Biosynthesis of purine and pyrimidine nucleotides

Introduction

Nucleotides consist of a nitrogenous base, a pentose and a phosphate. The pentose sugar is D-ribose in ribonucleotides of RNA while in deoxyribonucleotides of DNA, the sugar is 2-deoxy D-ribose.

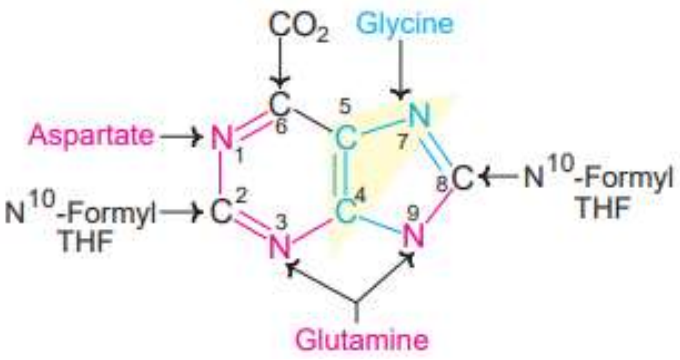
Nucleotides participate in almost all the biochemical processes, either directly or indirectly.

They are the structural components of nucleic acids (DNA, RNA), coenzymes, and are involved in the regulation of several metabolic reactions.

Biosynthesis of Purine Ribonucleotides

Many compounds contribute to the purine ring of the nucleotides.

1. N_1 of purine is derived from amino group of aspartate.
2. C_2 and C_8 arise from formate of N10- formyl THF.
3. N_3 and N_9 are obtained from amide group of glutamine.
4. C_4, C_5 and N_7 are contributed by glycine.
5. C_6 directly comes from CO_2.



It should be remembered that purine bases are not synthesized as such, but they are formed as ribonucleotides. The purines are built upon a pre-existing ribose 5-phosphate. Liver is the major site for purine nucleotide synthesis. Erythrocytes, polymorphonuclear leukocytes and brain cannot produce purines.

Denovo pathway

1. Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphoribosyl pyrophosphate (PRPP).

2. Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce 5-phosphoribosylamine. The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleotides (IMP, AMP and GMP). This reaction is the 'committed step' in purine nucleotide biosynthesis.

3. Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide (GAR).

4. N10-Formyl tetrahydrofolate donates the formyl group and the product formed is formylglycinamide ribosyl 5-phosphate.

5. Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5-phosphate.

Denovo pathway

6. The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5-aminoimidazole ribosyl 5-phosphate.

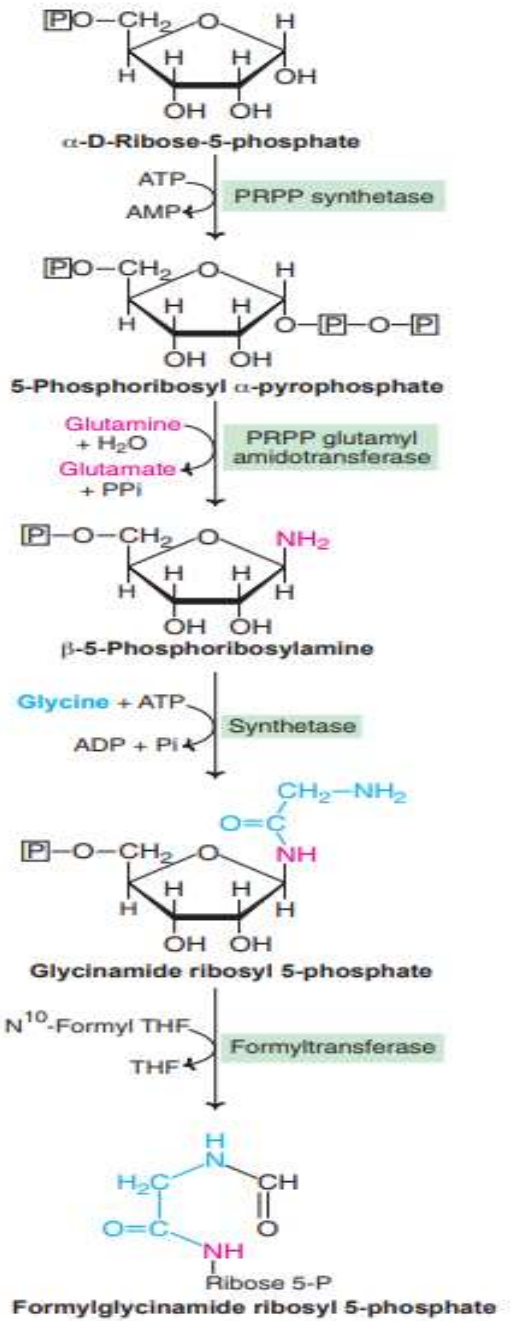
7. Incorporation of CO_2 (carboxylation) occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate. This reaction does not require the vitamin biotin and/or ATP which is the case with most of the carboxylation reactions.

