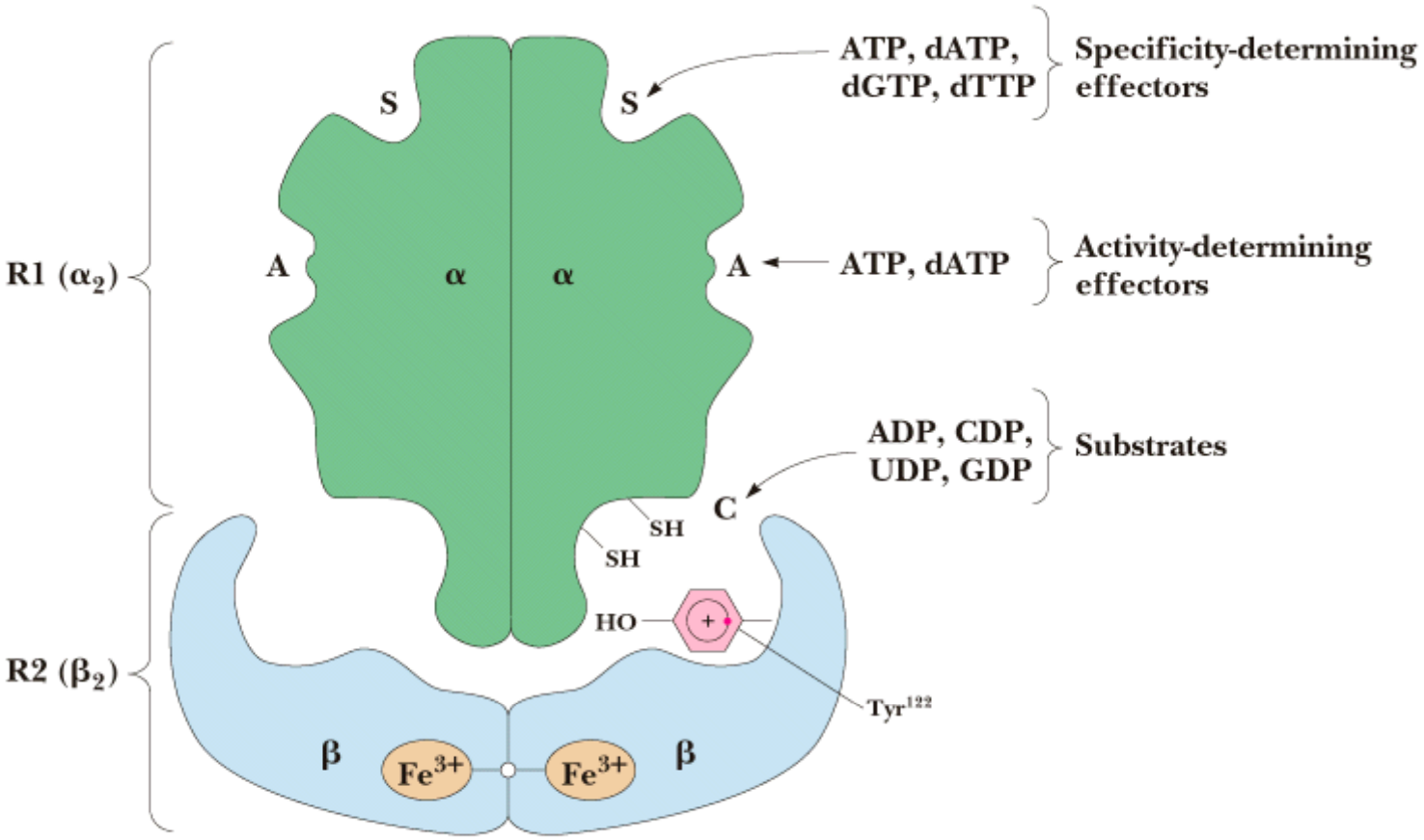
Figure 27.22



