MACQUARIE University

# Nucleotide metabolism

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# *Announcements*

- Tutorial 5 for Class 2 Room change
  - ***Wed May 26 2-4pm in 9WW 102.***
- Test 2 will feature case studies: please revise your Tutorial slides and case studies.
- Exam confirmed for Tue 15 June 9-11:10 AM

- All the very best!

# Objectives

- Nucleotides - Recap
  - *Nucleotide bases*
  - Nucleosides
  - Nucleotides
- Major synthetic pathways for nucleotides
- Purine nucleotide metabolism
  - *De novo synthesis of purine bases*
  - *Making IMP*
  - *AMP and GMP from IMP*
  - Salvage of purine nucleotides
  - Degradation of purine bases
- Pyrimidine nucleotide metabolism
  - *De novo* synthesis of pyrimidine bases
  - Degradation of pyrimidine bases
- • Making deoxyribonucleotides for DNA

# Two Types of Nucleic Acid: DNA and RNA

Nucleotide bases found in DNA (A, G, C, T) and RNA (A, G, C, U)

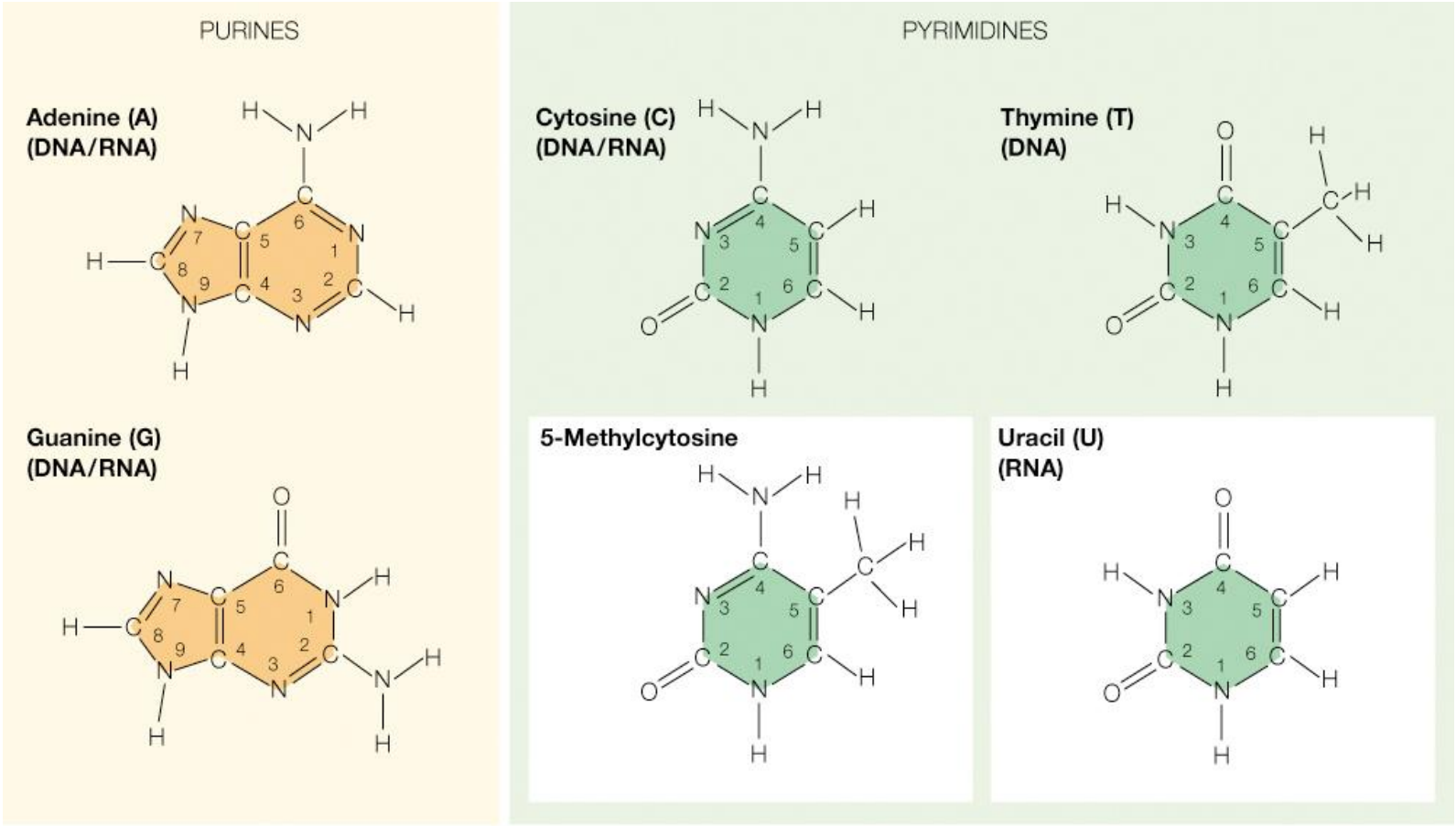



**FIGURE 4.3 Purine and pyrimidine bases found in DNA and RNA.**

# Purine Nucleosides and Nucleotides

Base + ribose sugar = nucleoside  Base + ribose sugar + phosphate = nucleotide

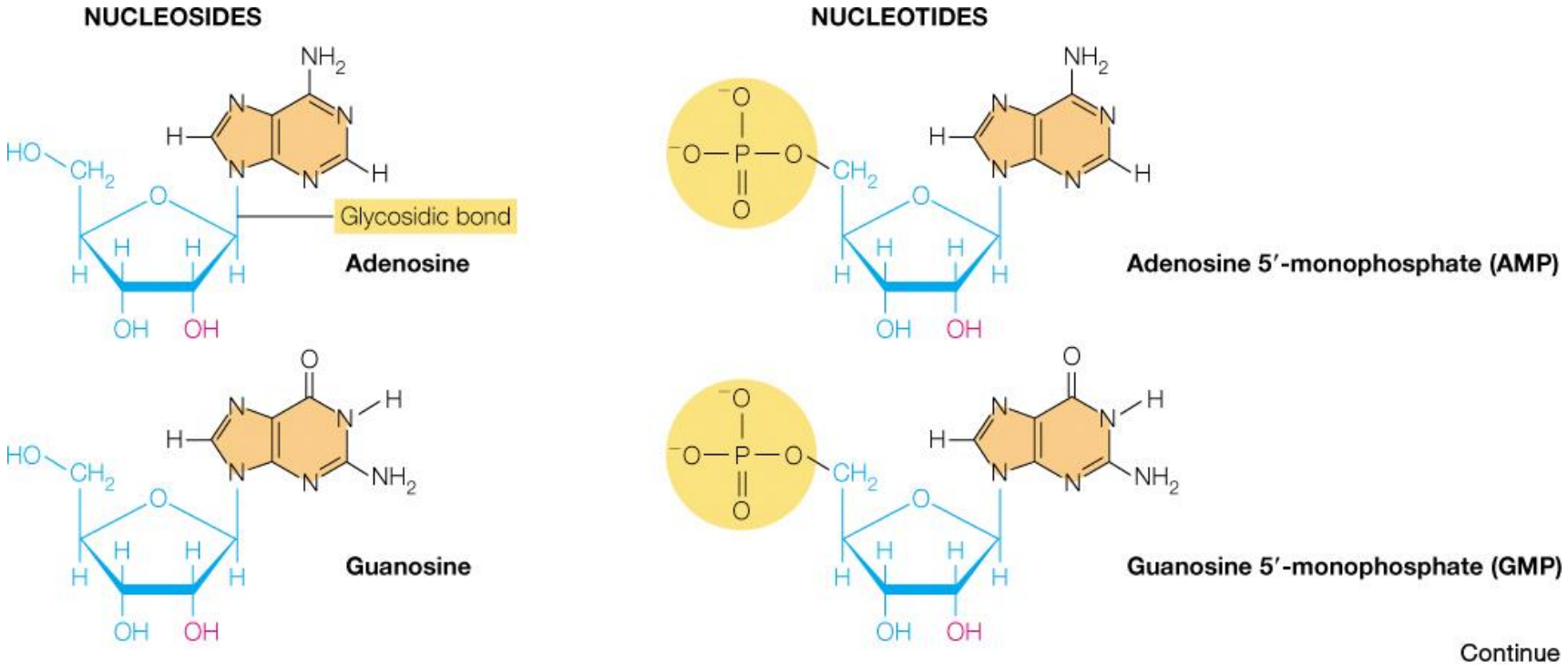



**FIGURE 4.4 Nucleosides and nucleotides.**

# Pyrimidine Nucleosides and Nucleotides

Base + ribose sugar = nucleoside

Base + ribose sugar + phosphate = nucleotide

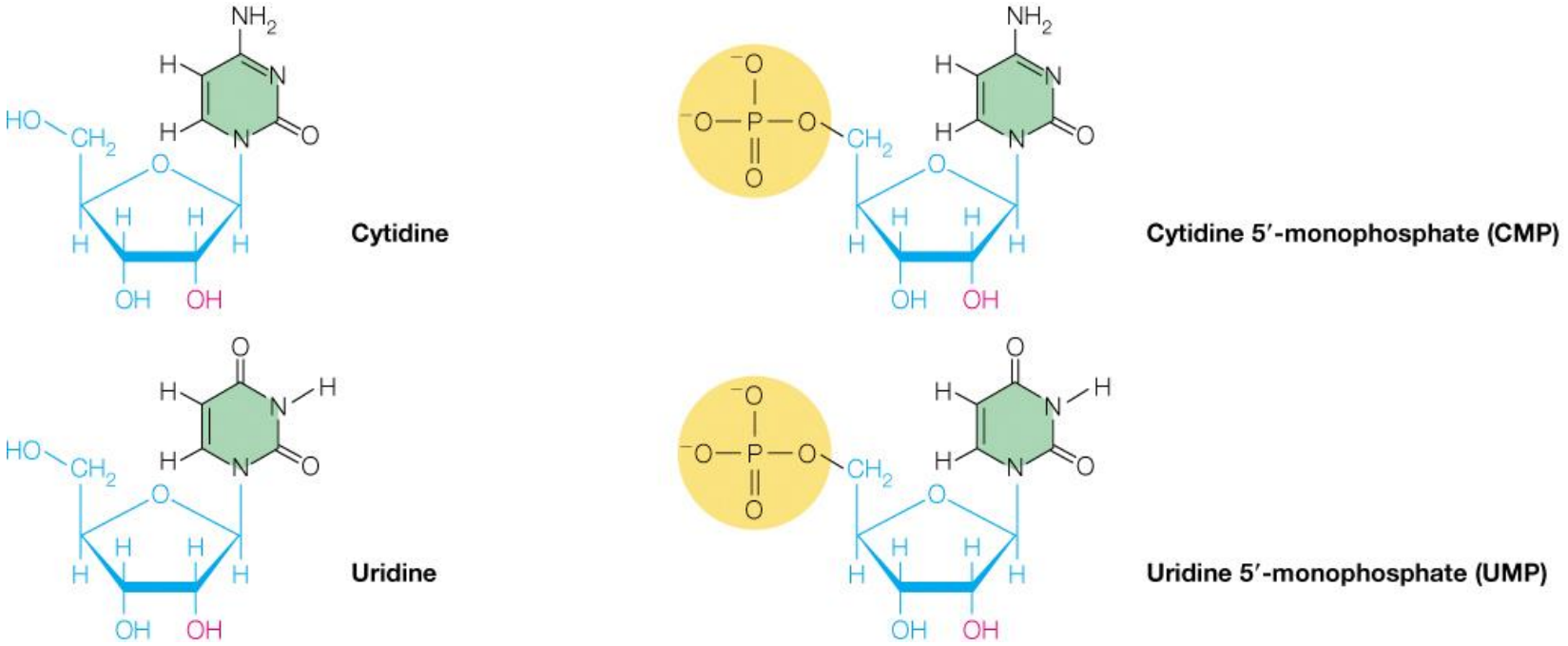
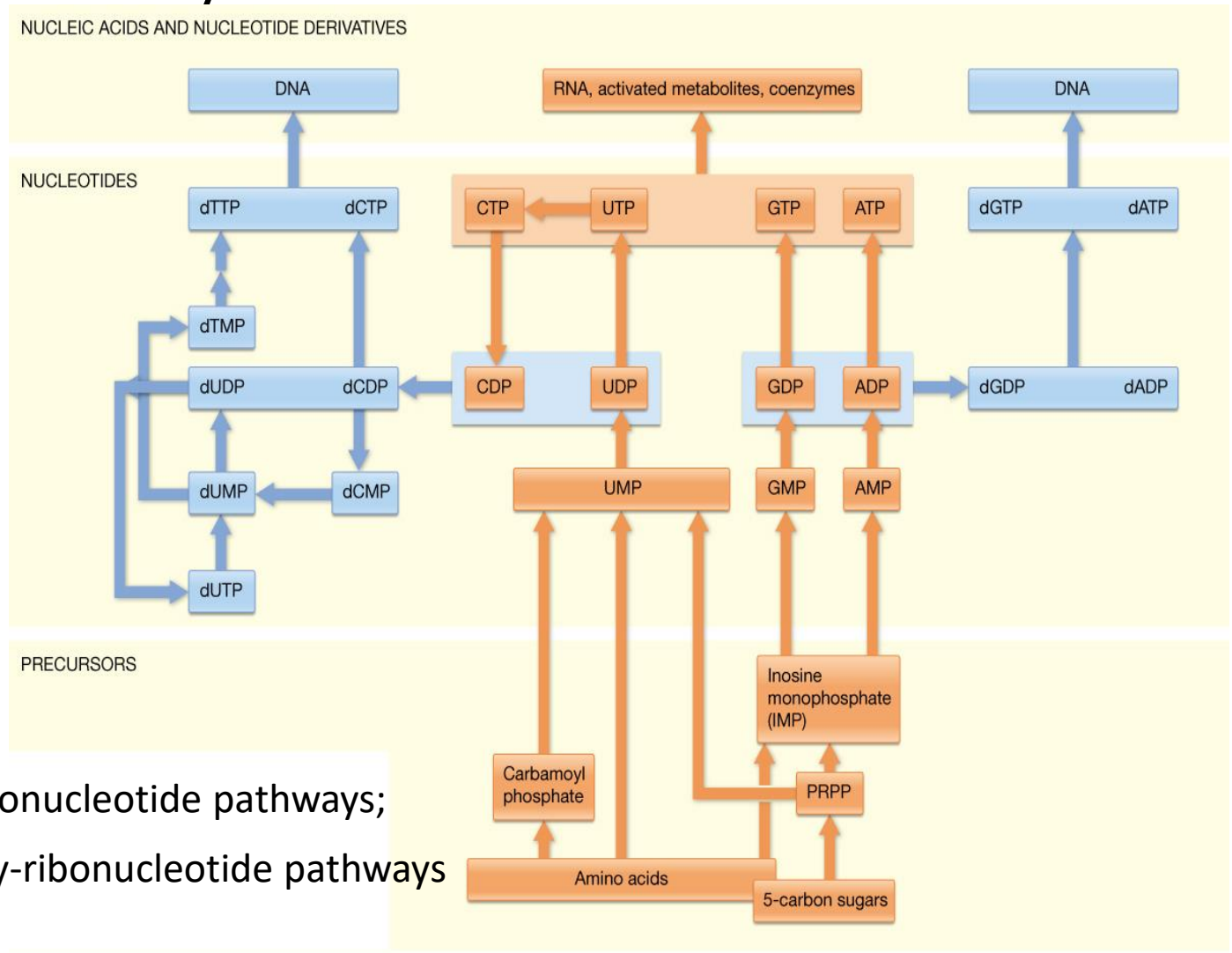



**FIGURE 4.4 Nucleosides and nucleotides.**

# Pathways in Nucleotide Metabolism

- Most organisms can synthesize both purine and pyrimidine nucleotides from low-molecular-weight precursors—amino acids, sugar phosphates, $CO_2$, and so forth. These are called ***de novo*** pathways, meaning they are newly synthesized - largely conserved throughout the biological world.
