Pyrimidine – Lecture

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Biochemistry & Molecular Biology

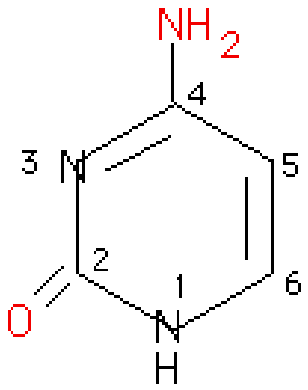
Pyrimidine-Lecture Overview

(i) **Biosynthesis pathways**

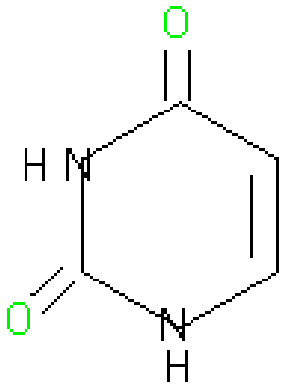
(ii) **Conversion of ribose bases to deoxyribose bases**

(iii) **Chemotherapeutics, anti-metabolites**

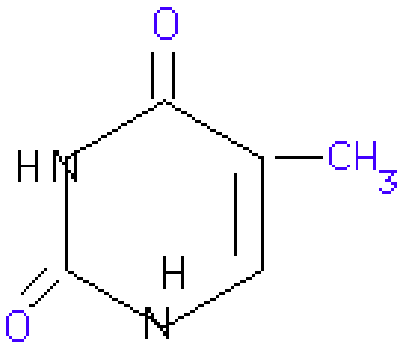
Structure of Pyrimidines



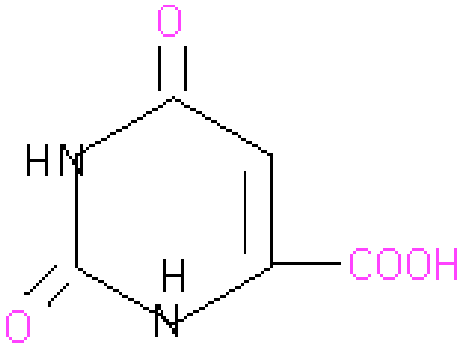
Cytosine



Uracil



Thymine

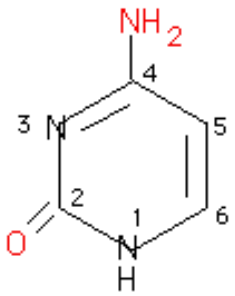


Orotic Acid

C= 2 oxy, 4 amino pyrimidine
T= 2,4 dioxy 5-methyl pyrmidine

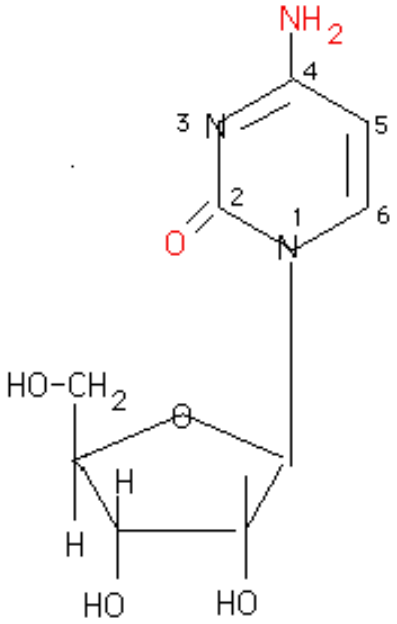
U= 2,4 dioxy pyrimidine
O= 2,4 dioxy 6 carboxy pyrimidine

The nomenclature of purines and pyrimidines depends on their linkage to a pentose



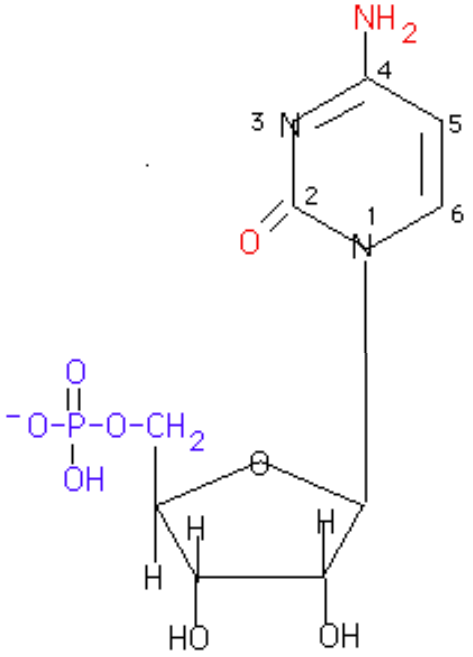
Cytosine

Base



Cytidine

Nucleoside*
Base

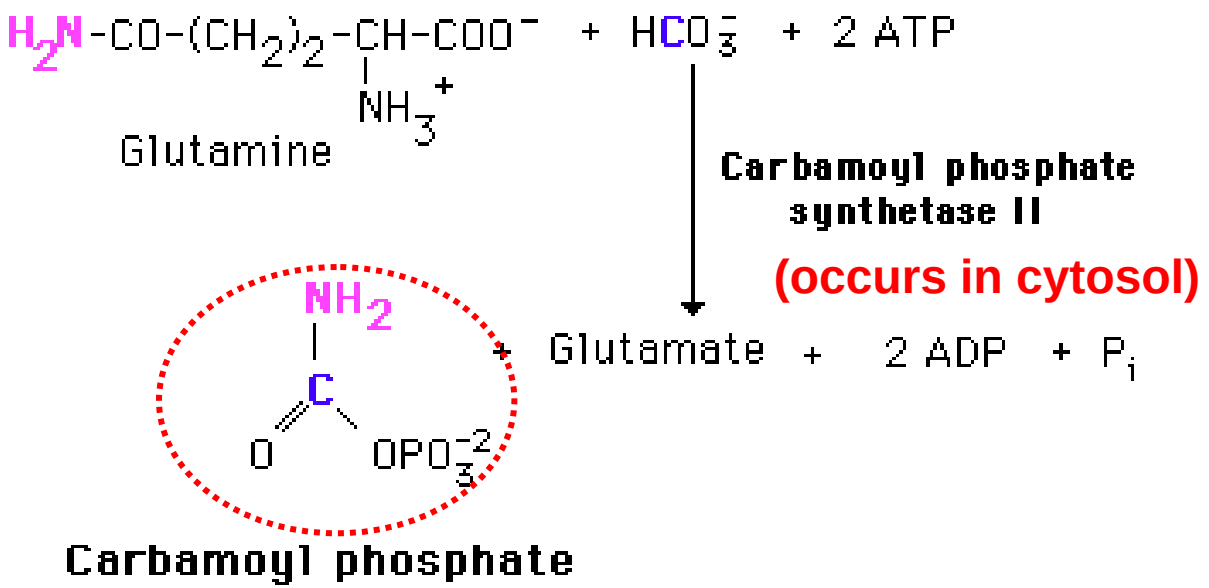


Cytidine Monophosphate

Nucleotide
Base ($P0_4$ ester)

*** when the base is purine, then the nucleoside ends in OSINE (AdenOSINE, GuanOSINE, InOSINE)**
when the base is pyrimidine, then the nucleoside ends in IDINE (UrIDINE, CytIDINE, ThymIDINE)

