Nucleotide Metabolism

Purines and pyrimidines

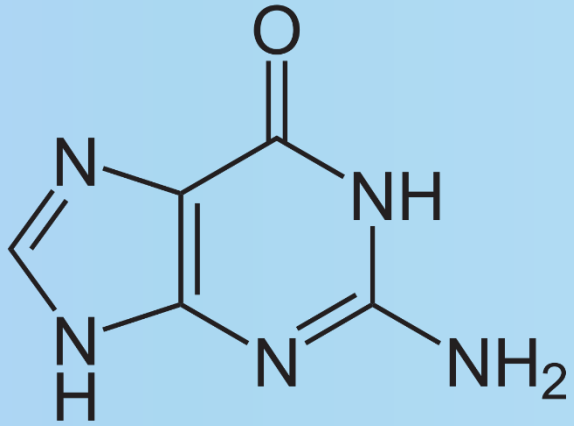
By: Adriana Nudga

Overview

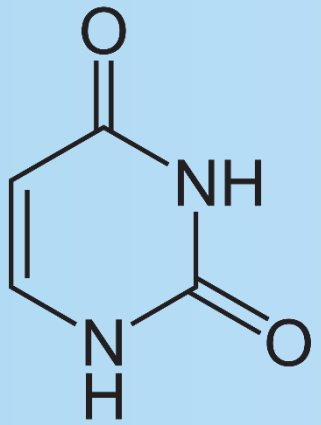
- Nucleobases and their function
- Ribonucleotide synthesis
  - Purine synthesis/degradation: “de novo” and salvage pathway
  - Pyrimidine synthesis/degeneration
- Deoxyribonucleotide synthesis

First a quick review!

Adenine

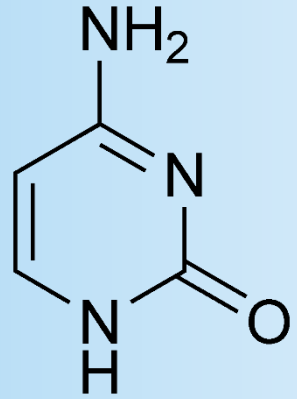


Guanine



Uracil

Thymine

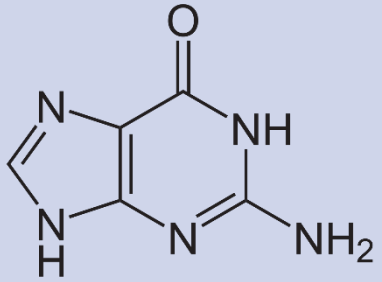
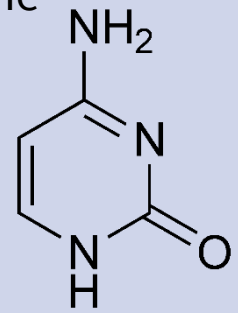
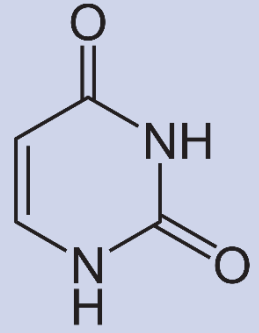


Cytosine

How to remember them

“TUC-TUC around the pyramids”

PURE AS GOLD

PURINES	PYRIMIDINES
Adenine 	Thymine 
Guanine 	Cytosine 
	Uracil (RNA) 

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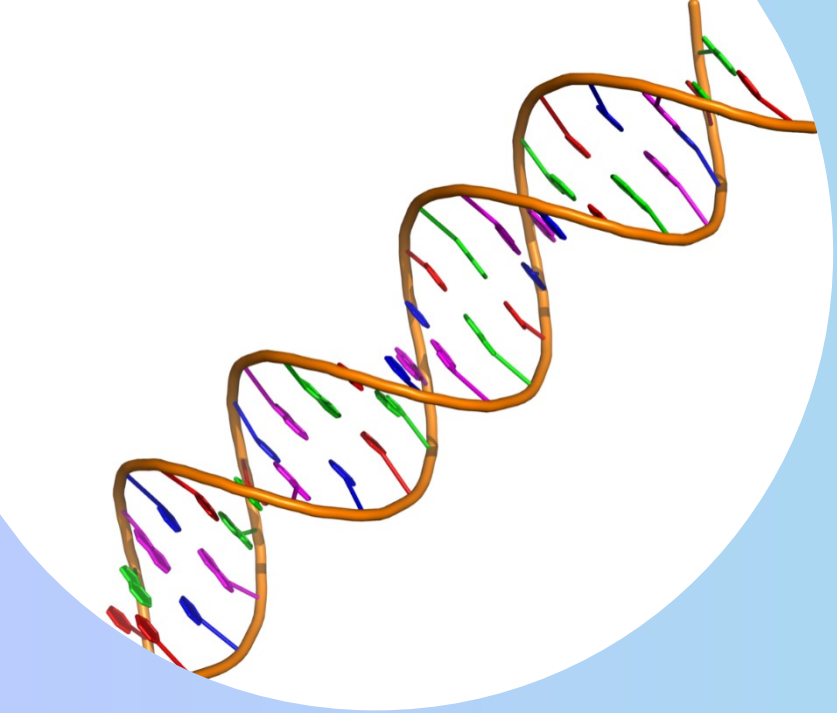
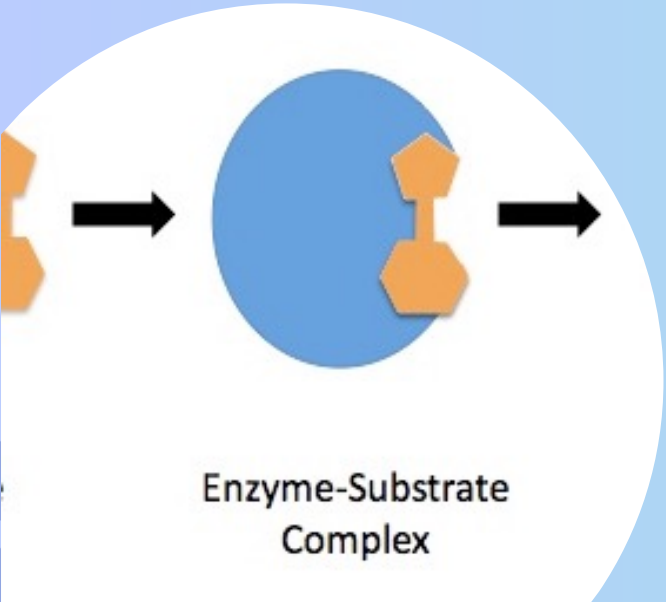




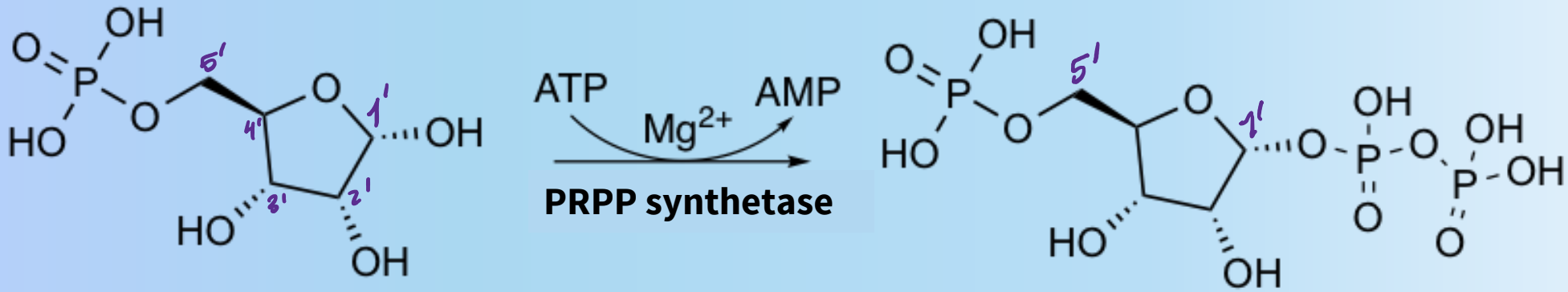
Functions:

- DNA and RNA
- Essential coenzymes:
  - Coenzyme A
  - FAD[H_2]
  - NAD[H]/NADP[H]
  - cAMP/cGMP
- Energy carriers:
  - ATP
  - GTP

Step one, the beginning. Creation of PRPP

Important, PRPP is needed for the synthesis of both purines and pyrimidines.

- PRPP is Synthesized from ATP and ribose 5-phosphate
- Catalyzed by *PRPP synthetase*
- Key substrate in <u>both</u> pyrimidine and purine synthesis



Ribose 5-phosphate

5-phosphoribosyl-1-pyrophosphate

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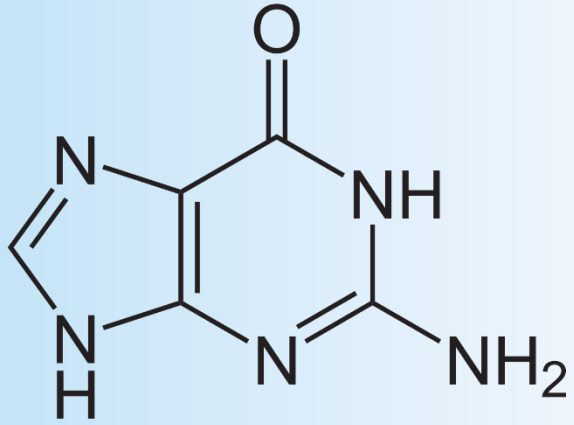
Adenine

Purine synthesis

“de novo”

This pathway creates nucleotides from scratch.



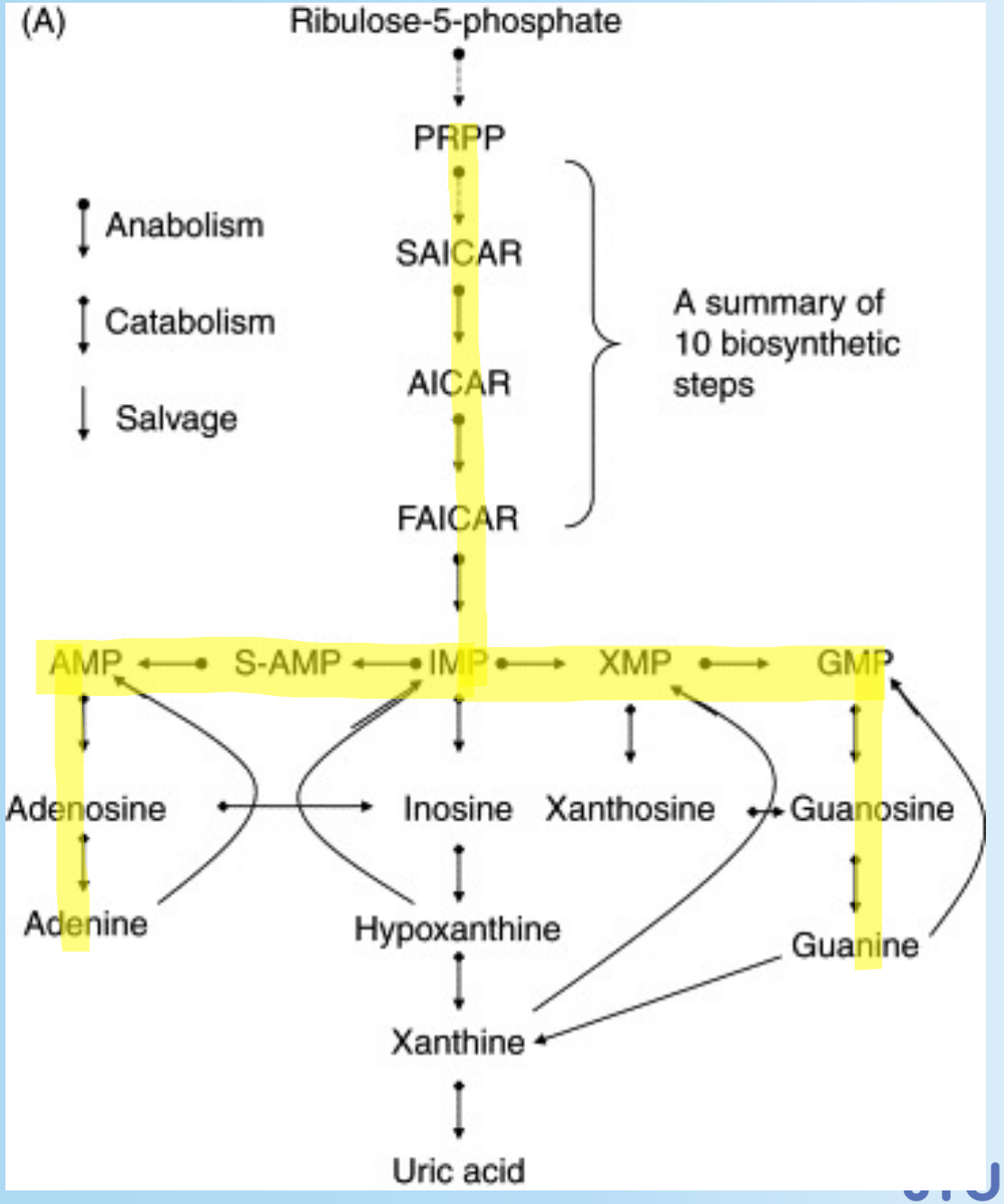
Guanine

QUICK OVERVIEW

“ de novo” synthesis is the creation of IMP then IMP turns to Adenine or Guanine.

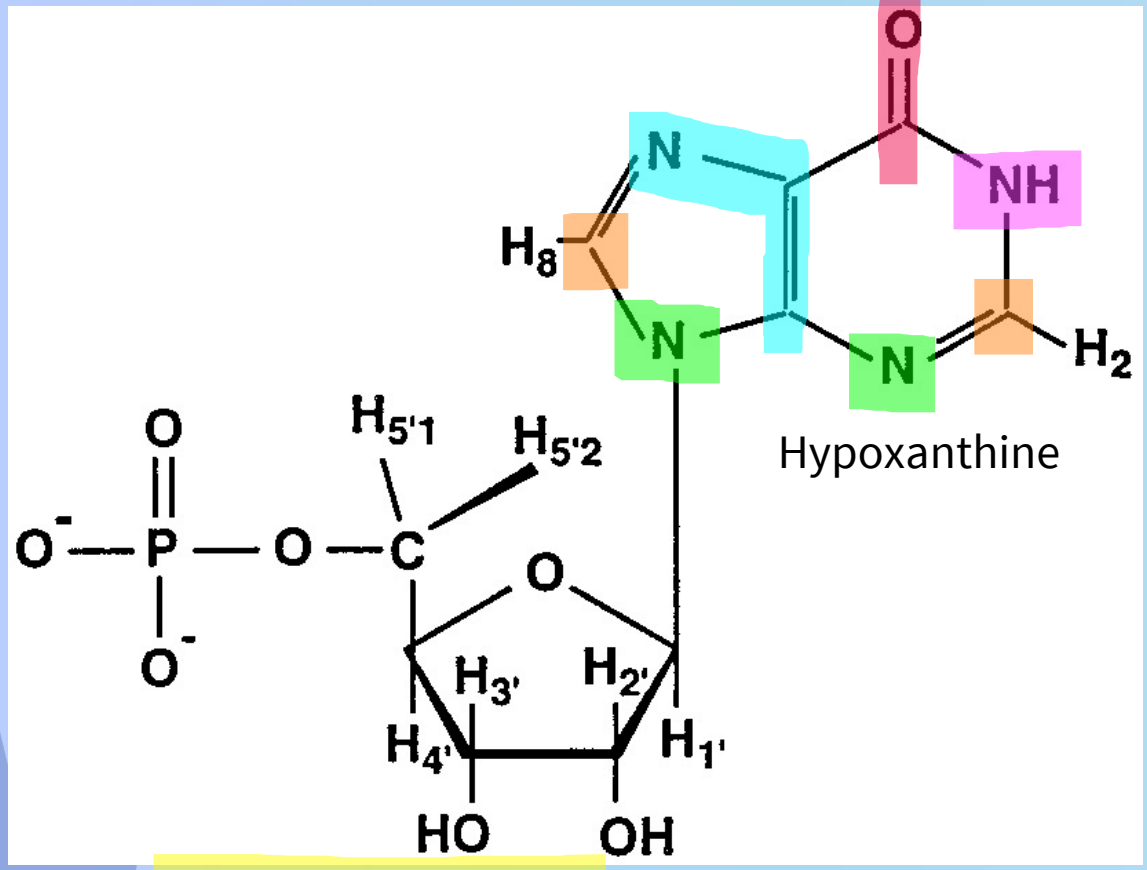
Rate limiting step is PRPP aminotransferase.

Remember GAG for glutamine, aspartate, and glycine



Synthesis of IMP for purines

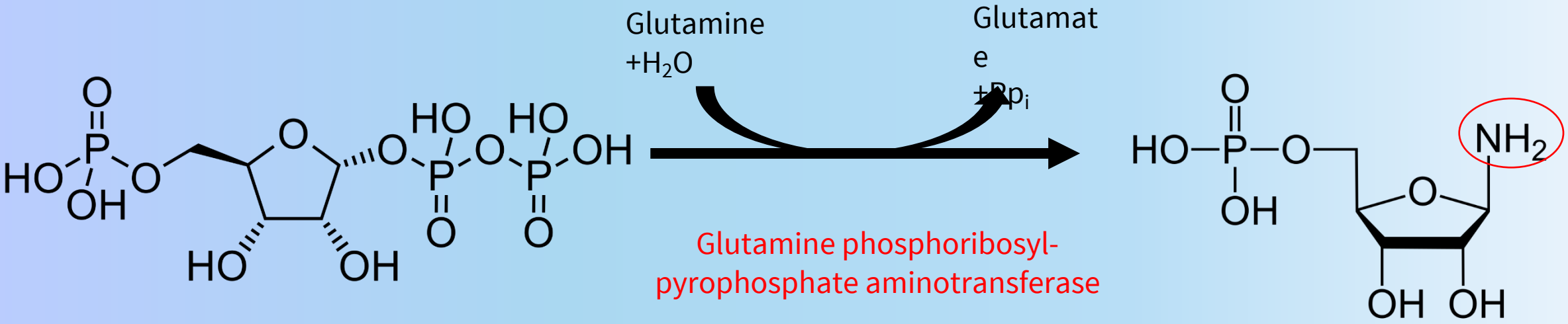
10 step reaction from PRPP to IMP



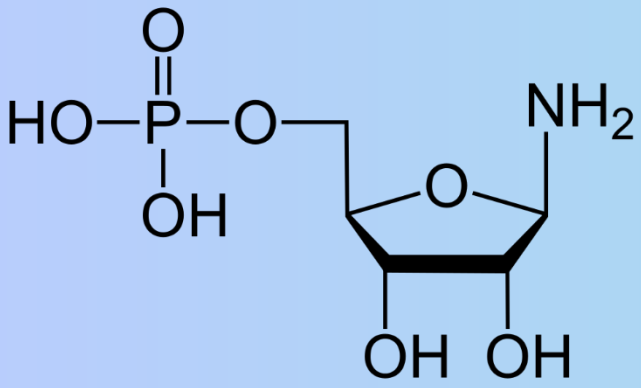
Used :

- 1 PRPP
- 2 glutamine
- 1 Glycine
- 1 aspartate
- 2 N^{10}-formyl-tetrahydrofolate
- 1 $HCO_3[CO_2]$
- 6 ATP

Step 1: rate limiting step

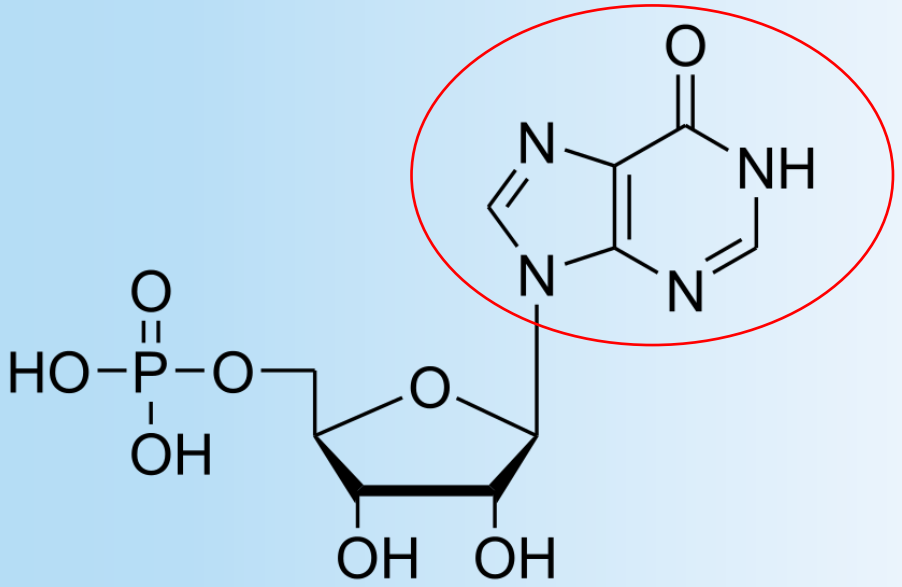


GOAL!