- Most organisms can also synthesize nucleotides from preformed nucleosides or nucleobases that become available either in the diet or from intracellular nucleic acid turnover - these are called **salvage pathways.**
- Parasites (e.g. malarial parasite) lack *de novo* pathways – this knowledge suggests strategies for treating parasitic diseases

# Pathways for the *De Novo* Nucleotide Synthesis




- Precursors are amino acids, carbamoyl phosphate and the pentose sugar, ribose.
- Ribonucleotides are made first
- NDPs are reduced to deoxyribonucleotide diphosphates

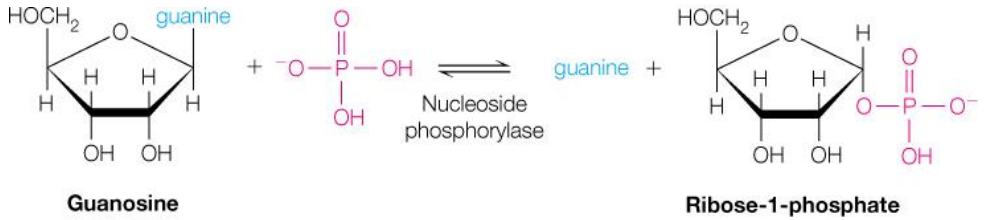
orange = ribonucleotide pathways;

blue = deoxy-ribonucleotide pathways

**FIGURE 19.1 Overview of nucleotide metabolism.**

# Reutilization of Purine and Pyrimidine Bases and Nucleosides

- Salvage, or reuse, of purine and pyrimidine bases involves molecules released by nucleic acid degradation
- Degradation can occur intracellularly (turnover of unstable mRNA species or as a result of DNA damage), as a result of cell death or, in animals, through digestion of nucleic acids in the diet
- Nucleic acids are degraded to oligonucleotides by **endonucleases**
- Further cleavage by **exonucleases** called phosphodiesterases to nucleoside monophosphates
- **Nucleotidases** remove the phosphate group (**nucleoside kinase** catalyses the ATP-dependent phosphorylation of nucleosides)
- **Nucleoside phosphorylase** catalyzes the reversible phosphorolysis of the glycosidic bond



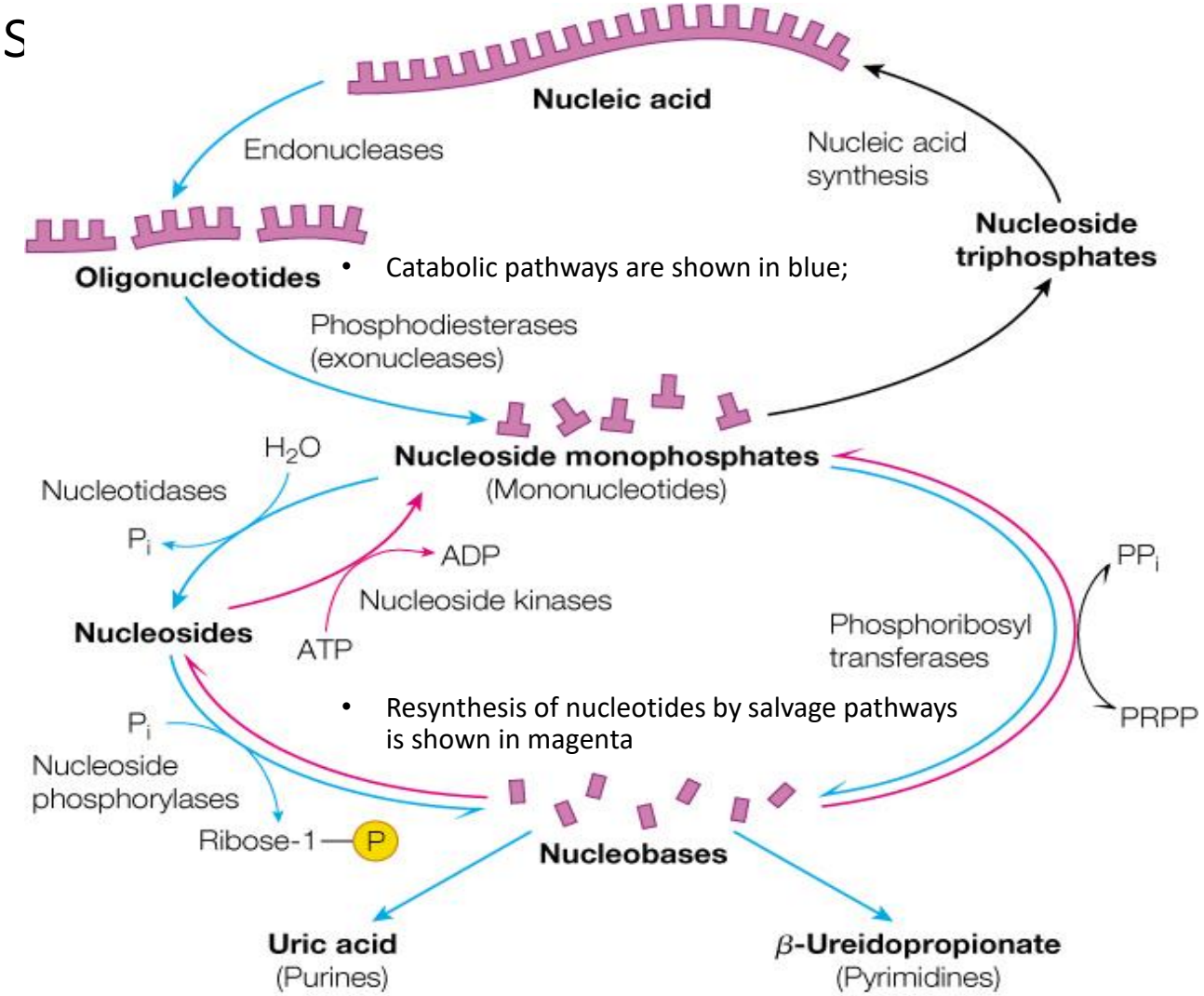
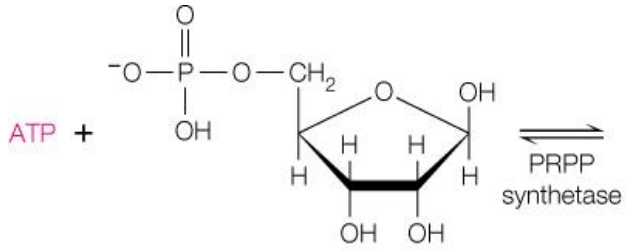