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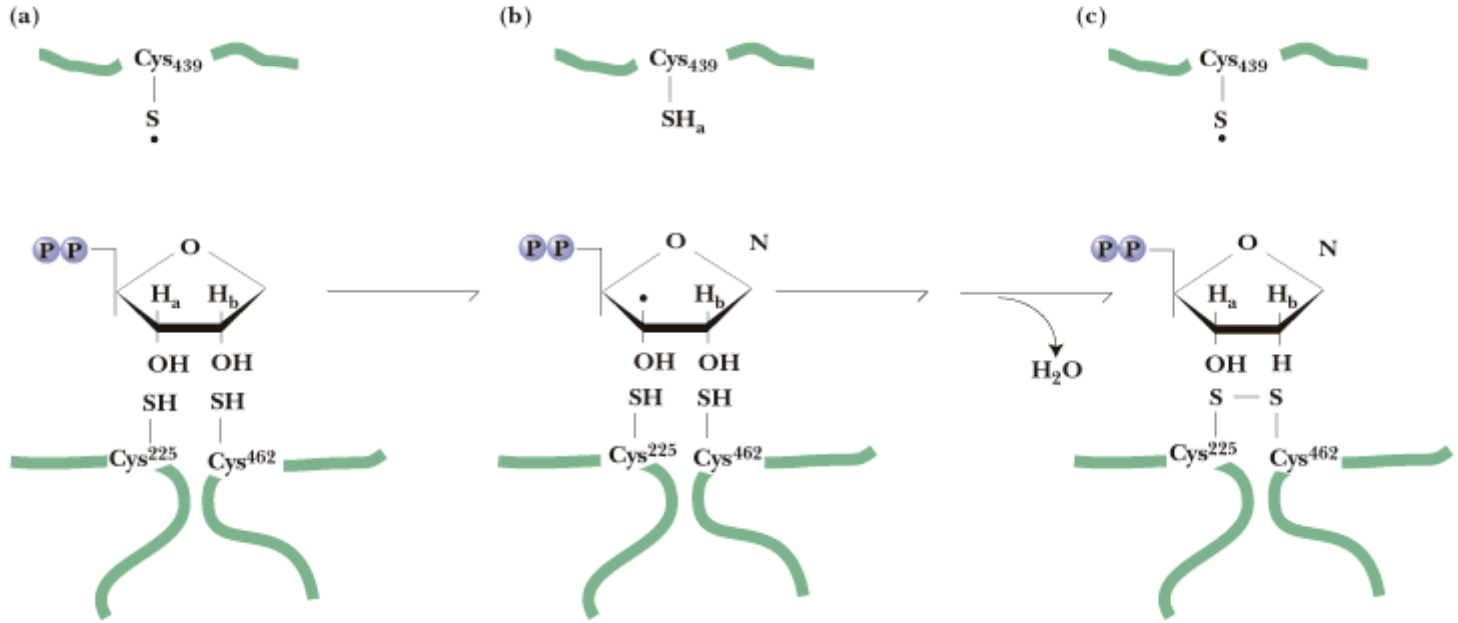