5-phosphorybosylamine

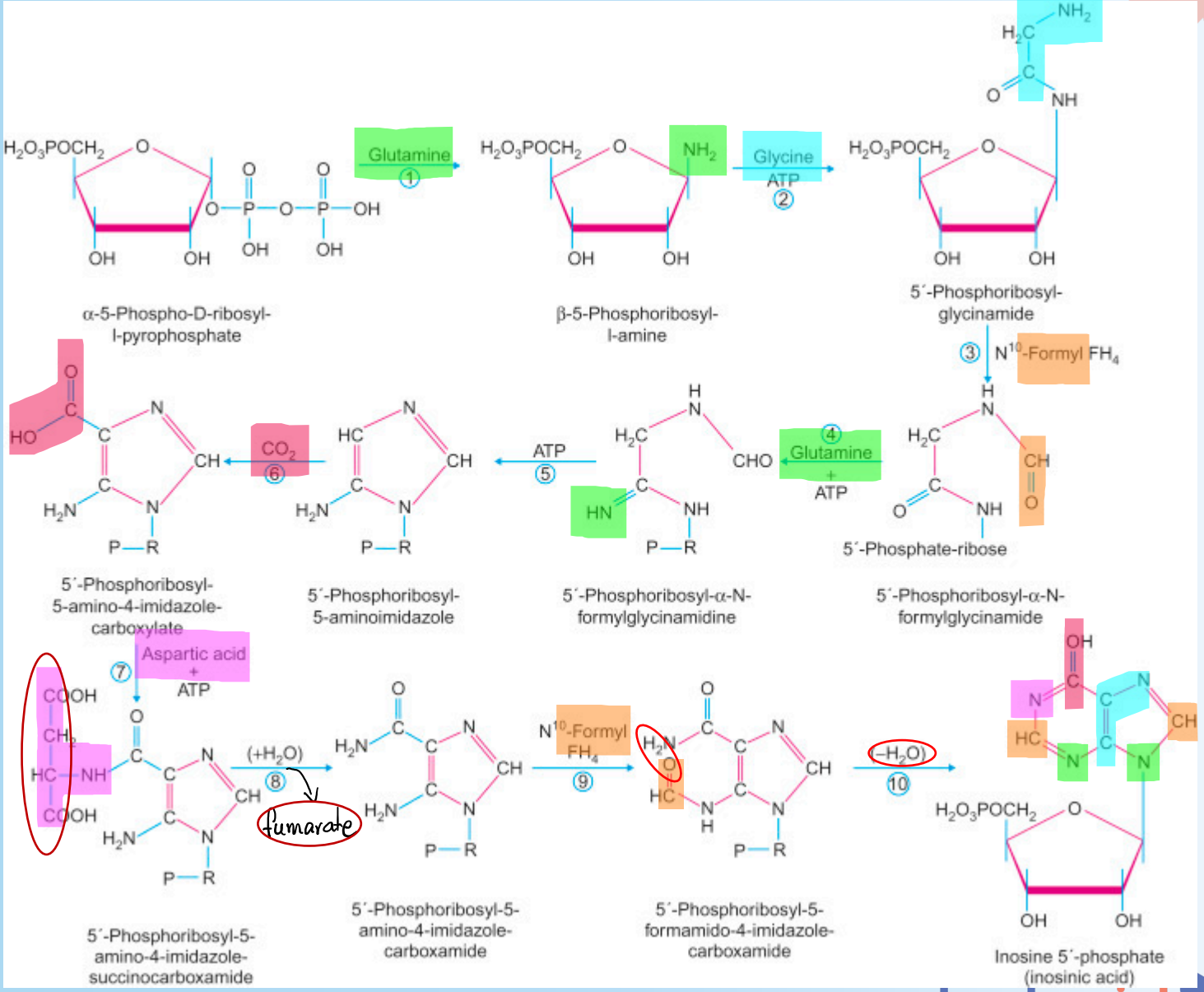
10 steps to reach our goal of IMP

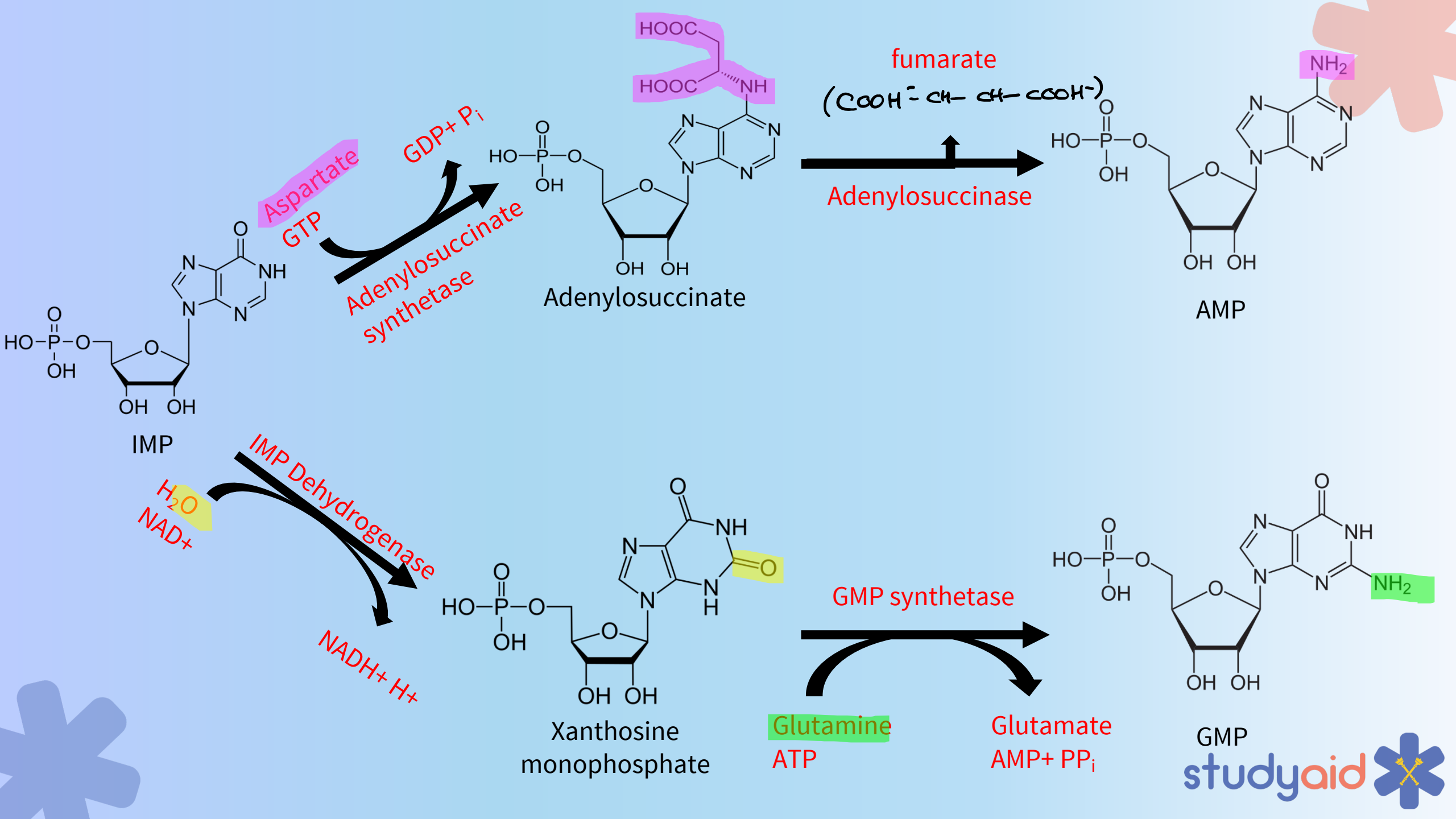


Inosine 5’ monophosphate (IMP)

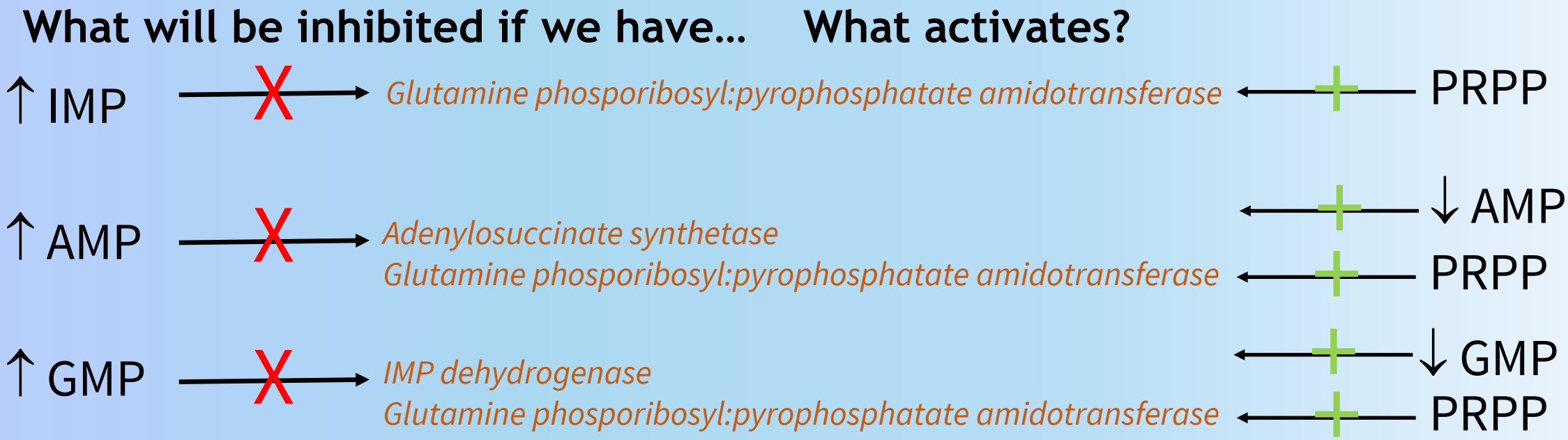
The 10 step process

1. Glutamine:phosphoribosyl pyrophosphate amindotransferase
2. GAR synthetase
3. Formyltransferase
4. Synthetase
5. Synthetase
6. Carboxylase
7. Synthetase
8. Adenylsuccinate lyase
9. Fromyltrransferase
10. Synthase

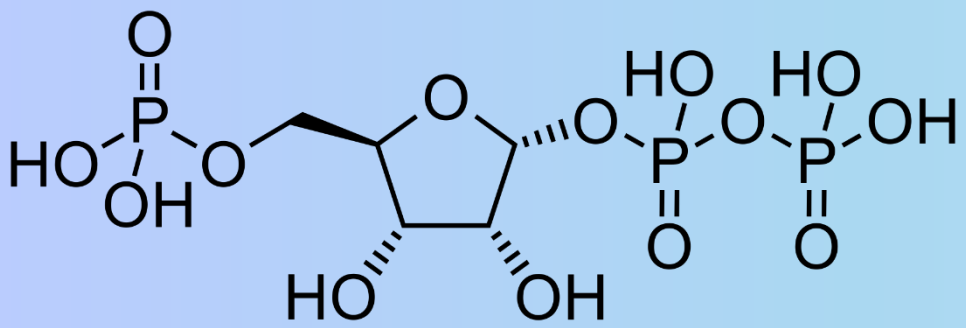



IMP
Aspartate
GTP
GDP+ P_i
Adenylosuccinate synthetase
Adenylosuccinate
fumarate
(COOH-CH-CH-COOH)
Adenylosuccinase
AMP
H_2O
NAD^+
IMP Dehydrogenase
$NADH + H^+$
Xanthosine monophosphate
GMP synthetase
Glutamine
ATP
Glutamate
$AMP + PP_i$
GMP

Regulation

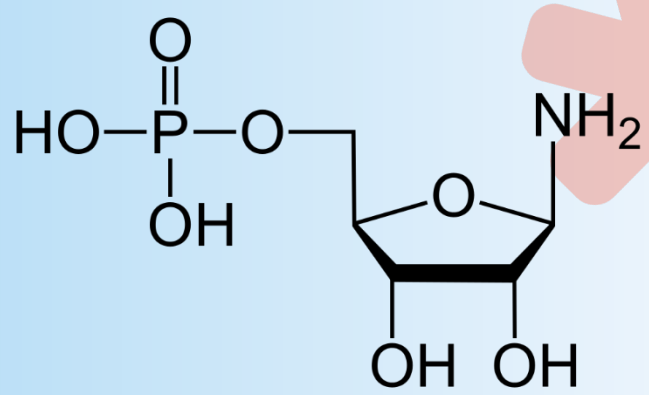


*negative feedback inhibition

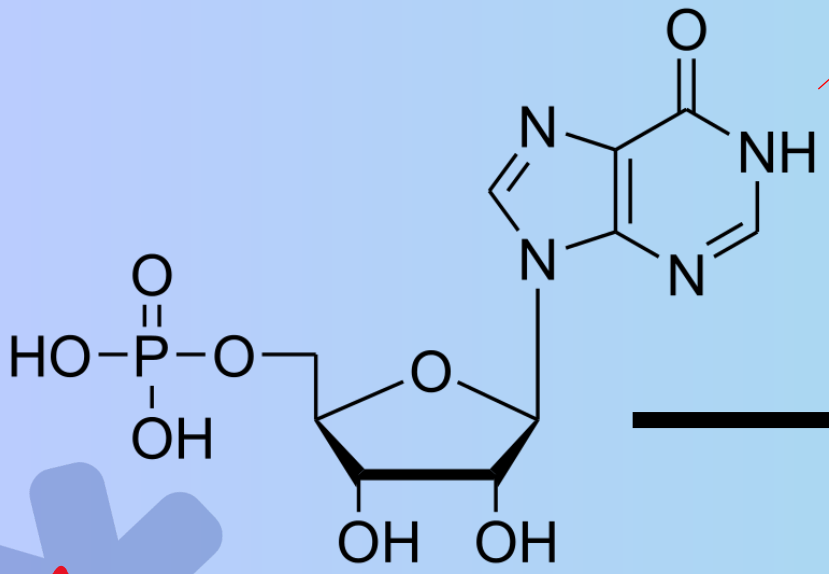


PRPP

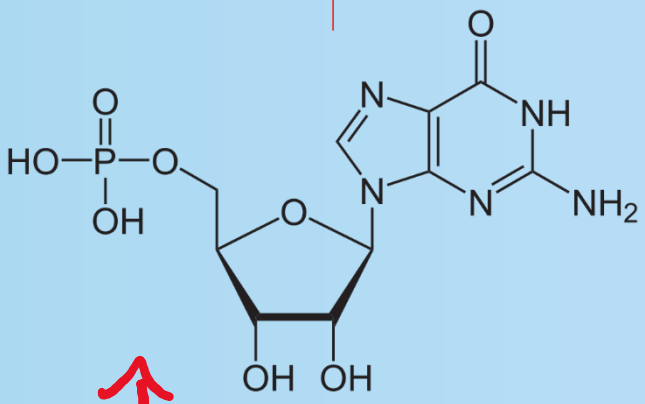
Glutamine phosphoribosyl-pyrophosphate aminotransferase



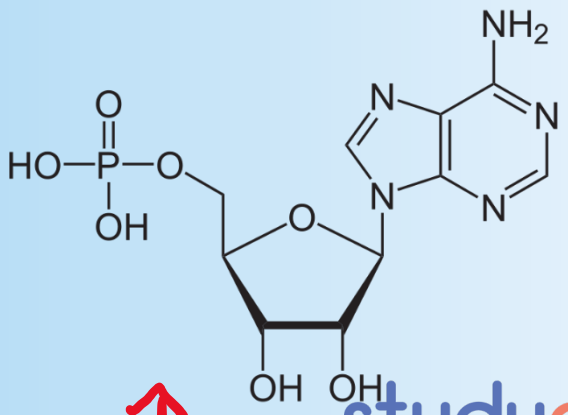
5-phosphorybosylamine



Inosine 5' monophosphate (IMP)