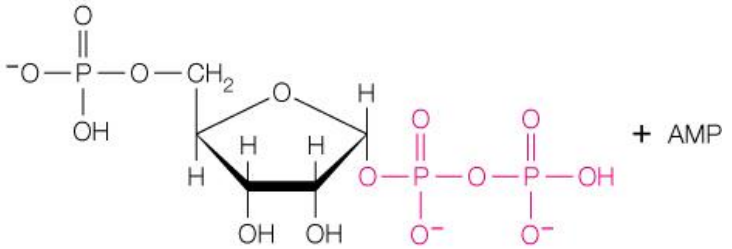

**FIGURE 19.2 Reutilization of purine and pyrimidine bases and nucleosides.**

# PRPP: important for the *De Novo* and Salvage Pathways

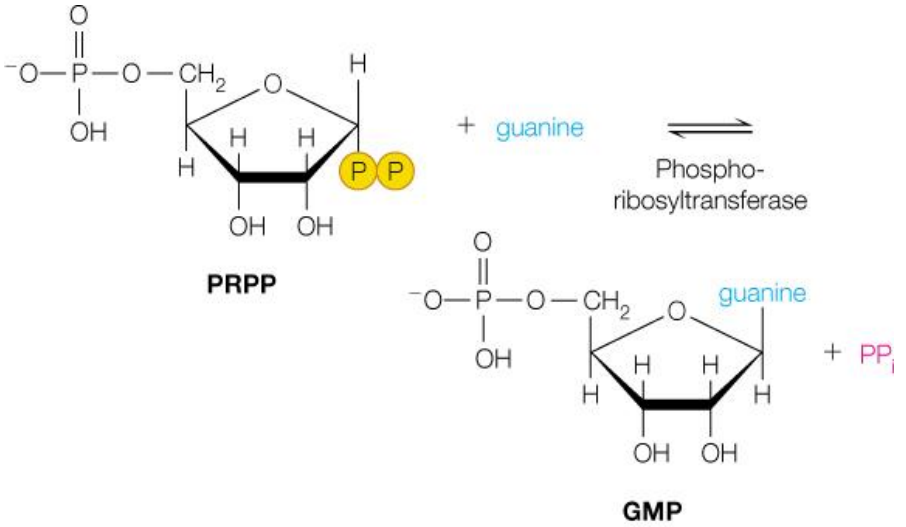
- PRPP: 5’-Phosphoribosyl-α-D-1-Pyrophosphate
- Utilizing nucleobases requires an activated sugar-phosphate donor, PRPP
- PRPP is synthesized from ribose 5-phosphate (R5P) and ATP by PRPP synthetase
- PRPP can then be used by phosphoribosyl transferases to synthesize nucleoside monophosphates




Ribose-5-phosphate



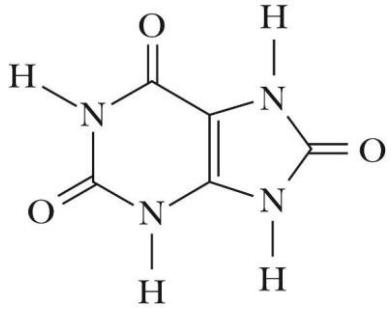

5-Phosphoribosyl-α-D-1-pyrophosphate (PRPP)

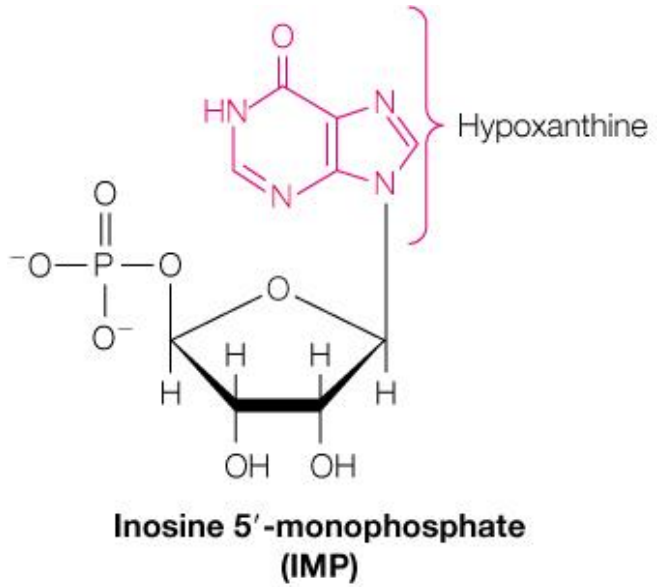



PRPP

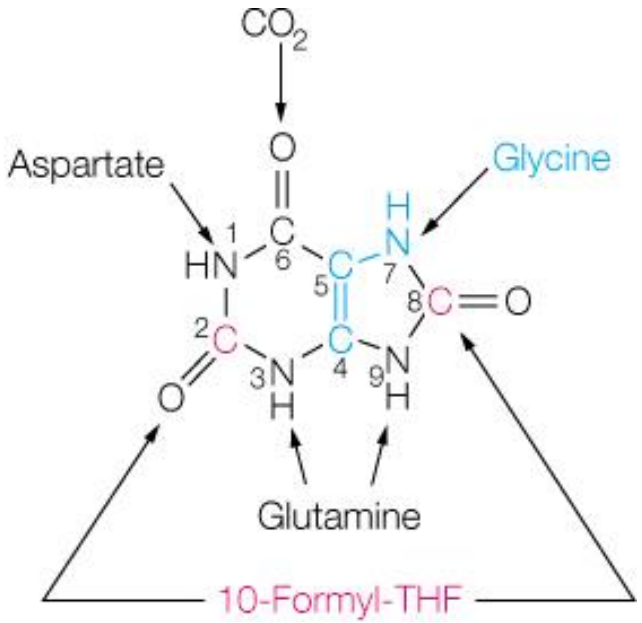
GMP

# *De novo* biosynthesis of Purine Ribonucleotides



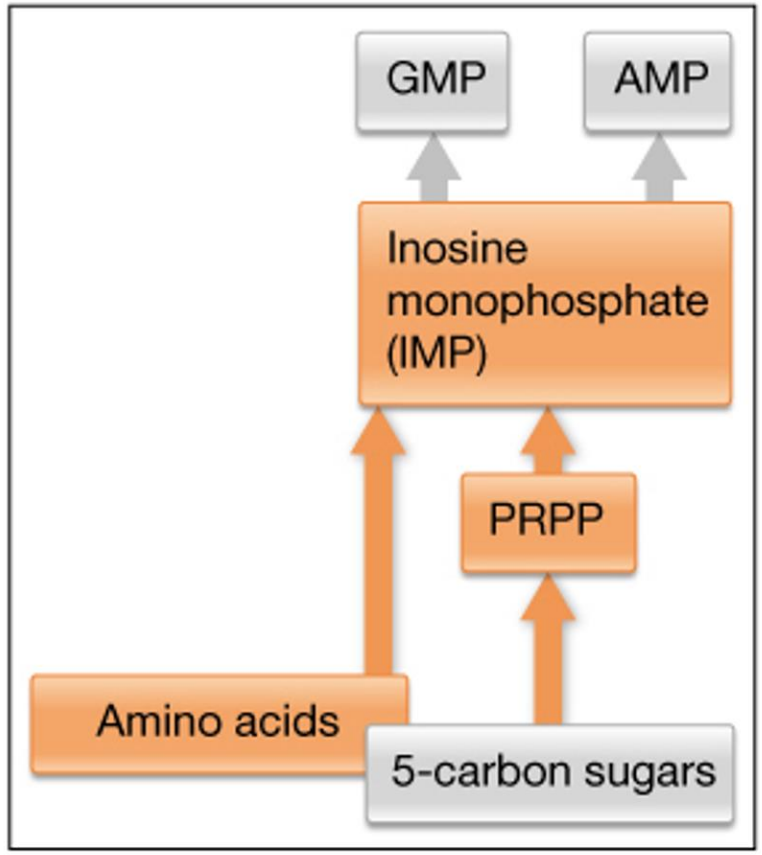



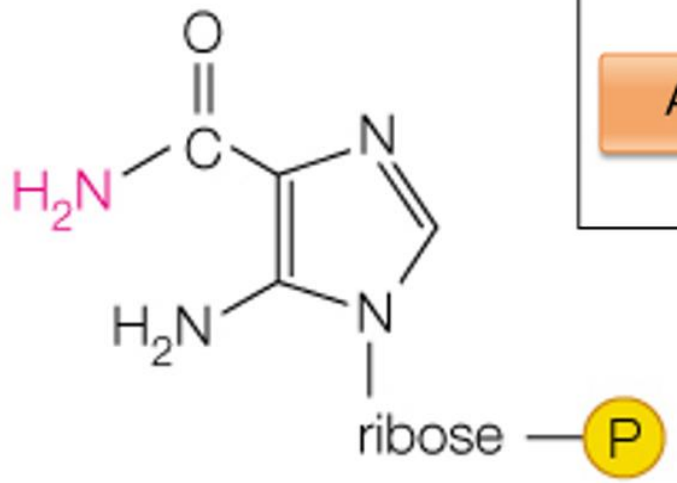

- Inosine 5’-monophosphate (IMP) is the branch point in purine nucleotide synthesis
- AMP (adenylate) and GMP (guanylate) are synthesized from IMP in short, separate pathways
- 10-step pathway from PRPP to IMP, containing the nucleotide base hypoxanthine
- Buchanan (1948) used **uric acid** (purine catabolism product) excreted by birds to trace the pathway
- Isotopically labelled compounds fed to pigeons
  - THF: tetrahydrofolate, a Vit B group coenzyme



# Synthesis of the Purine Nucleotide IMP

- Need PRPP + glutamine, glycine, 10-formyl-THF, aspartate, $CO_2$ + energy from 3 ATPs
- 10-step process to IMP
- Step 8 generates a metabolite called AICAR, which is a precursor for the amino acid, histidine!
- IMP formed is then converted to ATP or GTP (via AMP/GMP) for RNA synthesis
- ATP and GTP can also be reduced to the corresponding deoxynucleotides for DNA synthesis.