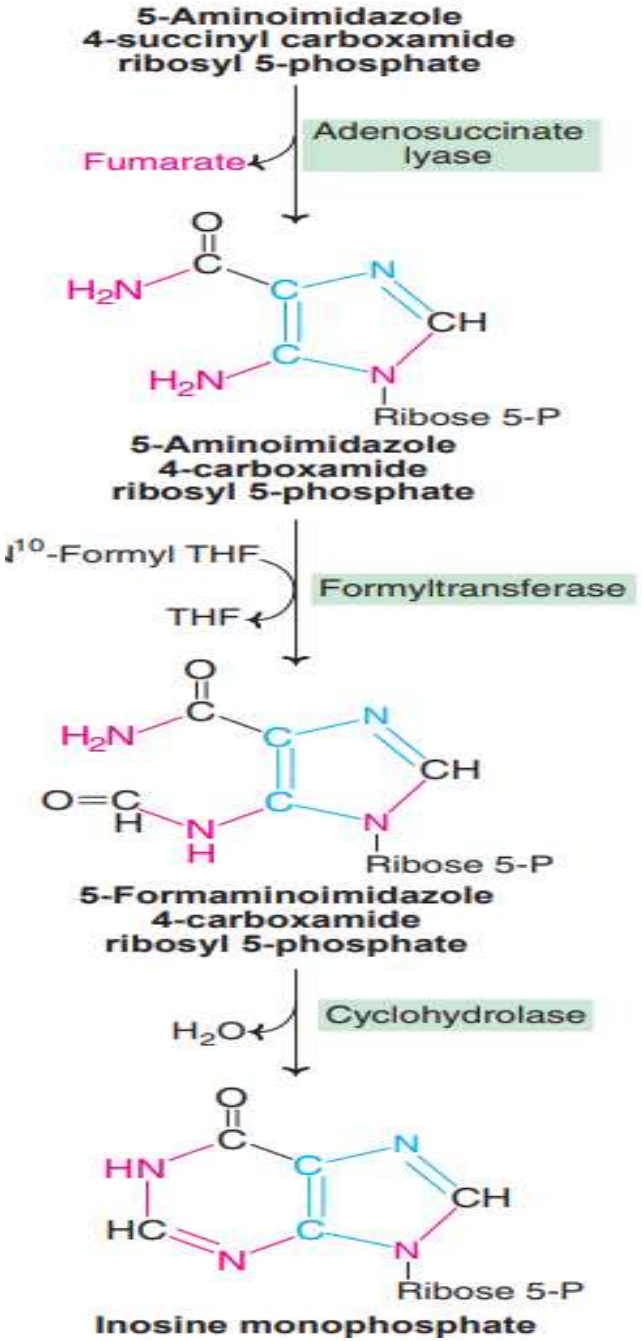
8. Aspartate condenses with the product in reaction 7 to form aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate.

9. Adenosuccinate lyase cleaves off fumarate and only the amino group of aspartate is retained to yield aminoimidazole 4-carboxamide ribosyl 5-phosphate.

10. N10-Formyl tetrahydrofolate donates a one-carbon moiety to produce formaminoimidazole 4-carboxamide ribosyl 5-phosphate. With this reaction, all the carbon and nitrogen atoms of purine ring are contributed by the respective sources.