GMP



AMP

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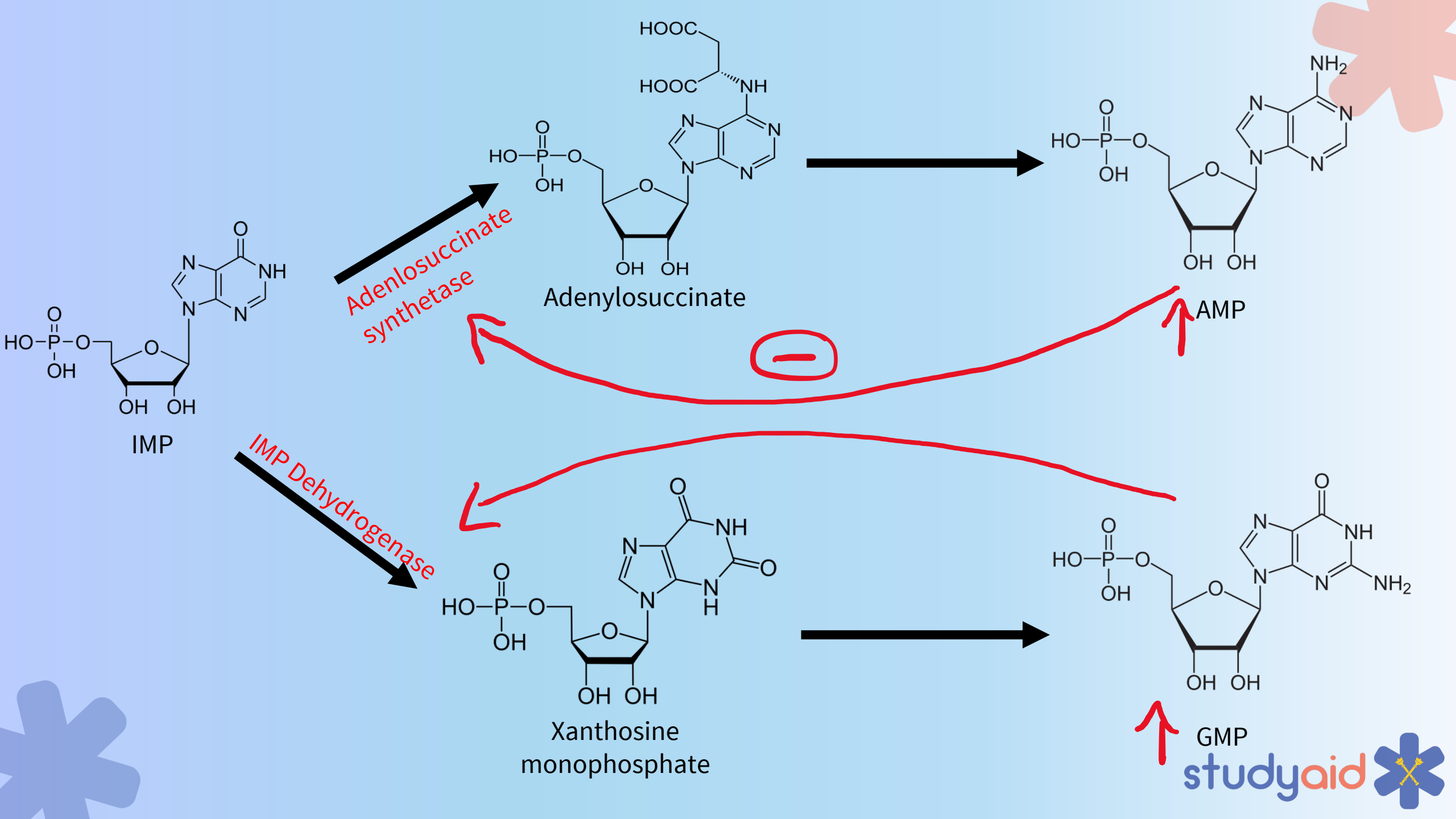
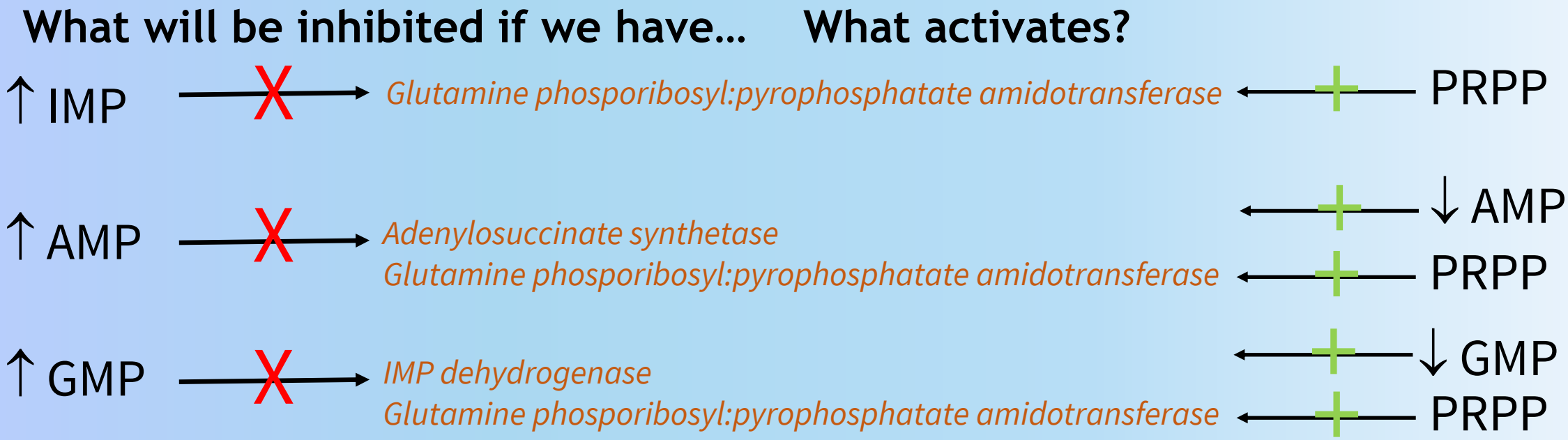
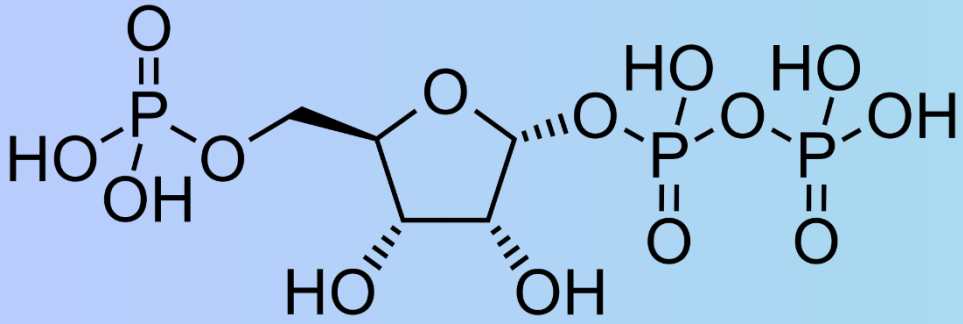

IMP
Adenlosuccinate synthetase
Adenylosuccinate
AMP
IMP Dehydrogenase
Xanthosine monophosphate
GMP

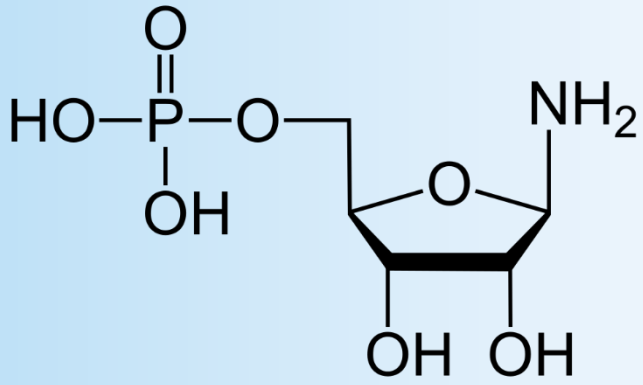
Regulation



*negative feedback inhibition

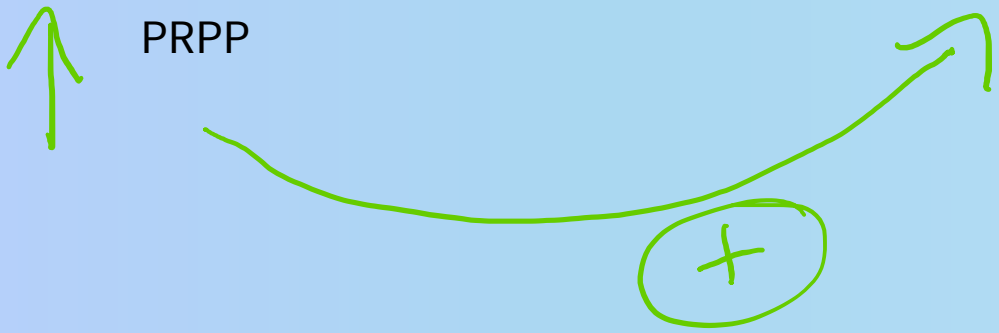


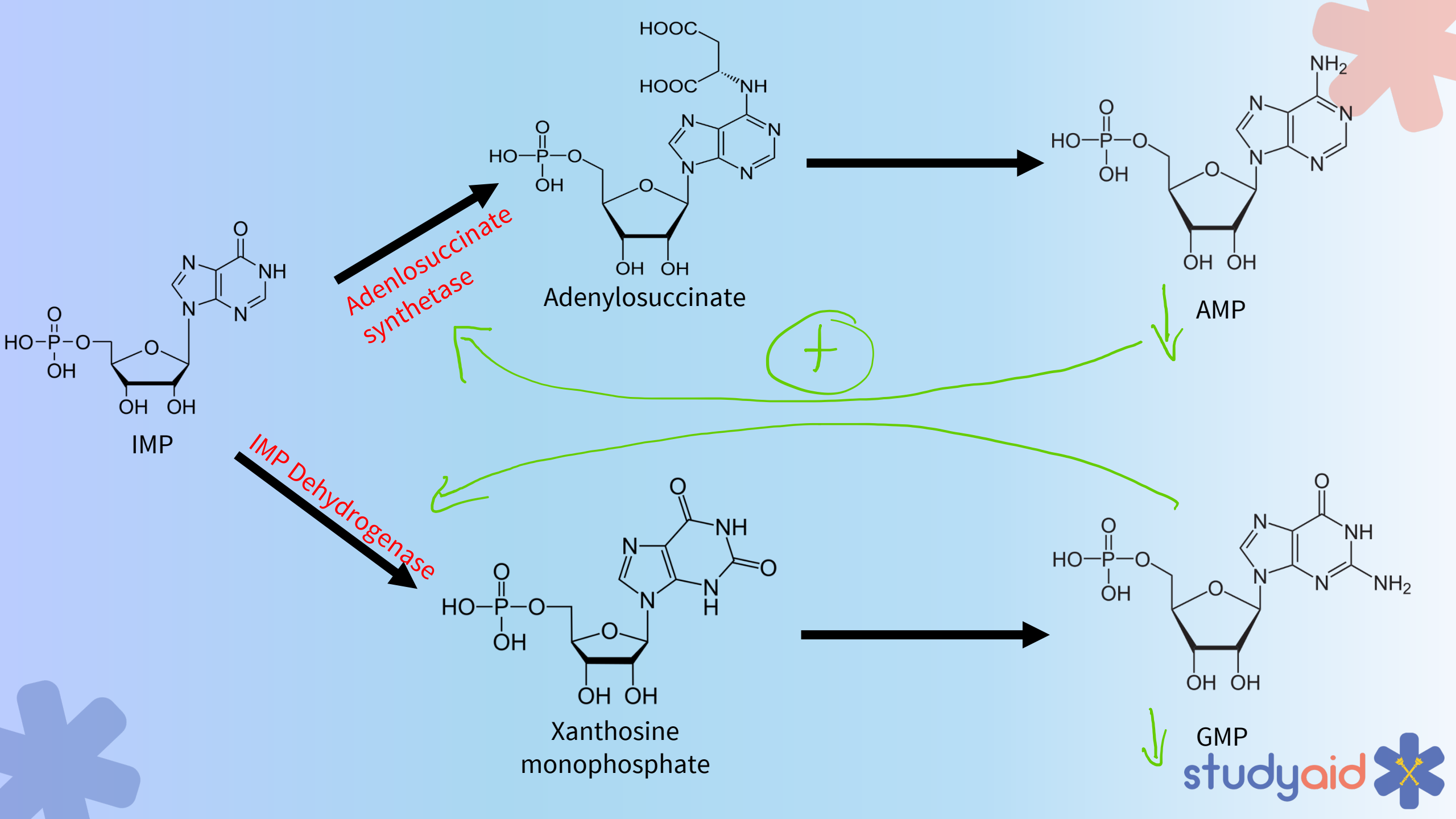
Glutamine phosphoribosyl-
pyrophosphate aminotransferase



5-phosphorybosylamine

PRPP

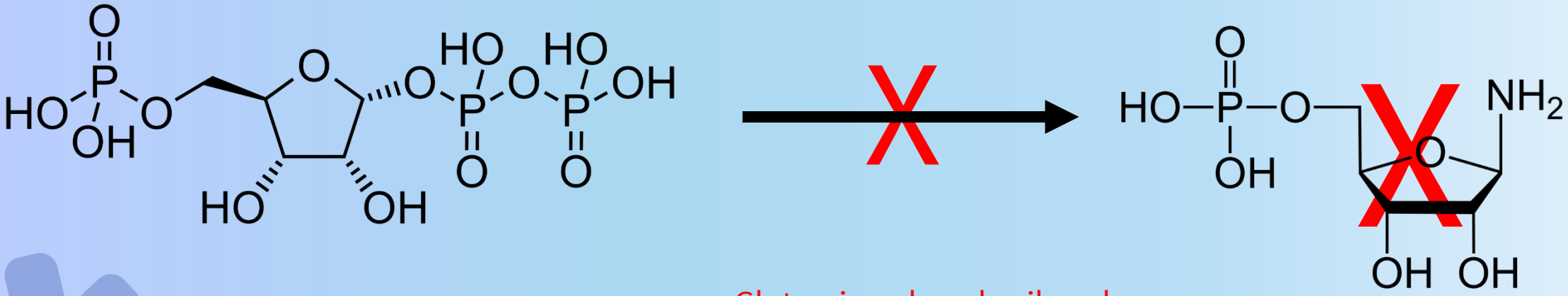



IMP
Adenlosuccinate synthetase
Adenylosuccinate
AMP
+
IMP Dehydrogenase
Xanthosine monophosphate
GMP

Clinical correlation

6-Mercaptopurine

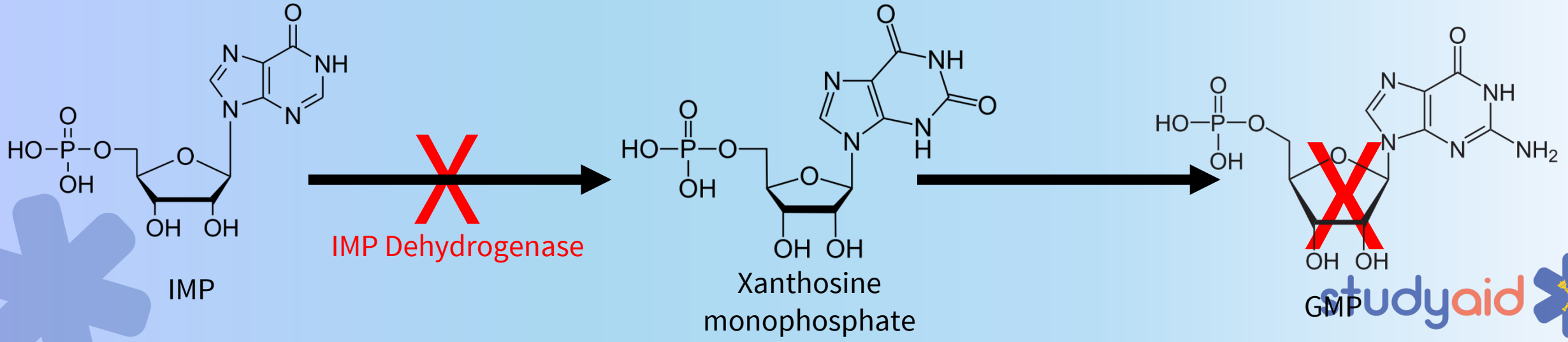
- Immunosuppressive drug
- Inhibits *PRPP amidotransferase*



Clinical correlation

Mycophenolic acid

- Immunosuppressive drug
- Inhibits *IMP dehydrogenase*
- Resulting in ↓ GMP production → ↓ production of T and B cells
- Clinical use: prevent graft rejection



Purine synthesis

Salvage pathway

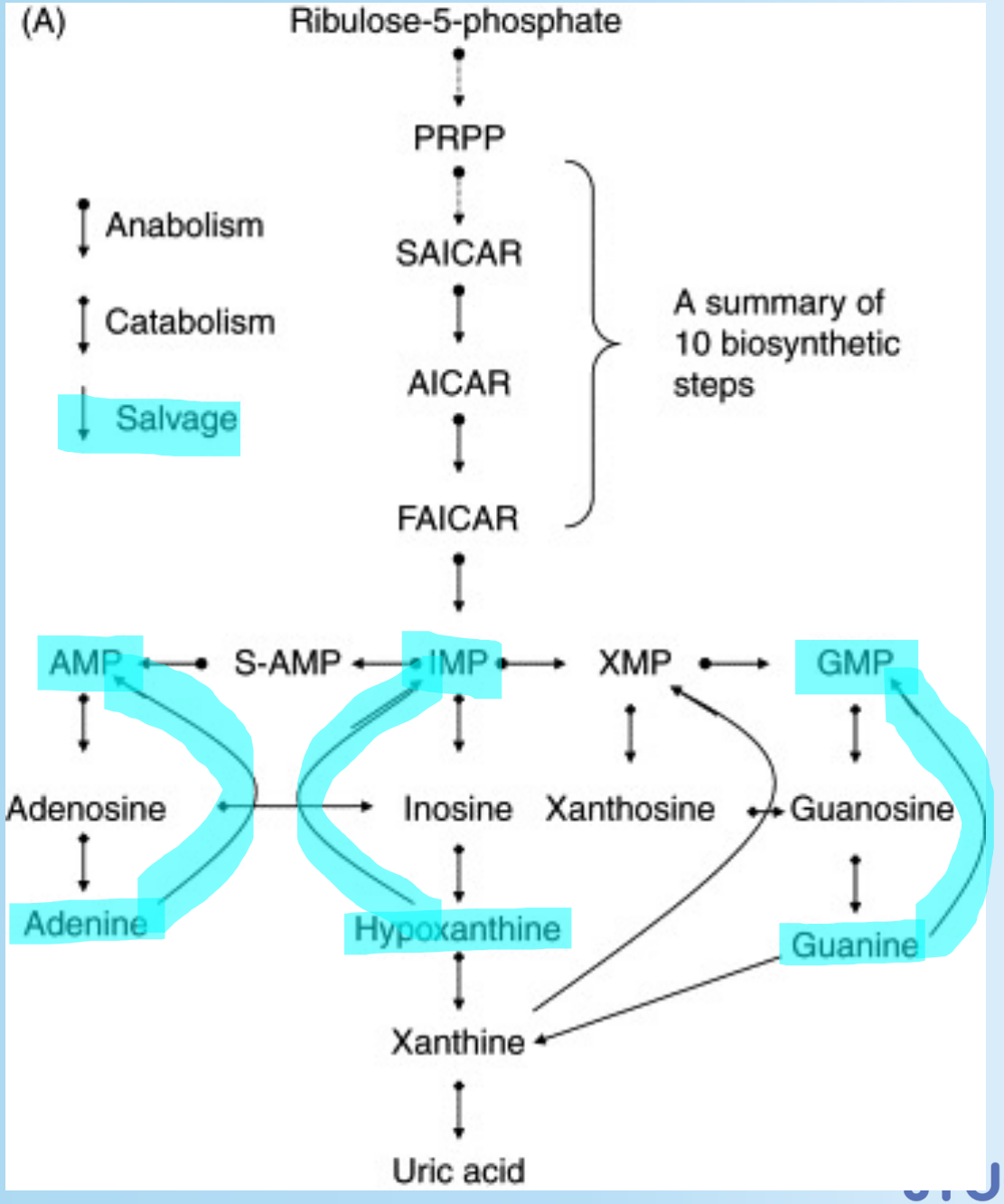
reuse of the performed base resulting from normal cell turnover, or from diet

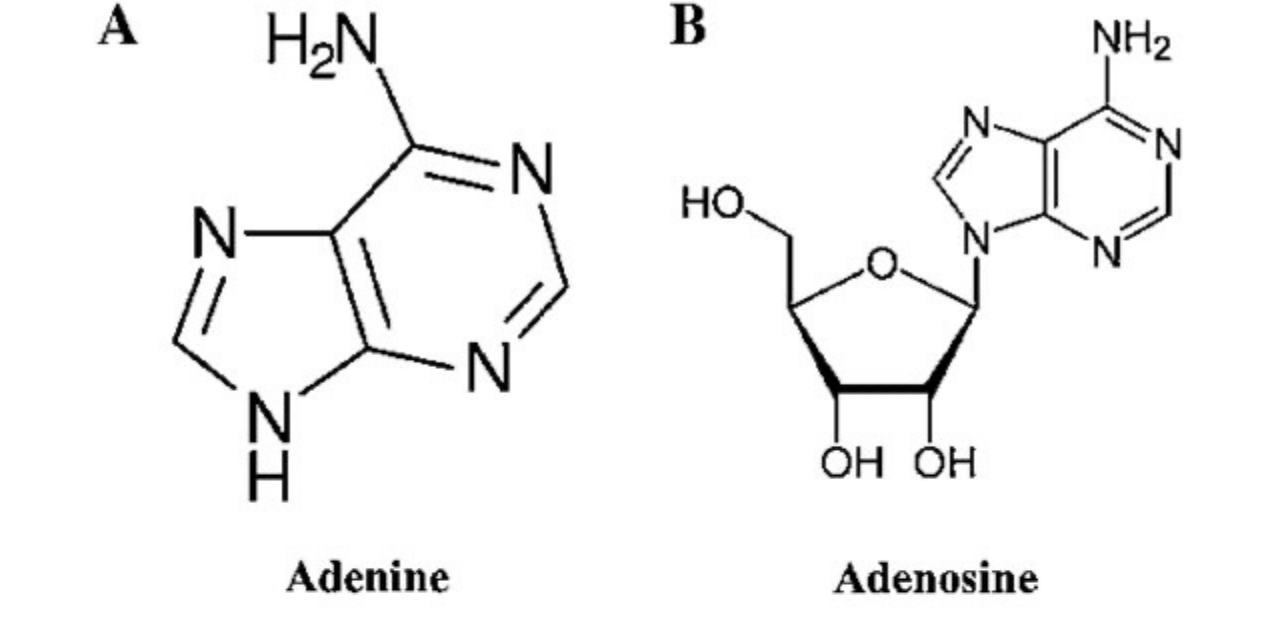
QUICK OVERVIEW

Salvage pathway: end products are used to create AMP, IMP, GMP

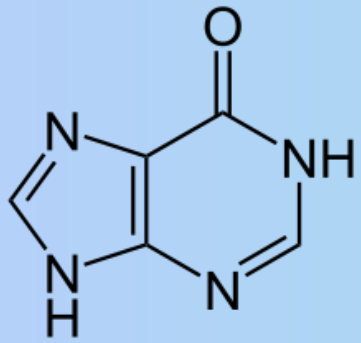
IMPORTANT ENZYMES:
- Adenine phosphorybosyltransferase (APRT)

-Hypoxanthine-guanine phosphorybosyltransferase (HGPRT)





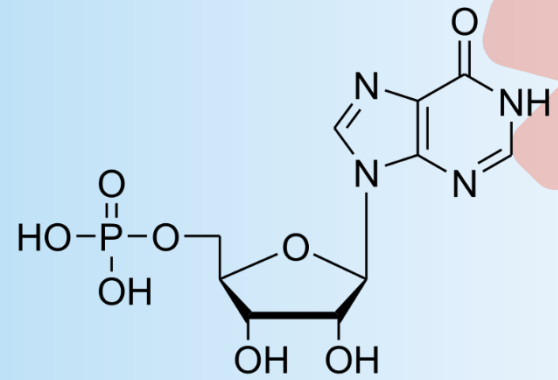
The difference of adenine vs adenosine and guanine vs guanosine is that the “–osine” is attached to the ribose sugar. This is important to distinguish when we start talking about the salvage pathway.



