Lecture 12 - Nucleotide Biosynthesis

Chem 454: Regulatory Mechanisms in Biochemistry

University of Wisconsin-Eau Claire

Introduction

Nucleotides perform a wide variety of functions

- Building blocks for nucleic acids
- Universal energy carriers (ATP, GTP)
- Activators (*e.g.* UDP-glucose)
- Components of signal transduction pathways (cAMP, cGMP)

Nucleotides contain

- Ribose or deoxyribose sugar
- One to three phosphate groups
- purine or pyrimidine hetercyclic nitrogen base.

Introductions

We will focus on the nucleotide bases

- Glycine and aspartate will provide a carbon scaffold.
- Aspartate and glutamine will provide the nitrogen.

We will look at

- *de novo* synthesis of pyrimidine bases
- *de novo* synthesis of purines bases
- Synthesis of deoxyribonucleotides
- Regulation of nucleotide synthesis

Introduction

de Novo versus salvage pathways

SALVAGE PATHWAY

Activated ribose (PRPP) + base

↓

Nucleotide

DE NOVO PATHWAY

Activated ribose (PRPP) + amino acids + ATP + CO_2 + . . .

↓

Nucleotide

Introduction

Nomenclature

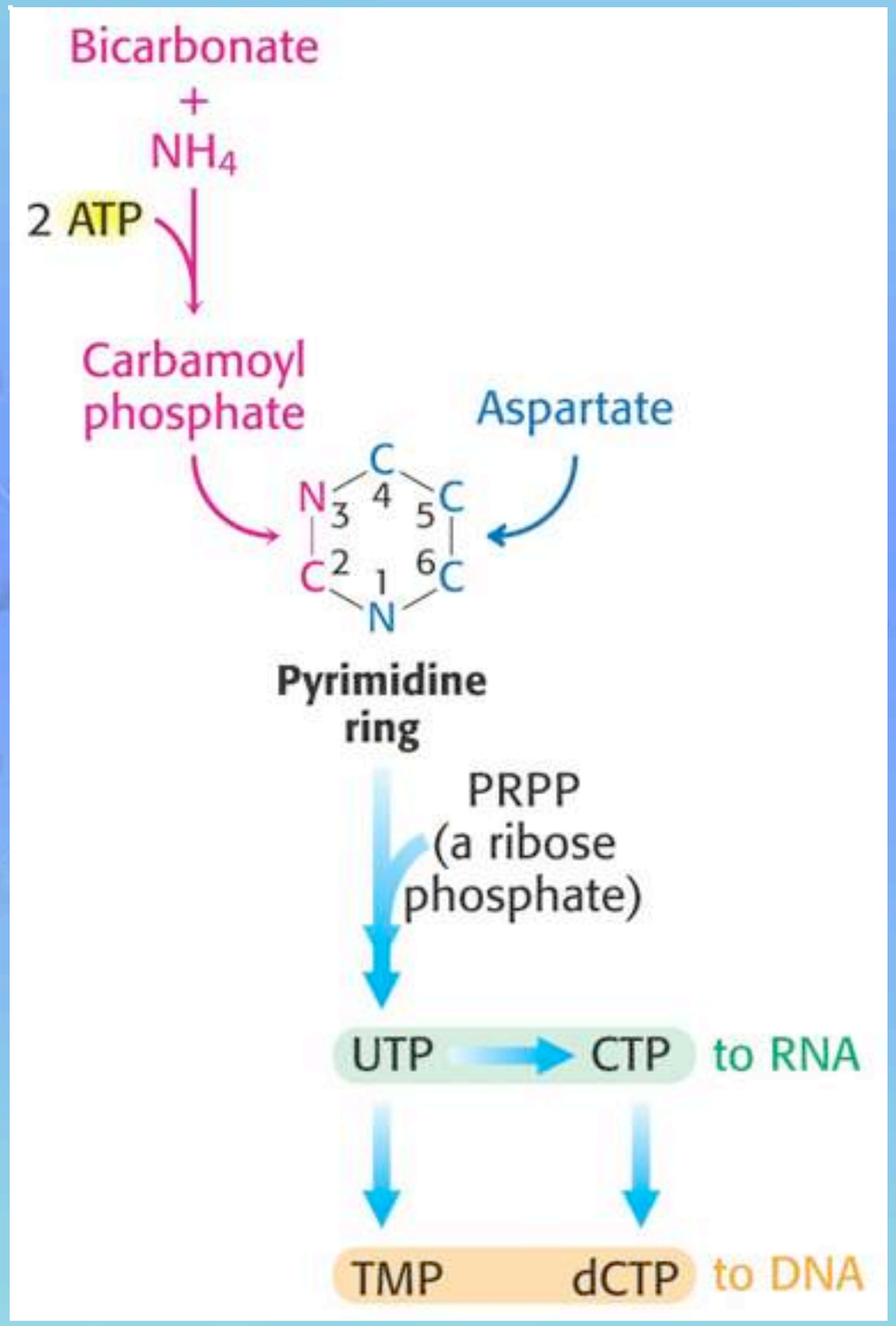
TABLE 25.1 Nomenclature of bases, nucleosides, and nucleotides

RNA		
Base	Ribonucleoside	Ribonucleotide (5′-monophosphate)
Adenine (A)	Adenosine	Adenylate (AMP)
Guanine (G)	Guanosine	Guanylate (GMP)
Uracil (U)	Uridine	Uridylate (UMP)
Cytosine (C)	Cytidine	Cytidylate (CMP)
DNA		
Base	Deoxyribonucleoside	Deoxyribonucleotide (5′-monophosphate)
Adenine (A)	Deoxyadenosine	Deoxyadenylate (dAMP)
Guanine (G)	Deoxyguanosine	Deoxyguanylate (dGMP)
Thymine (T)	Thymidine	Thymidylate (TMP)
Cytosine (C)	Deoxycytidine	Deoxycytidylate (dCMP)

1. *de Novo* Synthesis of Pyrimidines

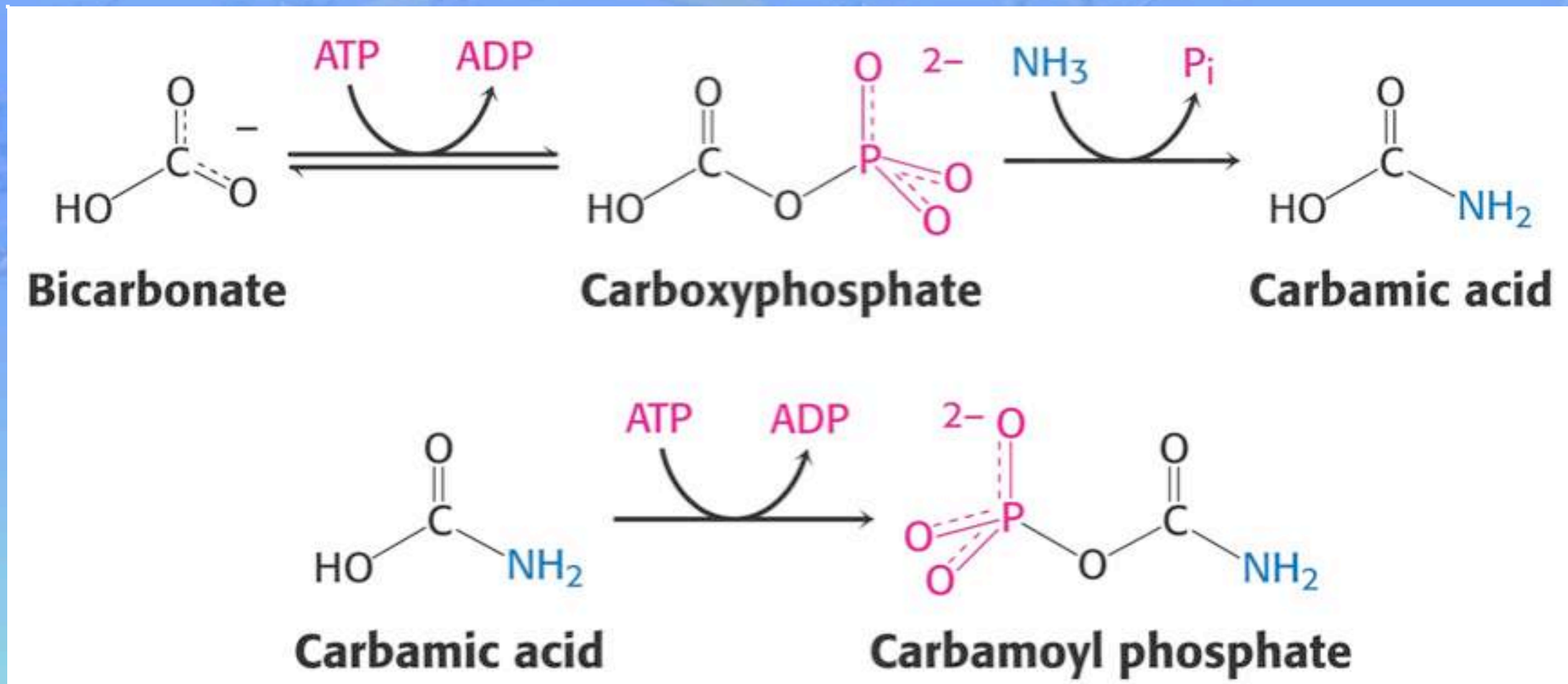
The ring is assembled from bicarbonate, aspartate and glutamate.