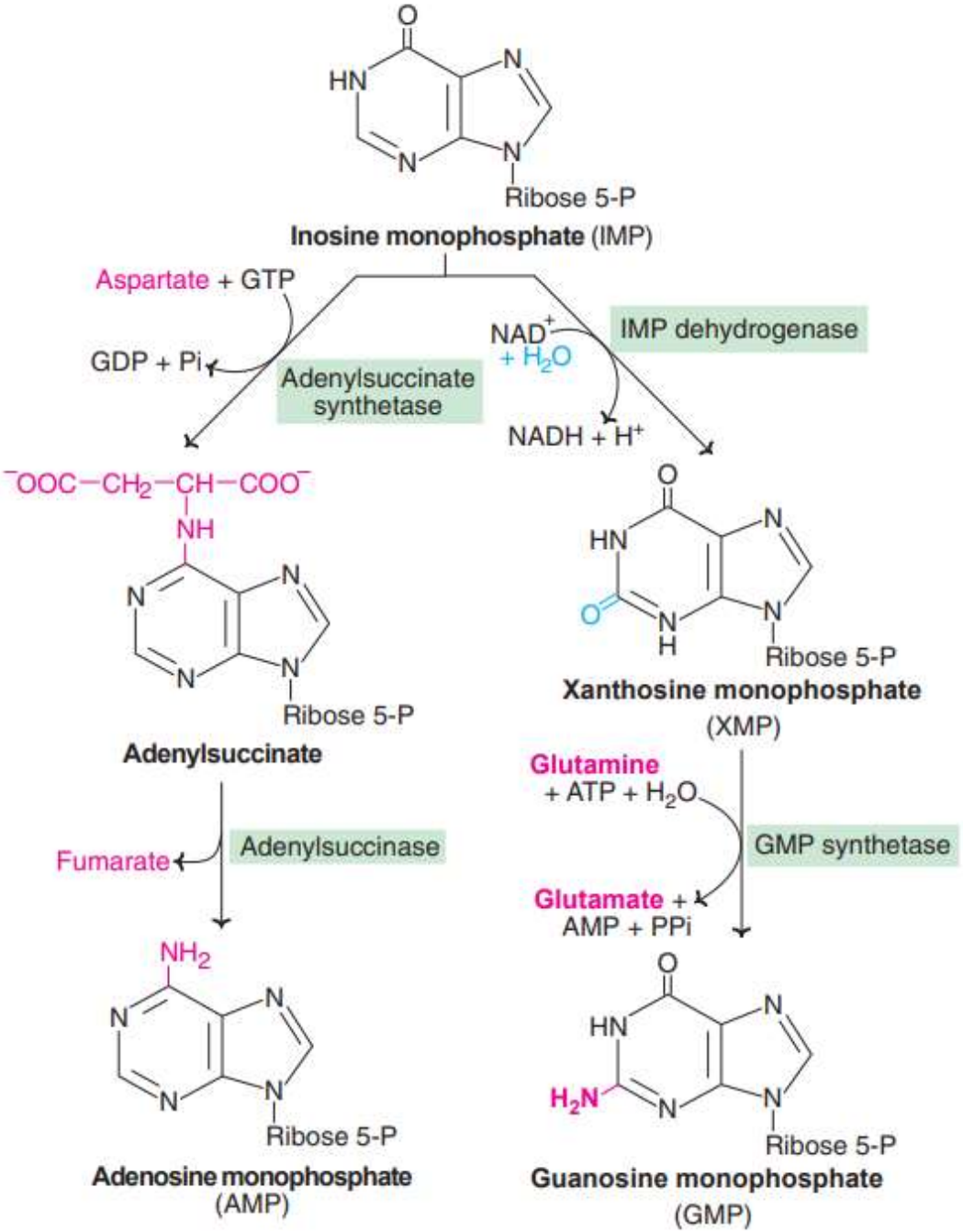
11. The final reaction catalysed by cyclohydrolase leads to ring closure with an elimination of water molecule. The product obtained is inosine monophosphate (IMP), the parent purine nucleotide from which other purine nucleotides can be synthesized.


α-D-Ribose-5-phosphate
ATP
AMP
PRPP synthetase
5-Phosphoribosyl α-pyrophosphate
Glutamine + H2O
Glutamate + PPi
PRPP glutamyl amidotransferase
β-5-Phosphoribosylamine
Glycine + ATP
ADP + Pi
Synthetase
Glycinamide ribosyl 5-phosphate
N10-Formyl THF
THF
Formyltransferase
Ribose 5-P
Formylglycinamide ribosyl 5-phosphate
Formylglycinamide ribosyl 5-phosphate
Glutamine + ATP
Glutamate + ADP + Pi
Synthetase
Ribose 5-P
Formylglycinamidine ribosyl-5-phosphate
ATP
ADP + Pi + H2O
Synthetase
Ribose 5-P
5-Aminoimidazole ribosyl-5-phosphate
CO2
Carboxylase
Ribose 5-P
5-Aminoimidazole carboxylate ribosyl 5-phosphate
Aspartate + ATP
ADP + Pi
Synthetase
Ribose 5-P
5-Aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate


5-Aminoimidazole
4-succinyl carboxamide
ribosyl 5-phosphate
Fumarate
Adenosuccinate lyase
Ribose 5-P
5-Aminoimidazole
4-carboxamide
ribosyl 5-phosphate
^{10}-Formyl THF
THF
Formyltransferase
Ribose 5-P
5-Formaminoimidazole
4-carboxamide
ribosyl 5-phosphate
H_2O
Cyclohydrolase
Ribose 5-P
Inosine monophosphate

Synthesis of AMP and GMP from IMP

- Inosine monophosphate is the immediate precursor for the formation of AMP and GMP.
- Aspartate condenses with IMP in the presence of GTP to produce adenylsuccinate which, on cleavage, forms AMP.
- For the synthesis of GMP, IMP undergoes NAD+ dependent dehydrogenation to form xanthosine monophosphate (XMP).
- Glutamine then transfers amide nitrogen to XMP to produce GMP. 6-Mercaptopurine is an inhibitor of the synthesis of AMP and GMP. It acts on the enzyme adenylsuccinase (of AMP pathway) and IMP dehydrogenase (of GMP pathway).


Ribose 5-P
Inosine monophosphate (IMP)
Aspartate + GTP
GDP + Pi
Adenylsuccinate synthetase
NAD+ + H2O
IMP dehydrogenase
NADH + H+
⁻OOC—CH2—CH—COO⁻
NH
Ribose 5-P
Adenylsuccinate
Xanthosine monophosphate (XMP)
Glutamine + ATP + H2O
GMP synthetase
Glutamate + AMP + PPi
Fumarate
Adenylsuccinase
NH2
Ribose 5-P
Adenosine monophosphate (AMP)
H2N
Ribose 5-P
Guanosine monophosphate (GMP)

Salvage pathway for purines

The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acids (particularly RNA), and also obtained from the dietary sources.

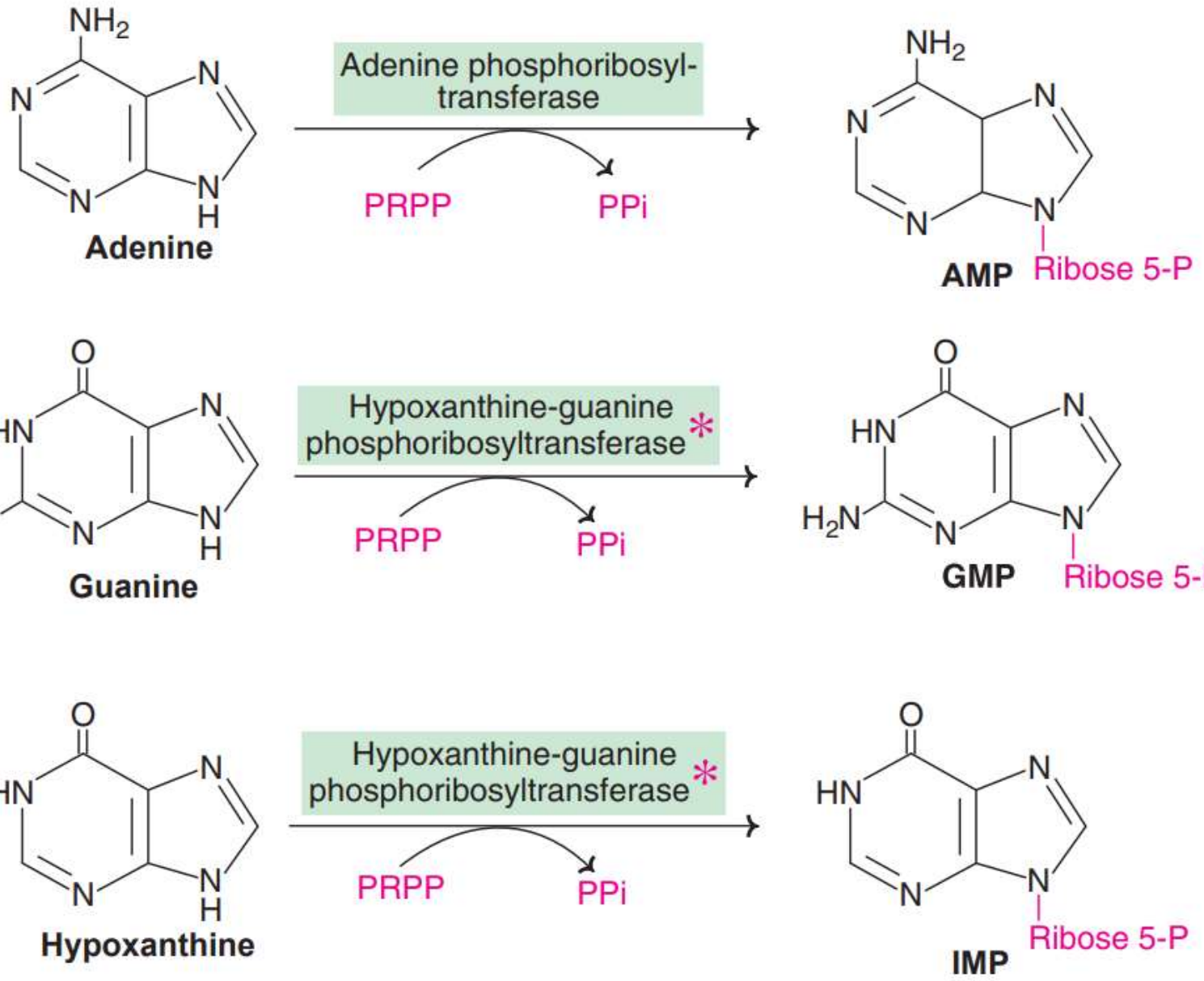
The purines can be directly converted to the corresponding nucleotides, and this process is known as ‘salvage pathway’.

Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts guanine and hypoxanthine, respectively, to GMP and IMP.

Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage pathway.

The salvage pathway is particularly important in certain tissues such as erythrocytes and brain where de novo (a new) synthesis of purine nucleotides is not operative.

Salvage pathway for purines



Biosynthesis of Pyrimidine Nucleotides

The synthesis of pyrimidines is a much simpler process compared to that of purines. Aspartate, glutamine (amide group) and CO_2 contribute to atoms in the formation of pyrimidine ring. Pyrimidine ring is first synthesized and then attached to ribose 5-phosphate. Glutamine transfers its amido nitrogen to CO2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalysed by cytosomal enzyme carbamoyl phosphate synthetase II (CPS II). CPS II is activated by ATP and PRPP and inhibited by UTP.

Carbamoyl phosphate synthetase I (CPS I) is a mitochondrial enzyme which synthesizes carbamoyl phosphate from ammonia and CO_2 and, in turn urea.

Carbamoyl phosphate condenses with aspartate to form carbamoyl aspartate. This reaction is catalysed by aspartate transcarbamoylase. Dihydroorotase catalyses the pyrimidine ring closure with a loss of H_2O.

The three enzymes—CPS II, aspartate transcarbamoylase and dihydroorotase are the domains (functional units) of the same protein. This is a good example of a multifunctional enzyme.

The next step in pyrimidine synthesis is an NAD+ dependent dehydrogenation, leading to the formation of orotate.