Pyrimidine Biosynthesis-I

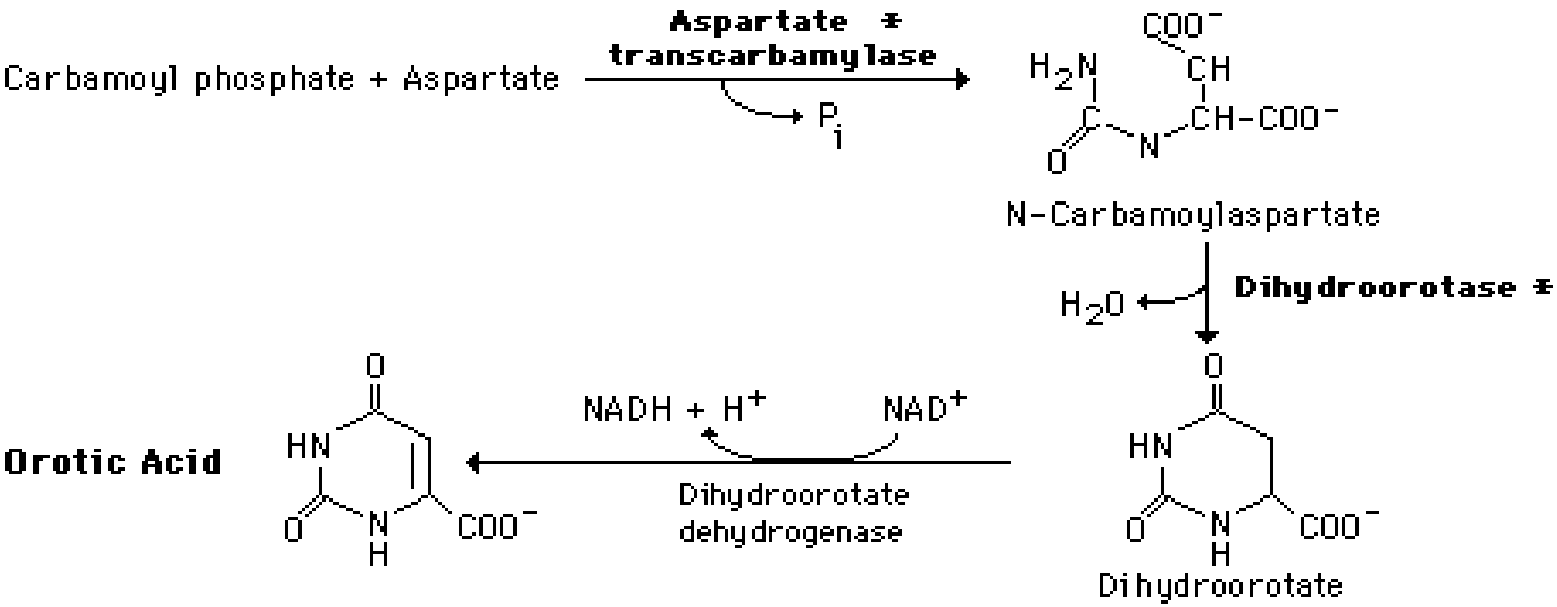


Pyrimidine biosynthesis begins with the assembly of the ring, then linked To ribose phosphate.

Precursors are Glutamine (NH2), Bicarbonate (C) , and ATP (PO_4).

Q. Why is it advantageous to generate carbamoyl phosphate in the cytosol rather than the mitochondria?

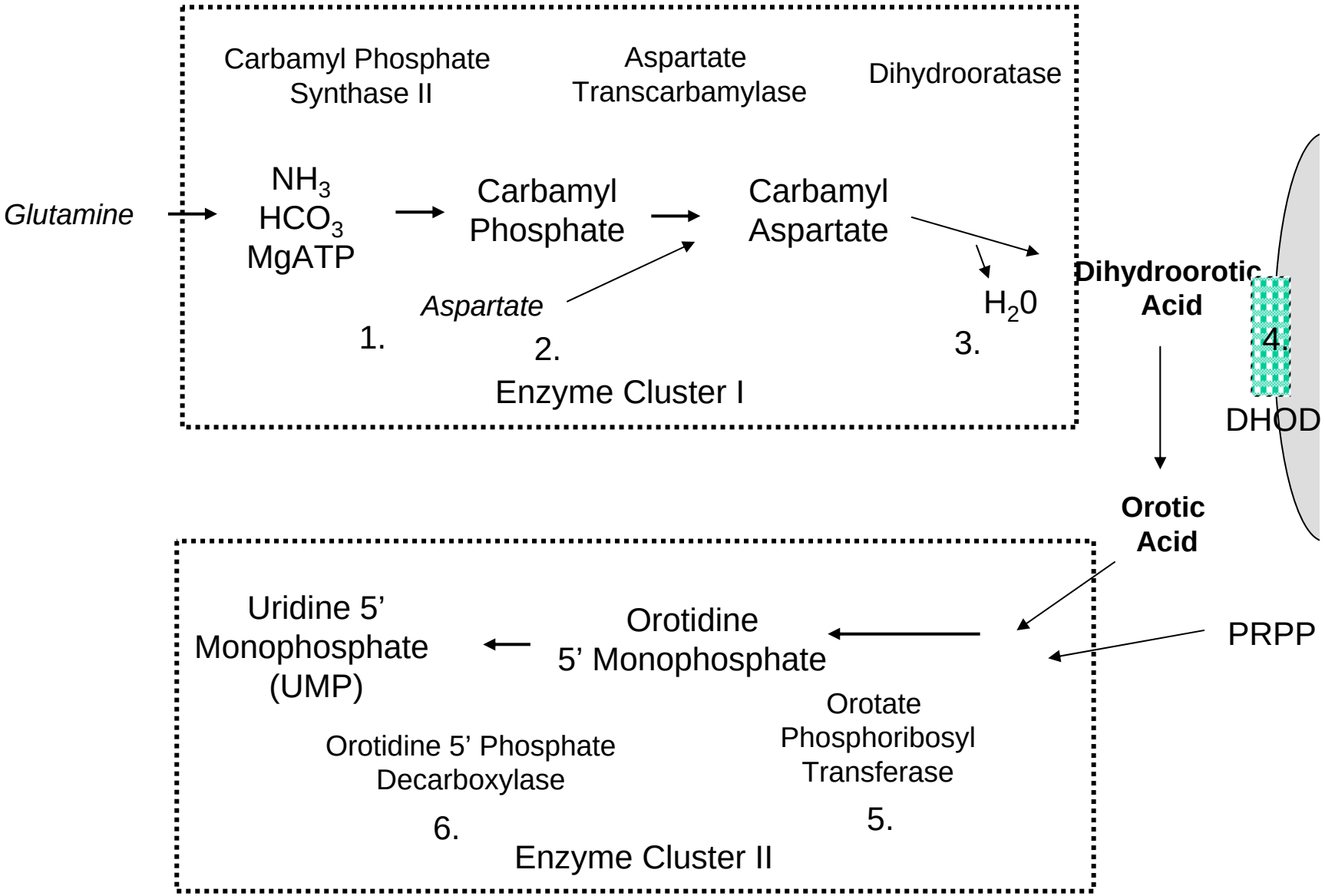
Pyrimidine Biosynthesis-II



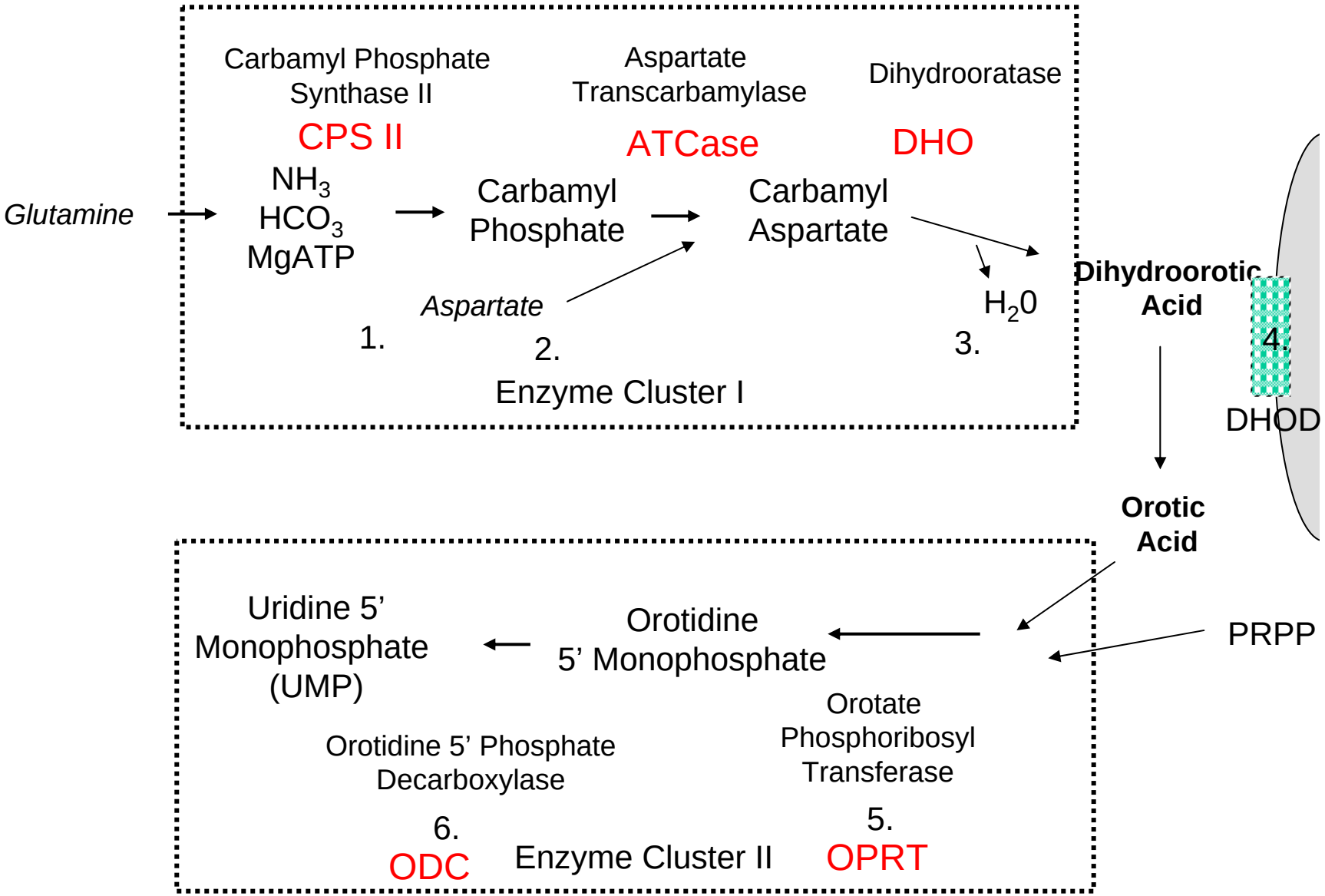
* Part of the same multi-functional protein