- The ring is synthesized first and then added to the ribose.
- The ammonia is produced from the hydrolysis of glutamine



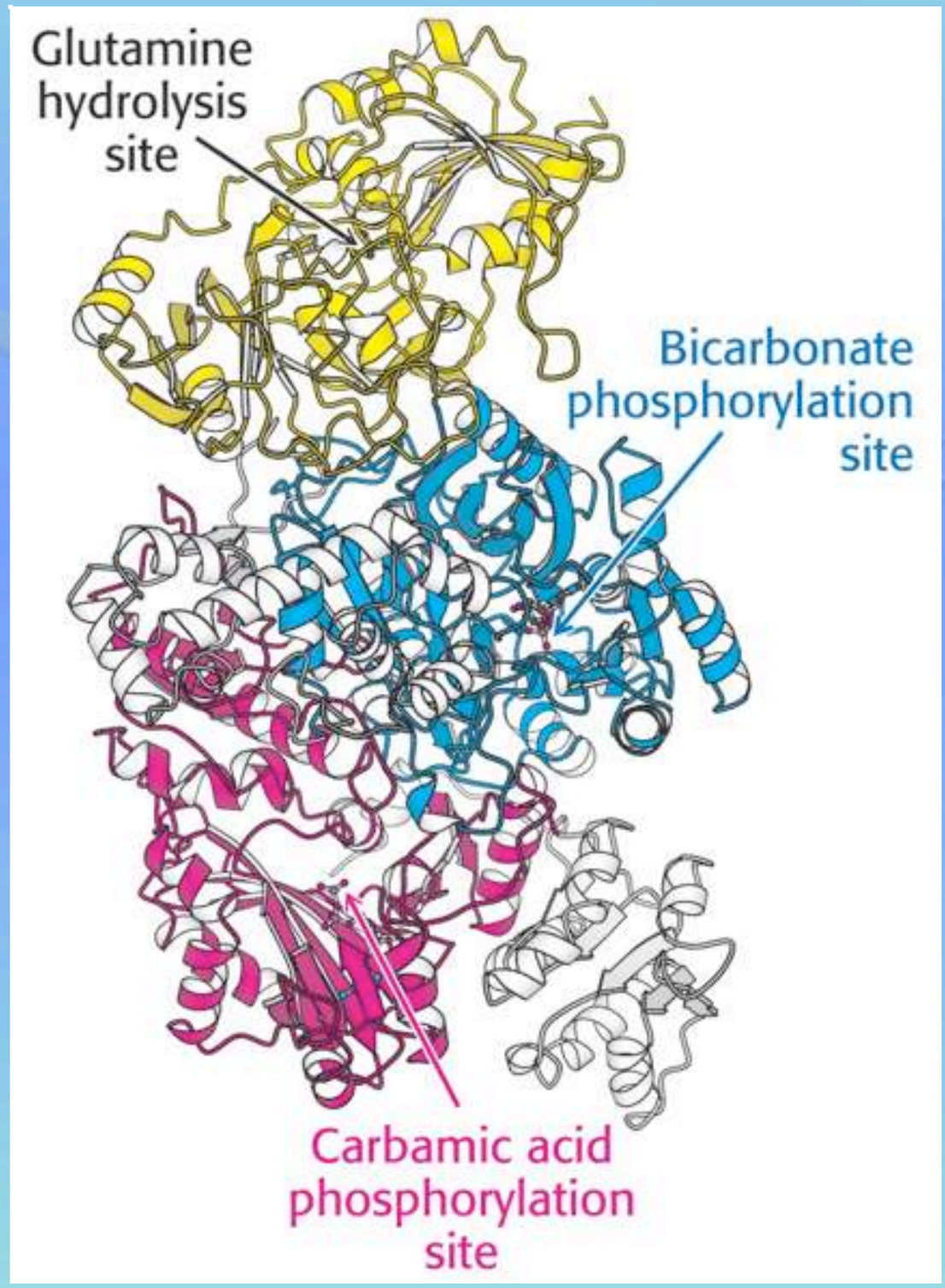
1.1 Pyrimidine Synthesis, First Step

Carbarmoyl phosphate is synthesized from bacarbonate and ammonia



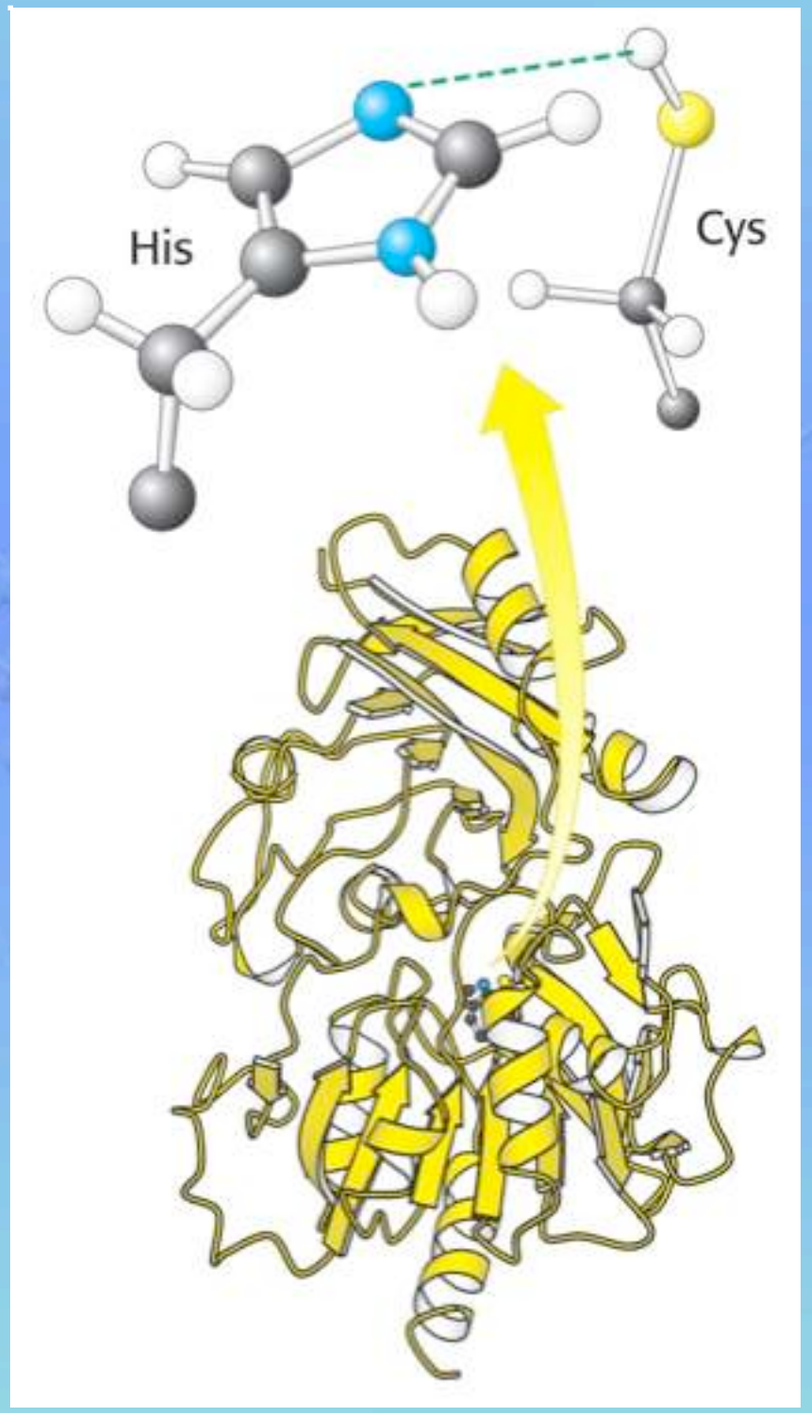
1.1 Pyrimidine Synthesis, First Step

Carbamoyl phosphate synthetate



1.2 Glutamine Hydrolysis

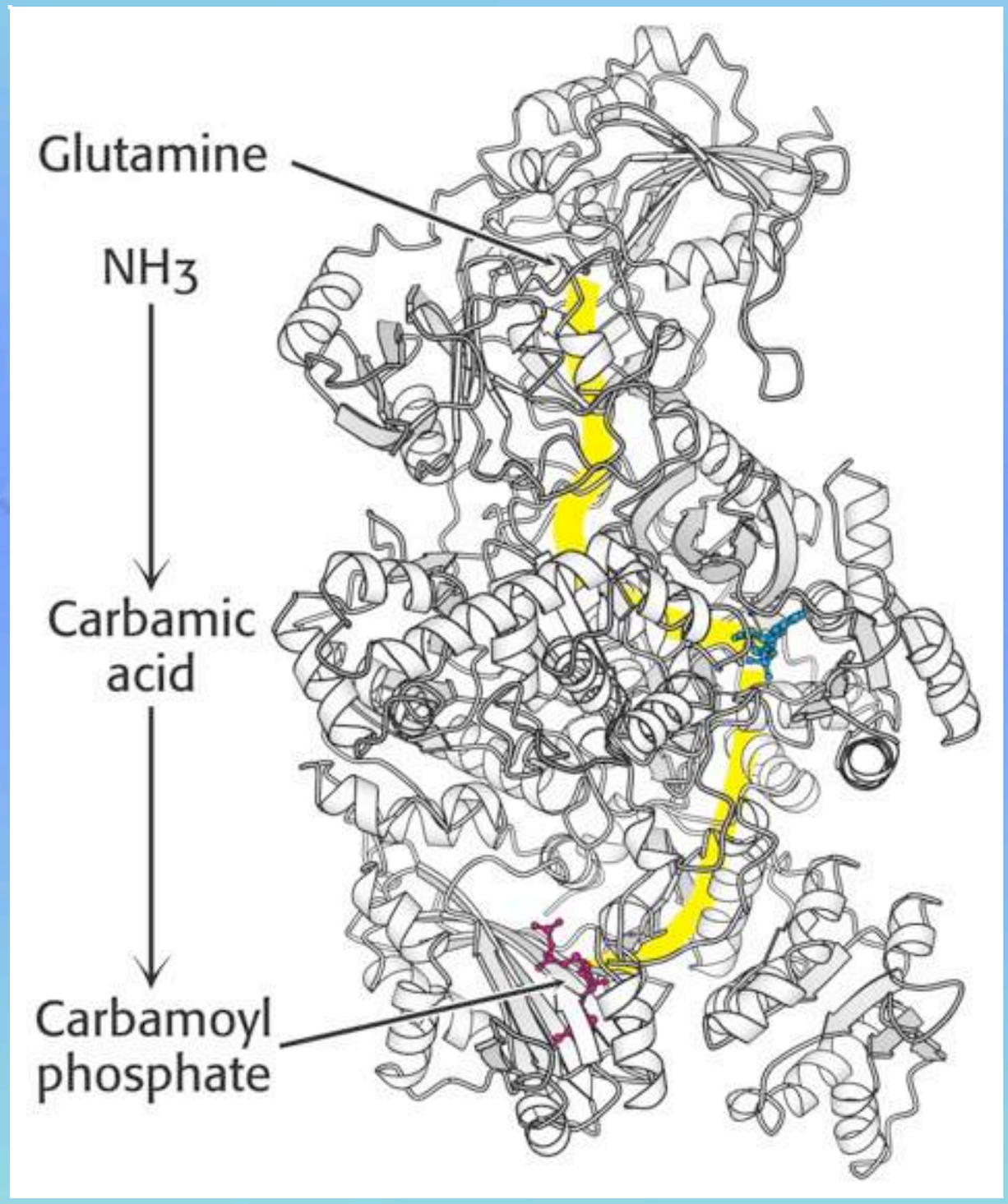
Carbamoyl phosphate synthetase also contains a glutamine hydrolysis domain