Hypoxanthine

PRPP → PP_I

HGPRT

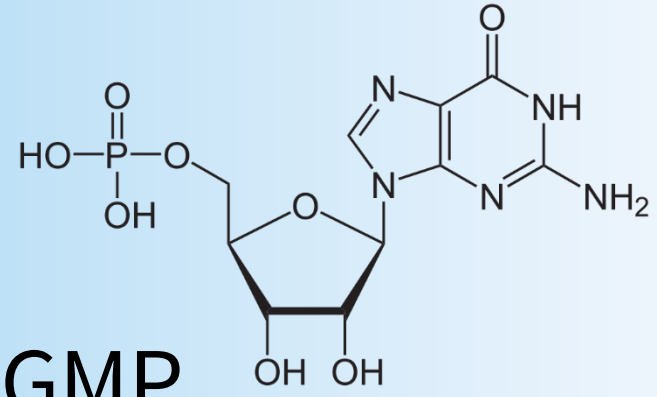


IMP

Guanine

PRPP → PP_I

HGPRT

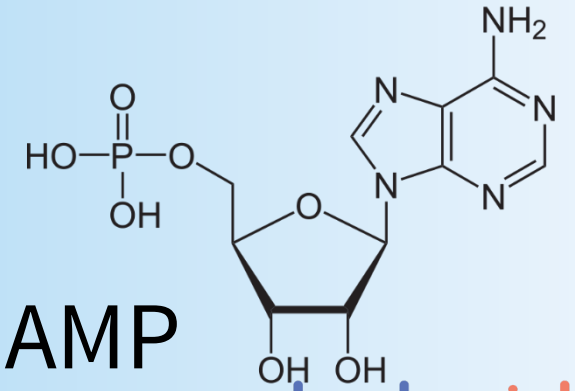


GMP

Adenine

PRPP → PP_I

APRT



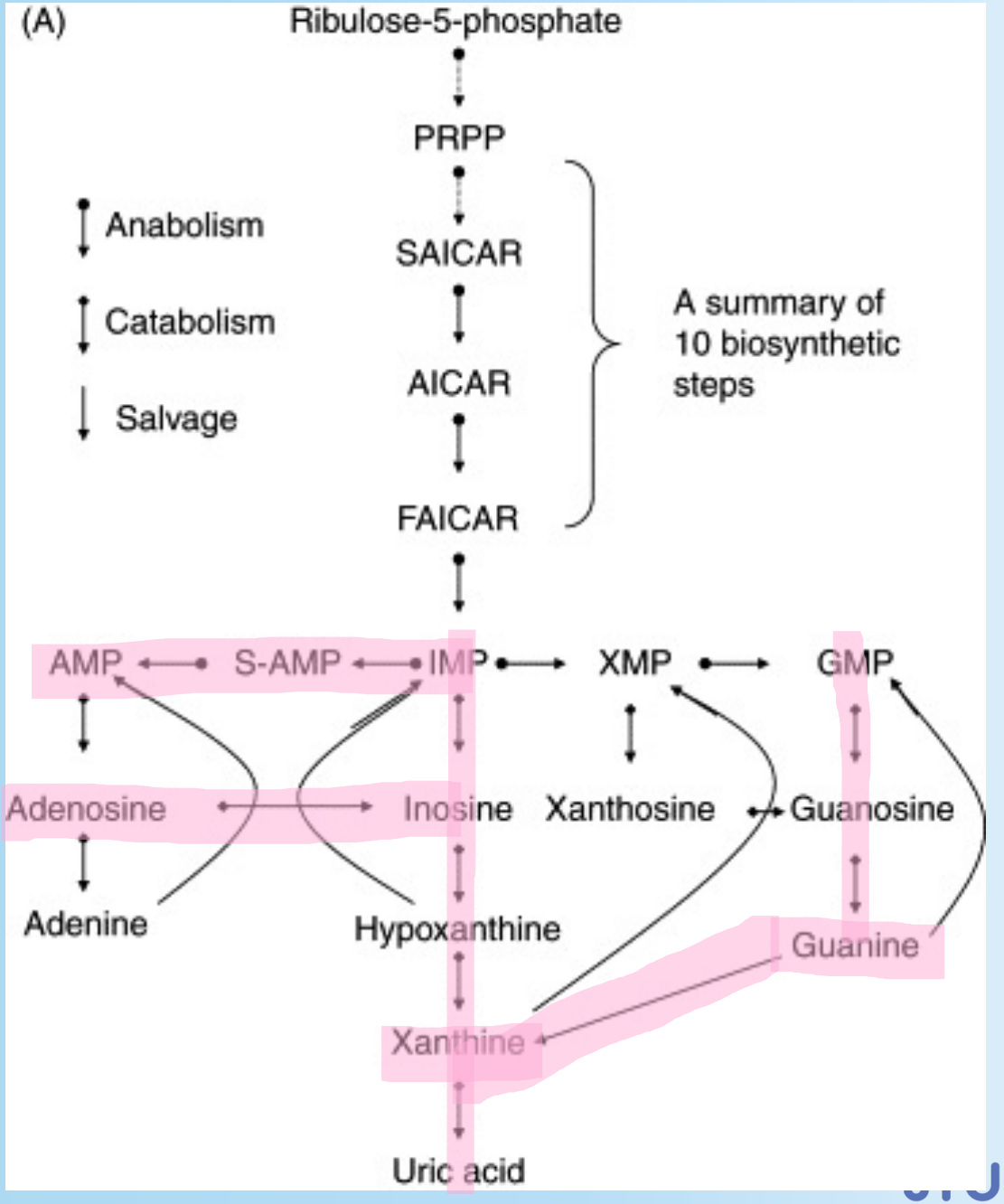
AMP

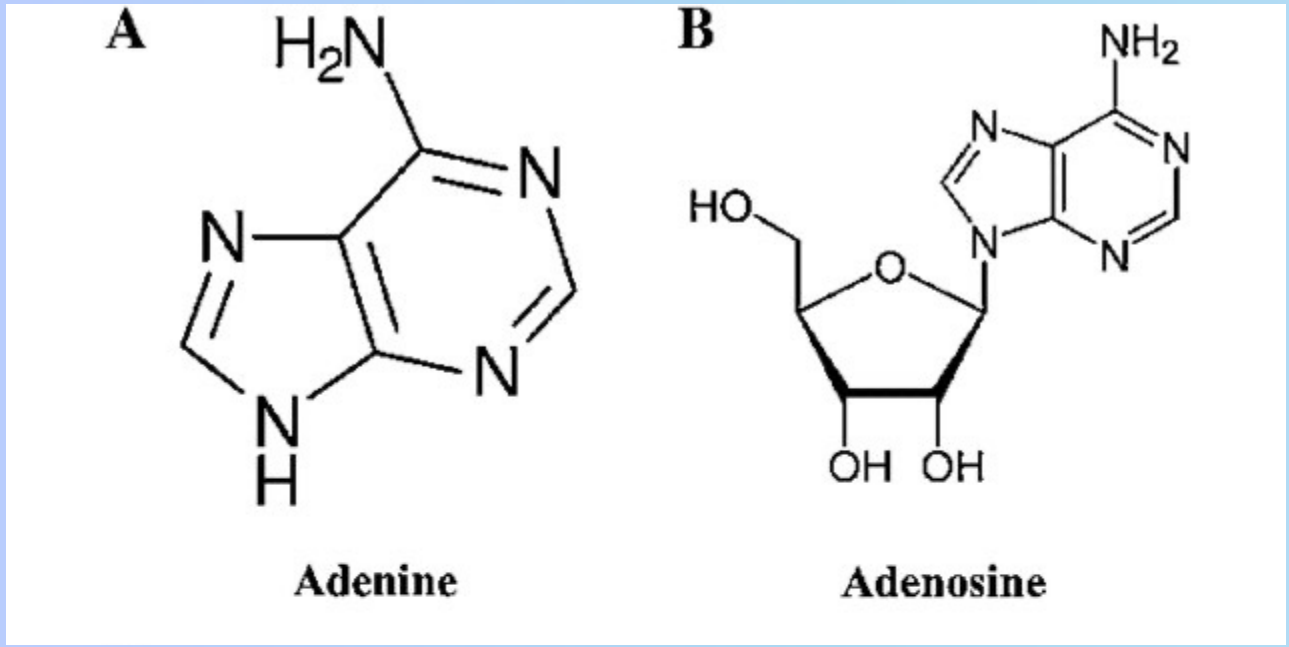
Purine degradation

QUICK OVERVIEW

- Degradation pathways:
  - IMP -> inosine -> uric acid
  - AMP -> IMP-> inosine -> uric acid
  - Adenosine -> inosine -> uric acid
  - GMP -> guanine -> xanthine -> uric acid

- Majority of URIC ACID is excreted in the urine.
- Purines formed in “de novo” are degraded in the liver. Then free bases are sent to peripheral tissue to join salvage pathway.



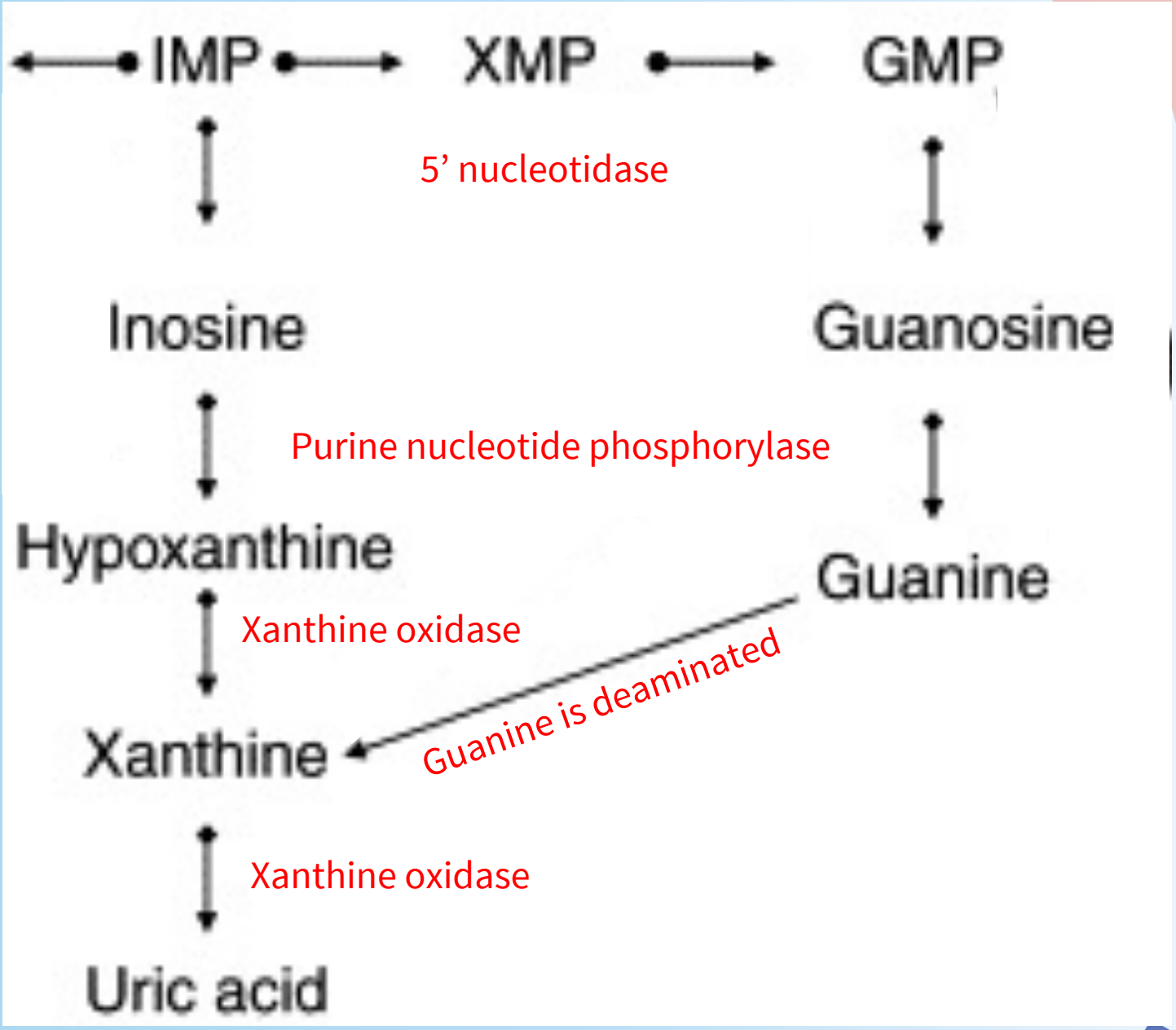


During degeneration of nucleotides Free purine and pyrimidine bases (adenine, guanine,) are released into the cell and are typically transported intercellularly across membranes and salvaged to create more nucleotides via nucleotide salvage. For example, adenine + PRPP --> AMP + PPi.

Formation of URIC ACID

IMP -> inosine -> uric acid

GMP -> guanine -> xanthine -> uric acid

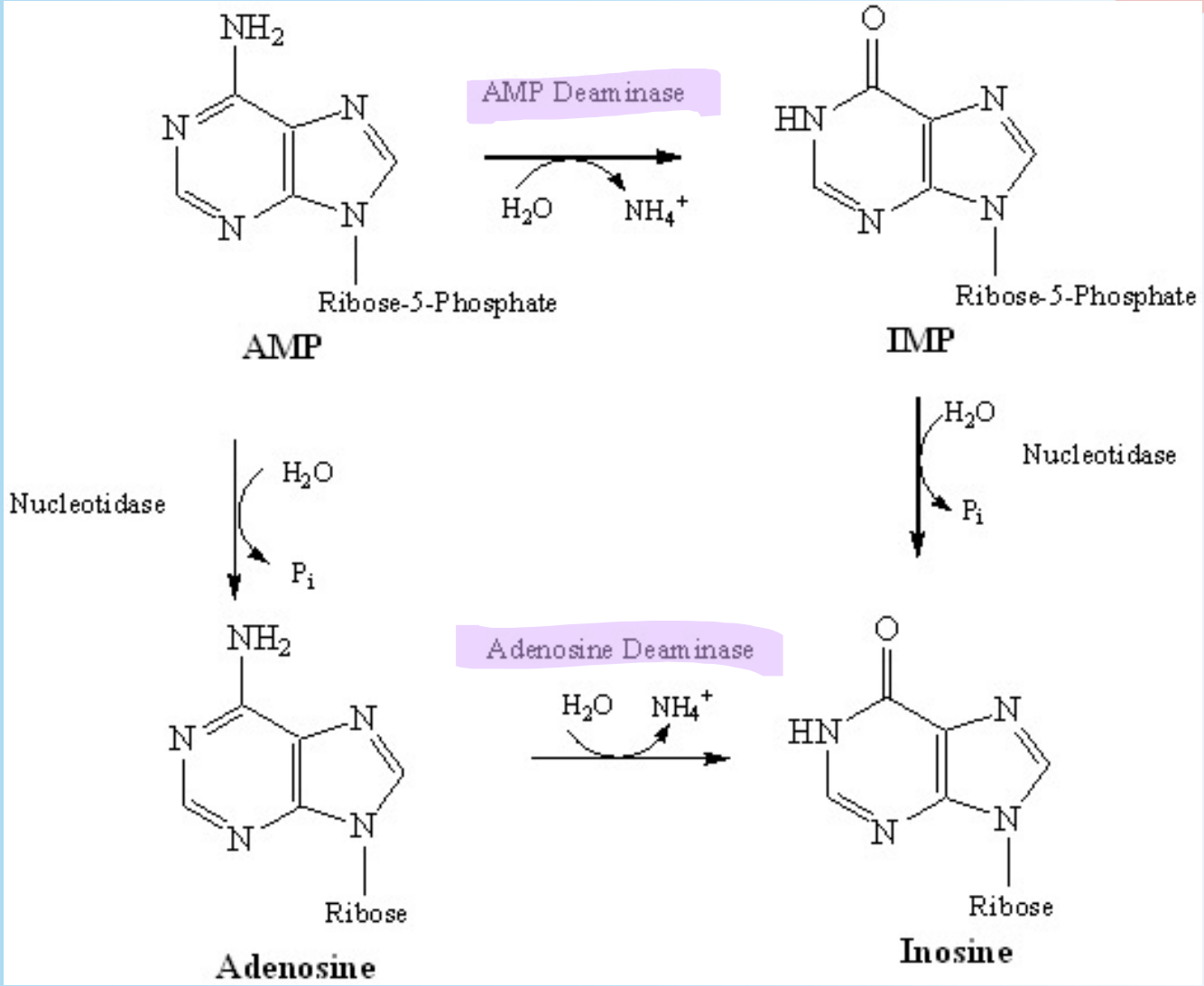


Formation of URIC ACID

AMP -> IMP-> inosine -> uric acid

Adenosine -> inosine -> uric acid

The anime group is removed from AMP to form IMP by AMP Deaminase. Or the amine groups is removed from adenosine to form inosine by adenosine deaminase.

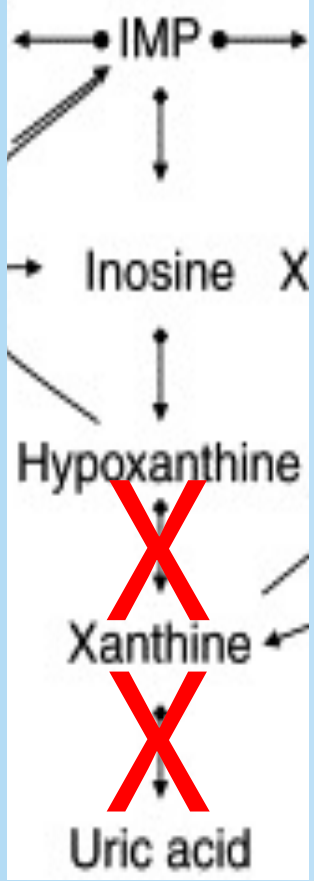


Clinical correlation

Gout

High levels of uric acid in blood (hyperuricemia)
-> deposits of monosodium urate(MSU) crystals in joints
-> inflamatory response

- Hyperuricemia results primarily from the UNDERexcretion of uric acid.
- OVERproduction of uric acid is rare.