Carbamoyl phosphate synthase II, ATCase, and Dihydrooratase are linked in a single 240 kD polypeptide chain. The enzyme is sometimes referred to as CAD.

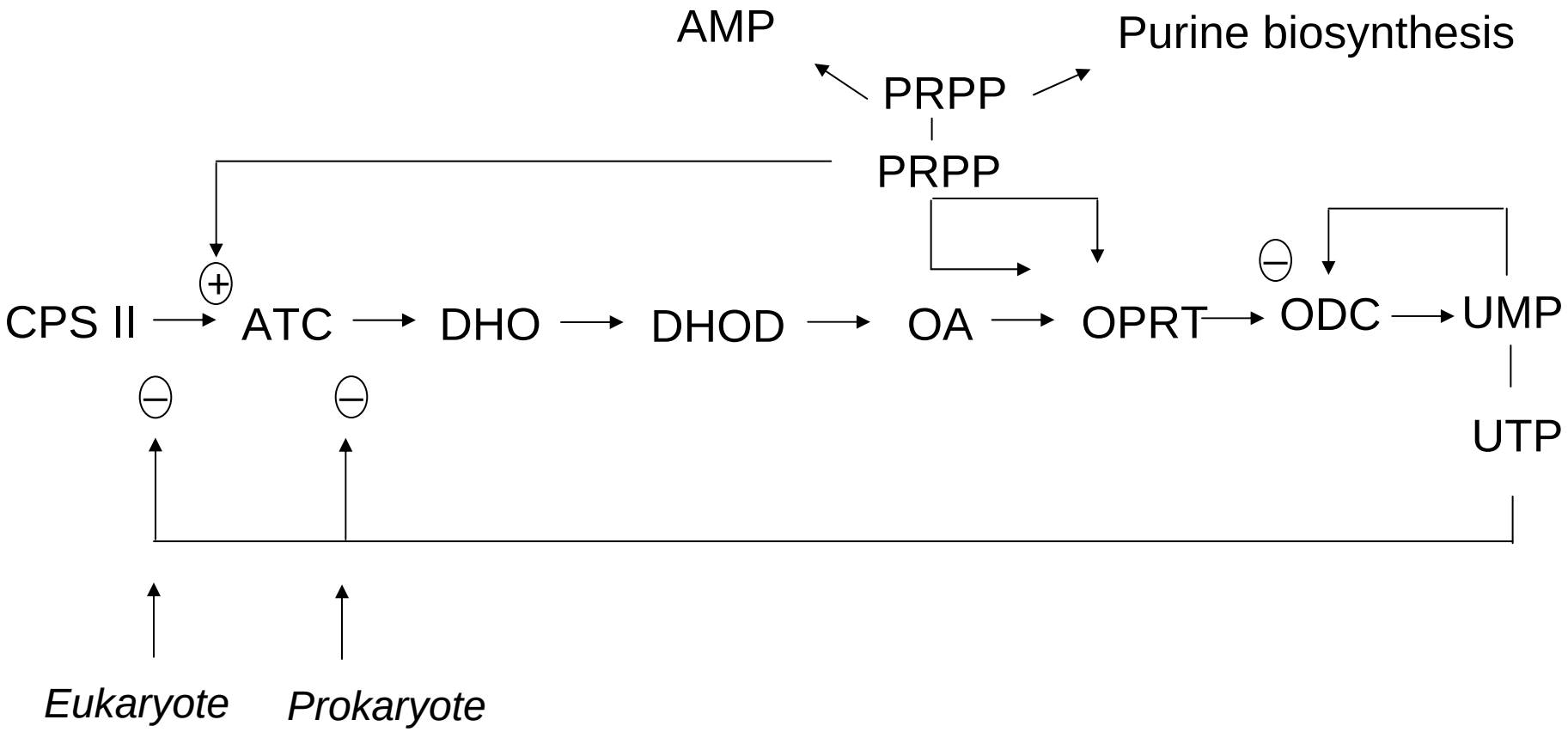
Pyrimidine Metabolism-III



Pyrimidine Metabolism-III

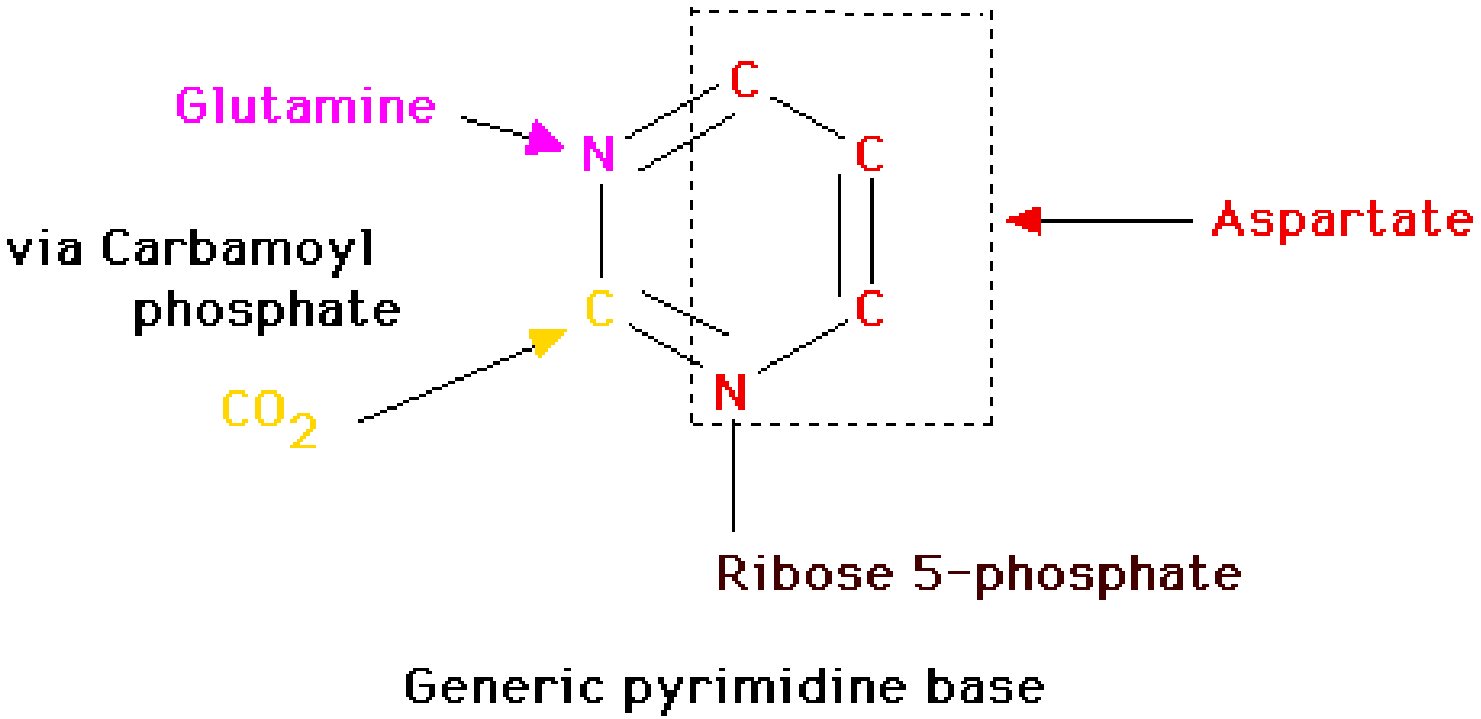


Pyrimidine Biosynthesis-IV



Committed Steps

Pyrimidines: where do the atoms come from?