1.3 Substrate Channeling

The ammonia is channeled 45Å to the carboxyphosphate

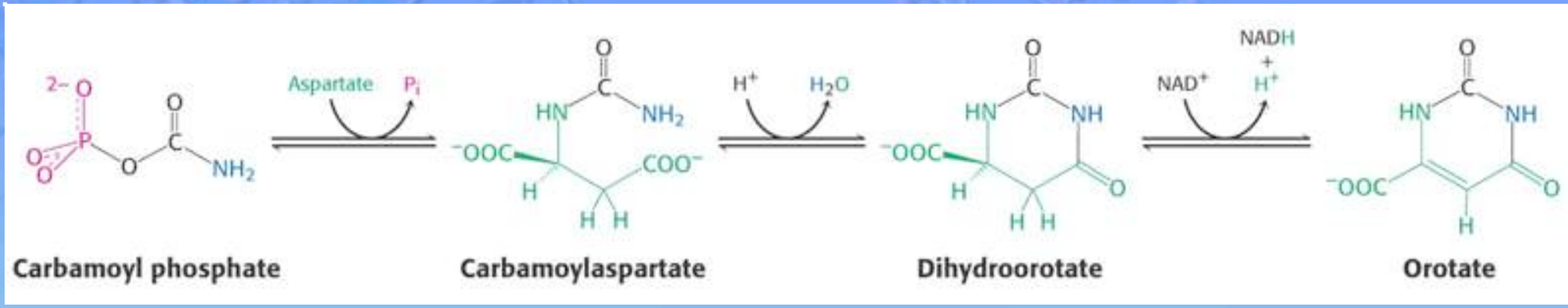
- The carbamic acid is channeled another 35Å to the site where it is phosphorylated



1.4 Pyrimidine Synthesis, Second Step

Synthesis of Orotate and attachment to ribose ring.

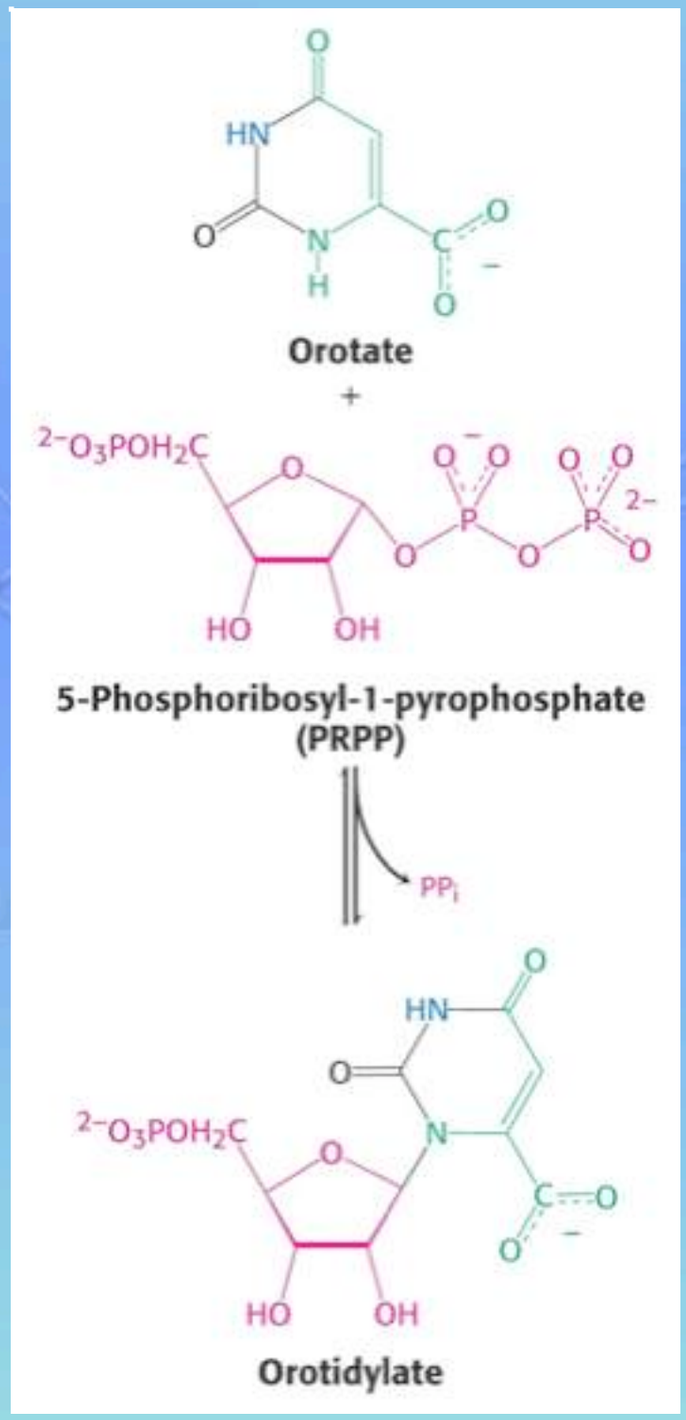
- The first reaction is catalyzed by aspartate transcarbamylase



1.4 Pyrimidine Synthesis, Second Step

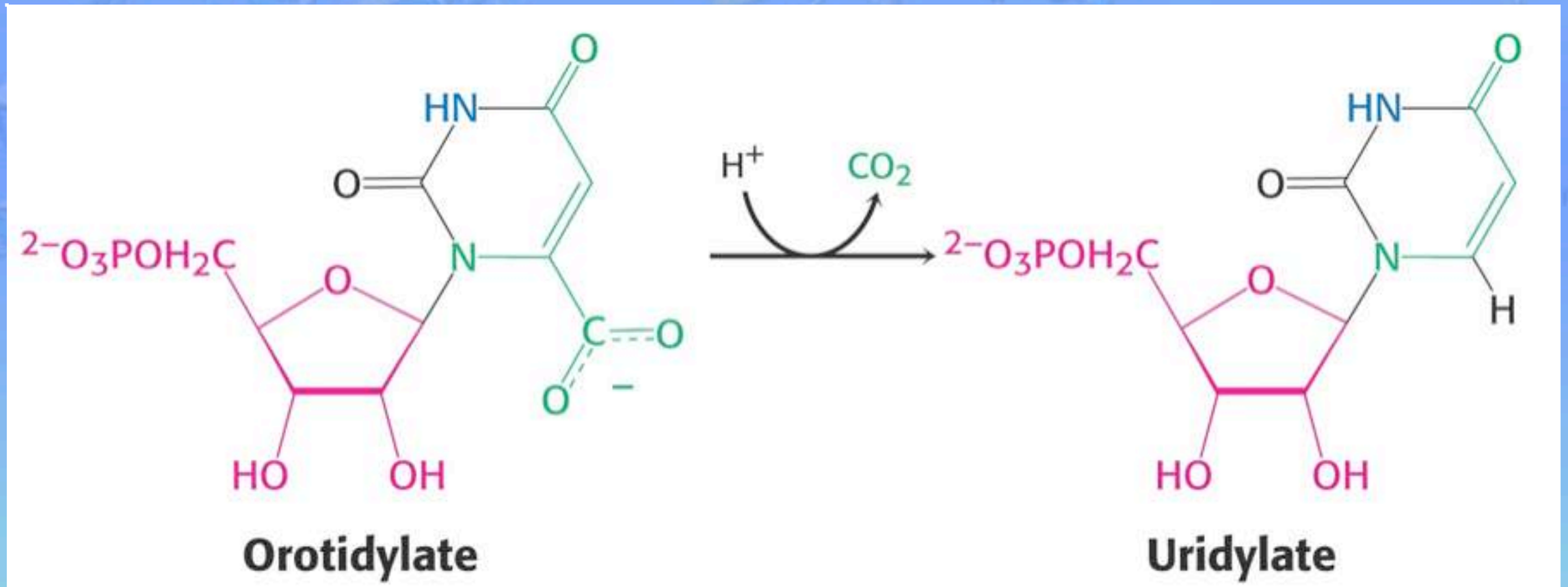
Synthesis of Orotate and attachment to ribose ring.

- Reaction is driven by the hydorlysis of pyrophosphate



1.4 Pyrimidine Synthesis, Second Step

Decarboxylation of orotidylate produces uridylate



Orotidylate **Uridylate**

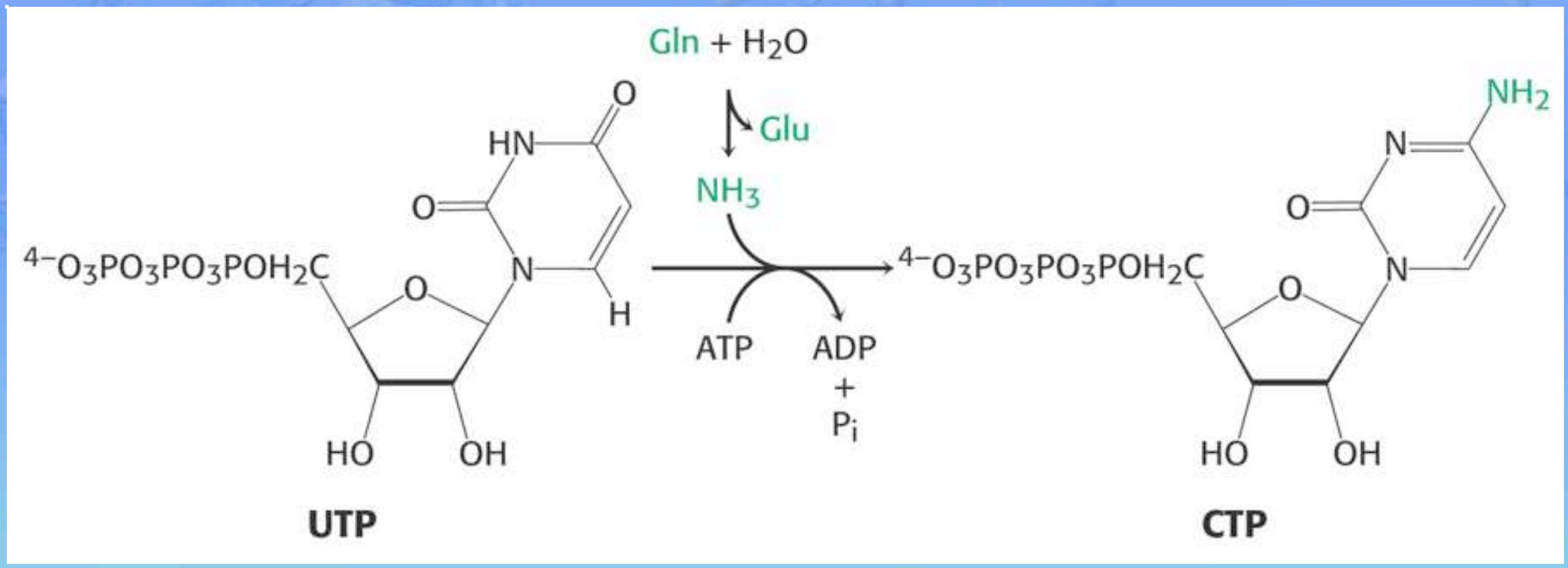
1.5 Nucleotides

Nucleotide mono-, di-, and triphospahtes are interconvertible

- Nucleoside monophosphate kinases
- UMP is converted to UTP before going on to produce CTP