Allopurinol (drug)

- Inhibits *Xanthine oxidase*
- Gout treatment
- Hypoxanthine analogue
- Inhibits uric acid synthesis

Clinical correlation

Lesch-Nyhan syndrome

Salvage pathway

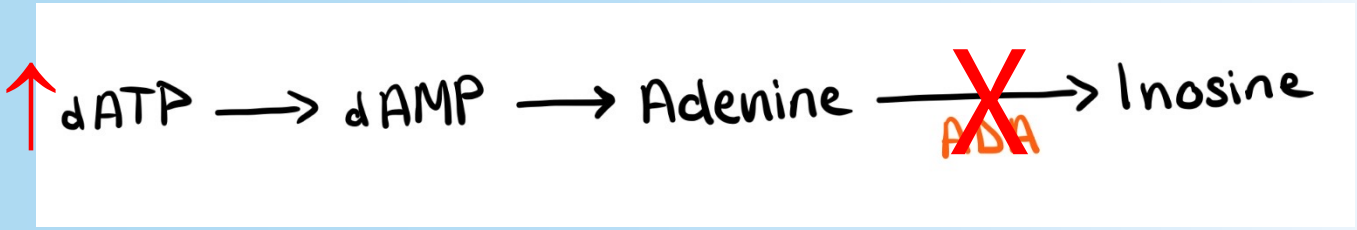
- *HGPRT* deficiency
- Excess uric acid production and de novo purine synthesis

Hyperuricemia
Gout
Pissed off (aggression, self mutilation)
Retardation
DysTonia

Adenosine deaminase deficien

Degradation pathway

- *ADA* deficiency
- One of the major causes of autosomal recessive SCID (severe combined immunedeficiency)
- Excess dATP, resulting in lymphotoxicity



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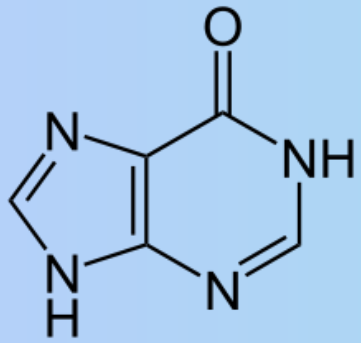
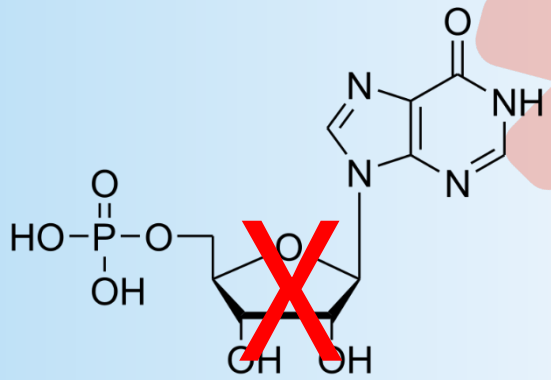


Hypoxanthine

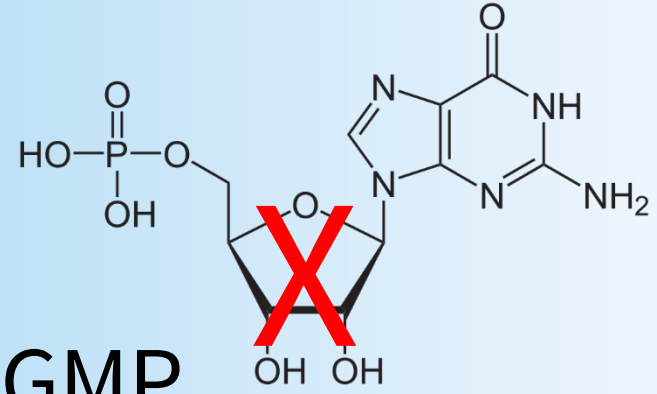
PRPP → PP$_I$ (HGPRT)



IMP

Guanine

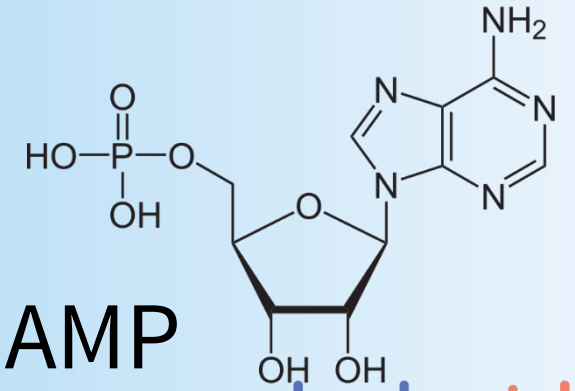
PRPP → PP$_I$ (HGPRT)



GMP

Adenine

PRPP → PP$_I$ (APRT)



AMP

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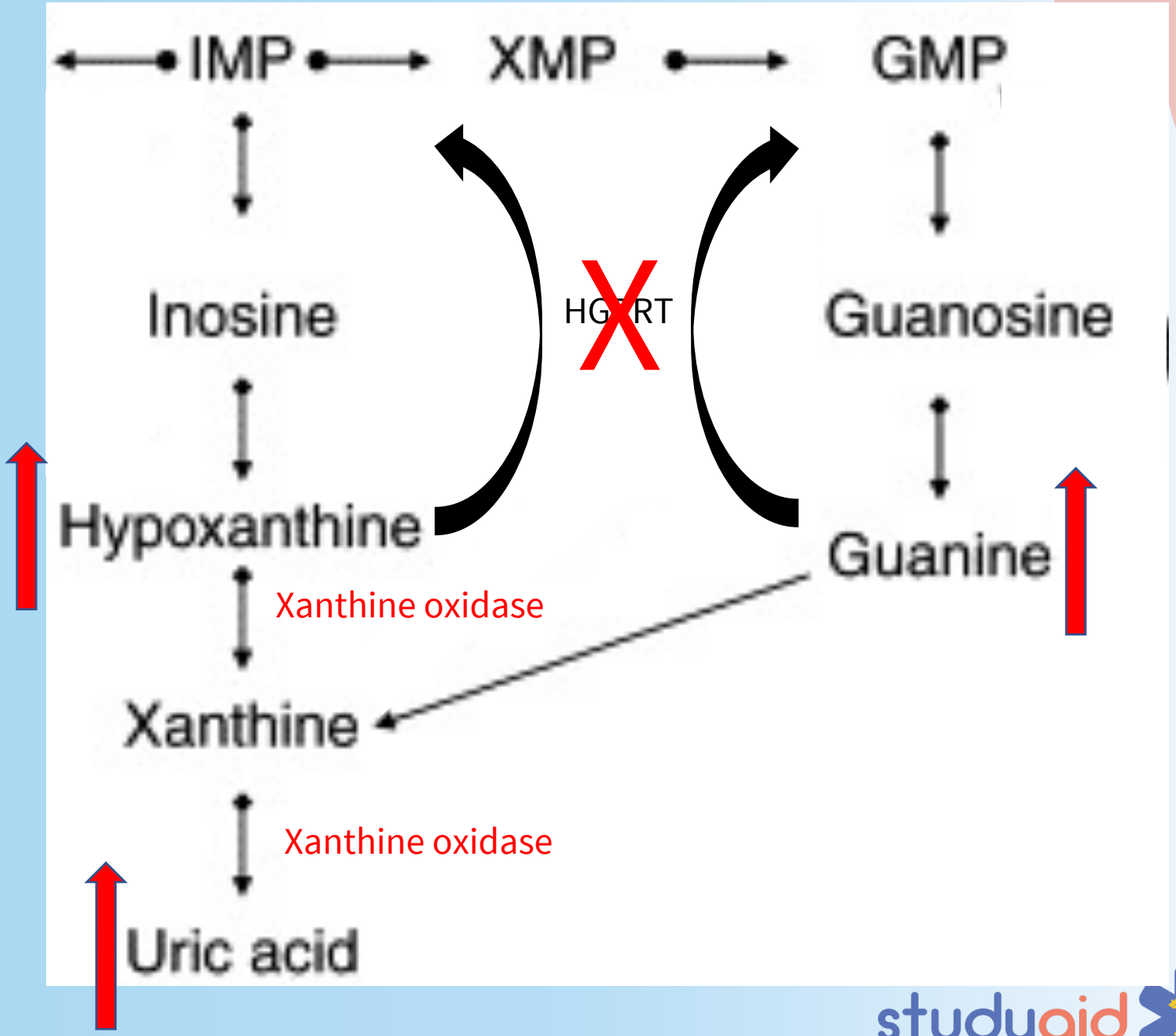
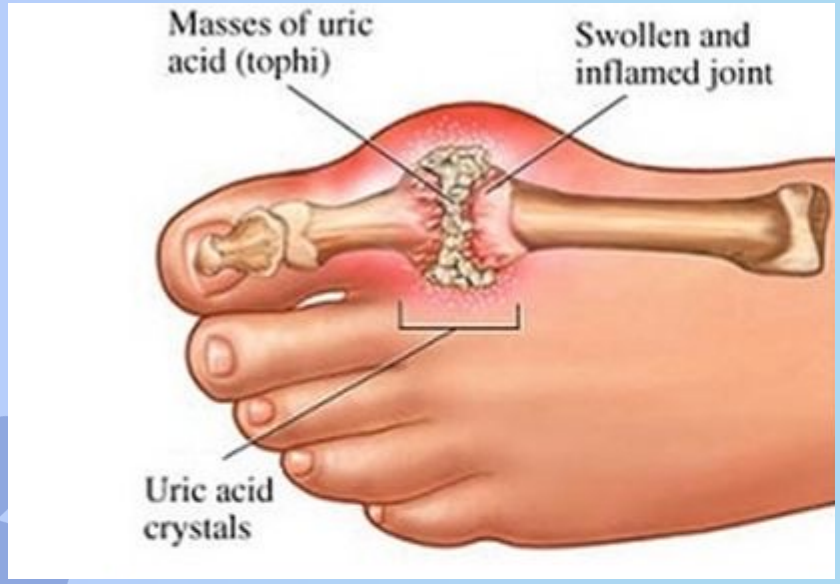
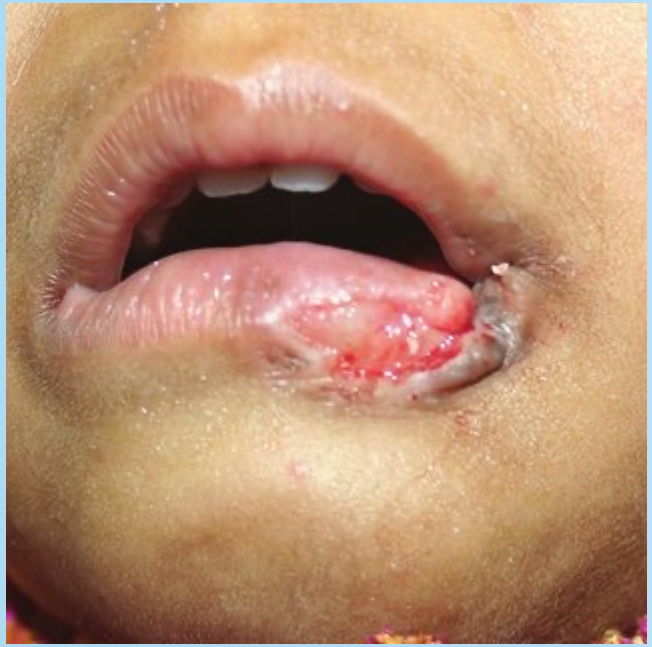
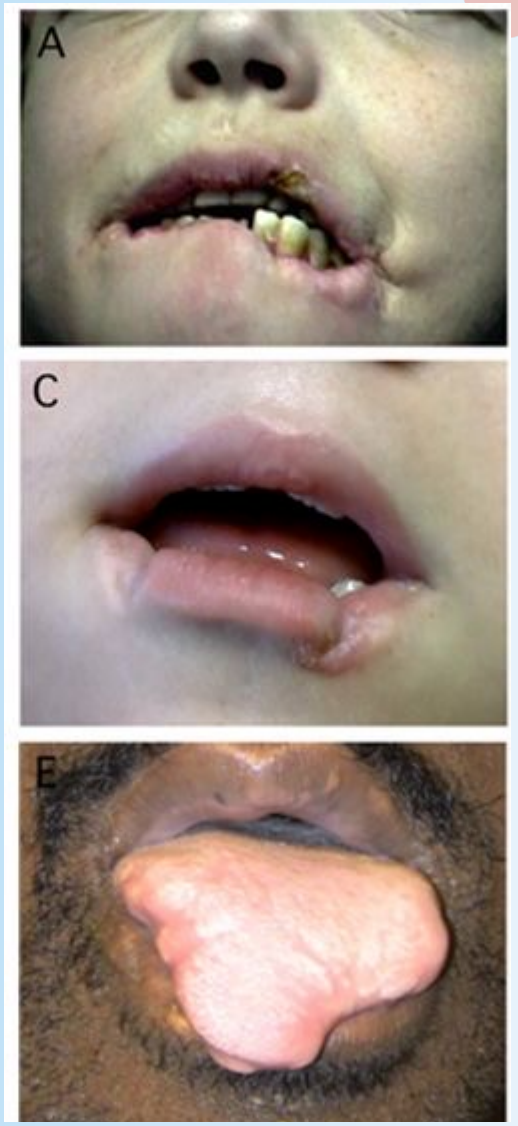
Lesch-Nyhan syndrome

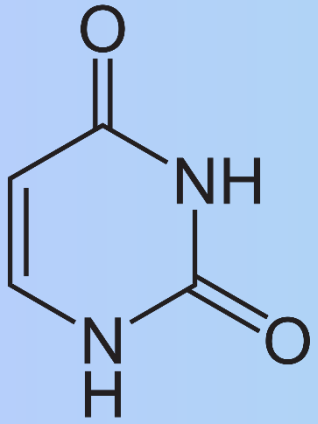
If HGPRT is no functioning then Hypoxanthine and guanine will rise thus pushing for the formation of uric acid



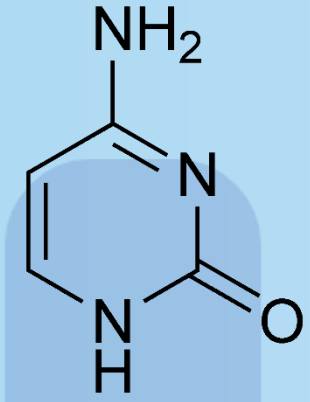

Masses of uric acid (tophi)
Swollen and inflamed joint
Uric acid crystals




A
C
E



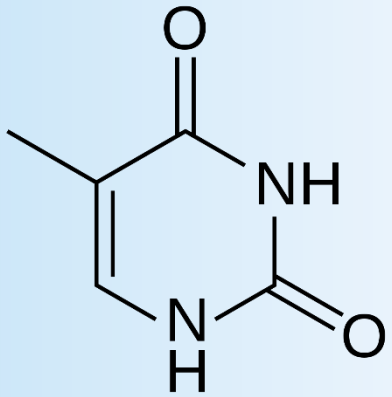
Uracil



Cytosine

Pyrimidine synthesis

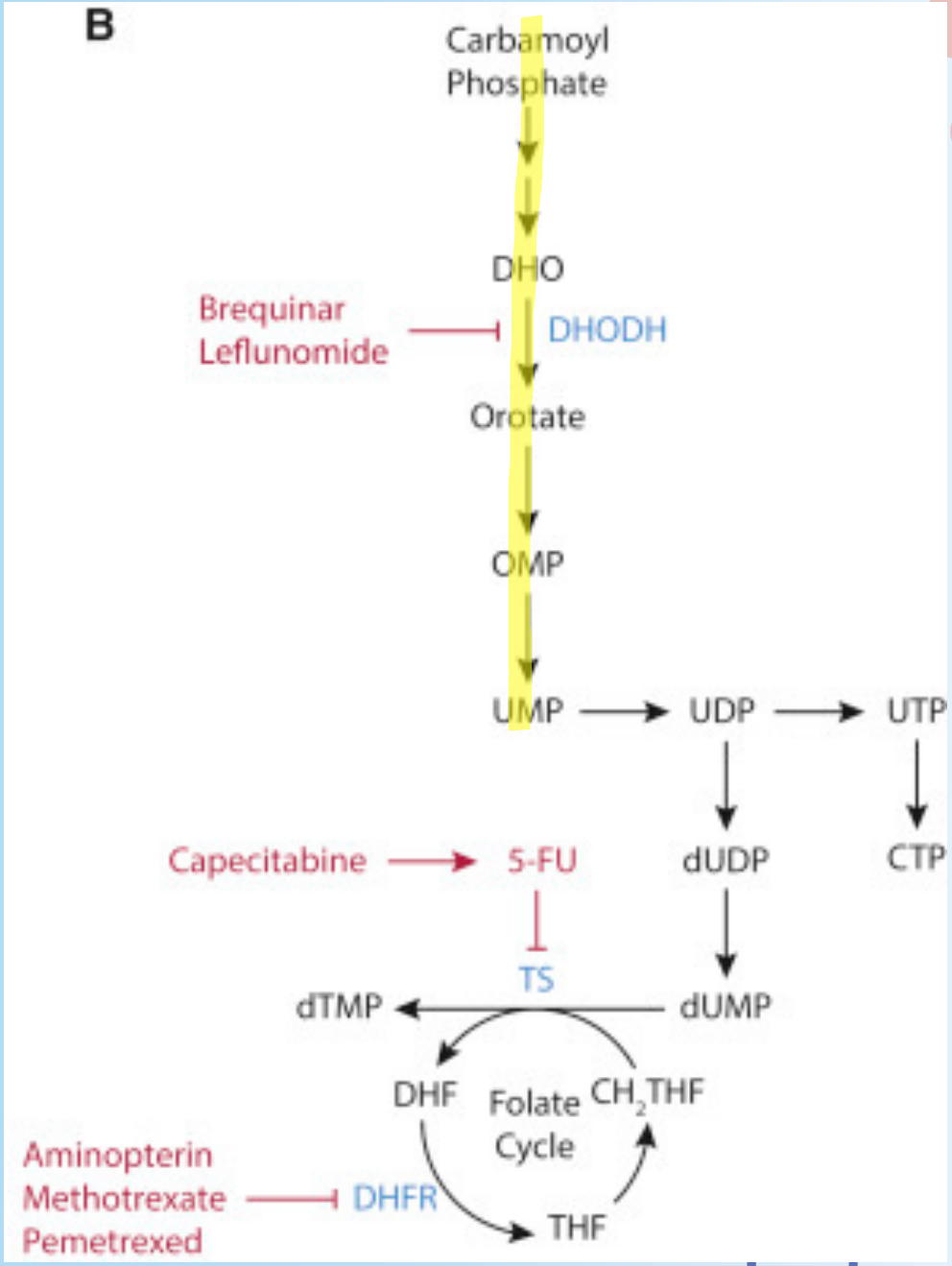
"de novo"



Thymine

QUICK OVERVIEW

- The synthesis of any pyrimidine nucleotide begins with the formation of uridine.
- Pyrmidines can be salvaged, however, their high solubility makes pyrimidine salvage less clinically significant than purines.