Generic pyrimidine base

The second phase of pyrimidine synthesis

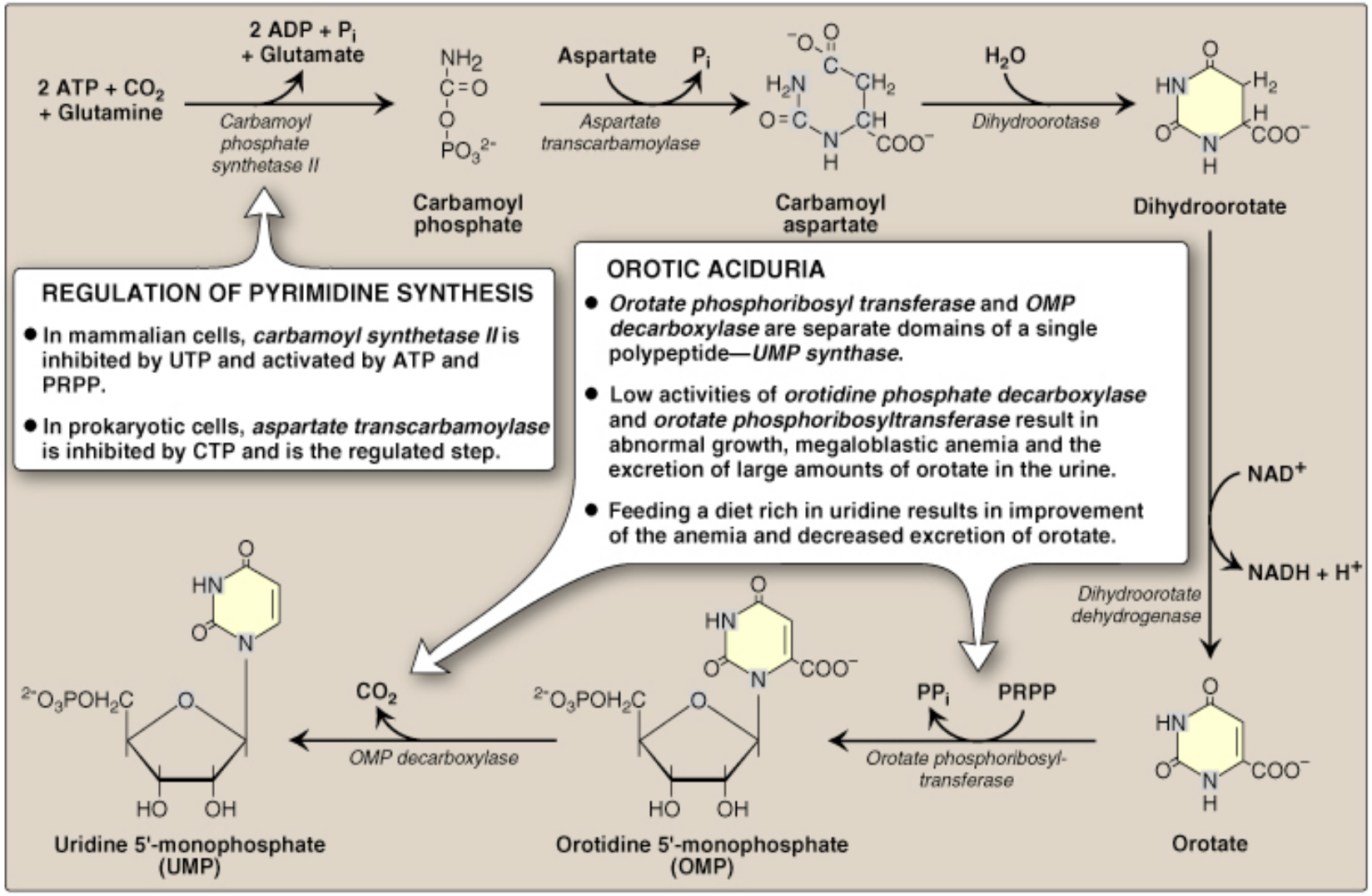
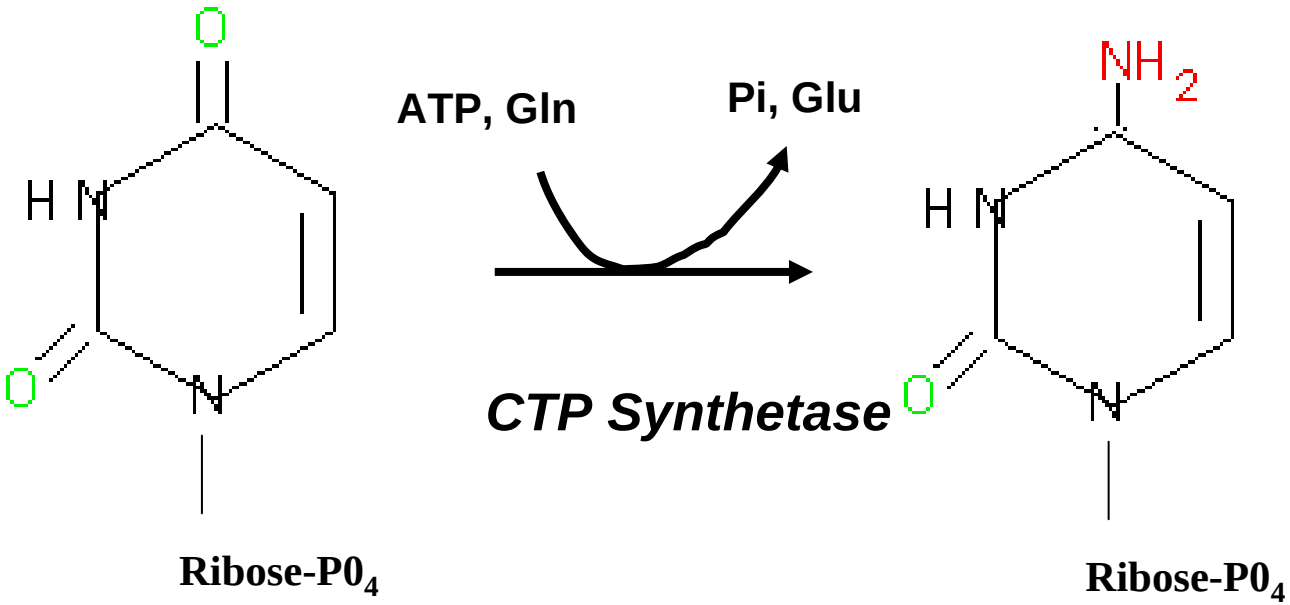


Figure 22.21
De novo pyrimidine synthesis.