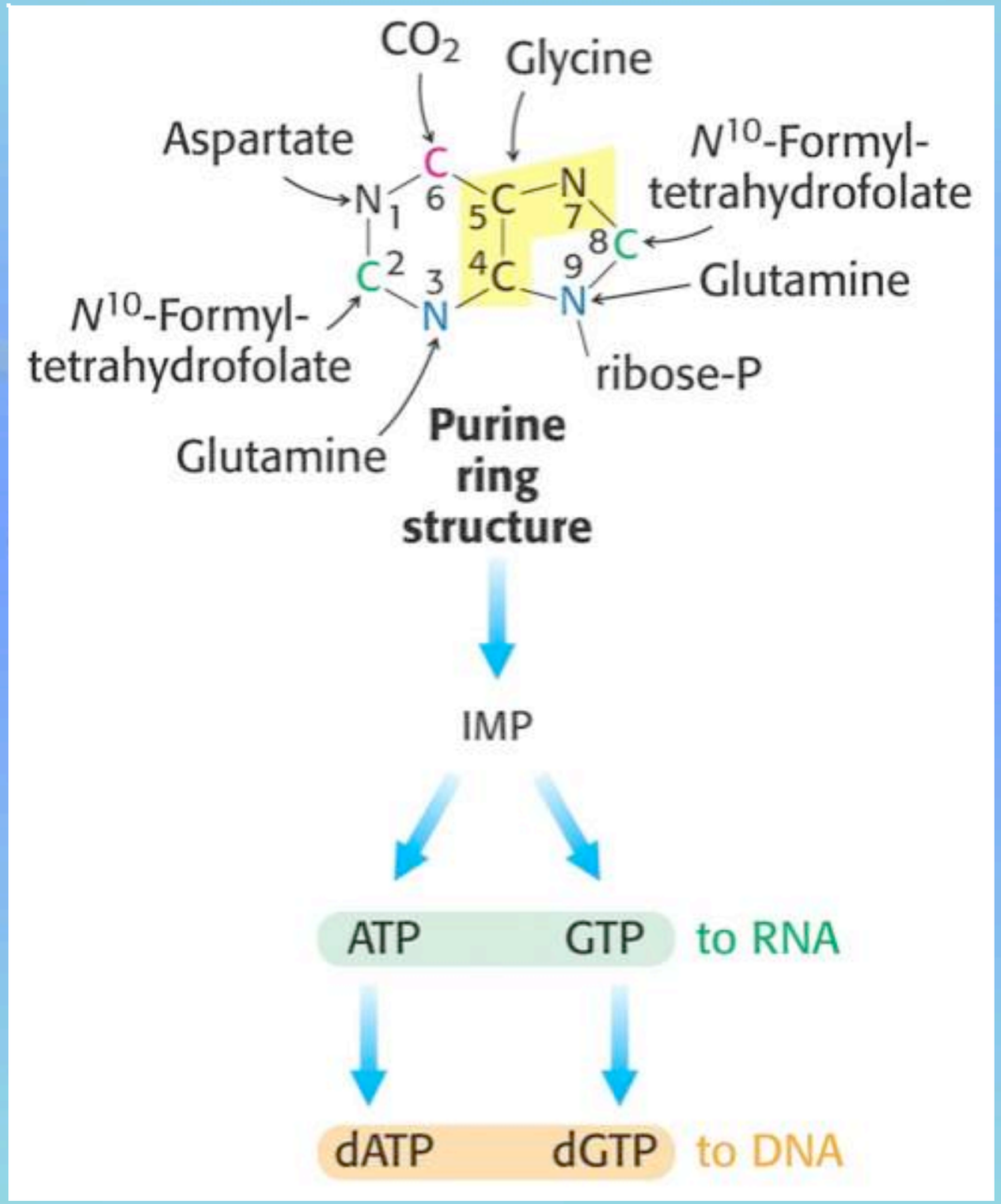
1.6 CTP

CTP is formed by amination (not animation!) of UTP.



2. *de Novo* Synthesis of Purines

Salvage *versus de Novo* synthesis



2.2 Purines Synthesis, Step One

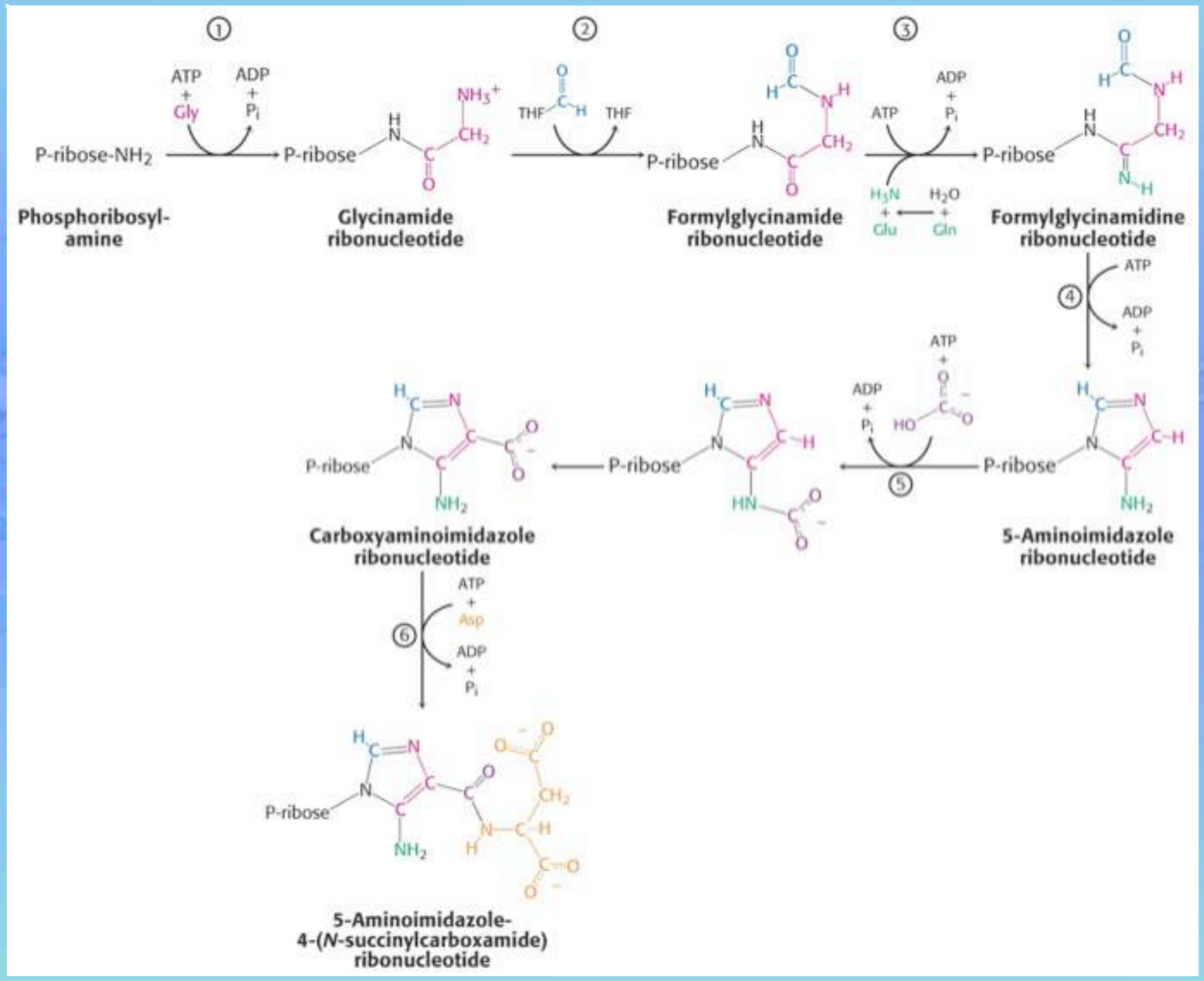
The purine ring system is assembled on a ribose phosphate.