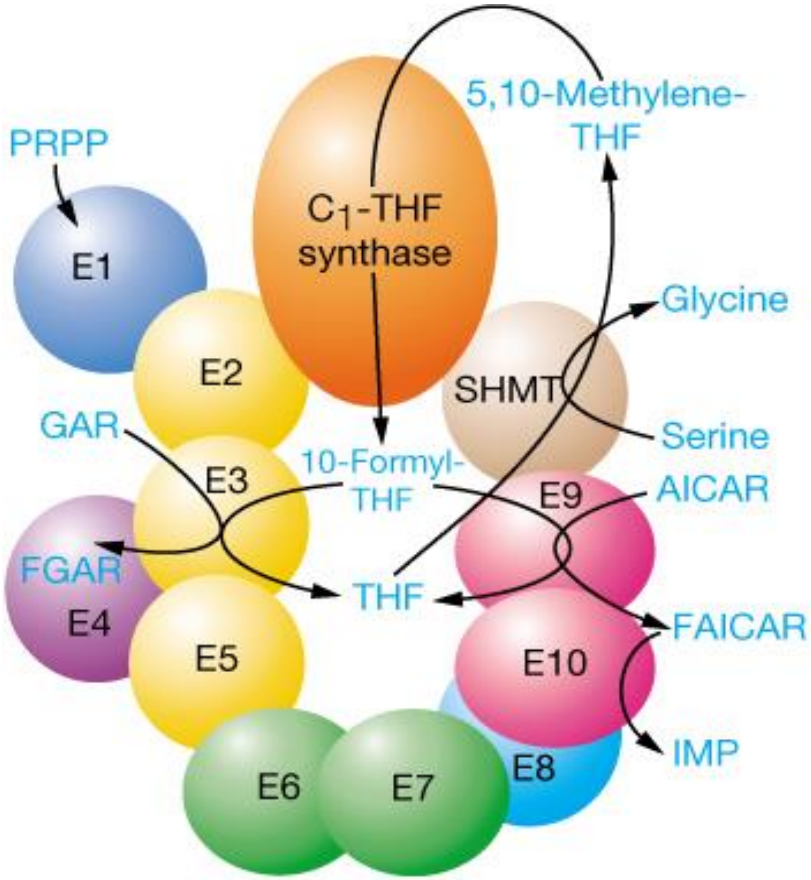



**5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)**

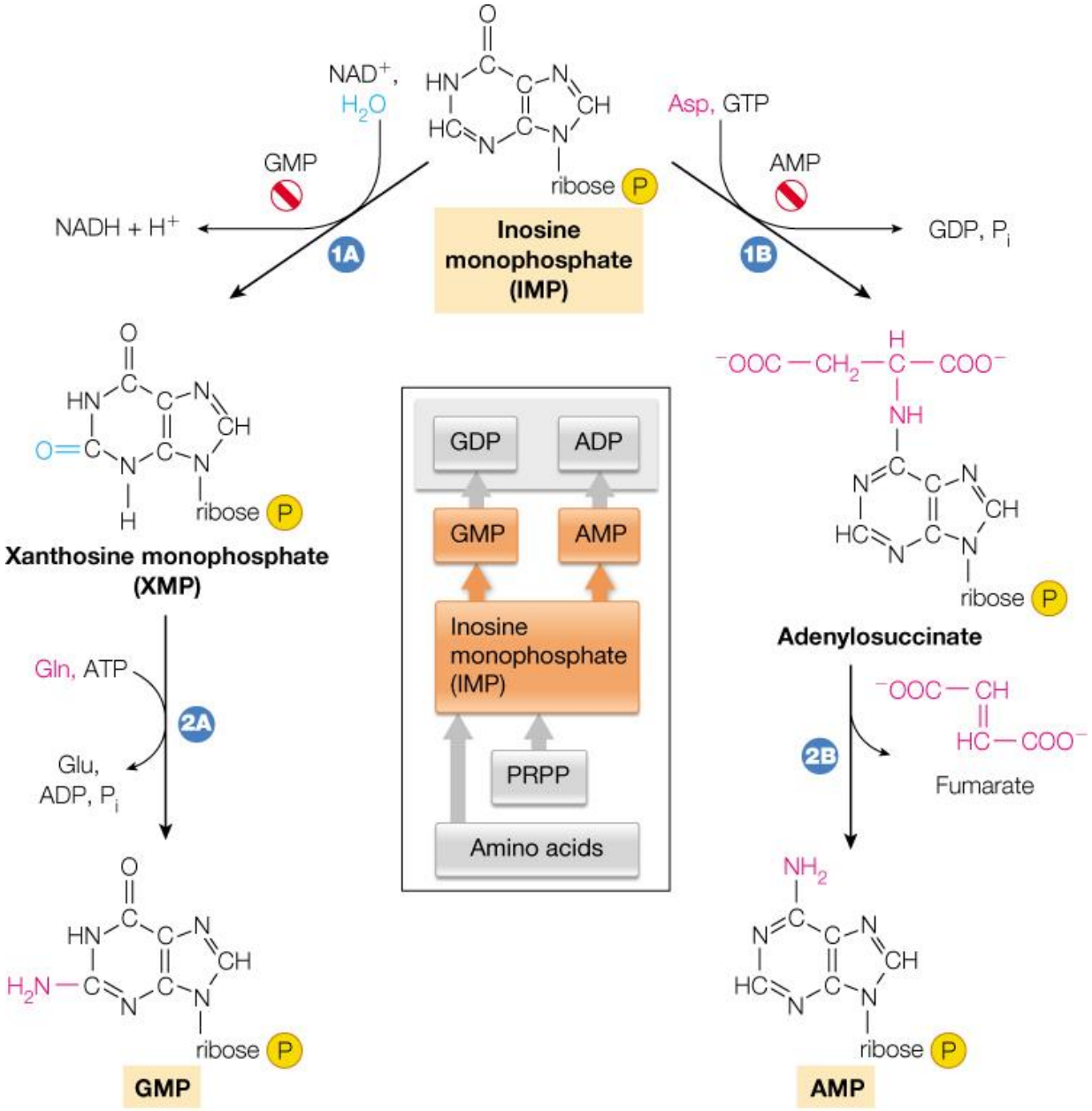
# Enzyme Organization in Purine Biosynthesis the Purinosome

- Example of a multi-enzyme complex!
- Enzyme organization facilitates the flow of intermediates through metabolic pathways
- The ten reactions of the *de novo* purine biosynthesis are catalyzed by six enzymes that associate reversibly into the purinosome



# Pathways from IMP to GMP and AMP

- Activities of the two pathways are linked in two ways:
  - **ATP** is required for ***GMP*** synthesis and **GTP** is required for ***AMP*** synthesis
  - GMP allosterically inhibits the reaction from IMP to XMP and AMP inhibits the conversion of IMP to adenylosuccinate
- These effects combine to promote AMP synthesis when GMP is abundant and vice versa



# Regulation of purine biosynthesis

- Allosteric regulation occurs in the first two steps
  - G- and A-nucleotides inhibit this step at distinct sites
- AMP and GMP are <u>competitive inhibitors</u> of the two branches at the end.

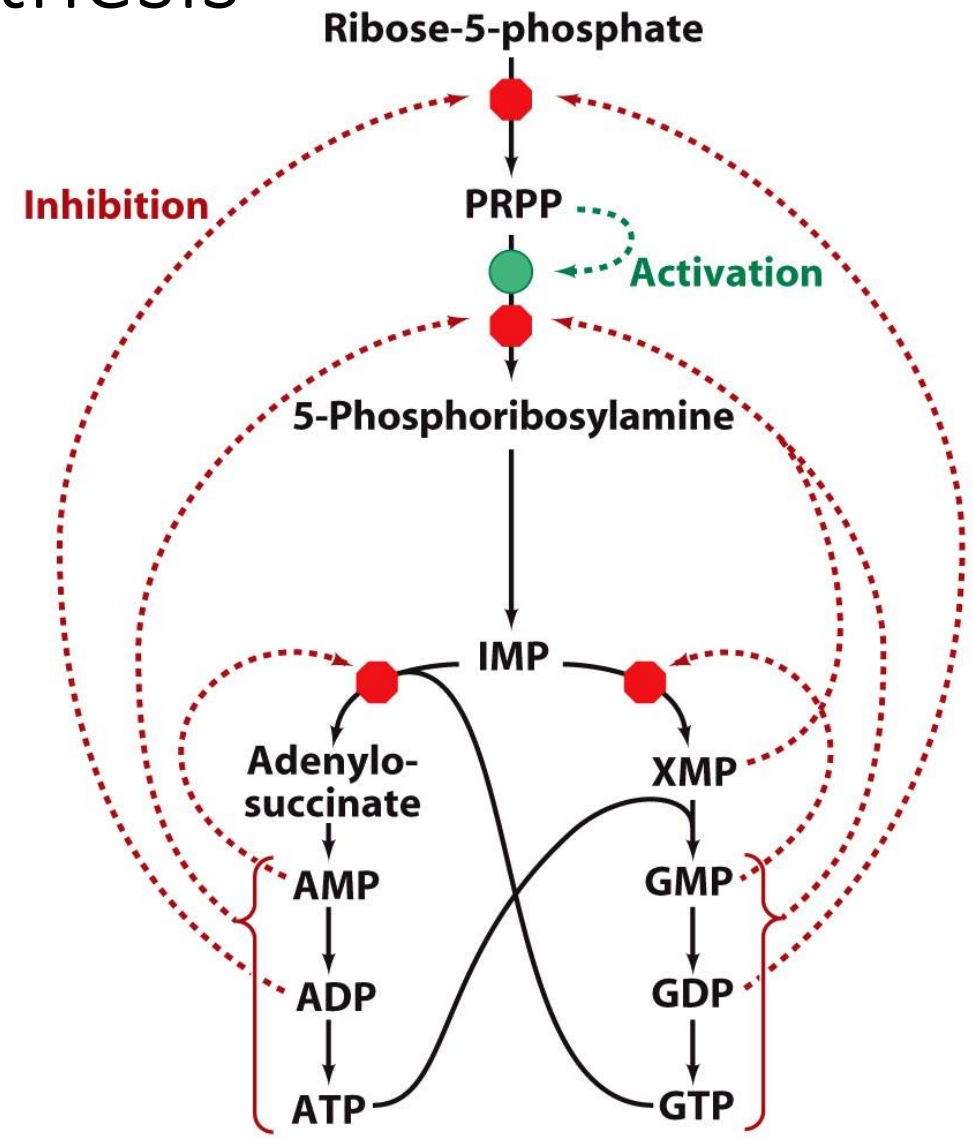
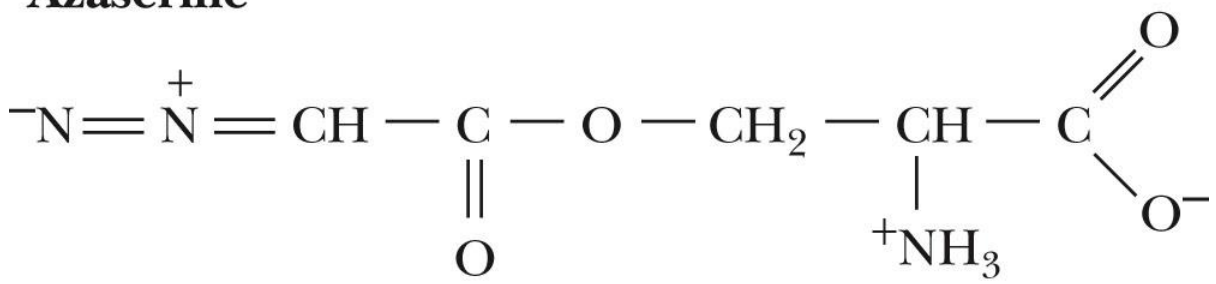



Figure 23-4

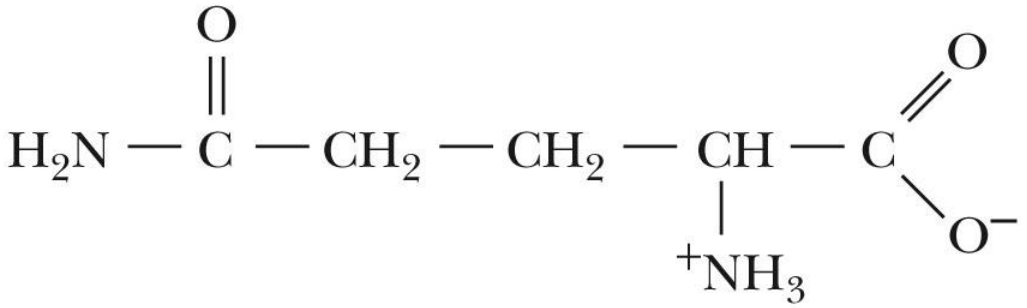
# Azaserine - Gln analog – purine synthesis inhibitor/anti-tumor agent

Azaserine acts as an irreversible inhibitor of glutamine-dependent enzymes by covalently attaching to nucleophilic groups in the glutamine-binding site.