URACIL

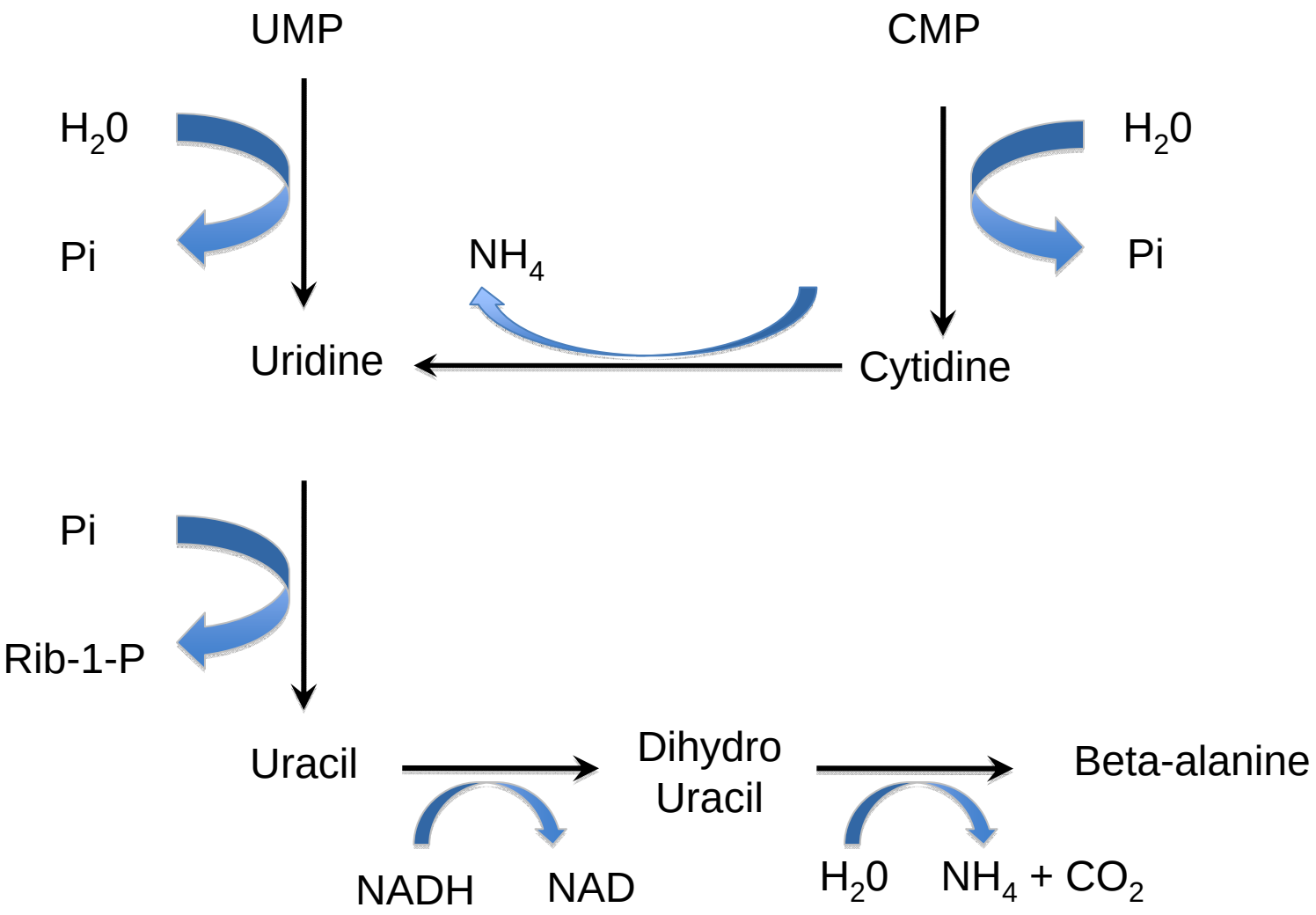
Cytosine



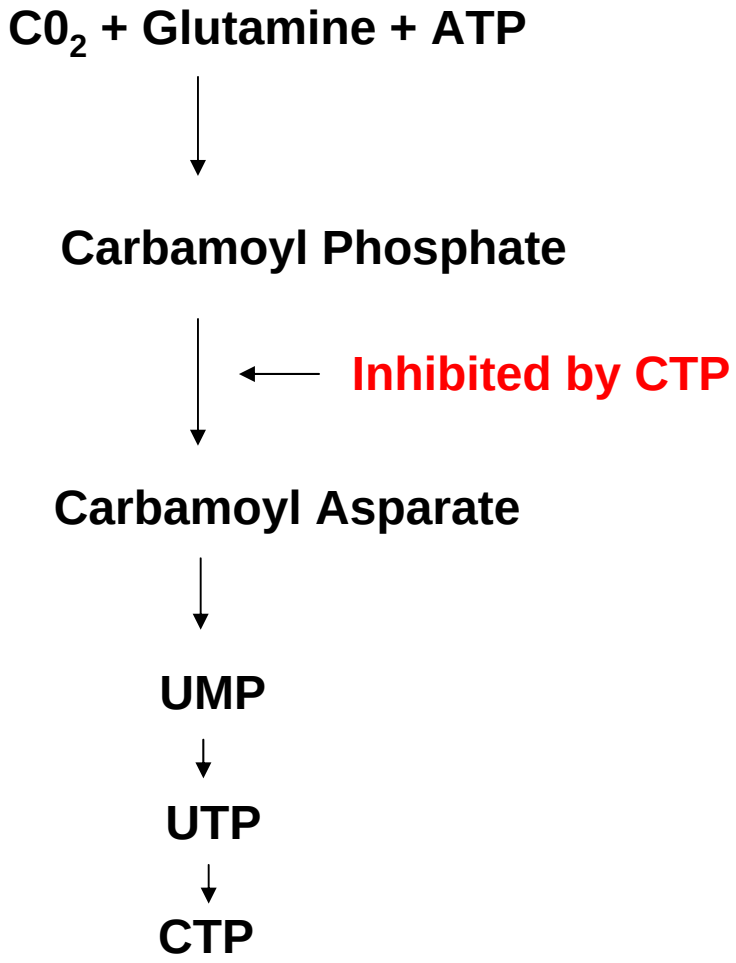
UTP

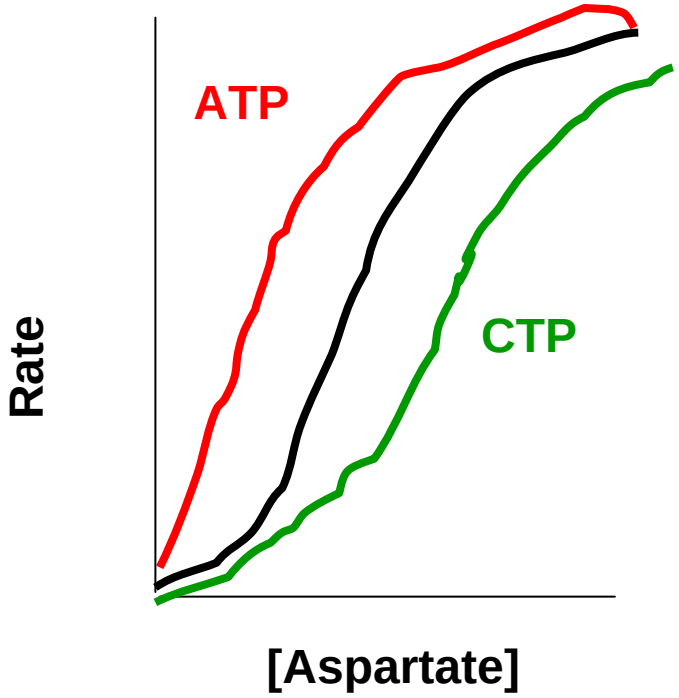
CTP

Pyrimidine Nucleotide Degradation (UMP and CMP)



ATCase is feedback inhibited by the end-products of pyrimidine biosynthesis

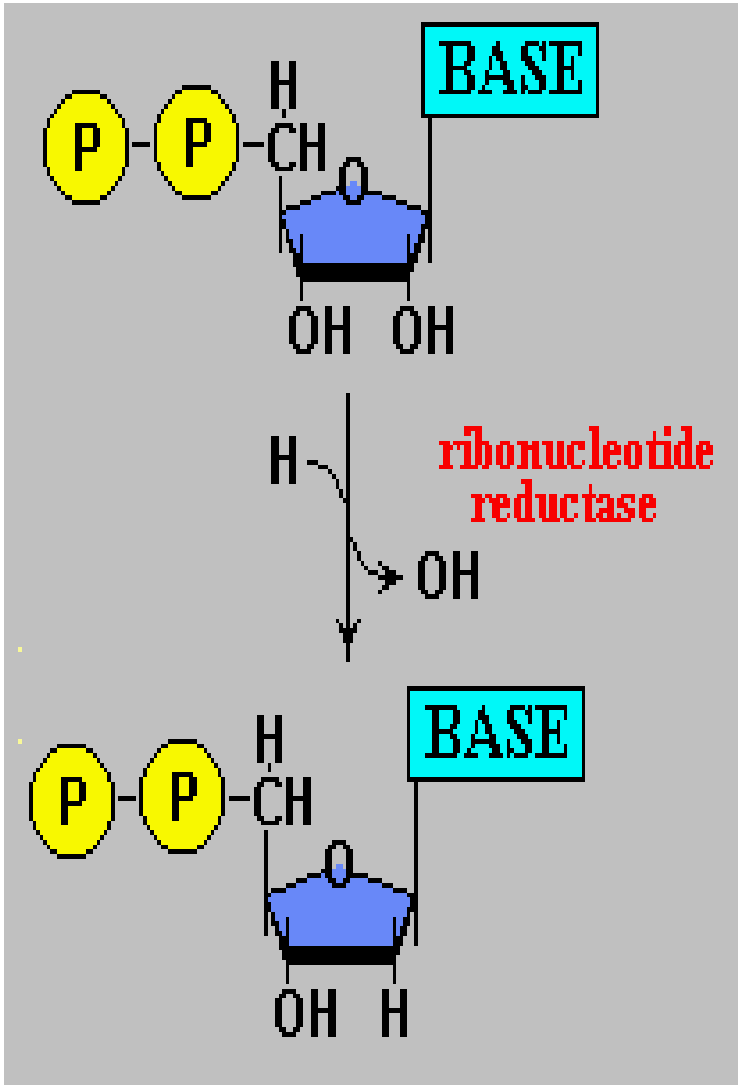




Conversion of RNA to DNA

1. Thymidylate Synthetase

2. Ribonucleotide Reductase



RIBONUCLEOTIDE REDUCTASE

1. Complex enzymatic reaction whereby electrons are transferred from NADPH through a series of sufhydryl groups at the catalytic site of Ribonucleotide Reductase.