Used:

1 PRPP

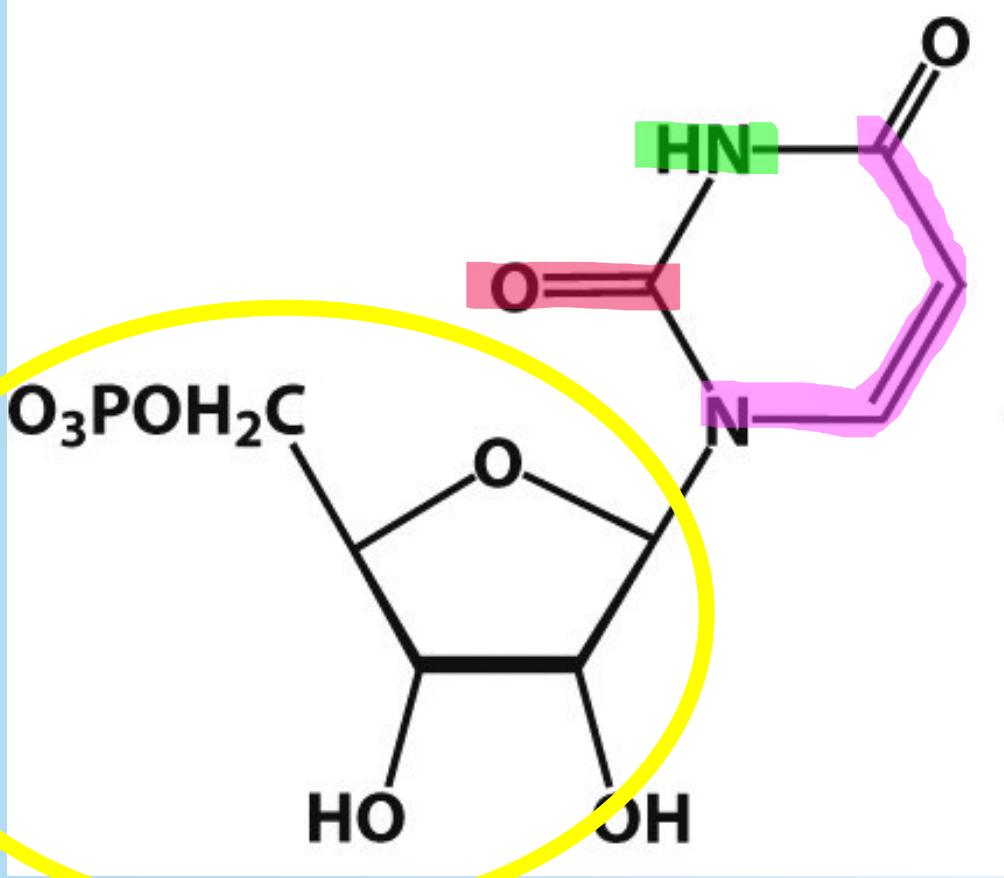
1 Glutamine

1 Aspartate

1 HCO_3 (CO_2)

1 NAD^+

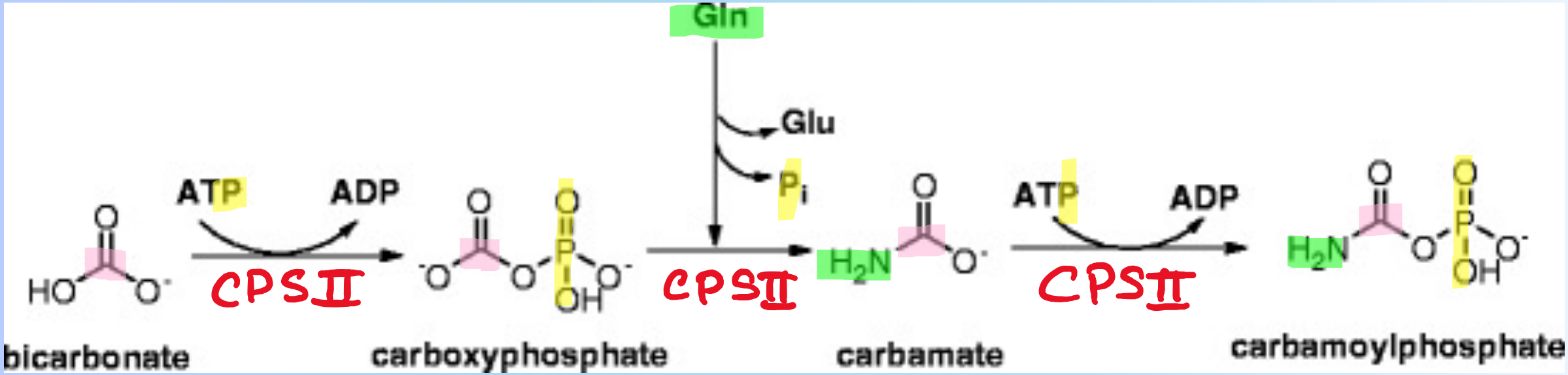
4 ATP



Carbamoyl phosphate synthetase II

	CPS-I	CPS-II
Location	Mitochondria	Cytosol
Pathway	Urea cycle	Pyrimidine ”de novo” synthesis
Regulation	+ N-acetylglutamate	+ PRPP X UTP
Source of nitrogen	ammonia	glutamine

Step 1 creating carbamoyl phosphate

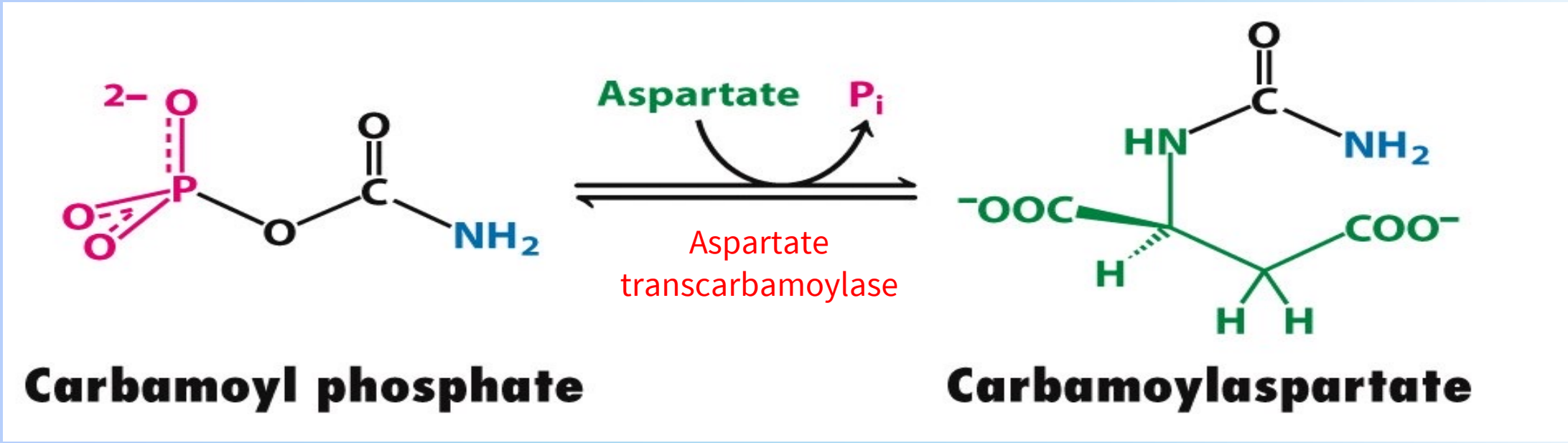


(carbamic) acid

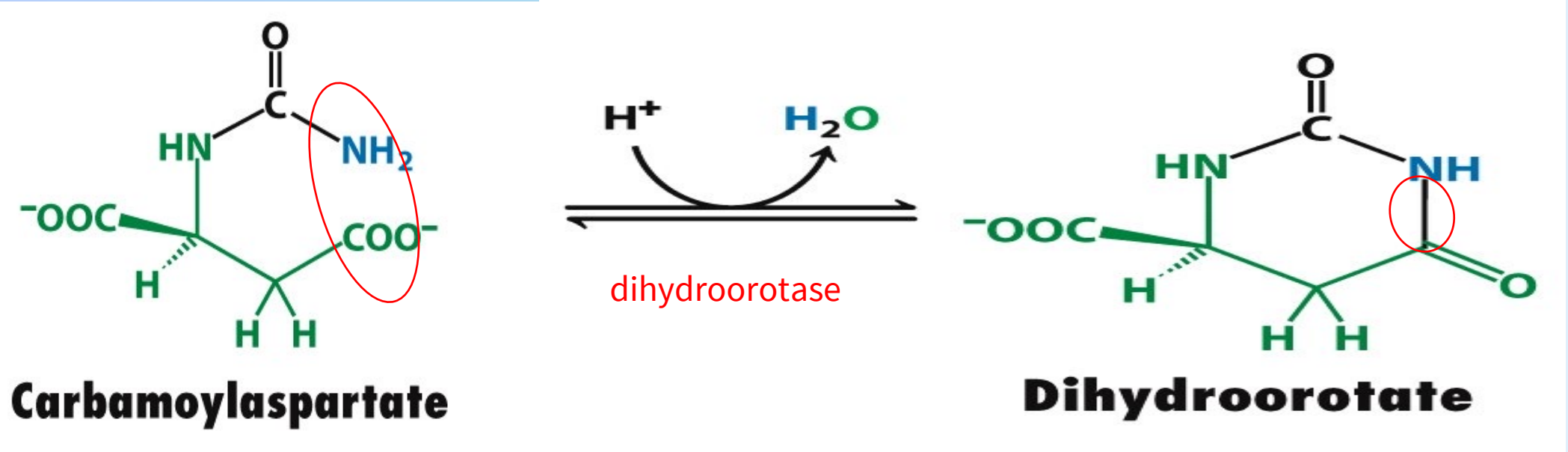
Gln = glutamine

Glu = glutamate

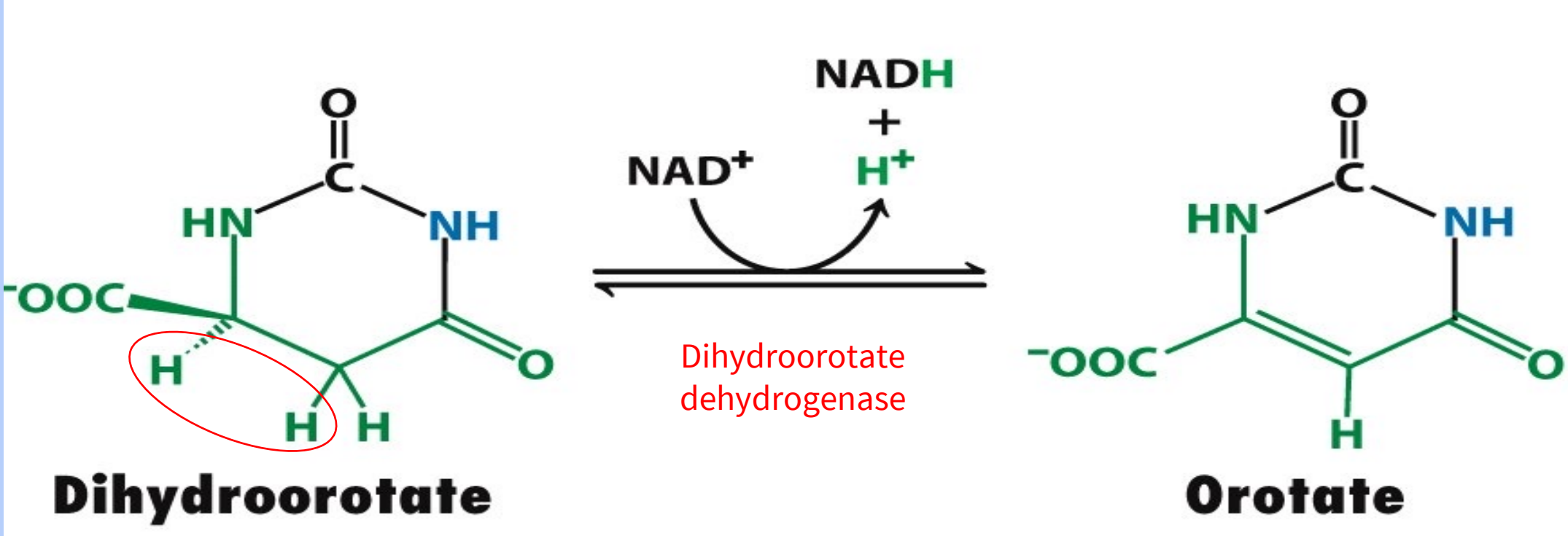
Step 2: Aspartate transcarbamoylase



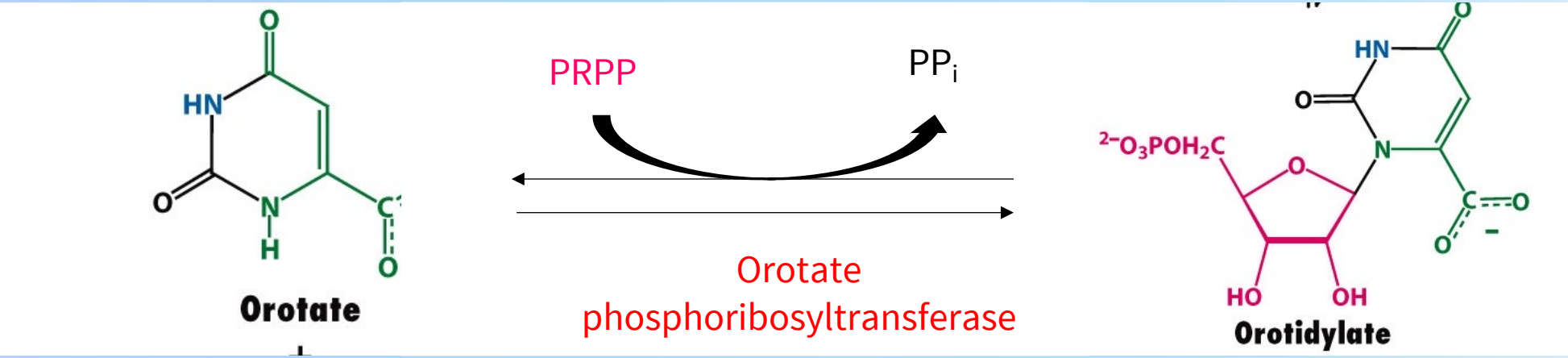
Step 3: Dehydratation



Step 4: Oxidation-reduction reaction

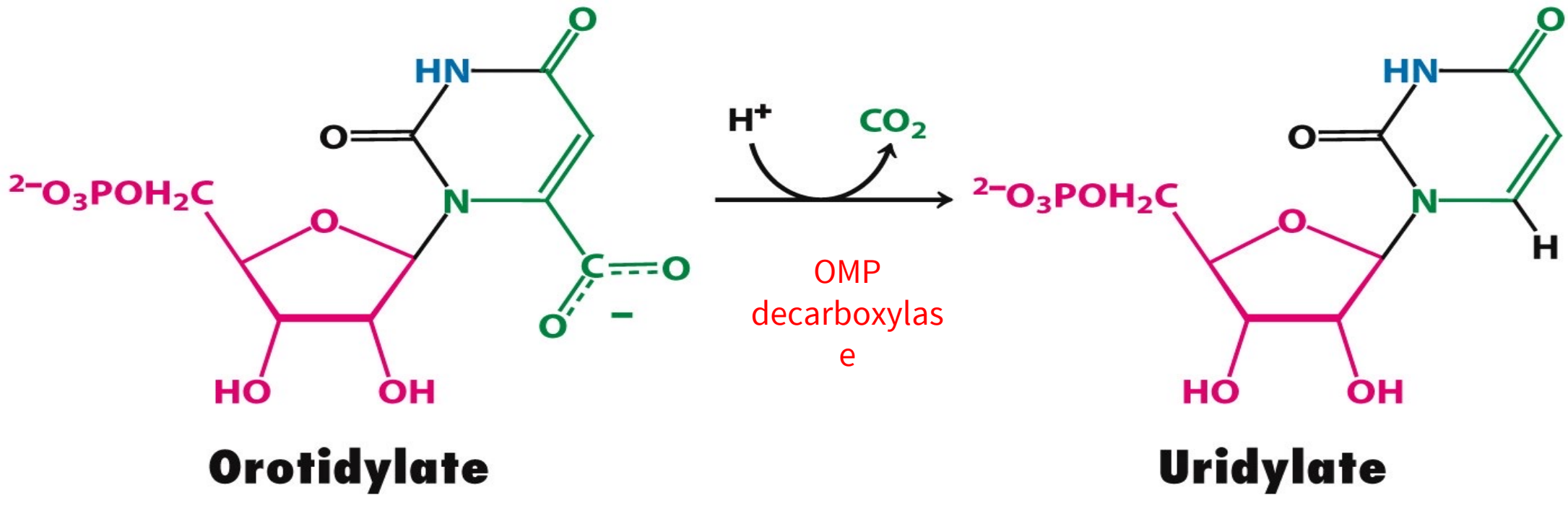


Step 5: adding PRPP



(orotidine 5'-monophosphate (OMP))

Step 6: finally formation of UMP!!!

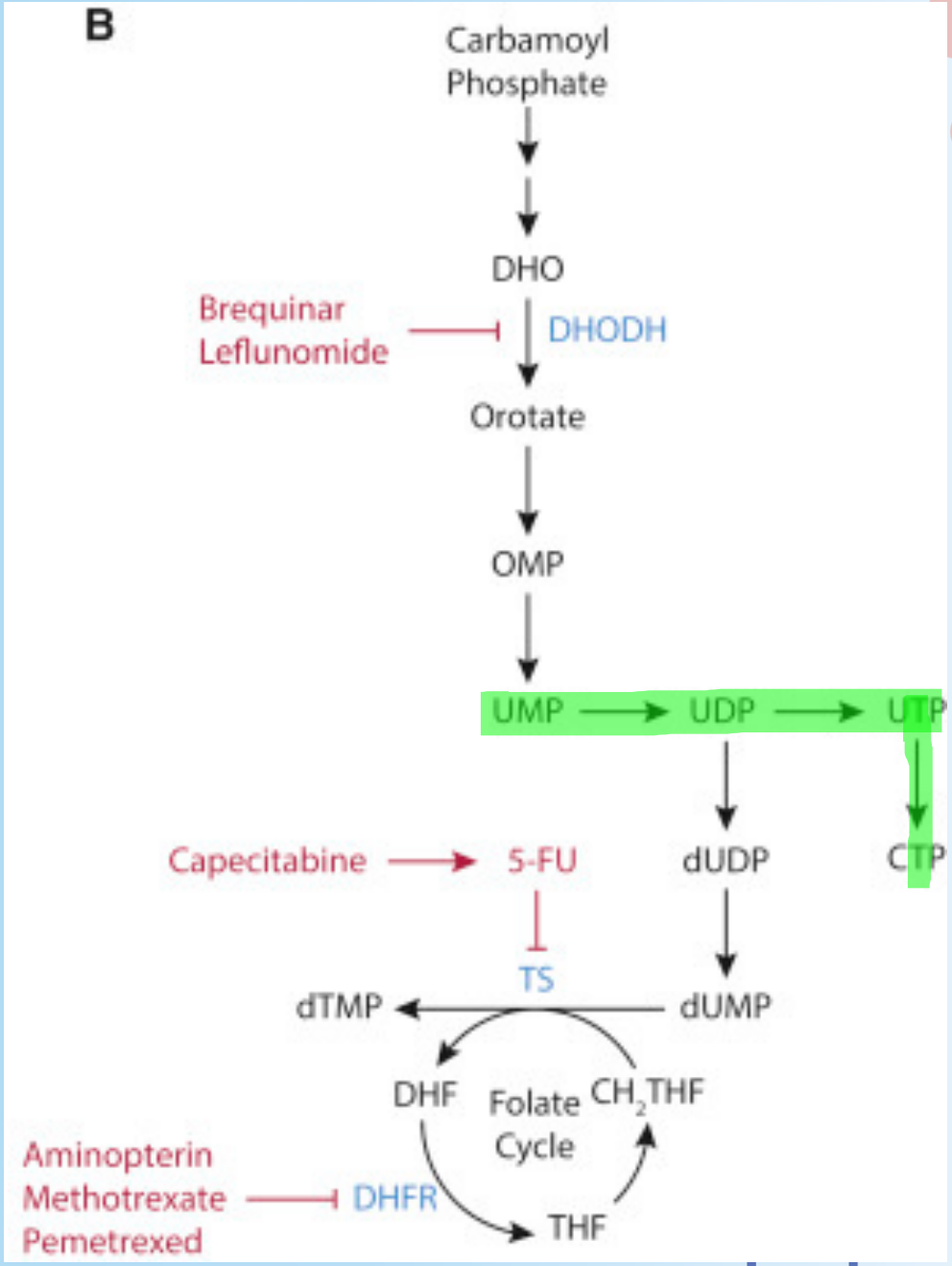


(orotidine 5'-monophosphate (OMP))

(uridine 5'-monophosphate (UMP))

Quick overview

- The convertion of uridine to cytidine happens only when UMP is converted to UTP. Only then can CTP be made.