**Azaserine**



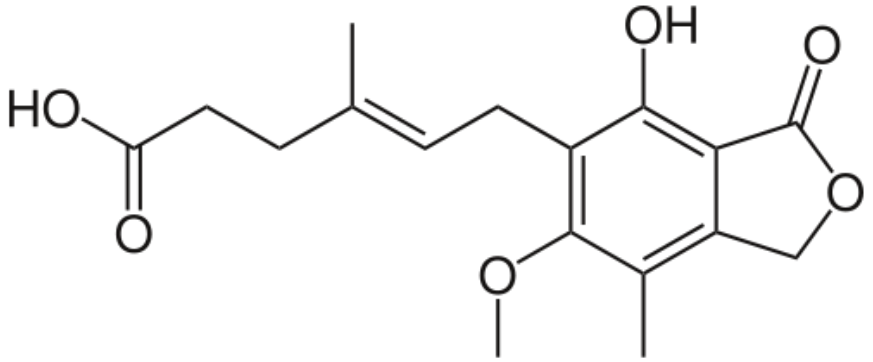
**Glutamine**

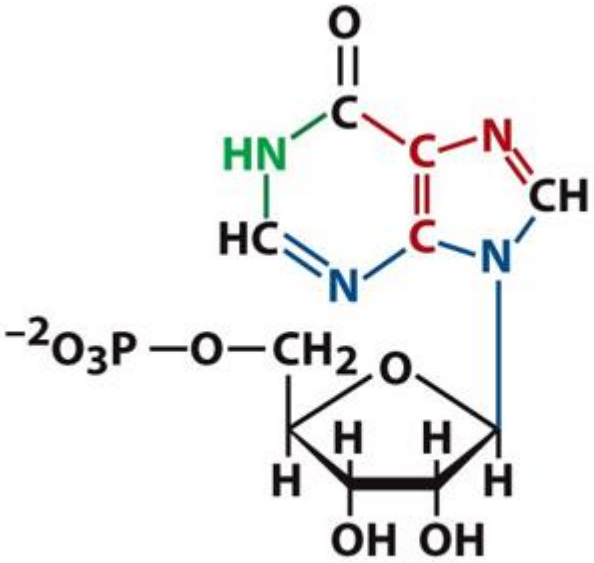


# GMP synthesis enzyme IMP dehydrogenase inhibitor

- The fungal compound, **mycophenolic acid**, acts as an irreversible inhibitor of IMP dehydrogenase.
- Used as an immuno-suppressant in kidney transplants agent

**Mycophenolic acid**



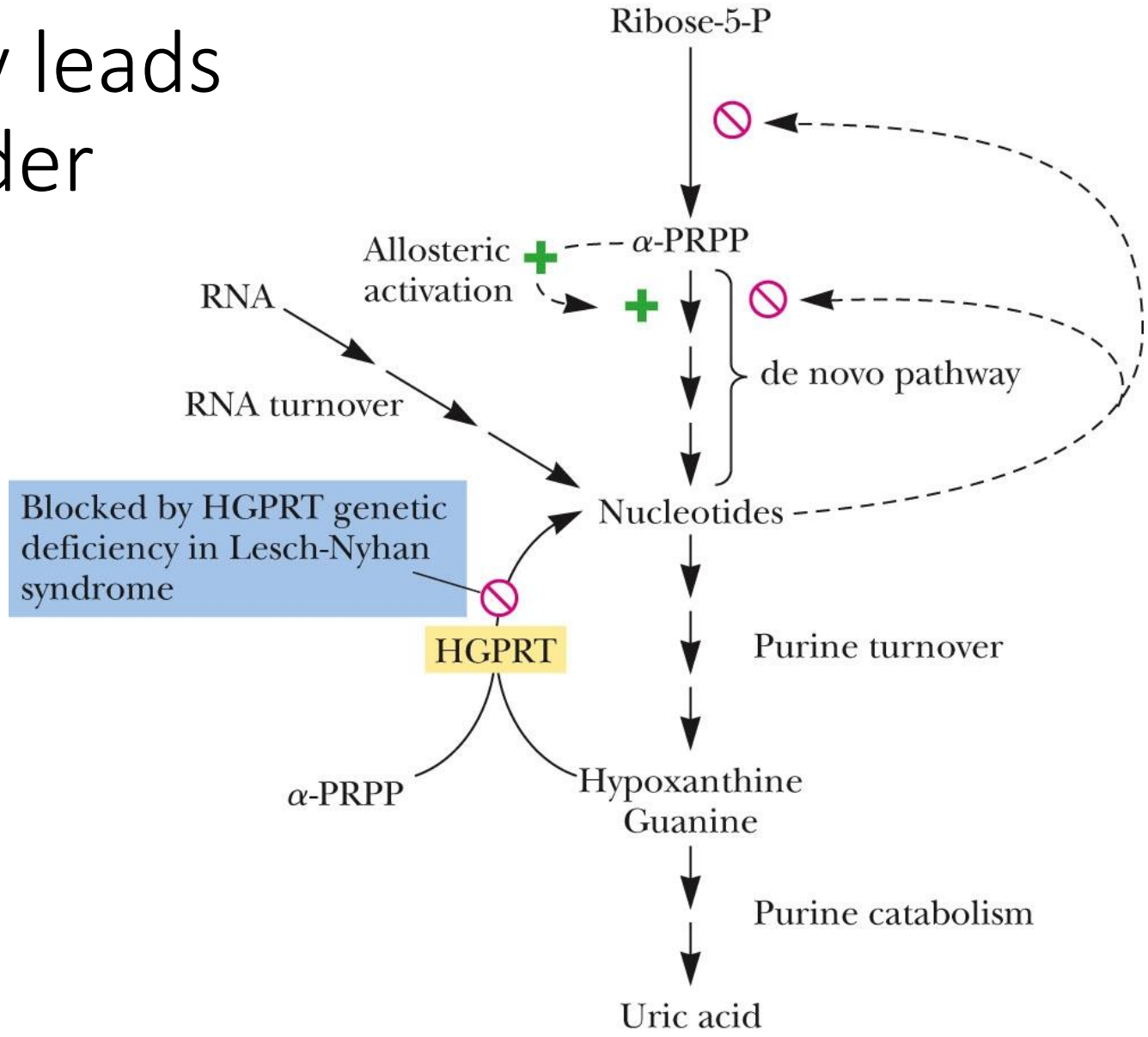

**Inosine monophosphate (IMP)**

# Purine salvage pathways

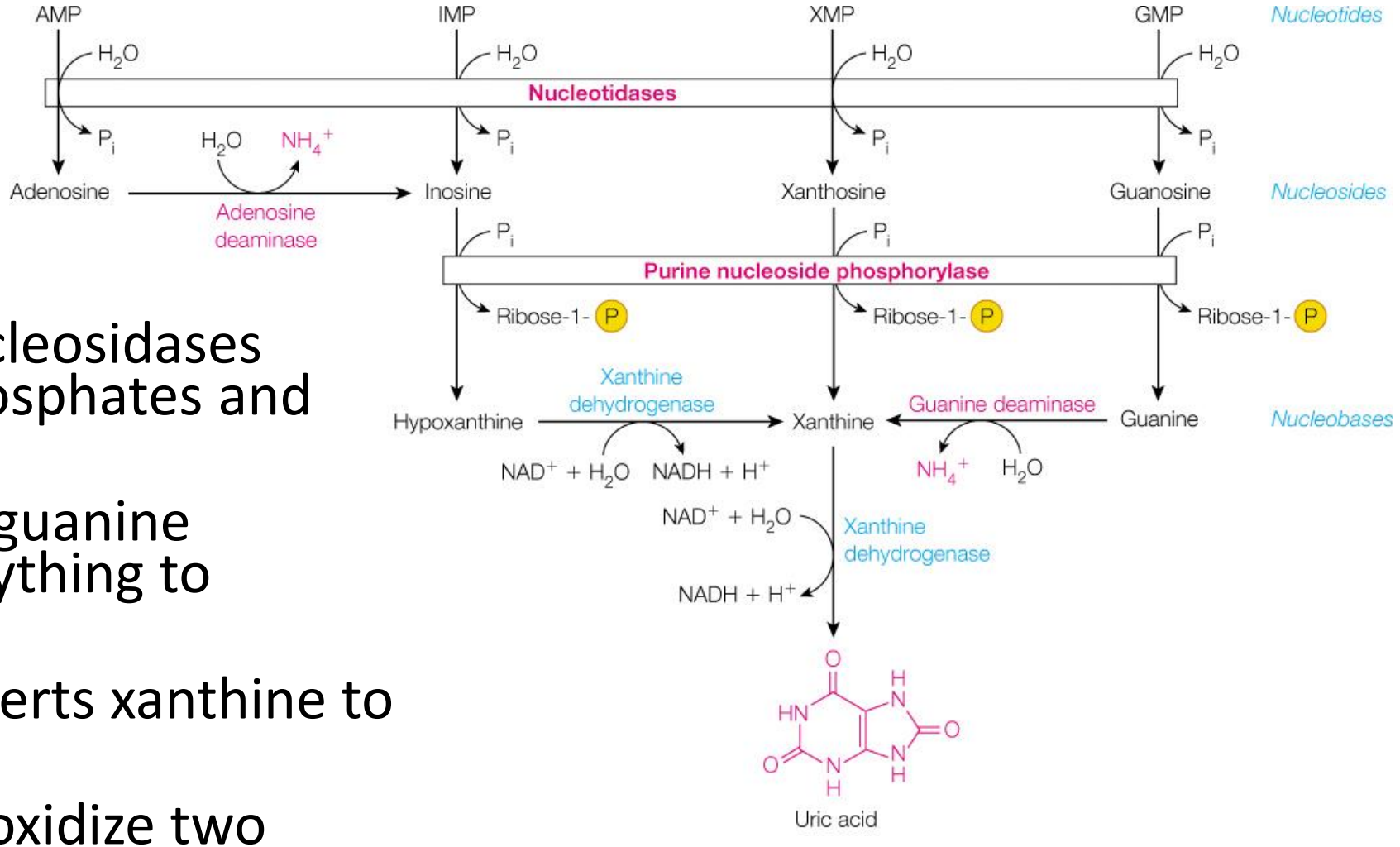
- Nucleic acid turnover (synthesis and degradation) is an ongoing process in most cells
- Salvage pathways collect hypoxanthine and guanine and recombine them with PRPP to form nucleotides in the hypoxanthine-guanine phosphoribotransferase (**HGPRT)** reaction.
- Absence of HGPRT is the cause of Lesch-Nyhan syndrome
  - 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.

# Lesch-Nyhan Syndrome – HGPRT deficiency leads to a severe disorder

Victims of Lesch-Nyhan syndrome experience severe arthritis due to accumulation of uric acid, as well as retardation, and other neurological symptoms.