2. Active site of RR contains thioredoxin, a 12 kD protein with two exposed cysteines, which become oxidized.

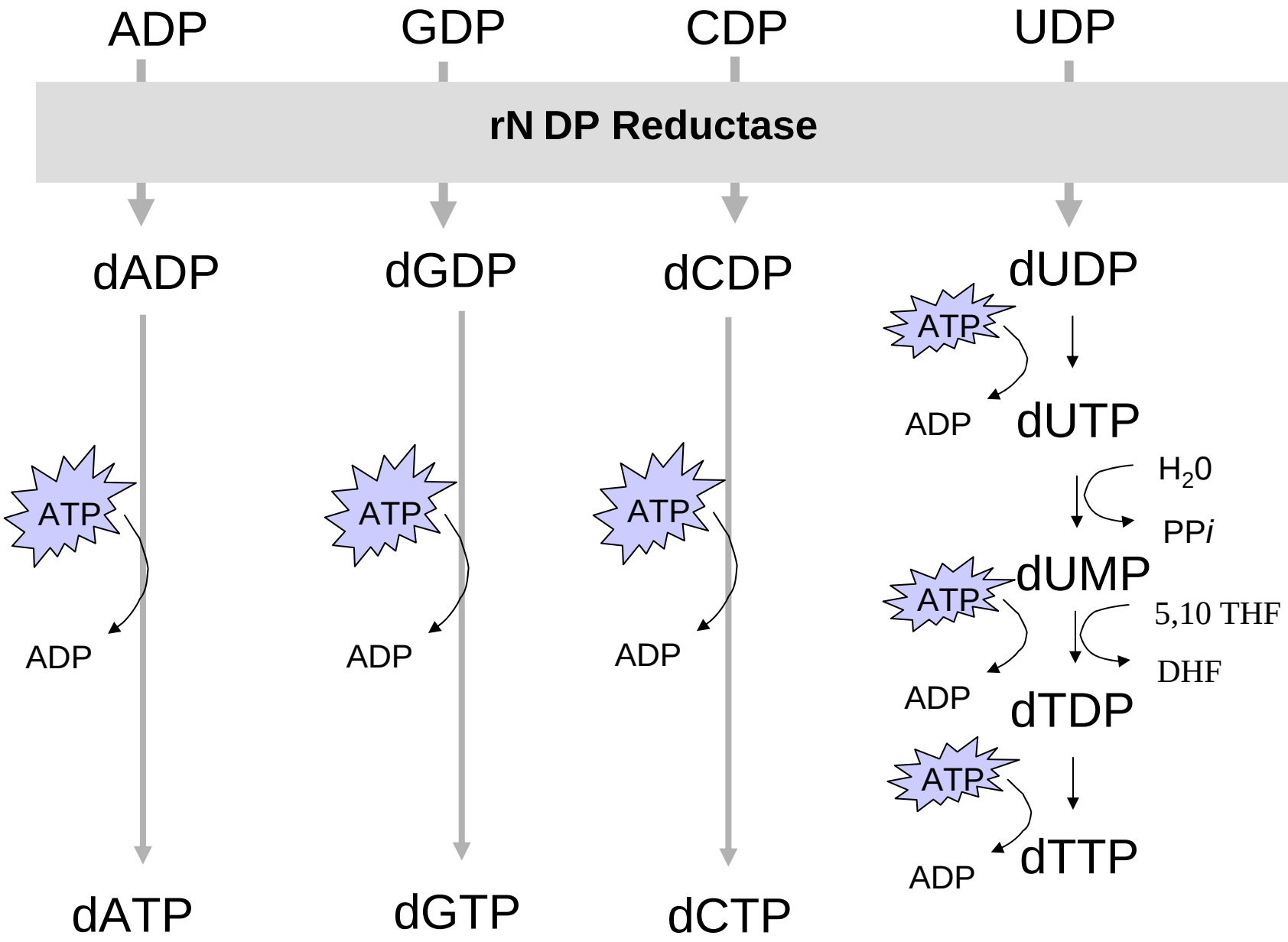
3. This ultimately allows for the reduction of ribose.

REGULATION

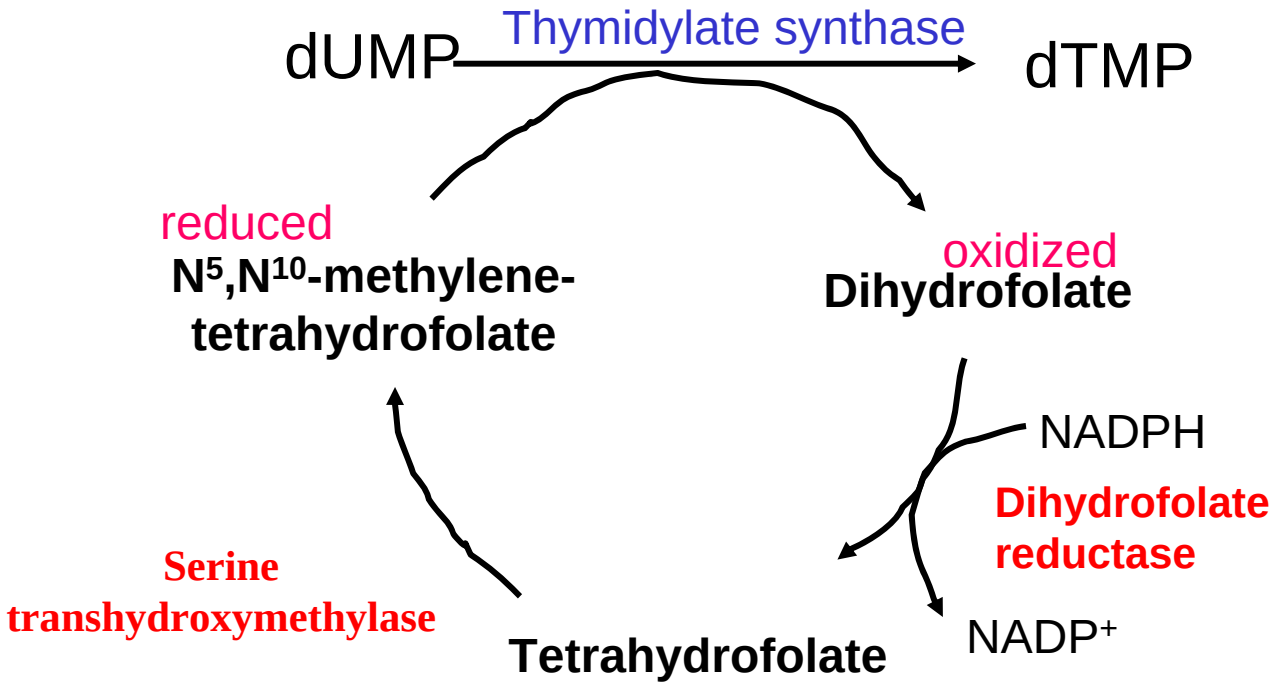
1. Based on the response to cellular need for dATPs.