- glutamine phosphoribosyl amidotransferase

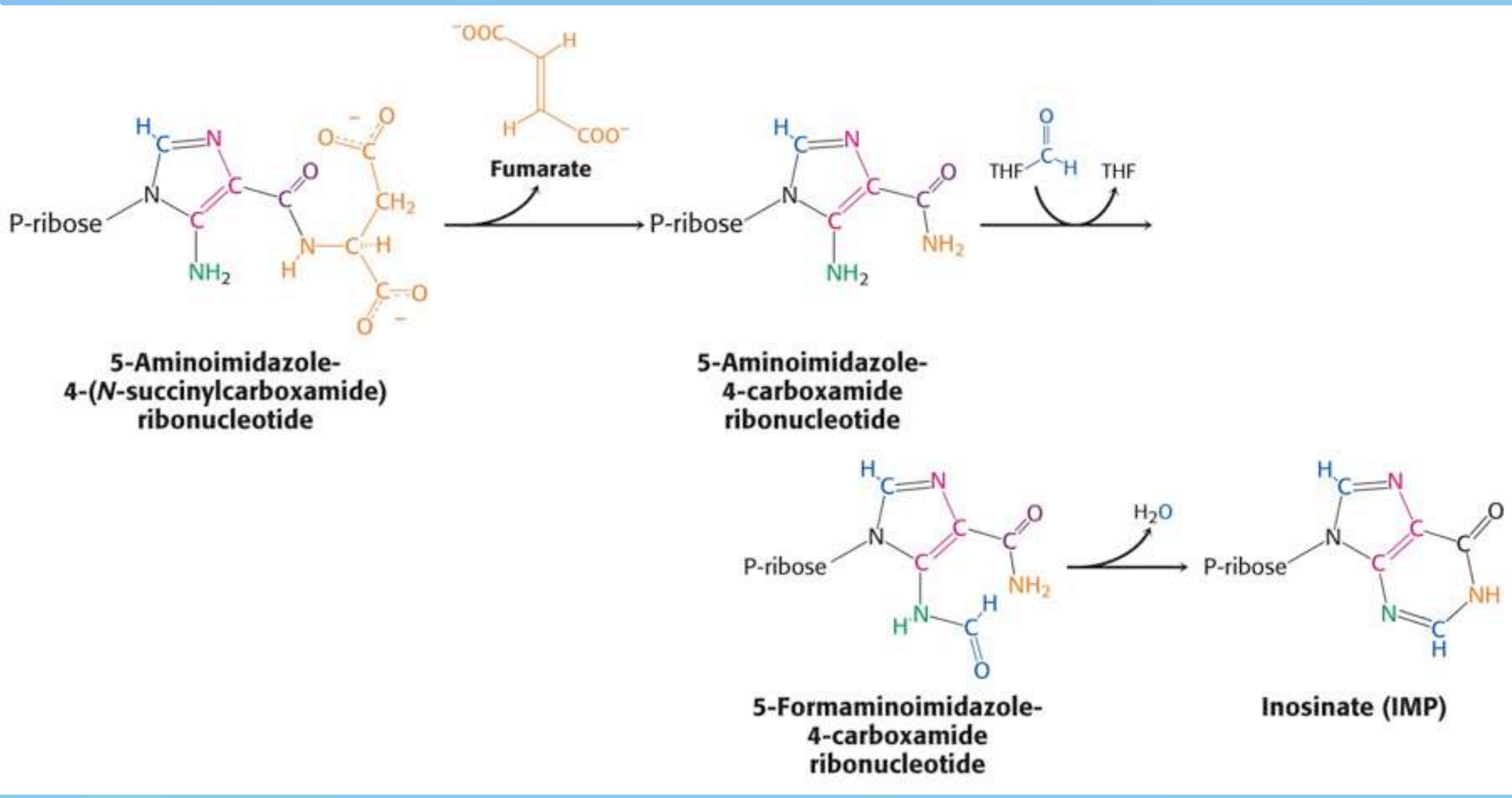
2.3 Purine Ring Synthesis

The purine ring is assembled by successive steps of activation by phosphorylation, followed by displacement.

2.3 Purine Ring Synthesis

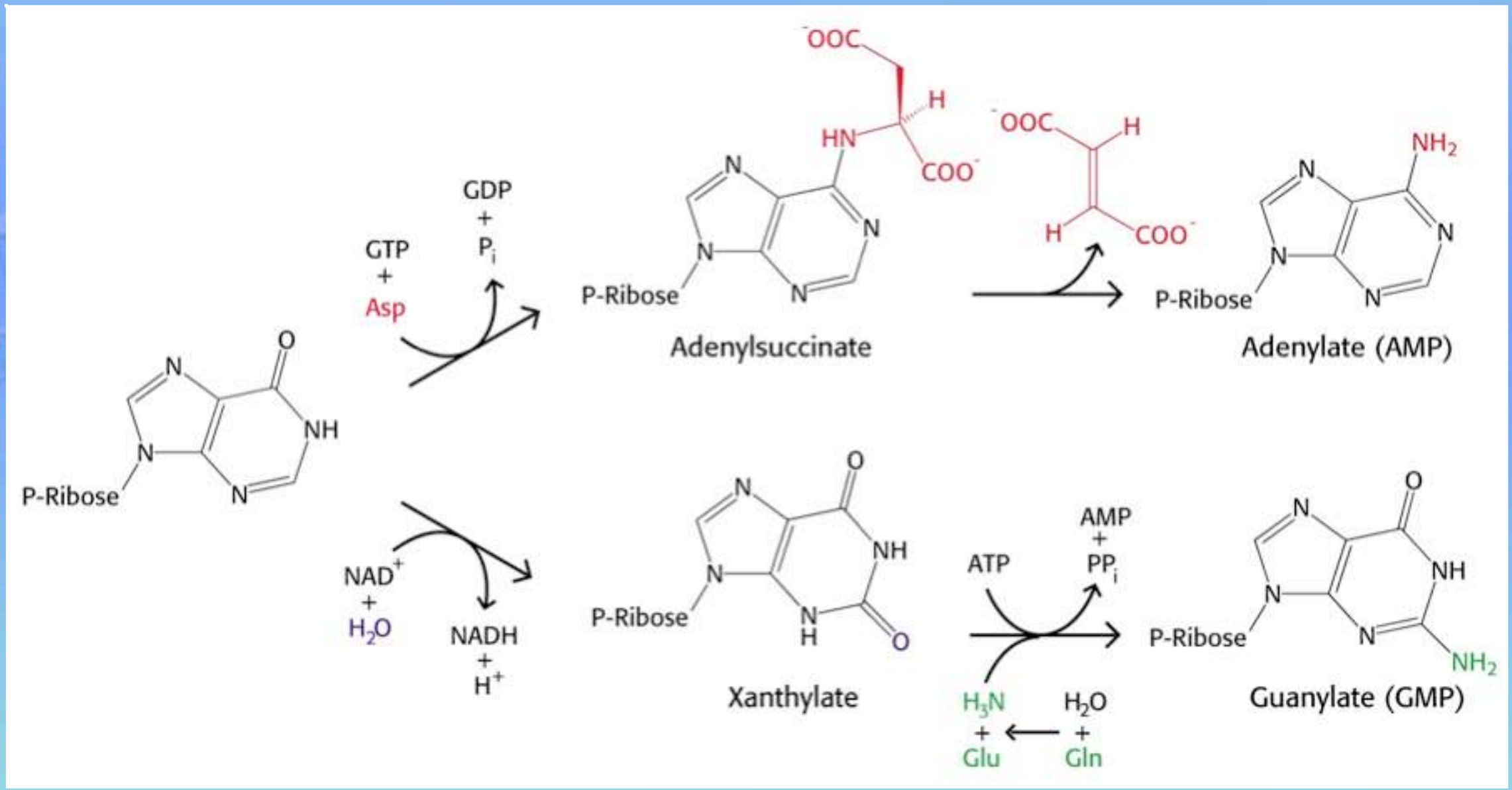


2.3 Purine Ring Synthesis



2.4 AMP and GMP

AMP and GMP are formed from IMP



3. Deoxyribonucleotides

Deoxyribonucleotides are produced form either ribonucleotide di- or triphophosphates

- The 2'-OH on the ribose sugar is reduced to a hydrogen.
- NADPH + H^+ is the reducing agent.
- The enzyme is called *ribonucleotide reductase*

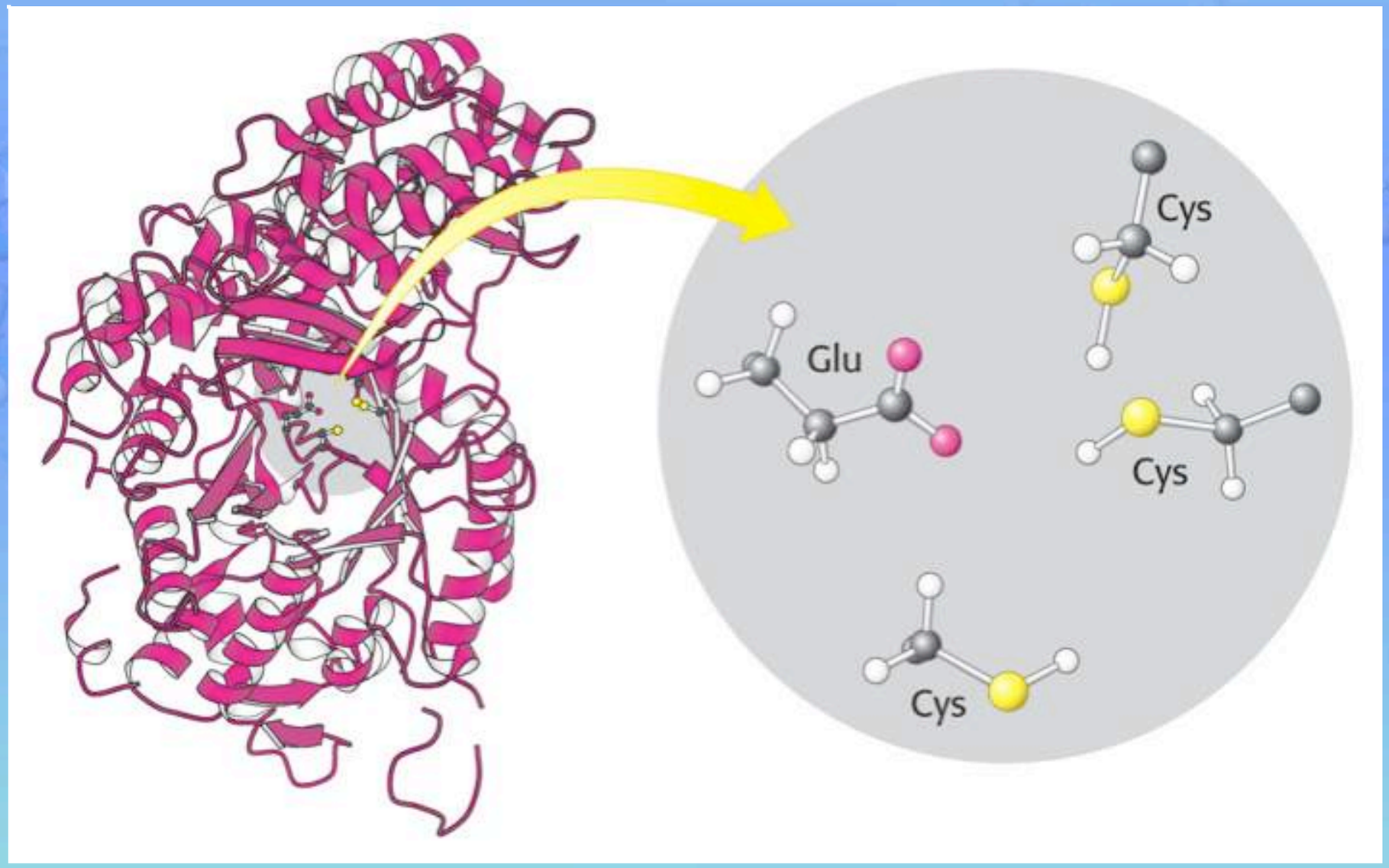
3. Deoxyribonucleotides

Ribonucleotide reductase

- R1 (87 kD dimer)
  - active site
  - allosteric sites
- R2 (43 kD dimer)