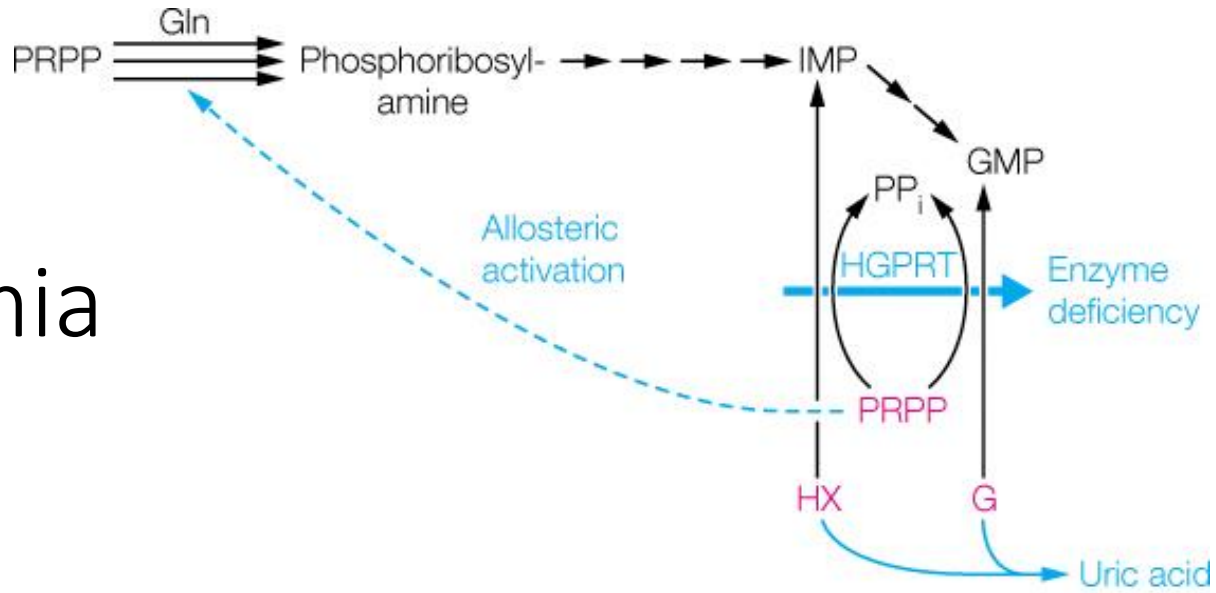


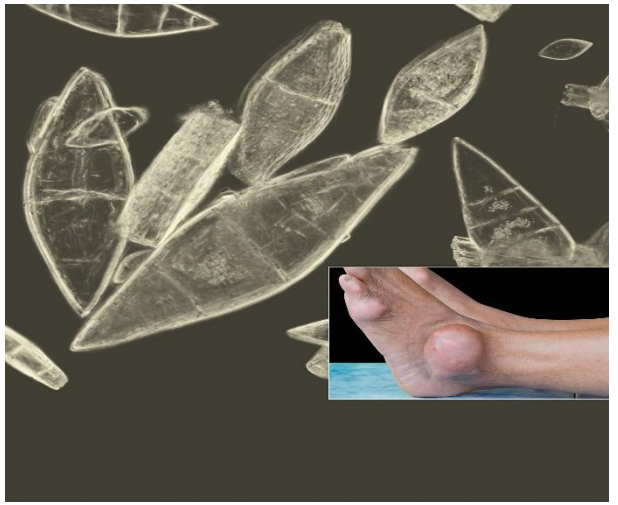
# Purine catabolism leads to uric acid




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

# Hyperuricemia and Gout

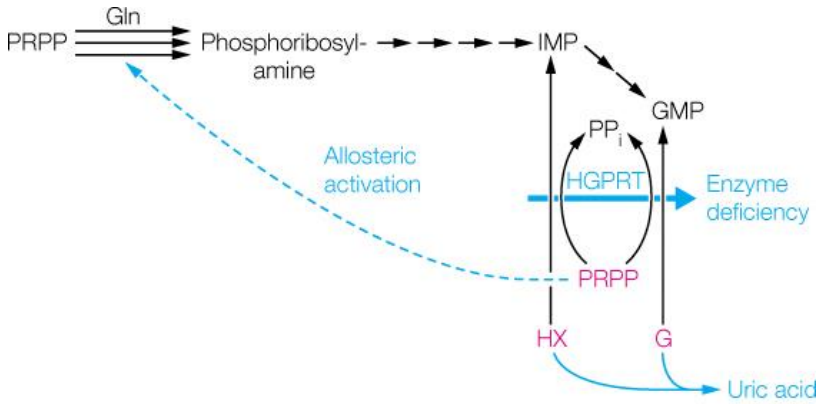


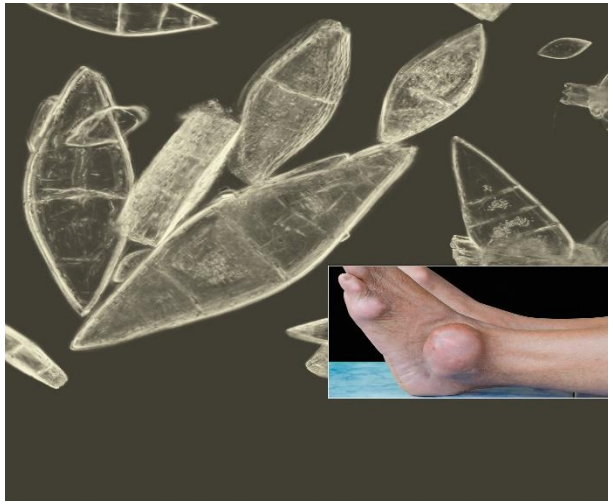



- In humans, purine catabolism ends in the excretion of uric acid, which is a good scavenger of free radicals, but is rather insoluble
- In North American and Europe, about 3 in 1000 individuals suffer from hyperuricemia, a disease characterized by very high uric acid levels in the blood, often due to a deficiency of hypoxanthine-guanine phosphoribosyltransferase (**HGPRT**)
- These very high uric acid levels lead to the formation of uric acid crystals and these crystals accumulate in joints (gout) causing inflammation and arthritis

# Hyperuricemia and Gout (2 of 2)

- These very high uric acid levels lead to the formation of uric acid crystals and these crystals accumulate in joints (gout) causing inflammation and arthritis

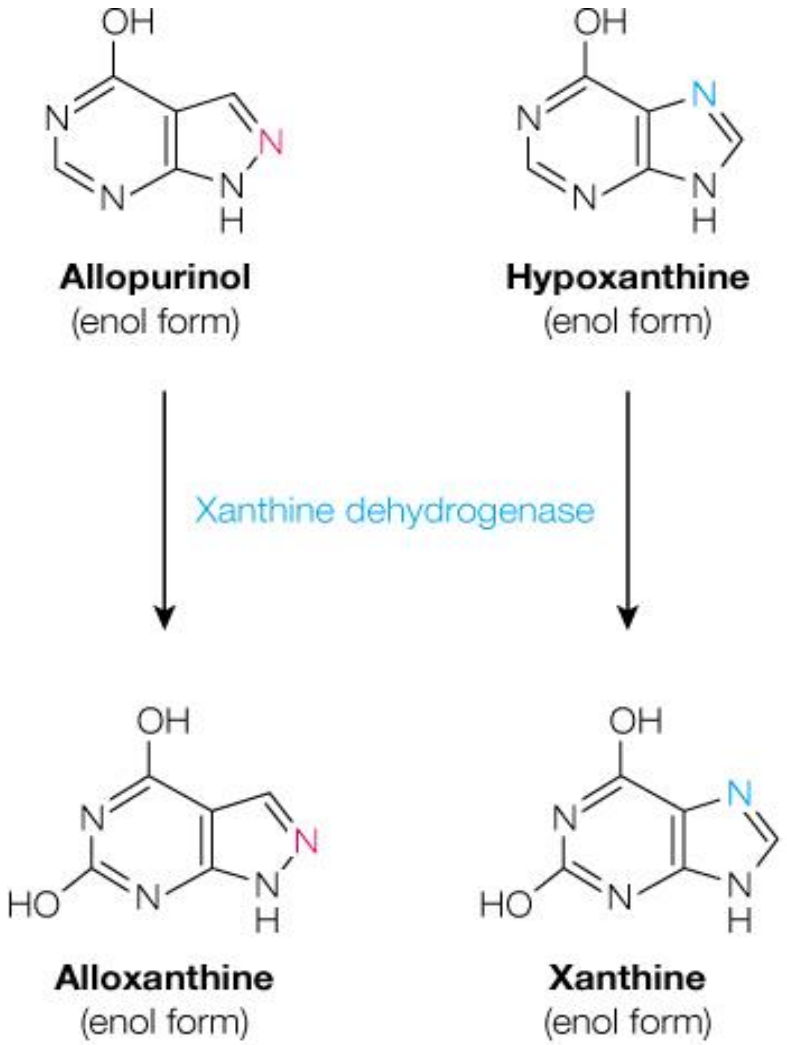


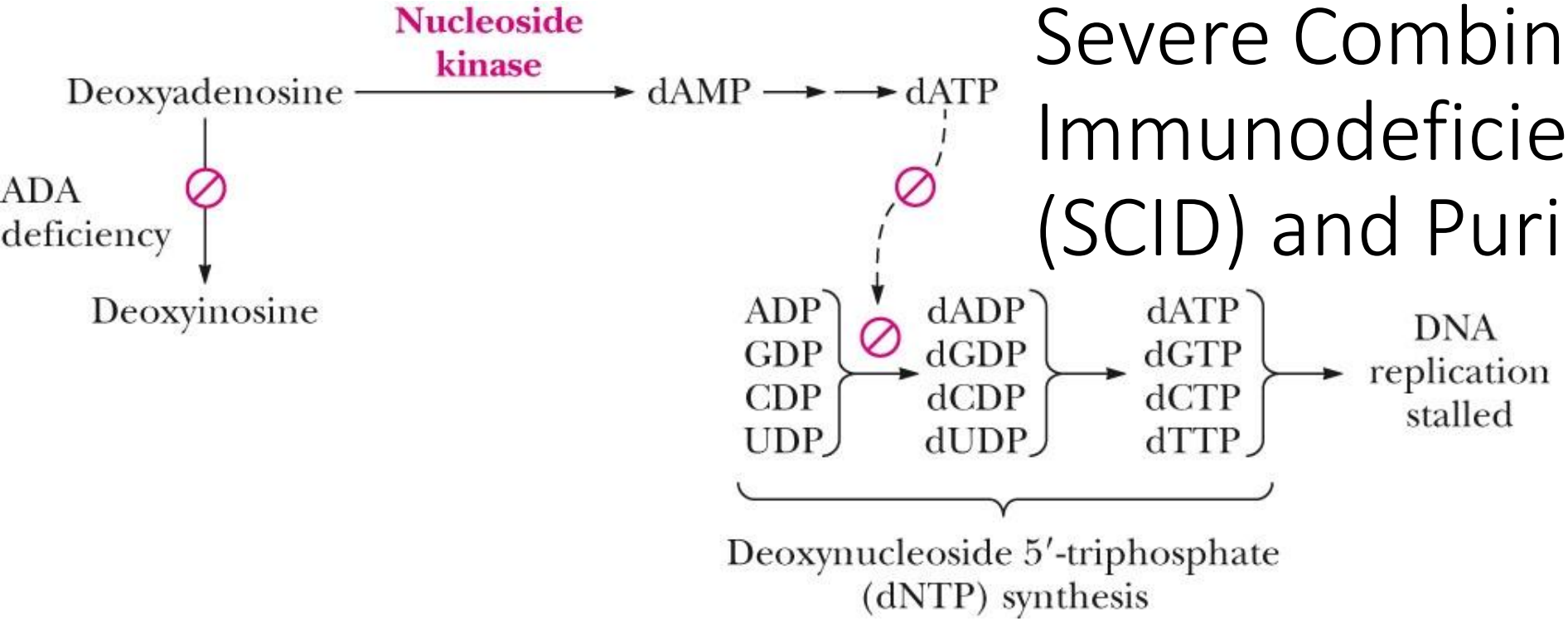
H: Hypoxanthine G: Guanine

# Treatment of Gout with Allopurinol

- Allopurinol is converted to alloxanthine by xanthine dehydrogenase
- Alloxanthine is a suicide inhibitor of xanthine dehydrogenase; it is converted to an active inhibitor by the target enzyme itself



# Severe Combined Immunodeficiency Disease (SCID) and Purine Catabolism




- SCID is a rare hereditary disease marked by an inability to react to immune challenges
- Deficiency of adenosine deaminase (ADA) in purine catabolism
- ADA acts on both adenosine and deoxyadenosine; when ADA is absent, deoxyadenosime accumulates and is converted to dATP, an allosteric inhibitor of ribonucleotide reductase (an enzyme that begins the flow of nucleotides into DNA)
- This causes other dNTPs to disappear and immune cells cannot proliferate
- ADA deficiency was one of the first genetic diseases to be treated by gene therapy