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Figure 27.24

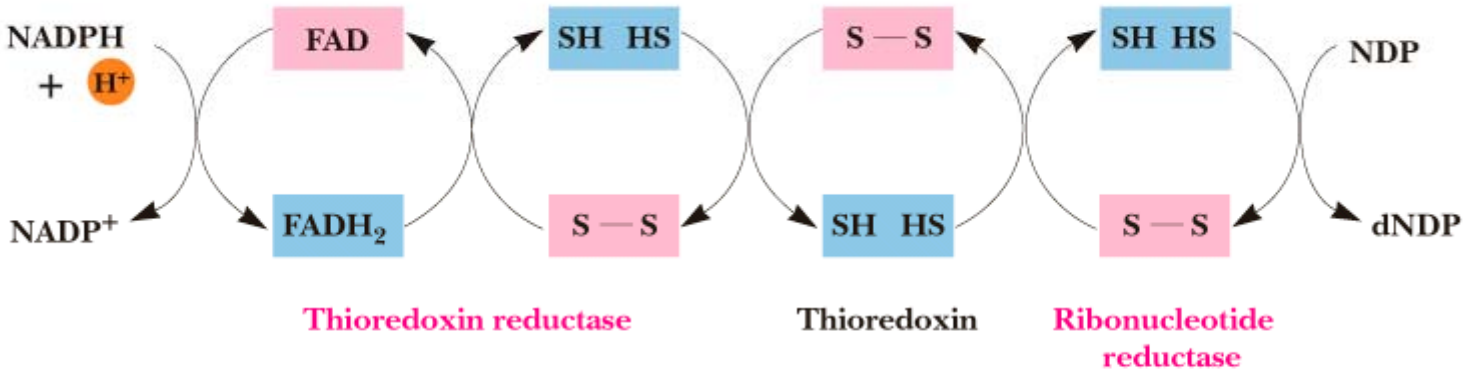

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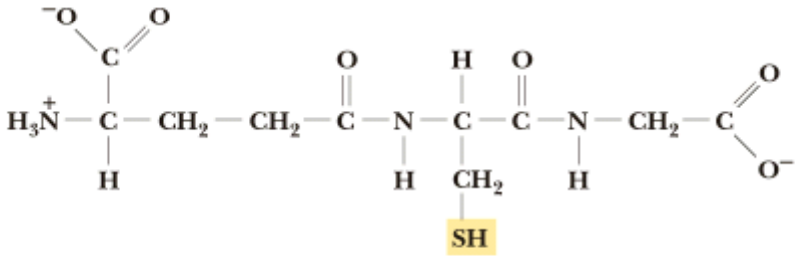
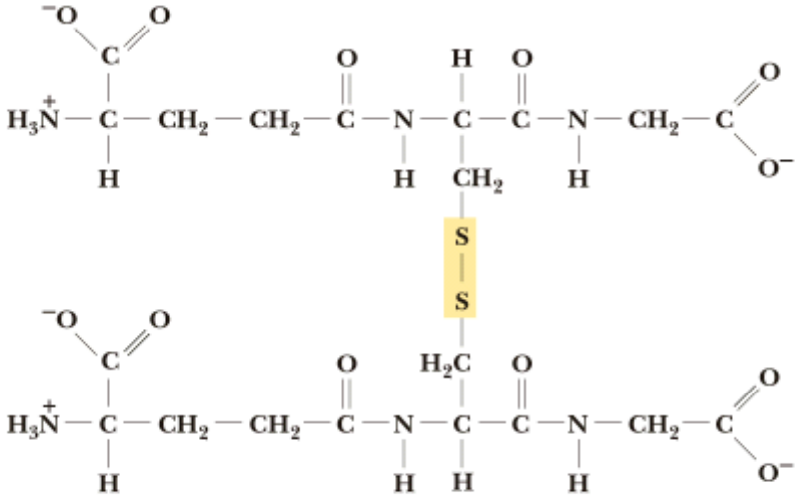
Figure 27.25



Reduced glutathione (γ-glutamylcysteinylglycine, GSH)



Oxidized glutathione (GSSG) or glutathione disulfide

Regulation of dNTP Synthesis

- The overall activity of ribonucleotide reductase must be regulated
- Balance of the four deoxynucleotides must be controlled
- ATP activates, dATP inhibits at the overall activity site
- ATP, dATP, dTTP and dGTP bind at the specificity site to regulate the selection of substrates and the products made