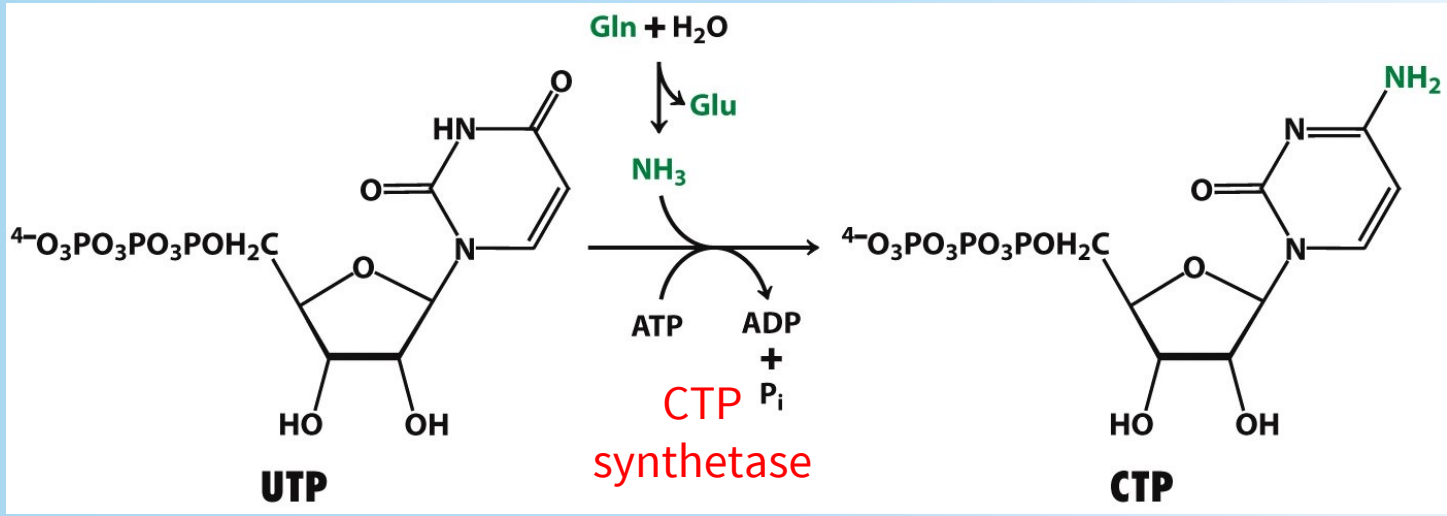
Nucleotide triphosphate formation

NMP → NTP

- Phosphorylation of NMP to NDP then TTP
- *Kinase activity*
- Usage: 2 ATP

- The same goes for both purines and pyrimidines

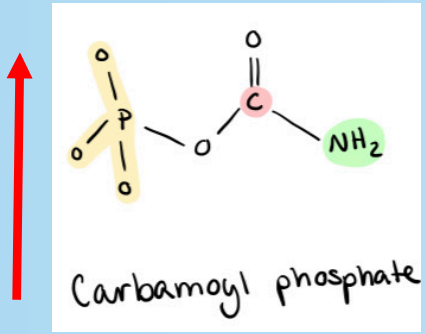
UTP → CTP

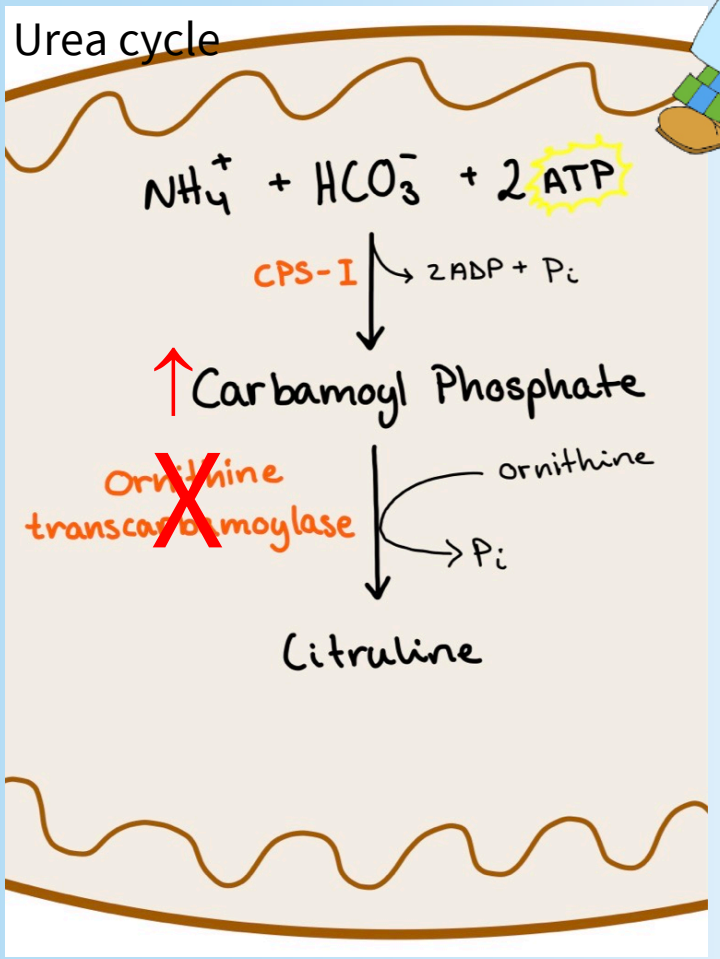


Clinical correlation

Ornithine transcarbamoylase deficiency

- ↑ Carbamoyl phosphate availability
- Carbamoyl phosphate leaks out into the cytoplasm
- ↑ pyrimidine synthesis
- Result: Orotic aciduria
- IMPORTANT! NO megaloblastic anemia



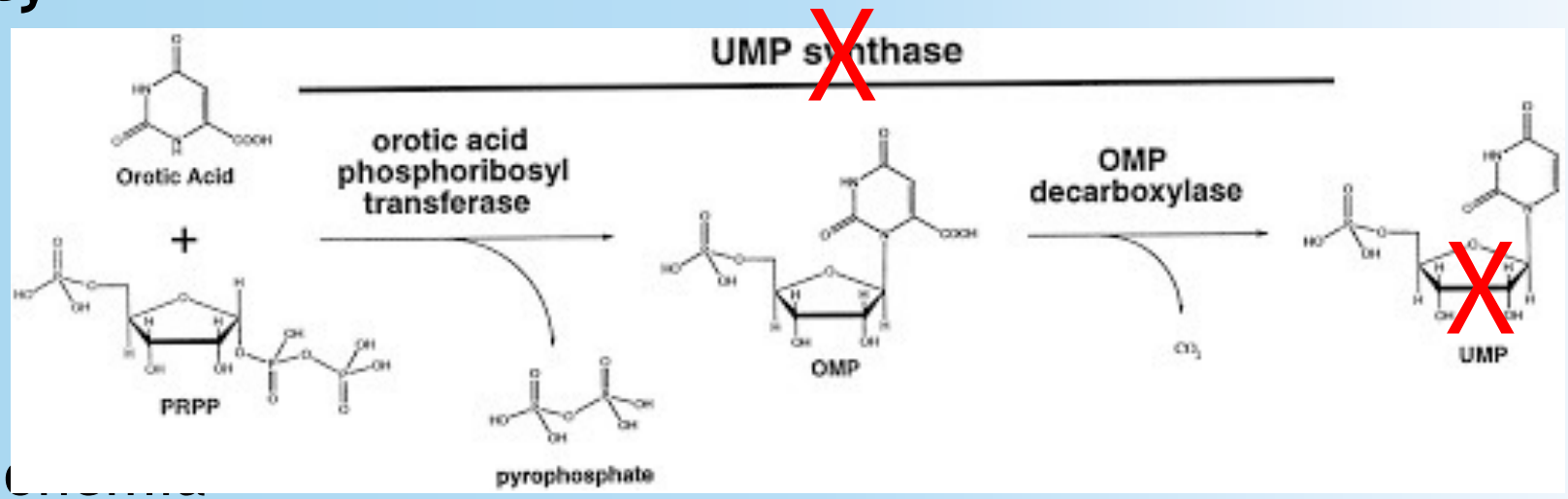


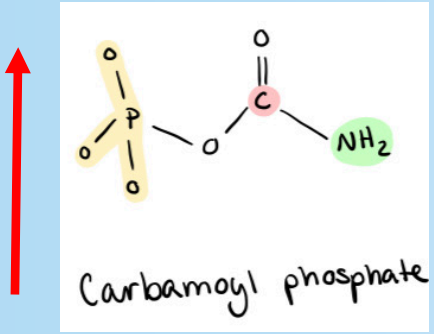
studyaid

Clinical correlation

UMP Synthase deficiency

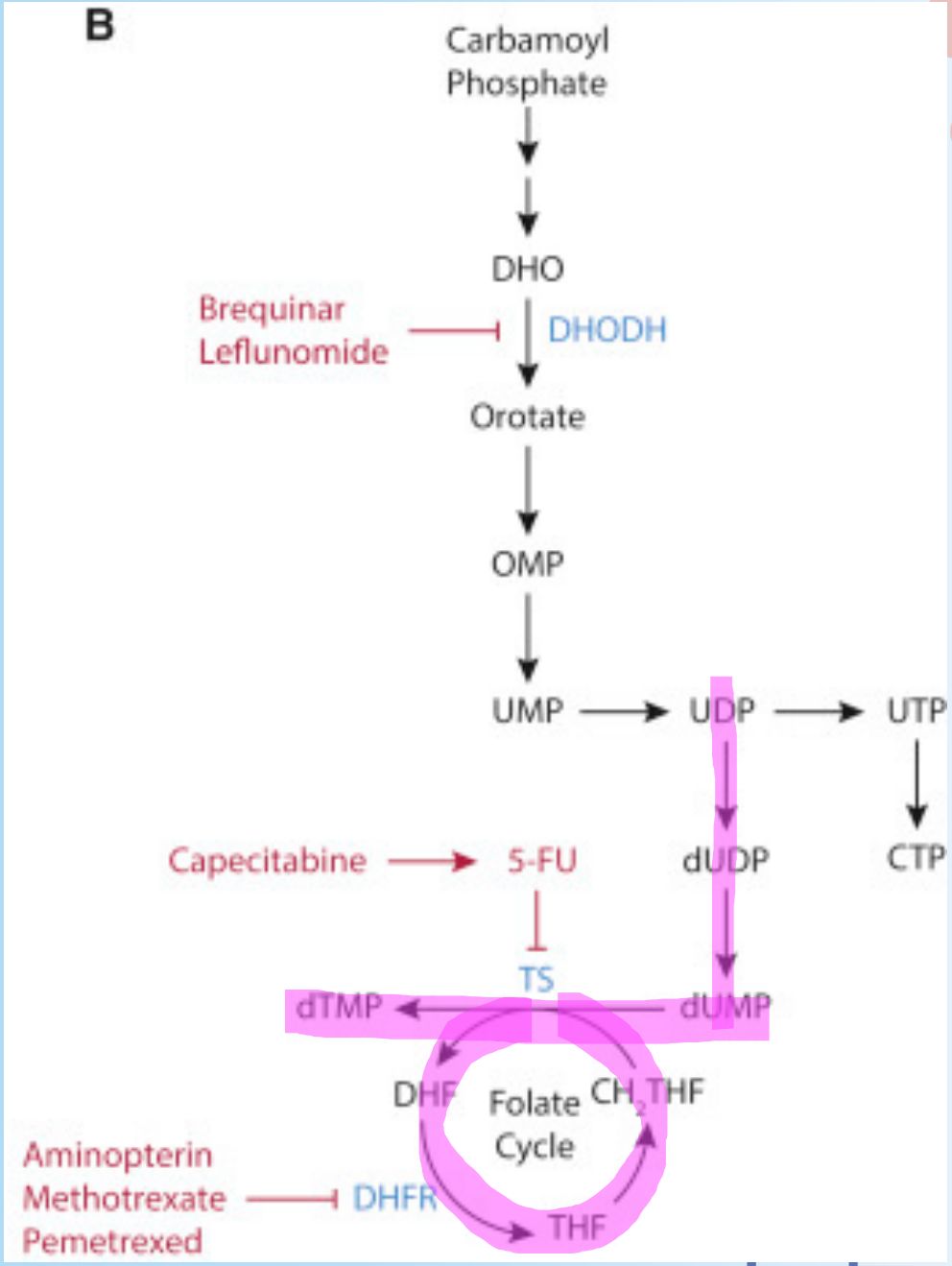
- ↑ Carbamoyl phosphate availability
- ↑ pyrimidine synthesis
- Result: Orotic aciduria
- IMPORTANT! NO hyperammonemia



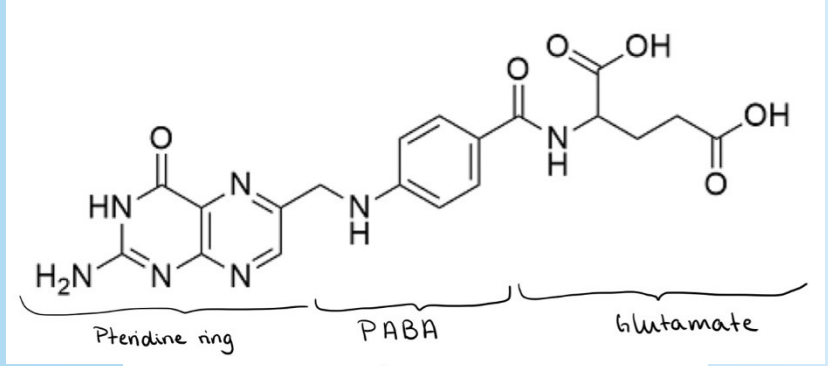


Quick overview

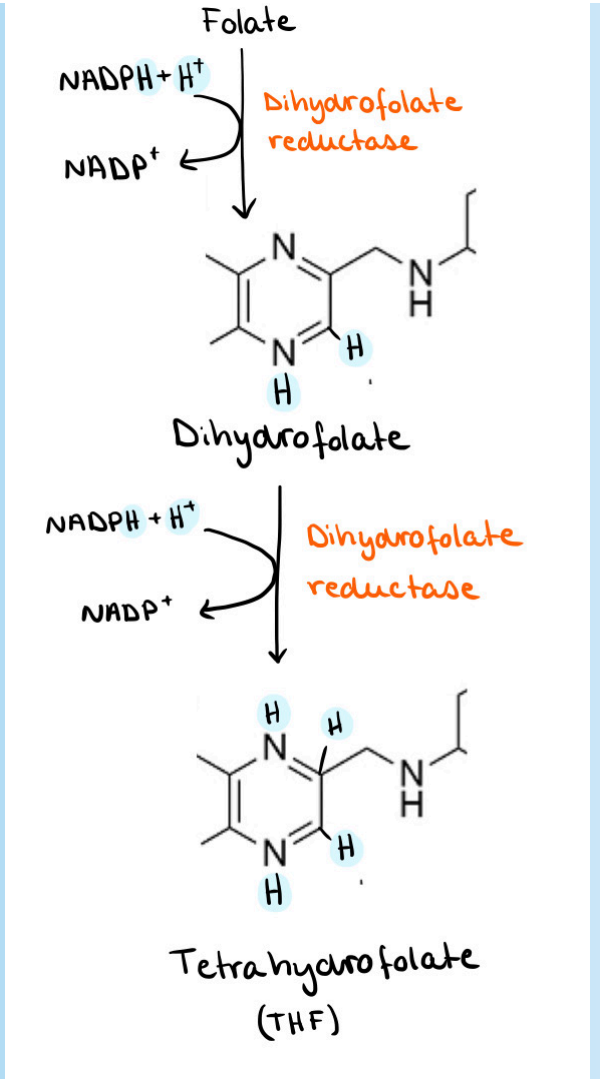
- dUMP is converted to dTMP by thymidylate synthase.
- It receives a methyl group from N^5,N^{10}-methylene tetrahyfrofolate
- Important pathways to note:
  - One Carbon Metabolism
  - Folate cycle



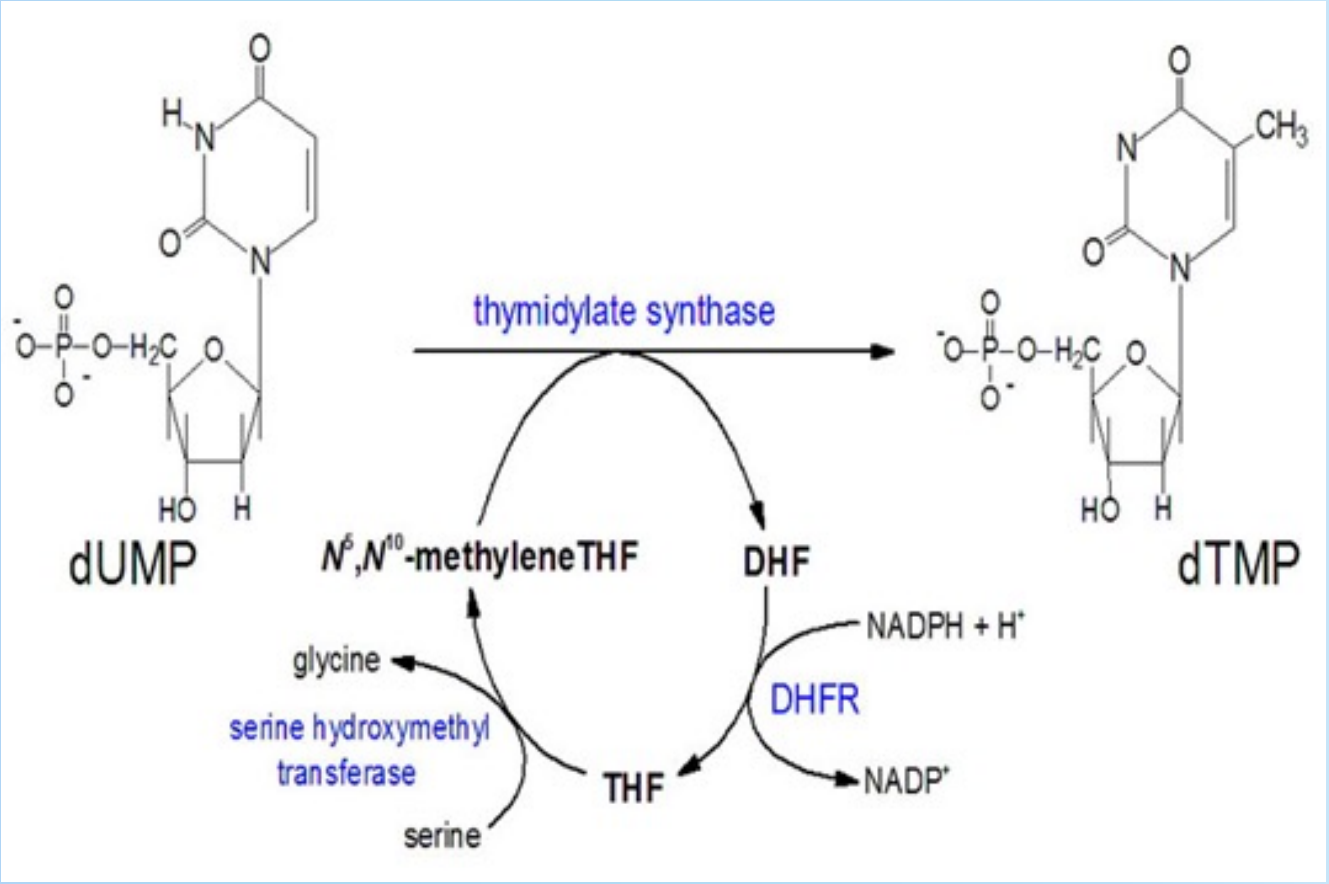
Folic acid (vitamin B_9)