# Summary of Nucleotide Metabolism

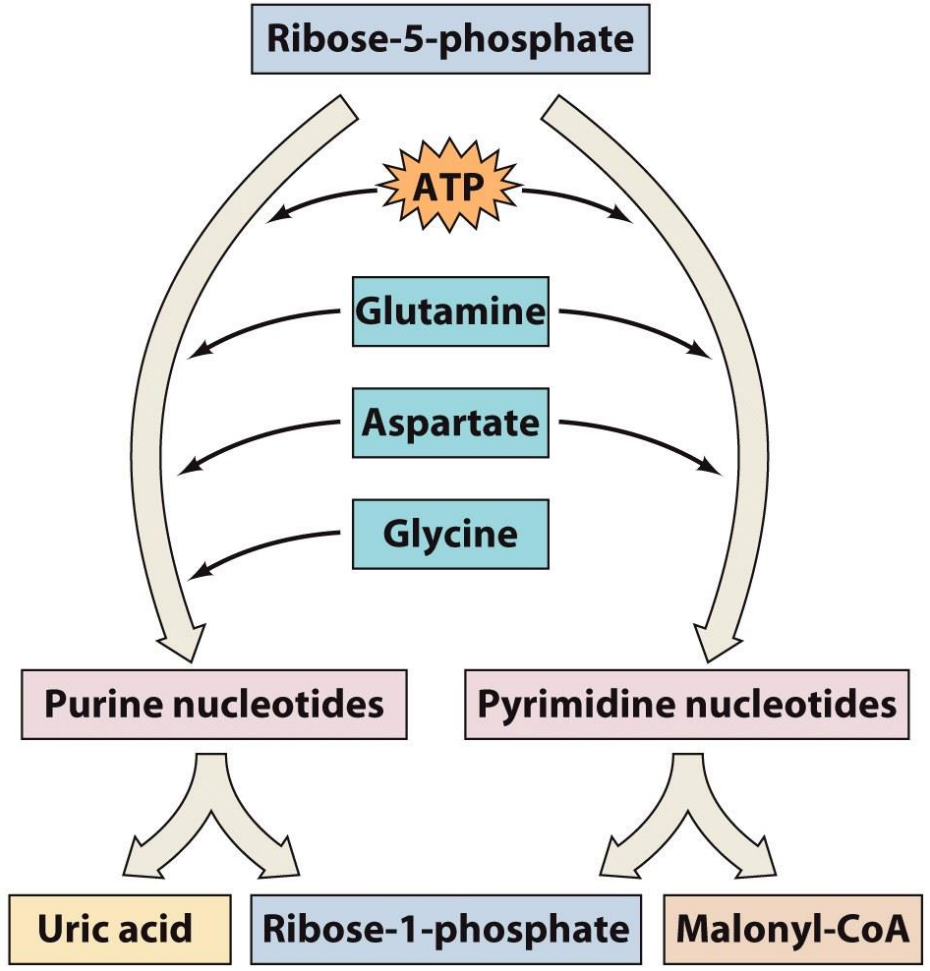
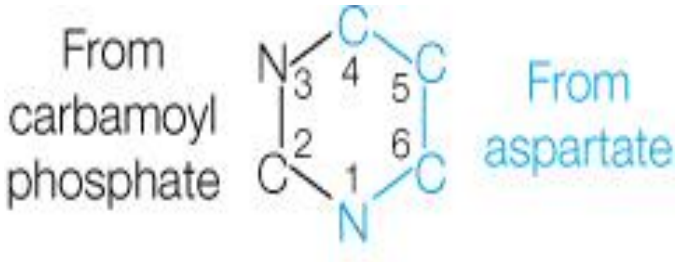
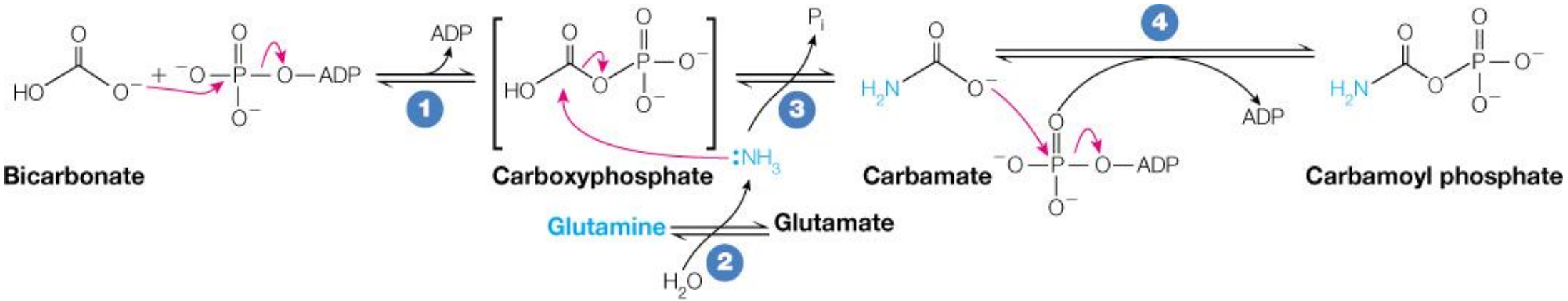



Figure 23-18

# Pyrimidine Synthesis

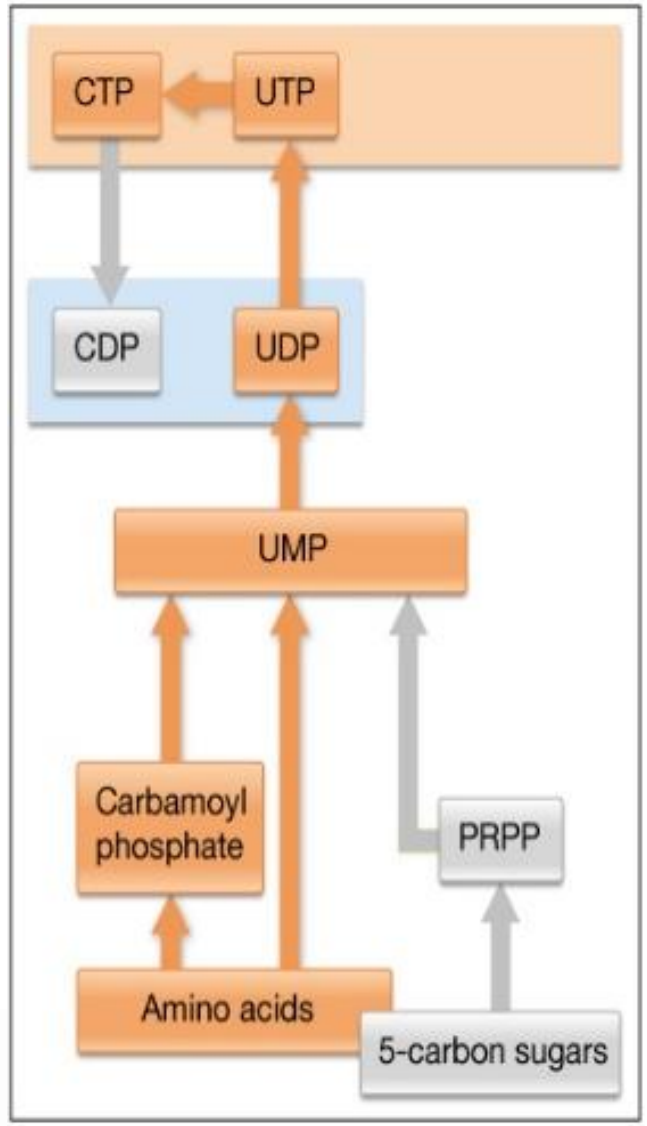


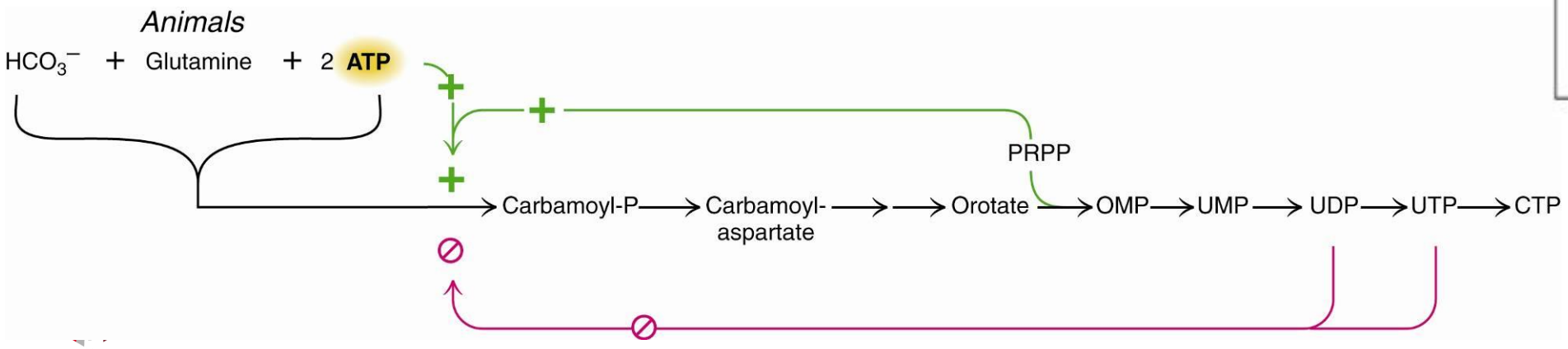

- occurs by an unbranched pathway, at the nucleobase level and with UTP serving as an end product and a precursor to CTP
- All six atoms in the pyrimidine ring are derived from only two precursors:
  - **Aspartate**
  - **Carbamoyl phosphate**
- The synthesis of pyrimidines begins with the synthesis of carbamoyl phosphate
- Carbamoyl phosphate synthetase joins ammonia (released from glutamine) with bicarbonate and phosphate (from ATP) – CPS II in animals



# *De Novo* Synthesis of Pyrimidine Ribonucleotides

- Needs glutamine and $CO_2$,to make carbomoyl phosphate with 2 ATPs
- Then another 5 steps with aspartate, PRPP and 1 ATP to UMP.
- UMP then goes to make other pyrimidine nucleotide phosphates.
- Pyrimidine biosynthesis is regulated at CPS II in animals.