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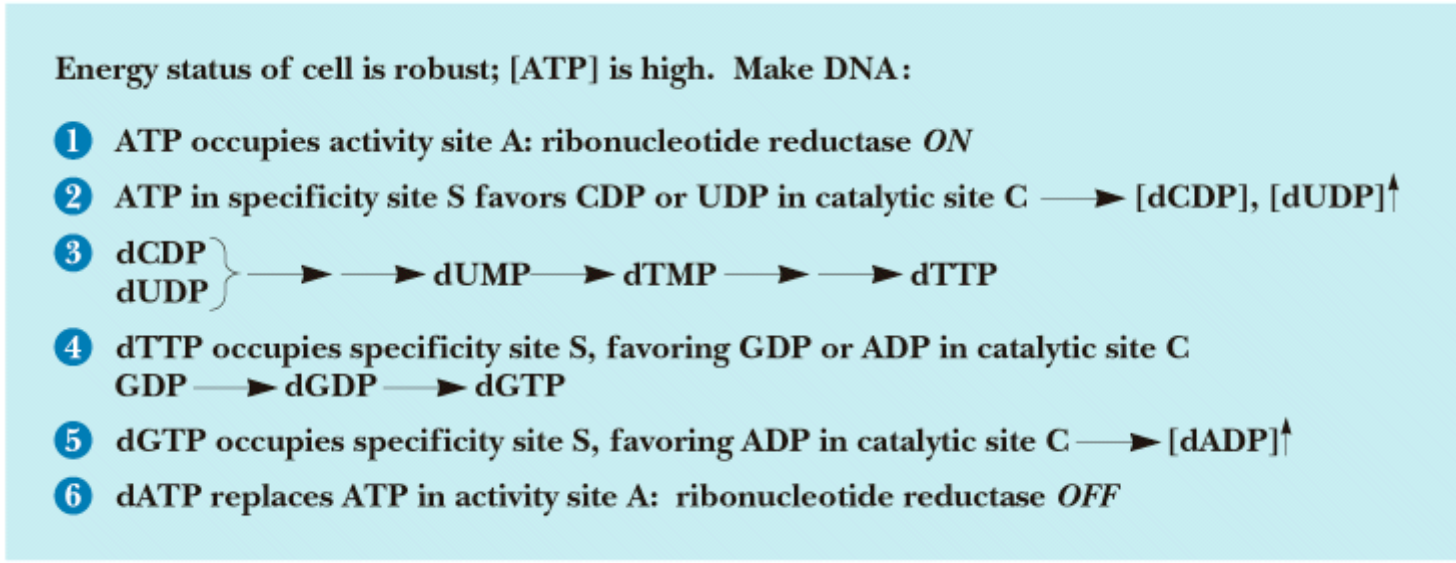
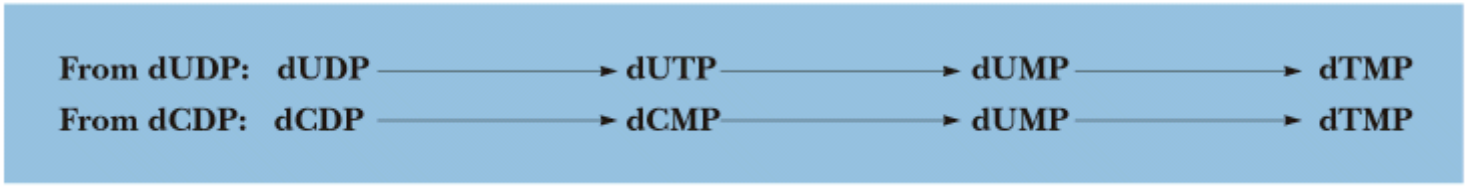
Figure 27.26



Garrett & Grisham: Biochemistry, 2/e
Figure 27.27



Synthesis of Thymine Nucleotides

- Thymine nucleotides are made from dUMP, which derives from dUDP, dCDP
- dUDP→dUTP→dUMP→dTMP
- dCDP→dCMP→dUMP→dTMP
- Thymidylate synthase methylates dUMP at 5-position to make dTMP
- N^5,N^{10}-methylene THF is 1-C donor
- Note role of 5-FU in chemotherapy