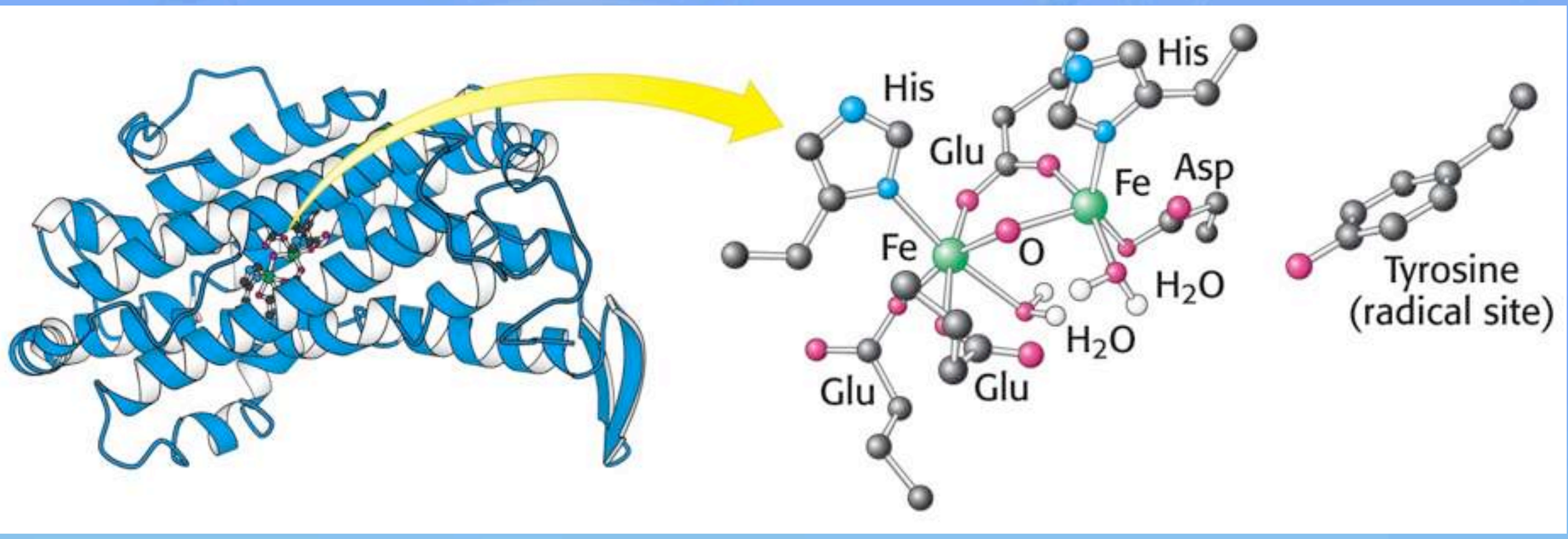
3. Deoxyribonucleotides

Ribonucleotide reductase: R1 subunit



3. Deoxyribonucleotides

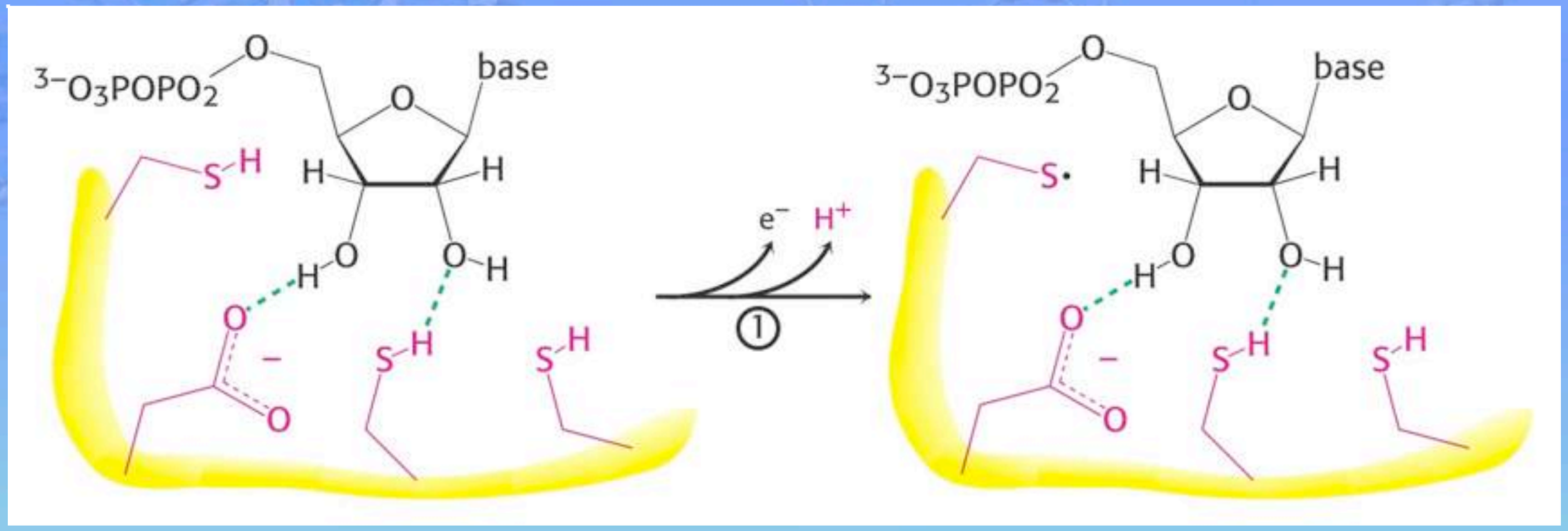
Ribonucleotide reductase: R2 subunit



3. Deoxyribonucleotides

Ribonucleotide reductase

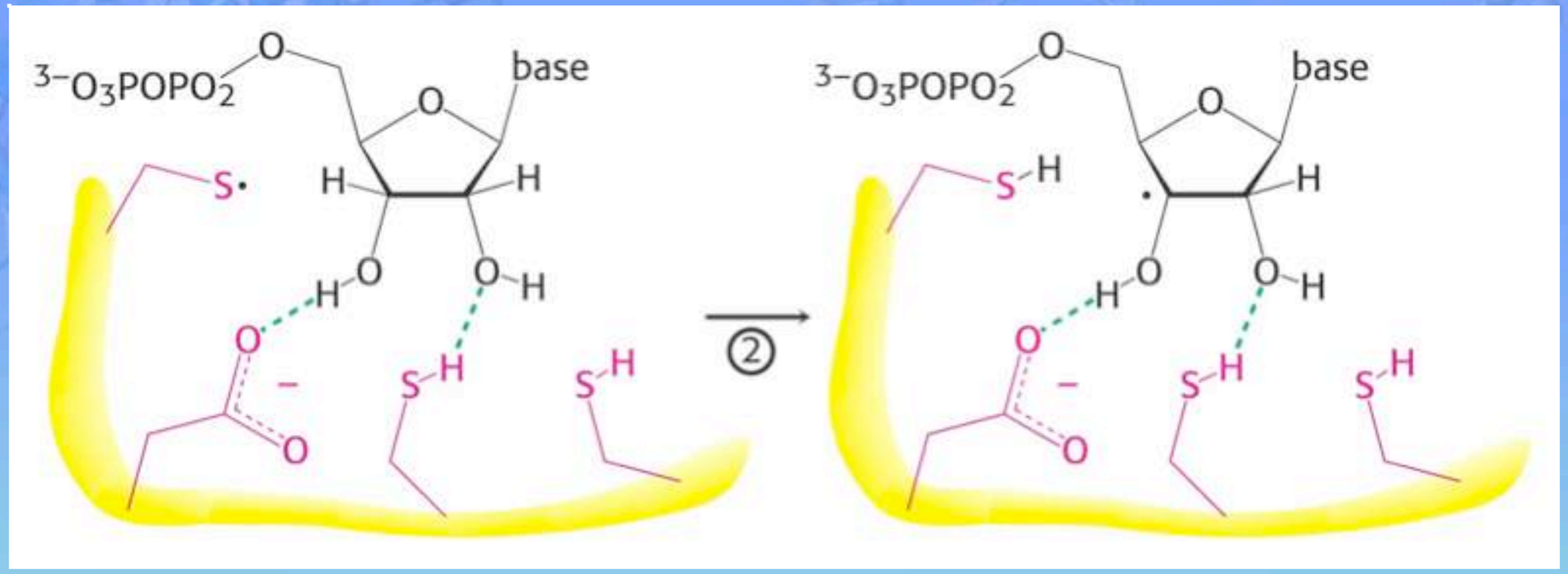
- 1. Transfer of a electron from a cysteine on R1 to the tyrosyl radical on R2



3. Deoxyribonucleotides

Ribonucleotide reductase

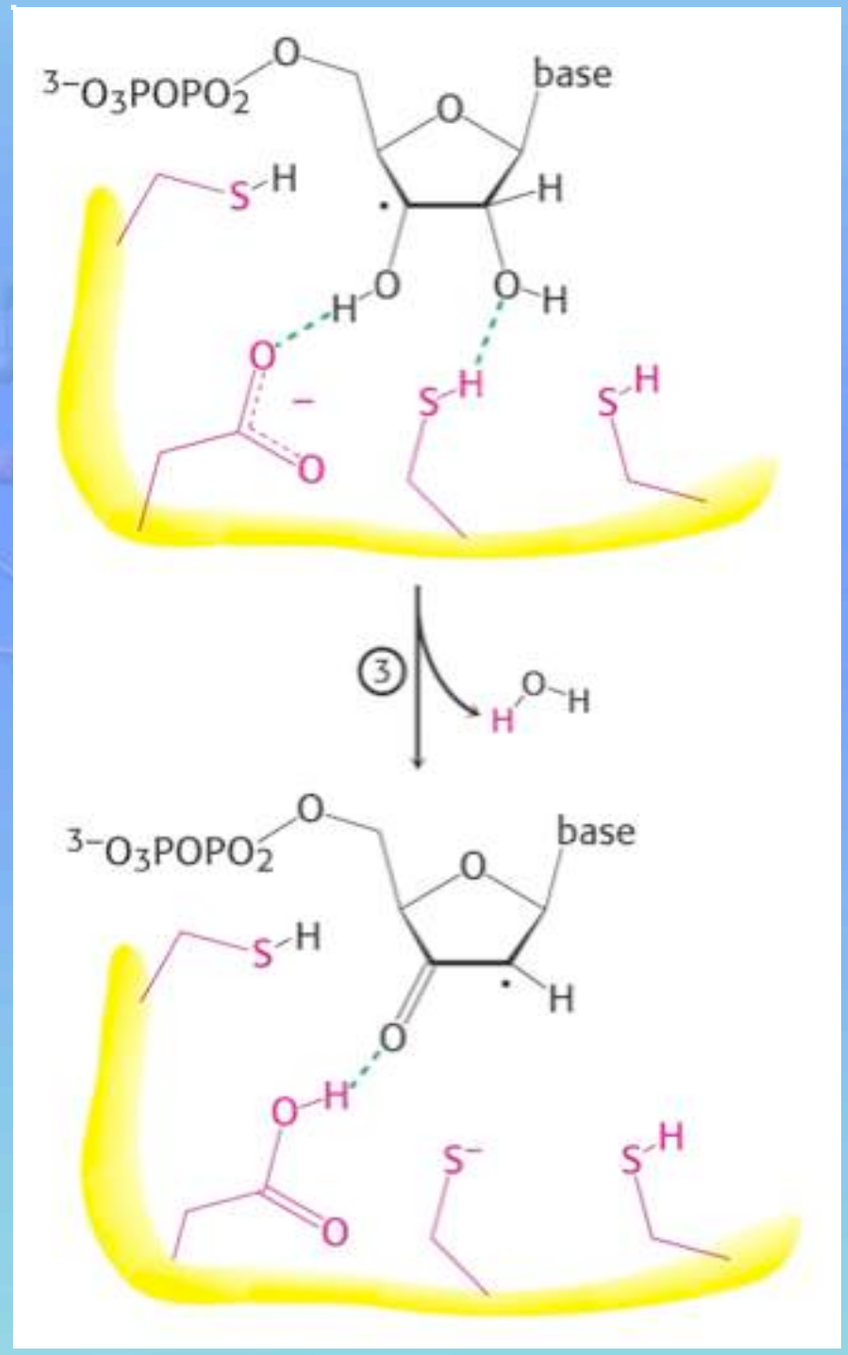
- 2. The cysteine thiyl radical produced on R1 abstracts a hydrogen from the C-3' of the ribose unit.