# How Are Pyrimidines Degraded?

- In some organisms, **free pyrimidines are salvaged** and **recycled** to form nucleotides via **phosphoribosyltransferase** reactions
- **In humans**, however, pyrimidines are recycled from **nucleosides**, but free pyrimidine bases are not salvaged
- **Catabolism of cytosine** and uracil yields β-alanine, ammonium ion, and $CO_2$
- **Catabolism of thymine** yields β-aminoisobutyric acid, $NH_4^+$ and $CO_2$

# Making di- (DP) and triphosphates (TP) from nucleotides

- Nucleoside monophosphates are converted into diphosphates by specific *nucleoside monophosphate kinases,* using a phosphate group from ATP.

$$UMP + ATP \rightleftharpoons UDP + ADP$$

- Nucleoside diphosphates are converted into triphosphates by *nucleoside diphosphate kinase,* an enzyme with broad specificity: X, Y below are any of the dinucleosides or even deoxydinuclosides:

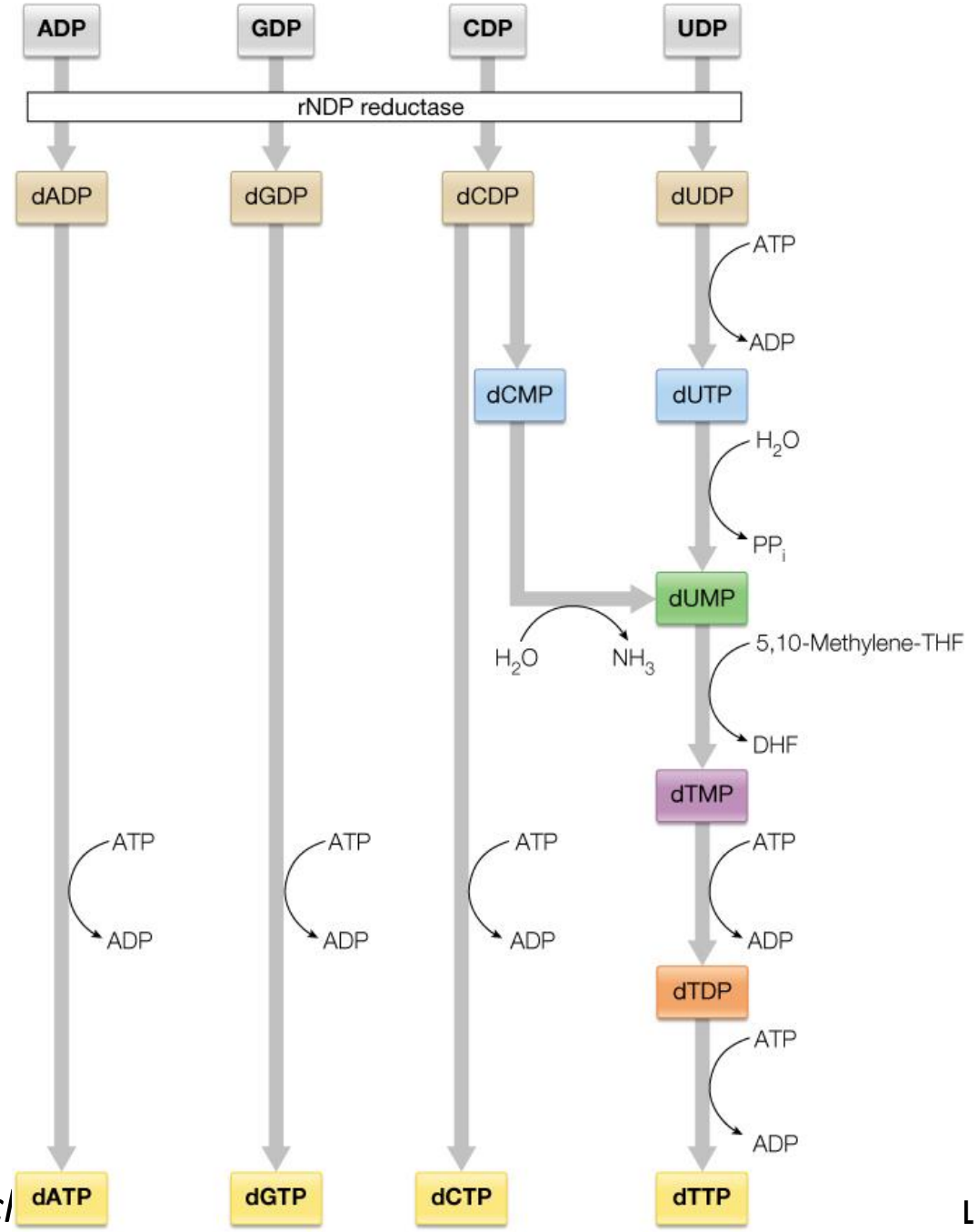
$$XDP + YTP \rightleftharpoons XTP + YDP$$

# Nucleoside 5'-triphosphates are carriers of chemical energy

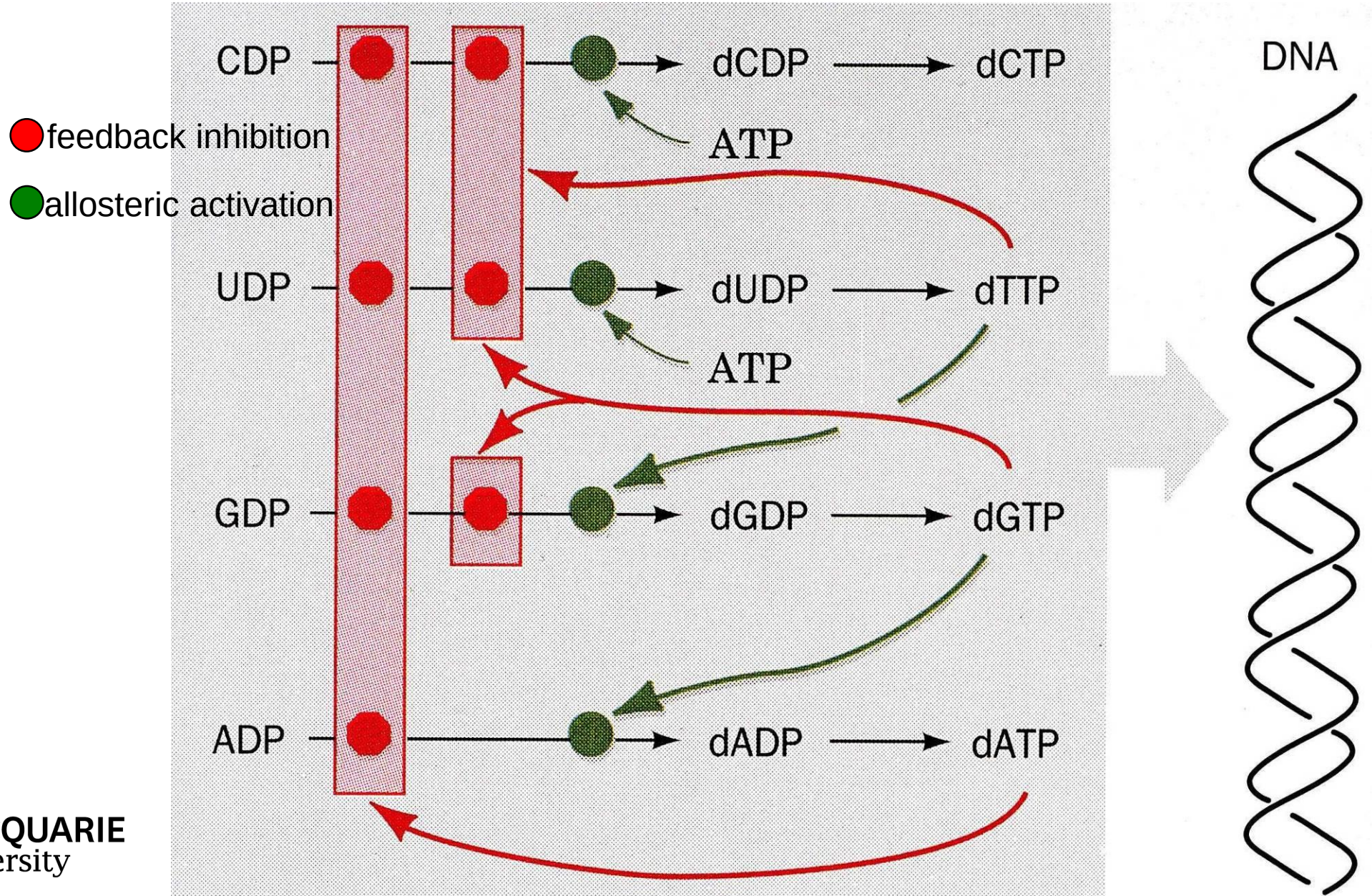
- Nucleoside 5’-triphosphates are indispensable agents in metabolism because their phosphoric anhydride bonds are a source of chemical energy
- Bases serve as recognition units
  - ATP is central to energy metabolism
  - GTP drives protein synthesis
  - CTP drives lipid synthesis
  - UTP drives glycogen synthesis.
- Cyclic nucleotides are signal molecules and regulators of cellular metabolism and reproduction

# Making Deoxyribonucleoside Triphosphates

- Most cells contain 5 to 10 times more RNA than DNA
- Ribonucleotides serve multiple metabolic roles, while deoxyribonucleotides only make up DNA
- Reduction at 2'-position commits nucleotides to DNA synthesis
- Replacement of 2'-OH with hydride is catalyzed by **ribonucleotide reductases**



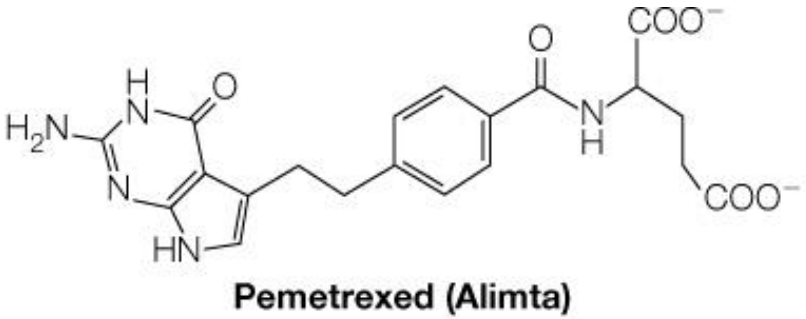
# Control network of dNTP biosynthesis



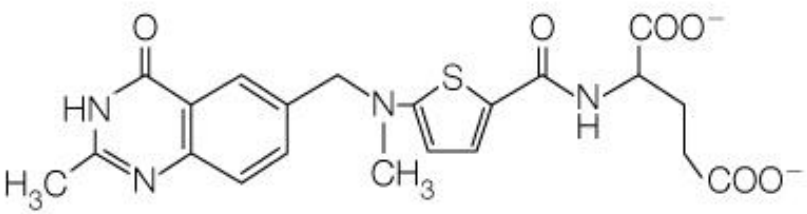

MACQUARIE University

# Structure-Based (Rational) Drug Design

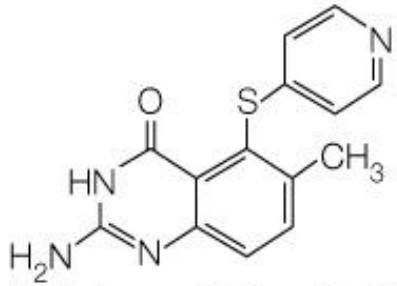
- In 1991 the crystal structure of the human thymidylate synthase enzyme was reported
- Analysis of substrate binding interactions has led to design and synthesis of folate cofactor analogs (**antifolates**)
- Three such inhibitors are shown here: Pemetrexed and raltitrexed are both used clinically to treat certain types of cancer




**Pemetrexed (Alimta)**




**Raltitrexed (Tomudex)**




**Nolatrexed (Thymitaq)**

# Chapter 19 Summary

- Purine and pyrimidine nucleotides arise within cells from nucleic acid breakdown, from reuse (or salvage) of performed nucleosides or nucleobases, or from *de novo* biosynthesis
- Purine nucleotides are synthesized via 5’-phospho-ribosyl-α-D-1-pyrophosphate (PRPP) to inosine monophosphate (IMP), where separate pathways lead to adenine and guanine nucleotides

# Chapter 19 Summary - 2

- Pyrimidine synthesis begins with the formation of carbamoyl aspartate, which is converted in four steps to uridine monophosphate (UMP); UMP is the branching point for distinct routes to cytosine and uridine nucleotides
- In most organisms, the ribonucleoside diphosphates are substrates for reduction of the ribose sugar *in situ*, yielding deoxyribonucleoside diphosphates
- Thymine nucleotides are synthesized from deoxy-uridine nucleotide (dUMP) via a methylene group transfer