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Figure 27.28

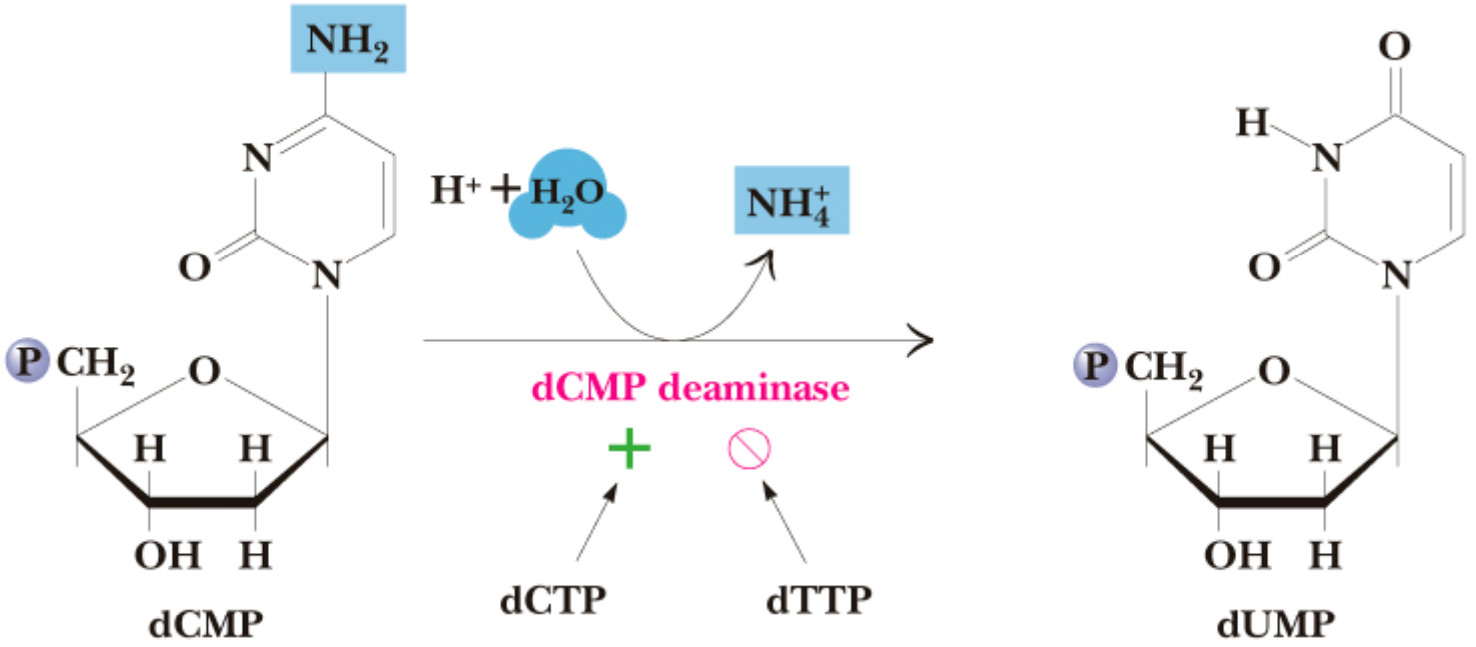

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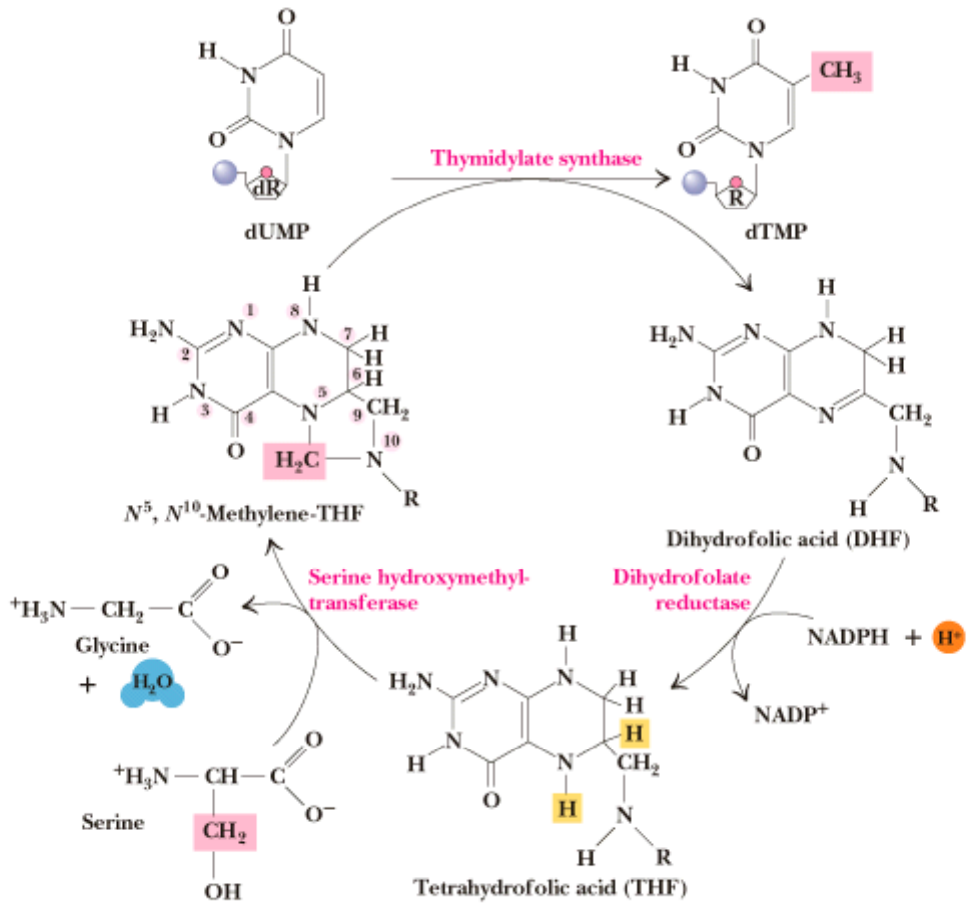
Figure 27.29