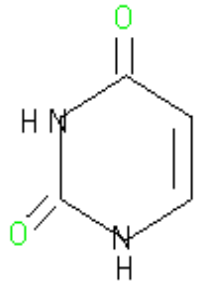
dATP is general inhibitor

ATP is a general activator


ADP
GDP
CDP
UDP
rN DP Reductase
dADP
dGDP
dCDP
dUDP
ATP
ADP
dUTP
H20
PPi
dUMP
5,10 THF
DHF
dTDP
dTTP
dATP
dGTP
dCTP

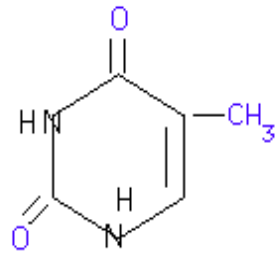
Thymidine biosynthesis is an important target for cancer therapeutics





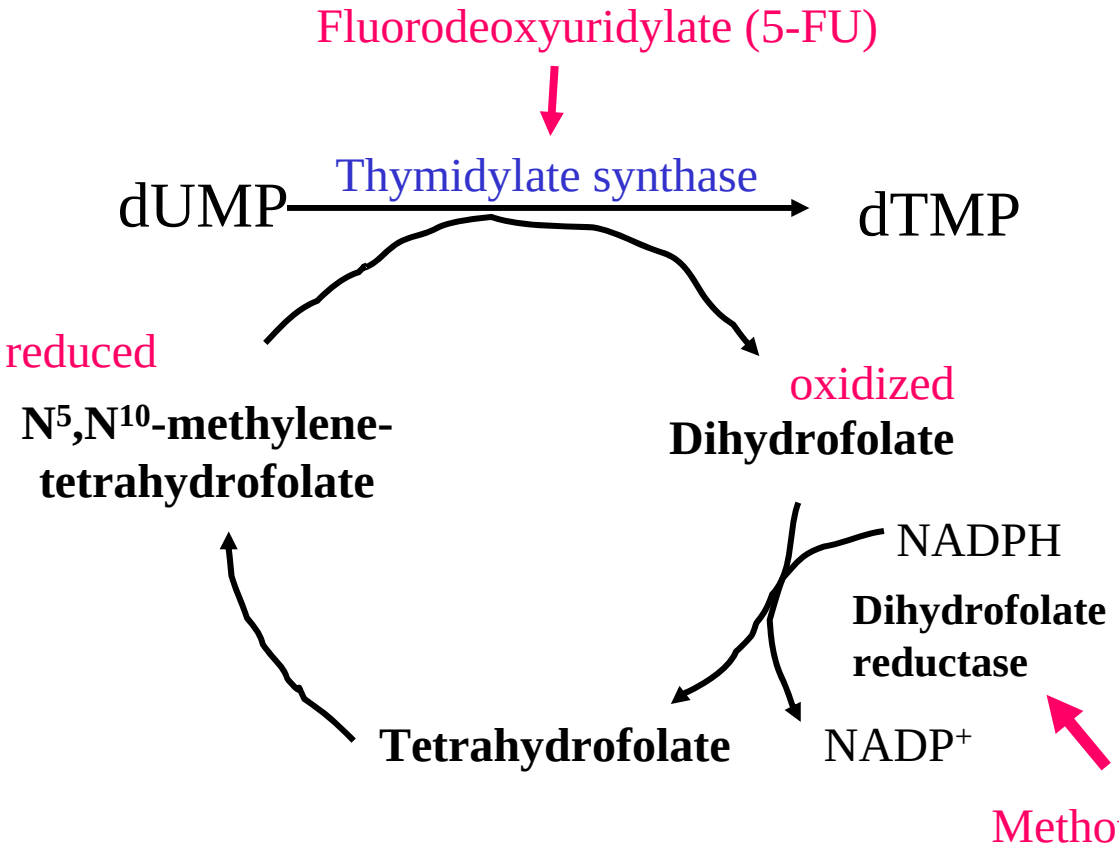
Uracil

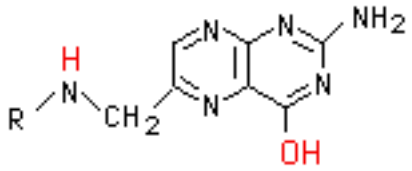
Thymidylate Synthase

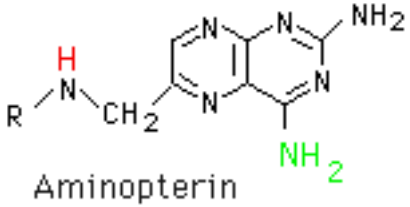


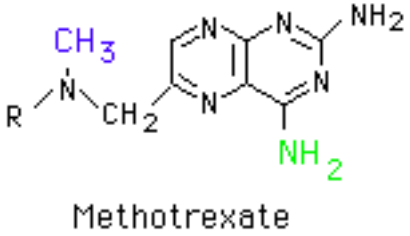
Thymine

Common molecular drugs targets of 5FU and Methotrexate.

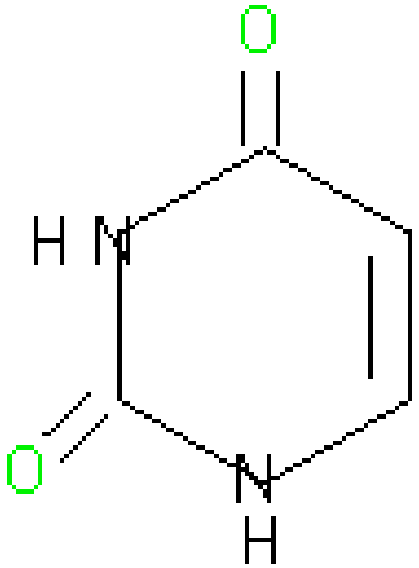




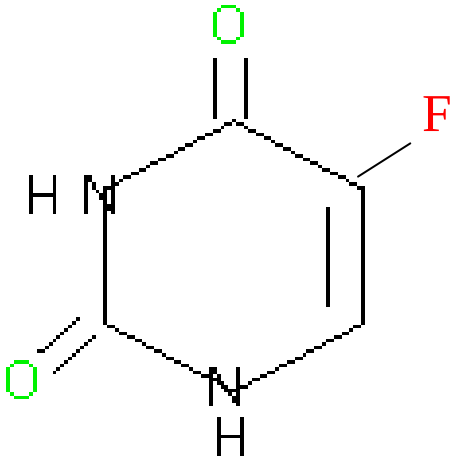




5FU is a simple derivative of Uracil



Uracil



5-Fluoro-Uracil (5FU)

THERAPEUTIC INDEX (Paul Ehrlich)

The basic idea behind cancer chemotherapeutics is to poison the tumor cell without killing the normal cells (or the patient) This is known as selective toxicity and is measured as "therapeutic index".

Selective toxicity = drug is more toxic to the tumor than to the host (patient).

Toxic dose = dose that harms the host
Therapeutic dose = dose that cures the disease.
Chemotherapeutic index = maximum tolerable dose divided by the minimum dose that will cure the disease.
The higher the chemotherapeutic index the better.

Most chemotherapeutics have a poor therapeutic Index:

Clinical Significances of Pyrimidine Metabolism

Degradation products are soluble, so no equivalent disorders as in Gout.

Two inherited Diseases results from last two steps in UMP Biosynthesis (Orotate phosphoribosyltransferase (OPRT) and OMP Decarboxylase). Loss of activity results in Orotic Aciduria.

Clinical condition is associated with retarded growth and Megablastic anemia (sometimes leukopenia).

Treatment includes IV injections of Uridine and Cytidine. They are converted to UMP by nucleoside kinase.

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$$\frac{\text{Drug [concentration] required to kill normal cells}}{\text{Drug [concentration] required to kill tumor cells}}$$

1: Science. 1994 Nov 4;266(5186):807-10.

p53 status and the efficacy of cancer therapy in vivo.

Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, Housman DE, Jacks T.

Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139.

The therapeutic responsiveness of genetically defined tumors expressing or devoid of the p53 tumor suppressor gene was compared in immunocompromised mice. Tumors expressing the p53 gene contained a high proportion of apoptotic cells and typically regressed after treatment with gamma radiation or adriamycin. In contrast, p53-deficient tumors treated with the same regimens continued to enlarge and contained few apoptotic cells. Acquired mutations in p53 were associated with both treatment resistance and relapse in p53-expressing tumors. These results establish that defects in apoptosis, here caused by the inactivation of p53, can produce treatment-resistant tumors and suggest that p53 status may be an important determinant of tumor response to therapy.