3. Deoxyribonucleotides

Ribonucleotide reductase

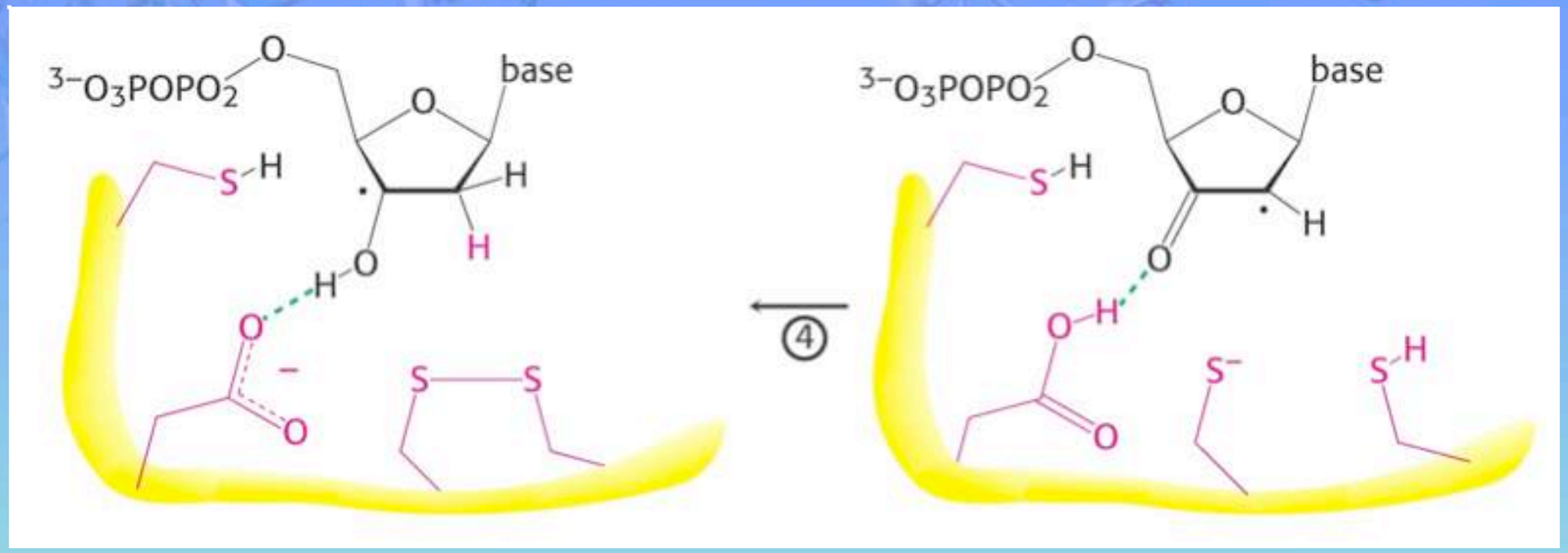
- 3. The carbon radical at C-3' promotes the release of a hydorxide ion on carbon-2.



3. Deoxyribonucleotides

Ribonucleotide reductase

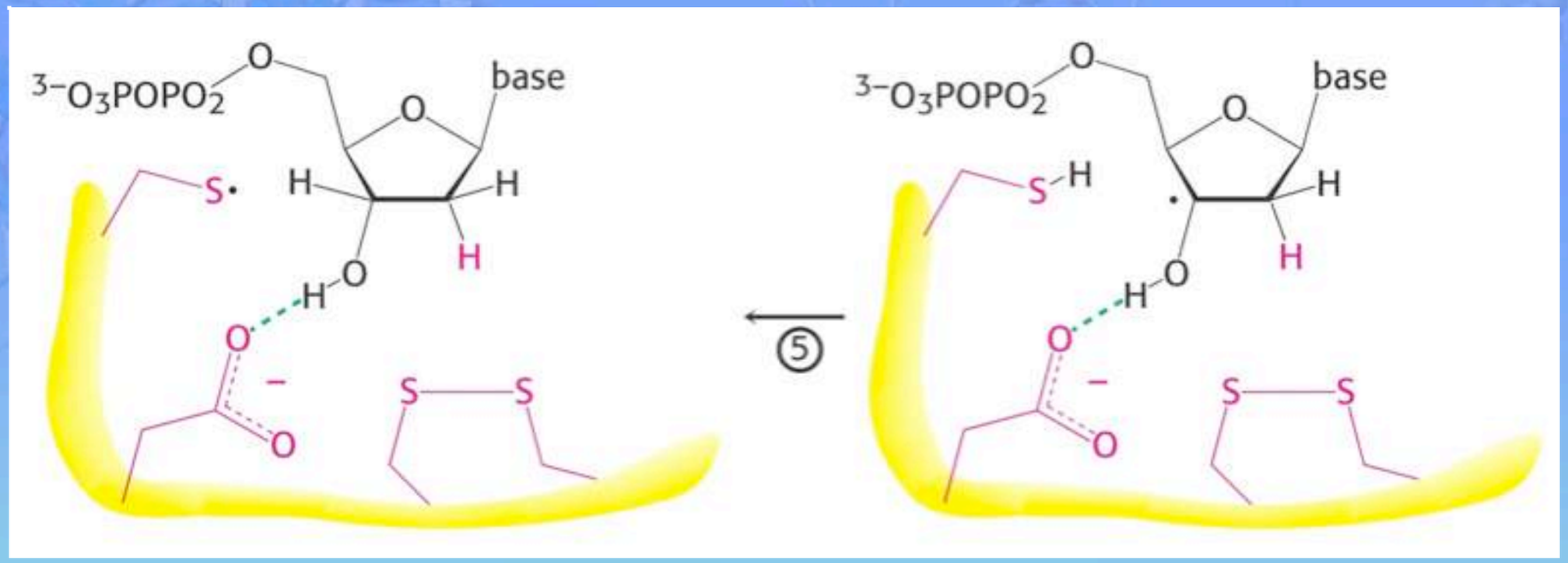
- 4. Hydride is transfered from a third cysteine residue to complete the reduction of the C–2' position.



3. Deoxyribonucleotides

Ribonucleotide reductase

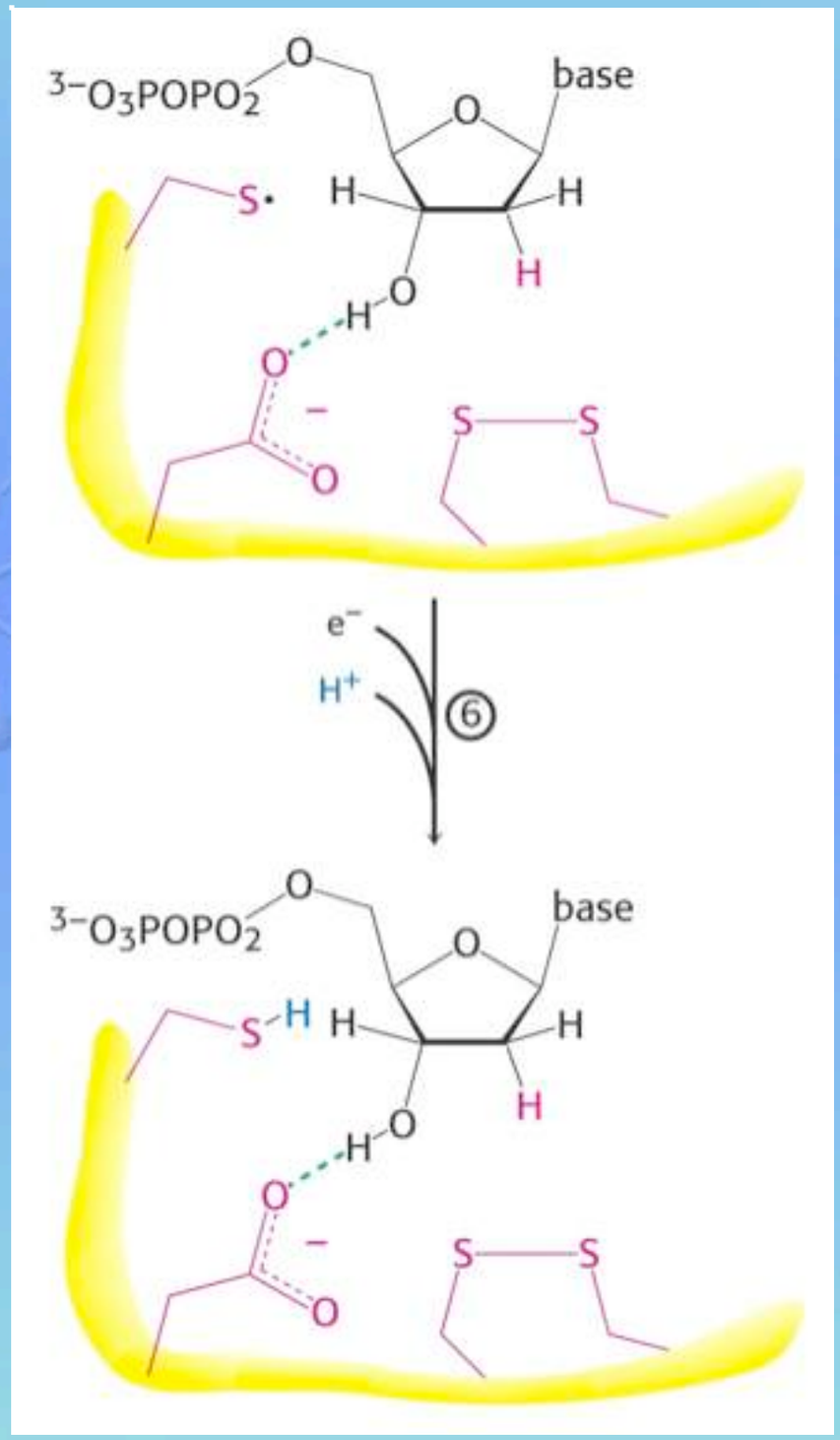
- 5. The C-3' radical recaptures the hydrogen that was abstracted by the first cysteine residue.