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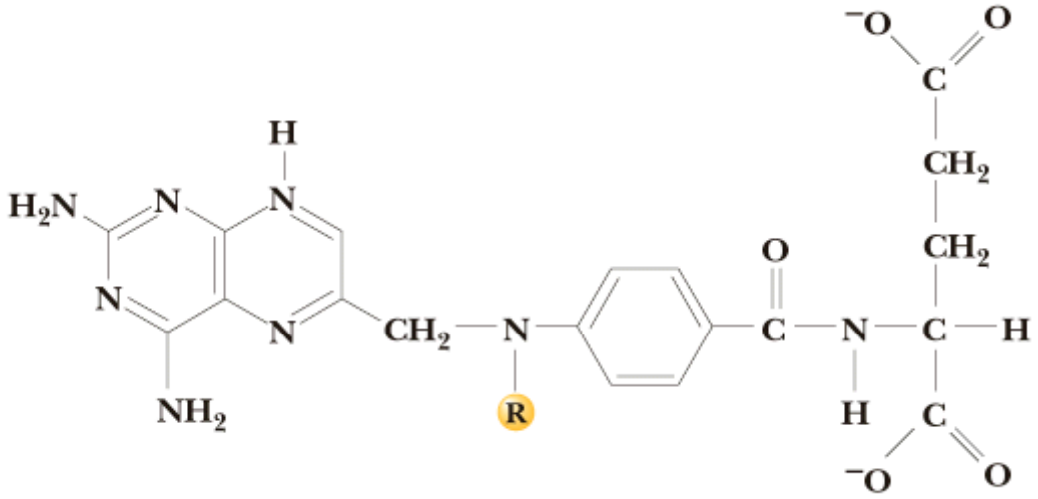
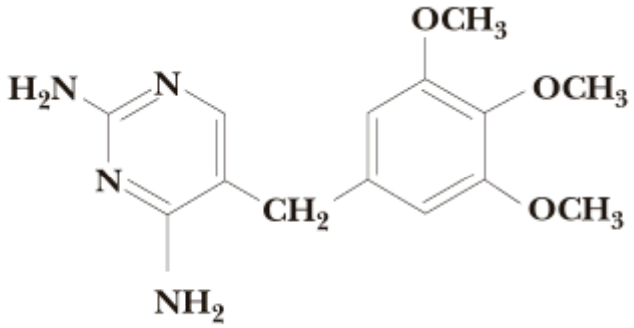