- THF is the active form of folic acid
- Requires 2 NADPH
- Essential enzyme: *Dihydrofolate reductase*
- A carrier of one-carbon units

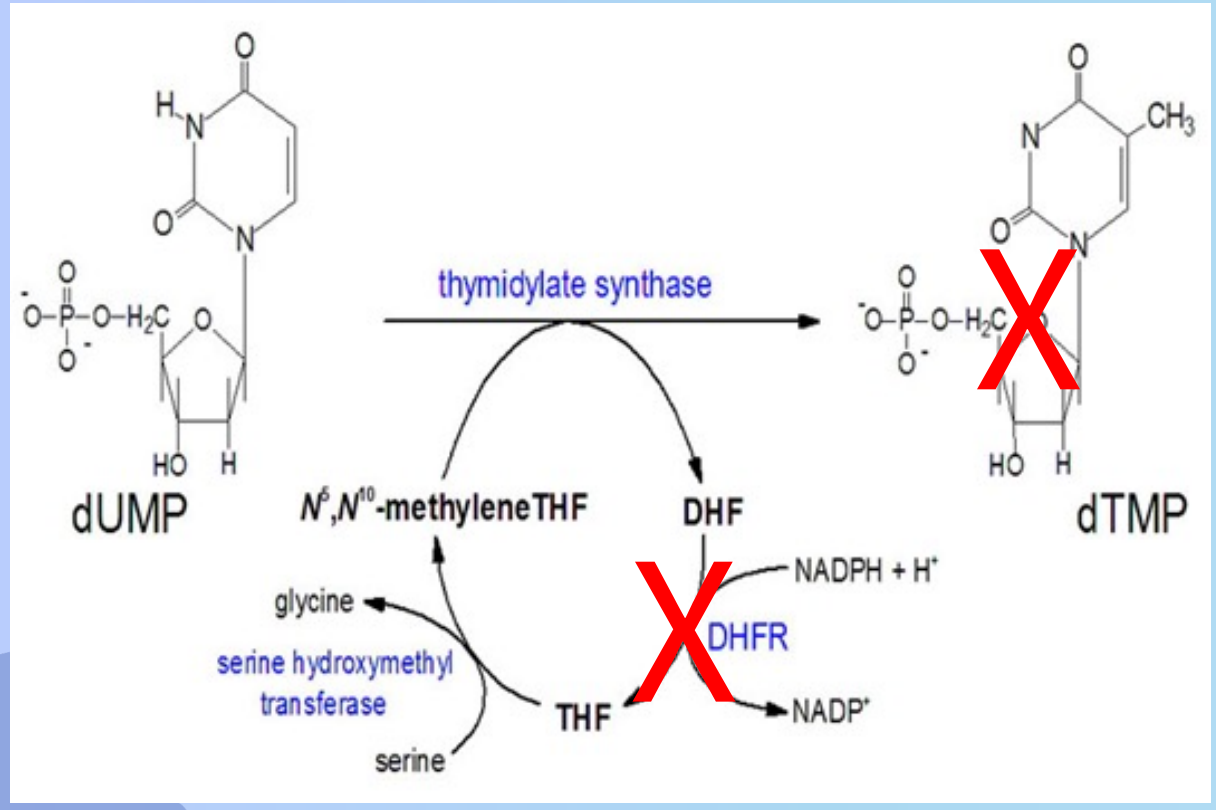


dUMP to dTMP

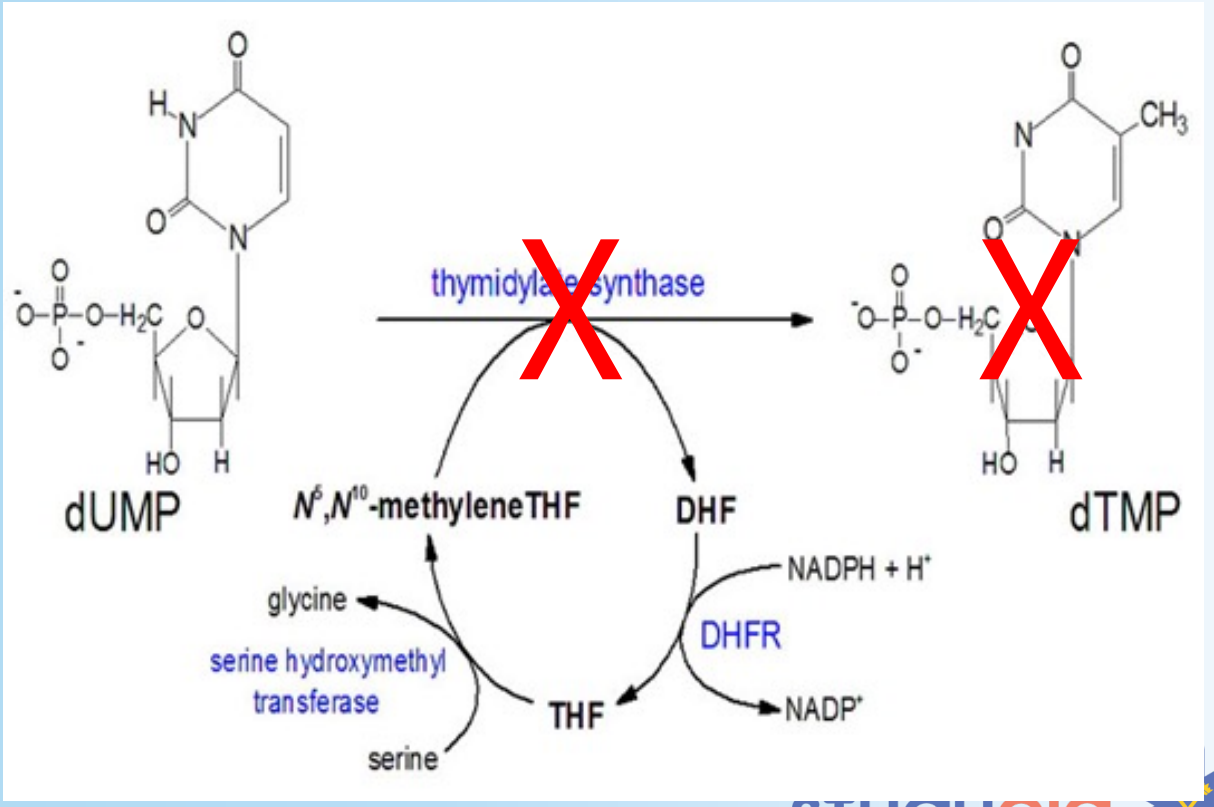

thymidylate synthase
dUMP
dTMP
N^5,N^{10}-methyleneTHF
DHF
THF
NADPH + H+
DHFR
NADP+
glycine
serine hydroxymethyl transferase
serine

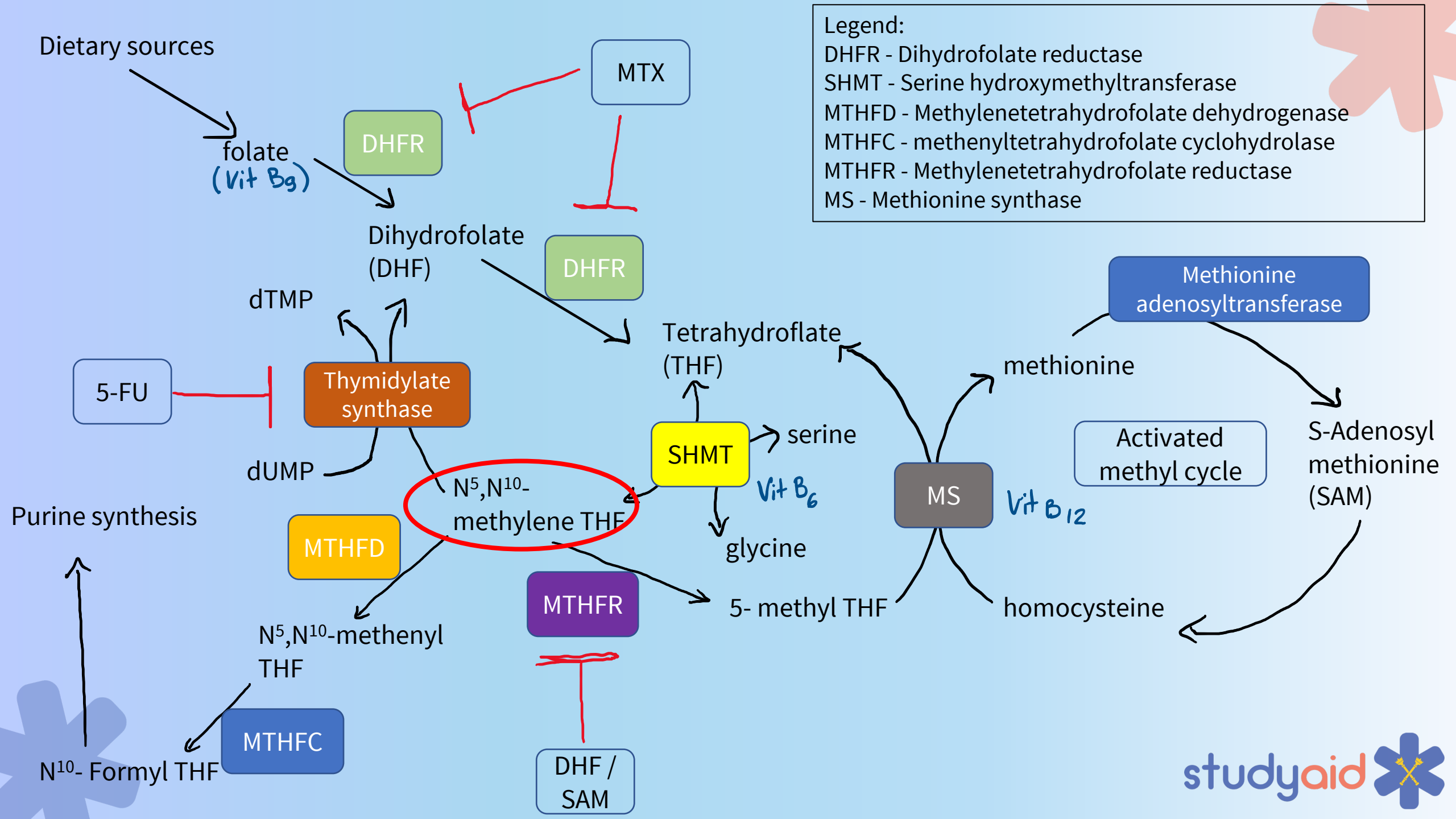
Clinical correlations
cancer drugs

Methotraxate (MTX)



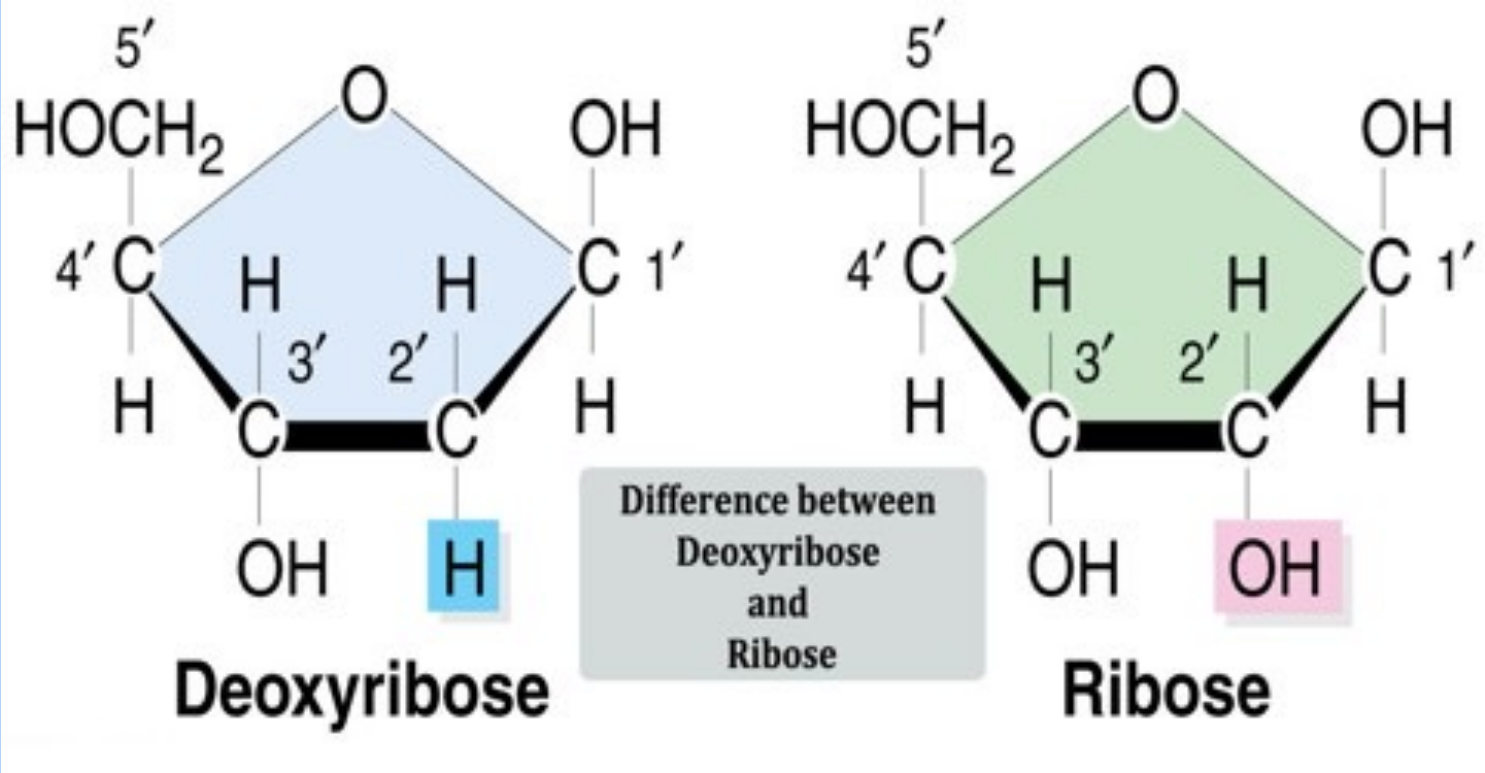
5 fluorouracil (5-FU)




Legend:
DHFR - Dihydrofolate reductase
SHMT - Serine hydroxymethyltransferase
MTHFD - Methylenetetrahydrofolate dehydrogenase
MTHFC - methenyltetrahydrofolate cyclohydrolase
MTHFR - Methylenetetrahydrofolate reductase
MS - Methionine synthase
Dietary sources
folate
(Vit B9)
DHFR
MTX
Dihydrofolate
(DHF)
DHFR
dTMP
5-FU
Thymidylate synthase
dUMP
Tetrahydroflate
(THF)
SHMT
serine
Vit B6
glycine
N5,N10-
methylene THF
MTHFD
N5,N10-methenyl
THF
MTHFR
5- methyl THF
MS
Vit B12
methionine
homocysteine
Methionine adenosyltransferase
Activated methyl cycle
S-Adenosyl methionine (SAM)
DHF / SAM
Purine synthesis
N10- Formyl THF
MTHFC
studyaid

Ribose to deoxyribose

What is the difference?



Essential enzyme:
Ribonucleotide reductase

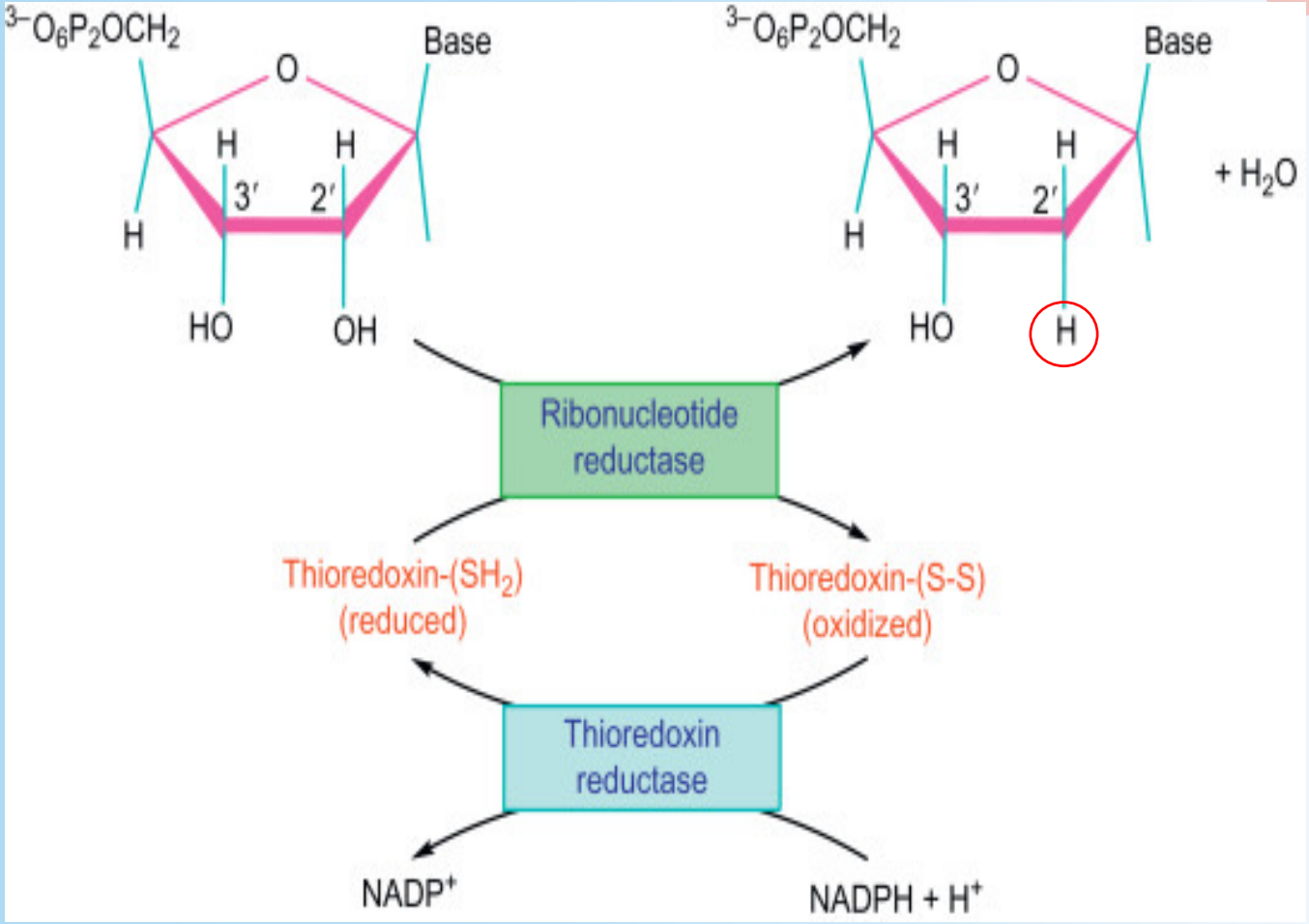
Regulation:

+ ATP

x dATP

x *Hydroxyurea* – anticancer drug

Inhibits ribonucleotide reductase



GOOD LUCK !!