PMID: 7973635 [PubMed - indexed for MEDLINE]

Common side effects of DNA inhibitor chemotherapeutics:

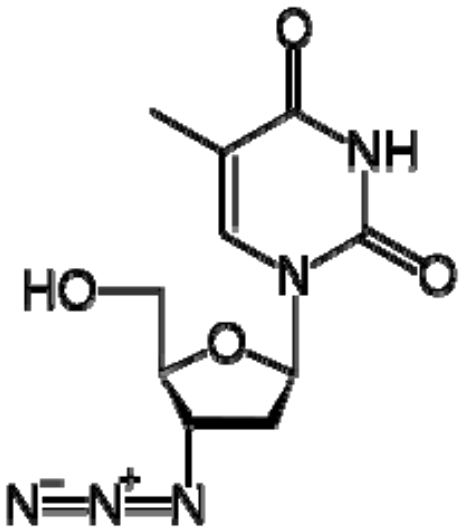
Diarrhea
Skin and eye sensitivity to sunlight
Abnormal liver function tests
Hair loss
Immuno-suppression

Skin rashes
Fatigue
Headache, backache,
Spinal cord irritation
Peripheral neuropathies

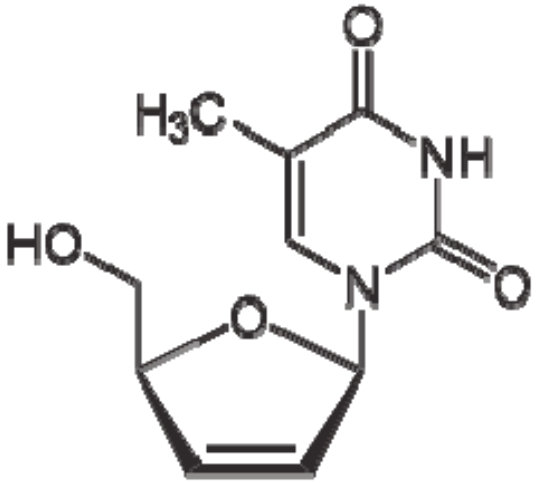
Nucleoside Analogs/Metabolites

Definitions: Wide range of antiviral products (anti-metabolites) that are used to inhibit spread of virus in infected cells. Less selective nucleosides are used for cancer chemotherapy.

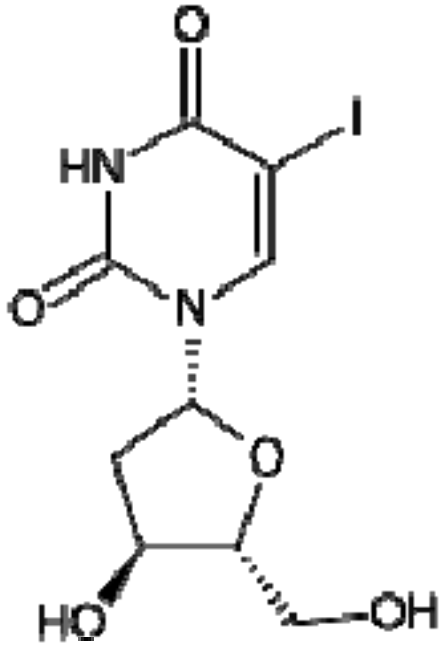
Activity based on trail and error from medicinal chemists. Classes include; Deoxyadenosine, Deoxycytidine, Deoxyguanosine, Deoxythymidine, and Deoxyuridine.



AZIDOTHYMIDINE (AZT)
(RETROVIR, RETROVIS)



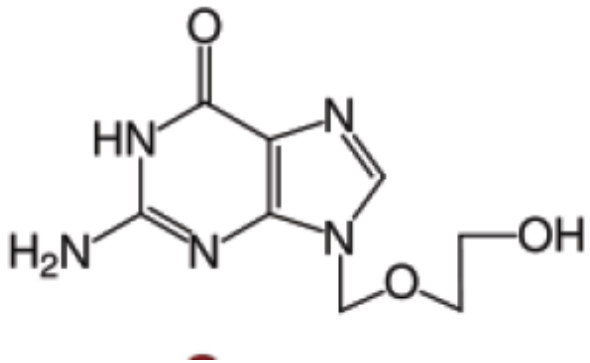
STAVUDINE (4DT))



IDOXURIDINE)

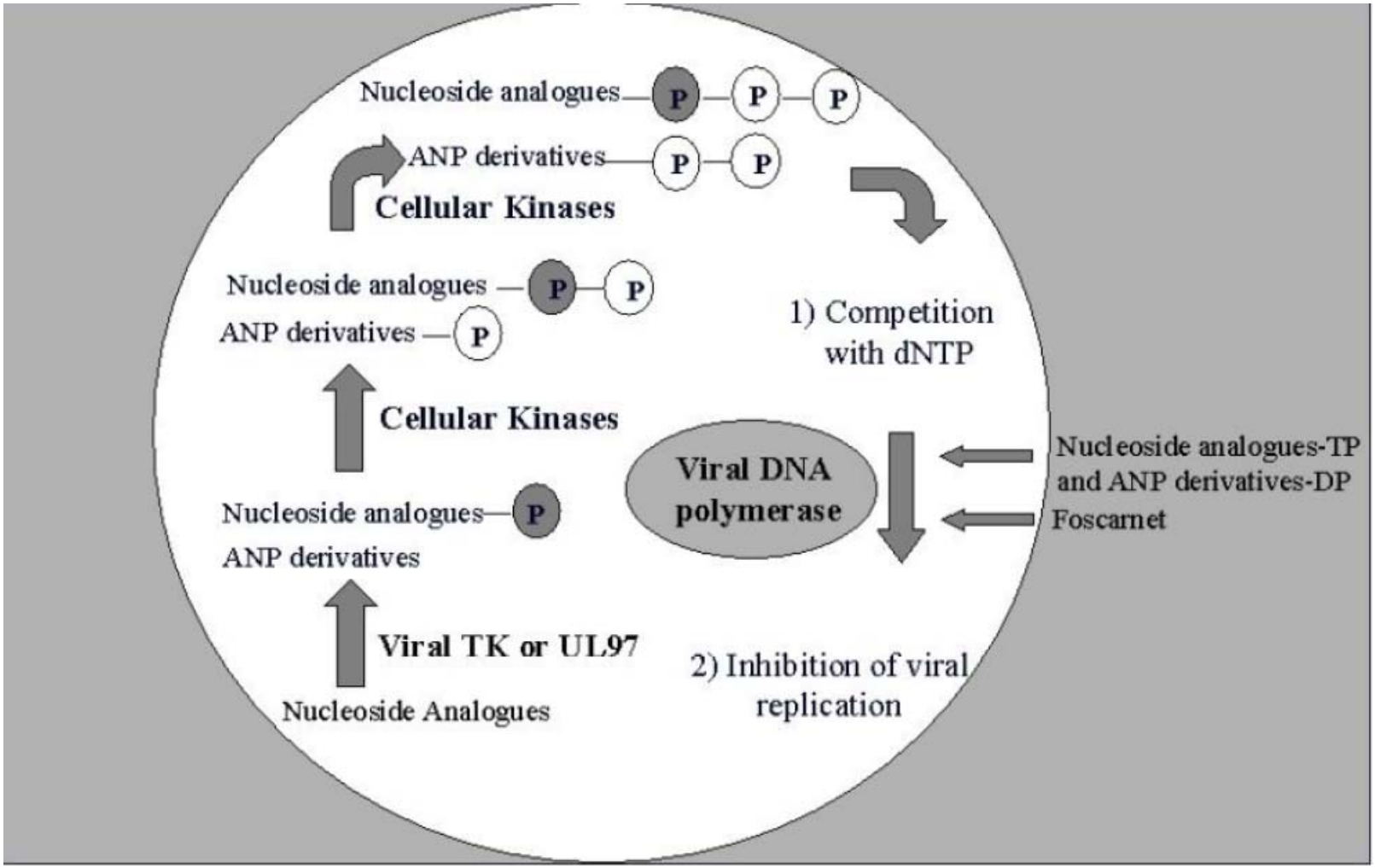
Function: Blocking reverse transcriptase and terminate DNA synthesis by a process called “chain-termination”

Acyclovir: Guanine analogue antiviral drug



Discovered by pharmacologist Gertrude B. Elion (nobel prize in 1988)
Low toxicity and high selectivity (high therapeutic index)
Contains partial nucleoside structure in that the sugar is an open-chained Structure (pro-drug)
It is selectively converted to acyclo-GMP by viral thymidine kinase.
(Acyclo-GMP -> Acyclo-GDP -> Acyclo-GTP by cellular kinases)
Trade names are Zovir or Zovirax

Activation of viral nucleoside pro-drugs and anti-viral activity



Summary:

1. **Recognize basic structures of purines and pyrimidines**

2. **Key regulatory enzymes and feedback networks**

3. **Targets for clinical interventions**