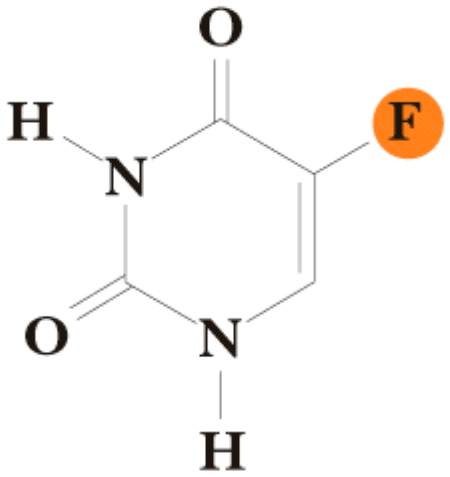
2-Amino, 4-amino analogs of folic acid

R = H Aminopterin

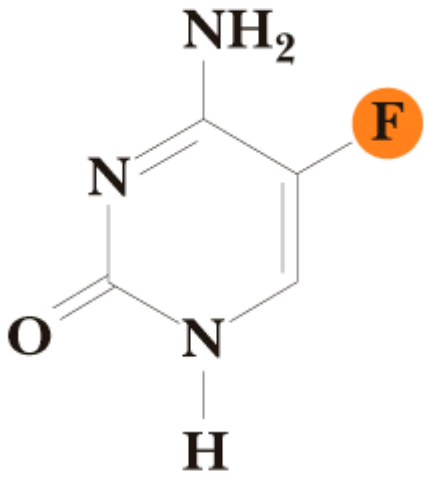
R = CH_3 Amethopterin (methotrexate)



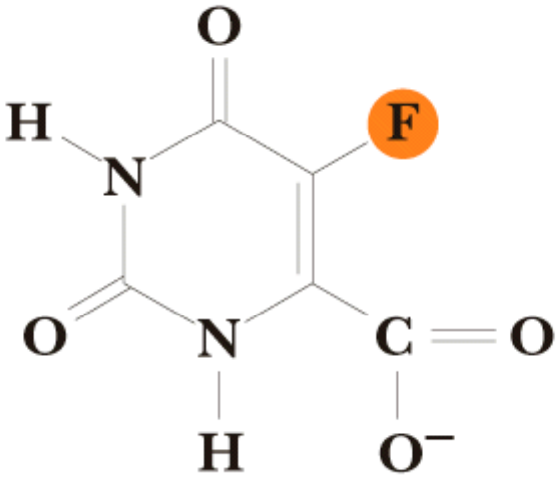
Trimethoprim



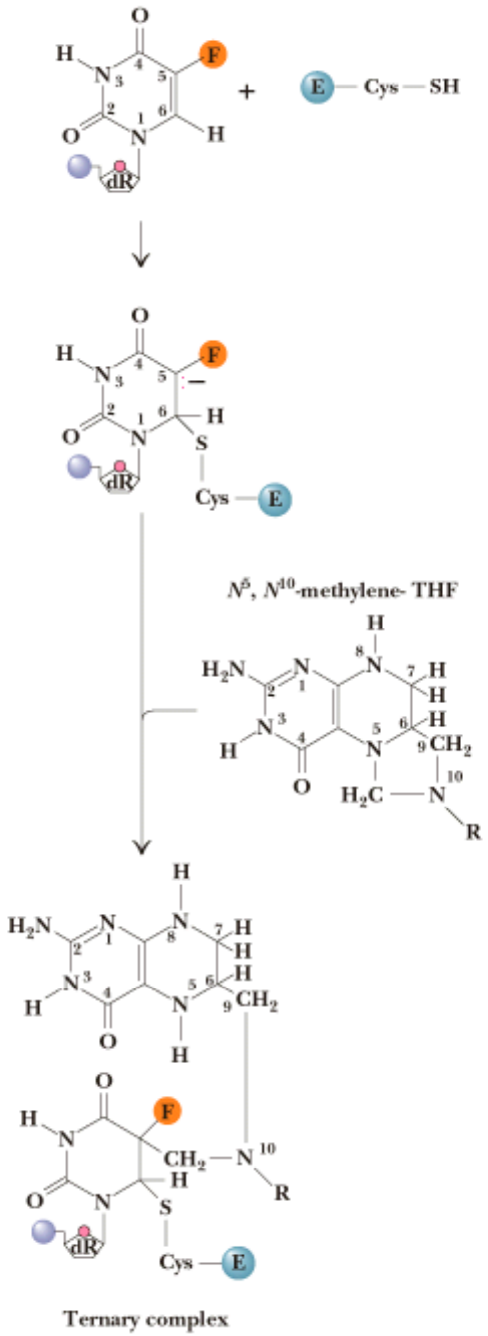
5-Fluorouracil



5-Fluorocytosine



5-Fluoroorotate


E — Cys — SH
Cys — E
N^5, N^{10}-methylene- THF
Ternary complex