3. Deoxyribonucleotides

Ribonucleotide reductase

- 6. The tyrosyl free radical is regenerated

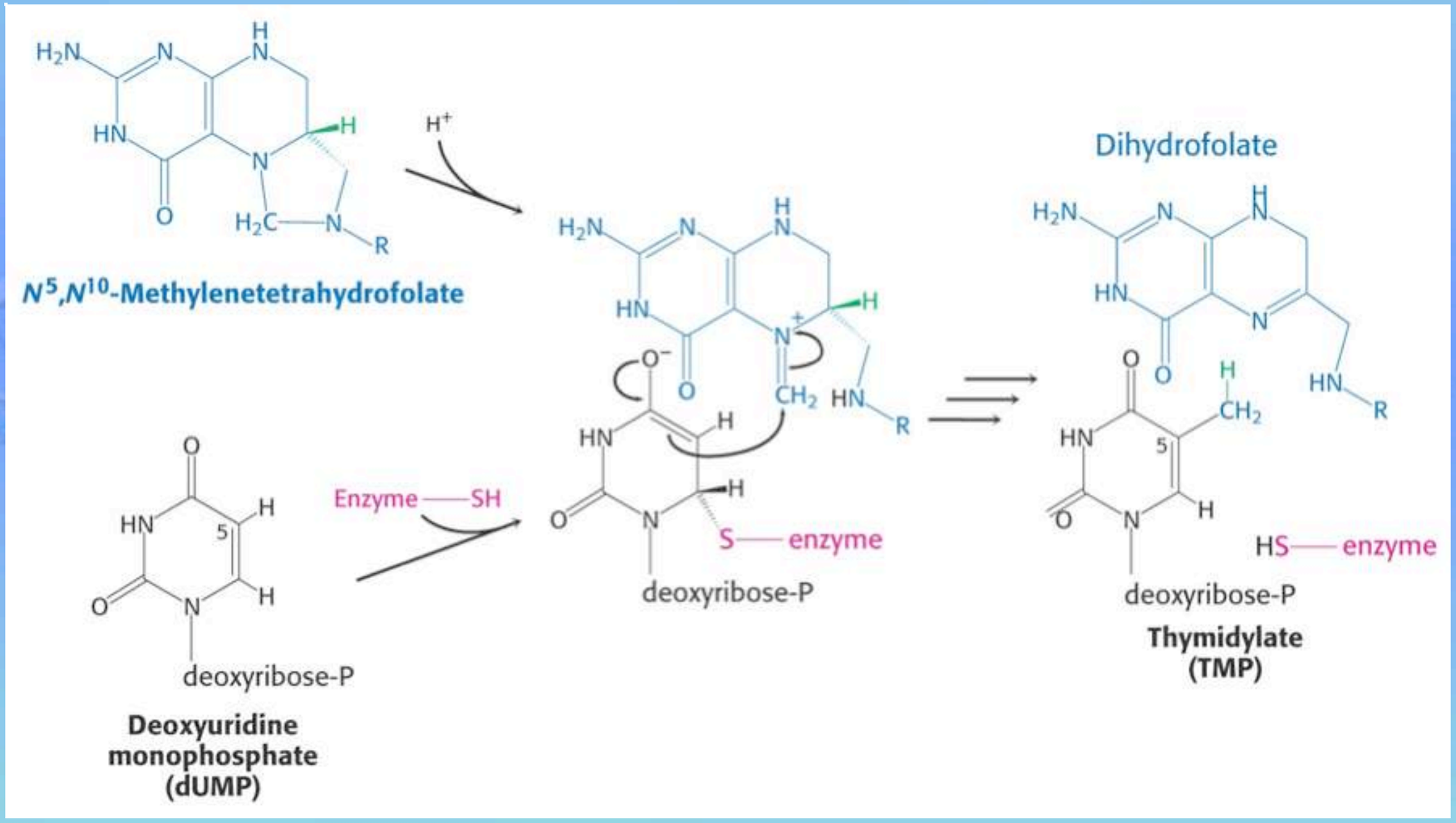


3. Deoxyribonucleotides

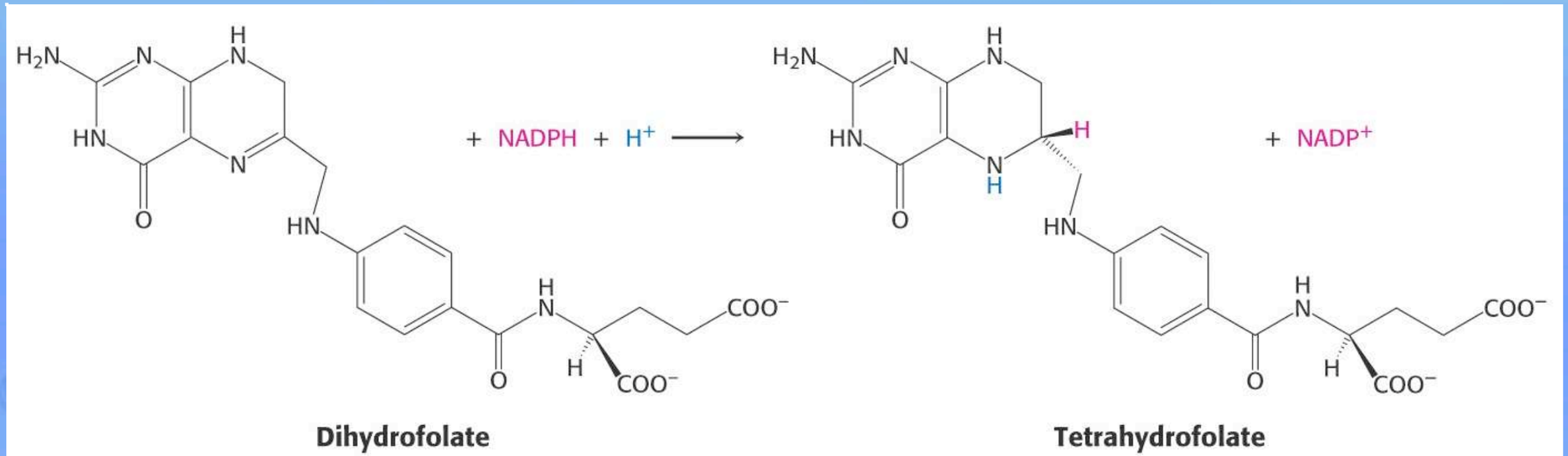
Ribonucleotide reductase

- 7. The disulfide is reduced by thioredoxin.
- 8. Thioredoxin is reduced by *thioredoxin reductase* using NADH + H+

3.1 Thymidylated Formed by Methylation



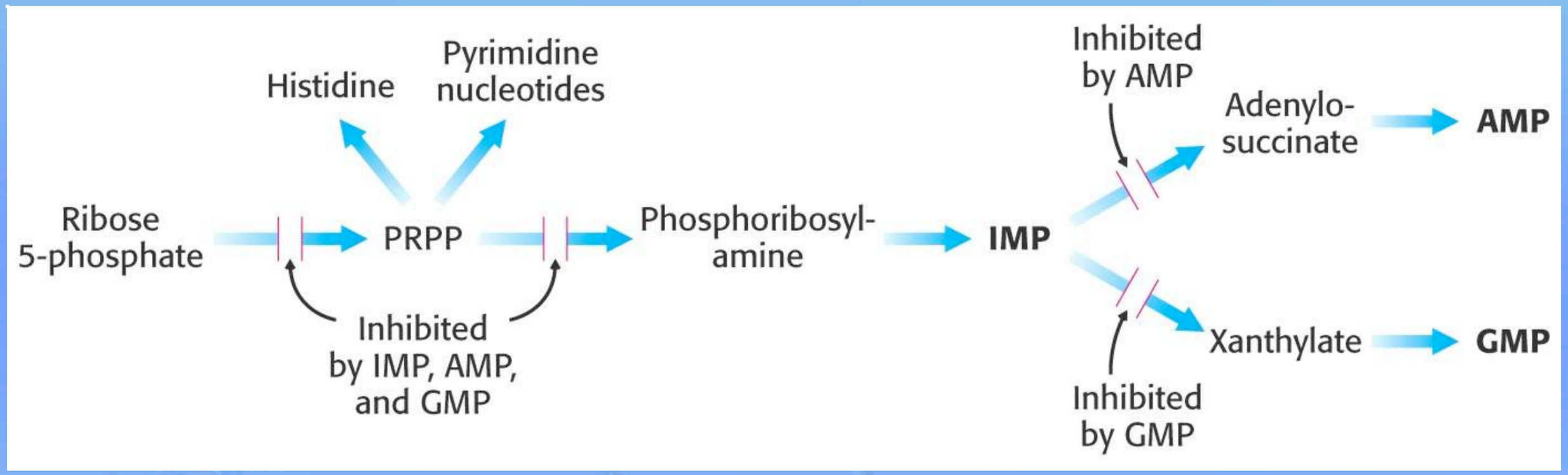
3.2 Dihydrofolate Reductase



3.3 Anticancer Drugs

Inhibition of the synthesis of dexoyribonucleotides or thymidylate will selectively inhibit fast growing cells.

4. Regulation of Nucleotide Biosynthesis



5. NAD^+, FAD and Coenzyme A (Skip)

6. Metabolic Diseases (Skip)

6.1 Purine Degradation (Skip)

6.2 Lesch-Nyhan Syndrome (Skip)