Biosynthesis of Pyrimidine Nucleotides

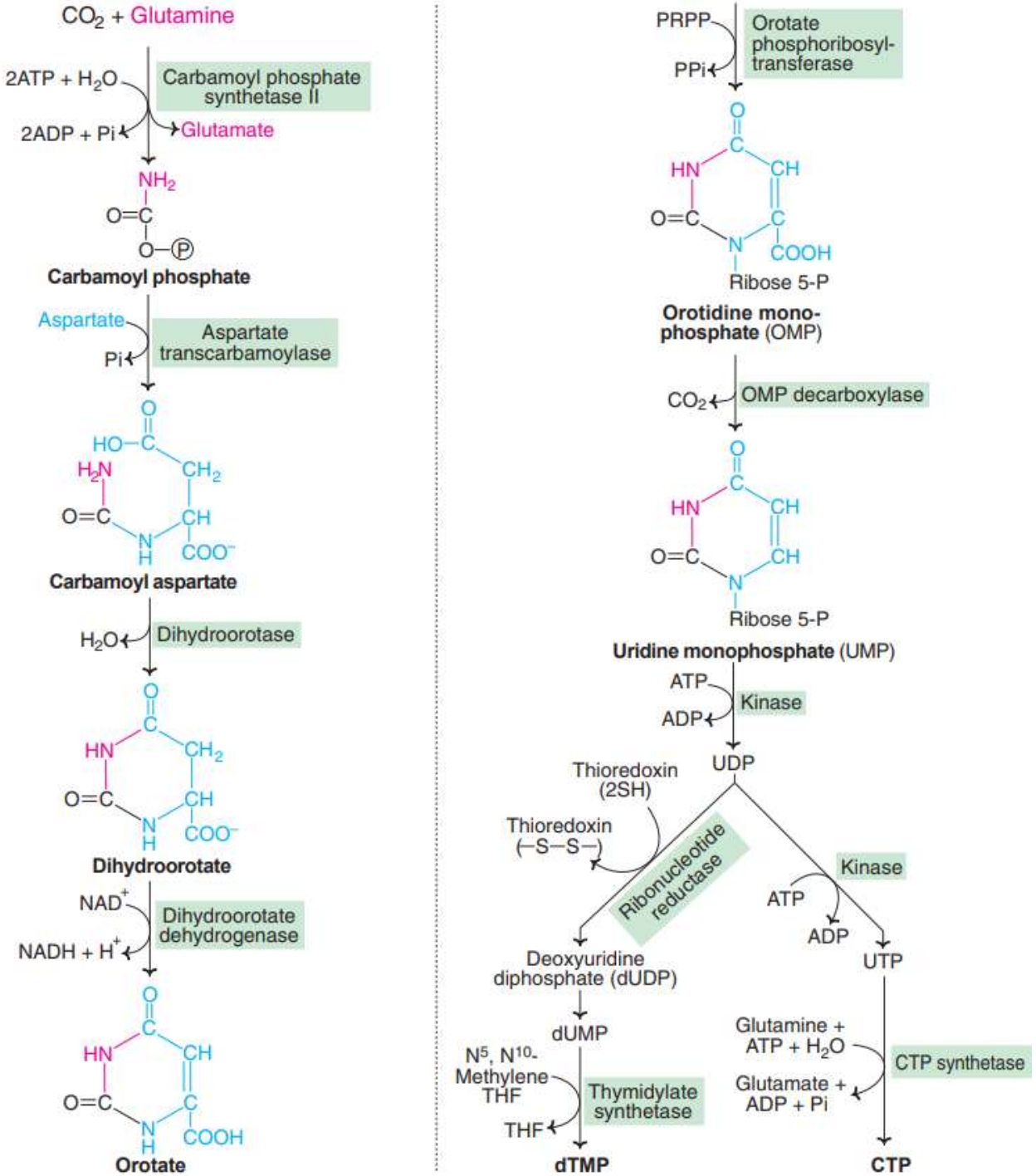
Ribose 5-phosphate is now added to orotate to produce orotidine monophosphate (OMP). This reaction is catalysed by orotate phosphoribosyltransferase, an enzyme comparable with HGPRT in its function.

OMP undergoes decarboxylation to uridine mono-phosphate (UMP). Orotate phosphoribosyltransferase and OMP decarboxylase are domains of a single protein.

By an ATP-dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP.

Ribonucleotide reductase converts UDP to dUDP by a thioredoxin-dependent reaction. Thymidylate synthetase catalyses the transfer of a methyl group from N5, N10-methylene tetrahydrofolate to produce deoxythymidine monophosphate (dTMP).

UDP undergoes an ATP-dependent kinase reaction to produce UTP. Cytidine triphosphate (CTP) is synthesized from UTP by amination. CTP synthetase is the enzyme and glutamine provides the nitrogen.


CO_2 + Glutamine
2ATP + H_2O
2ADP + Pi
Carbamoyl phosphate synthetase II
Glutamate
Carbamoyl phosphate
Aspartate
Pi
Aspartate transcarbamoylase
Carbamoyl aspartate
H_2O
Dihydroorotase
Dihydroorotate
NAD^+
$NADH + H^+$
Dihydroorotate dehydrogenase
Orotate
PRPP
PPi
Orotate phosphoribosyl-transferase
Ribose 5-P
Orotidine mono-phosphate (OMP)
CO_2
OMP decarboxylase
Ribose 5-P
Uridine monophosphate (UMP)
ATP
ADP
Kinase
UDP
Thioredoxin (2SH)
Thioredoxin (–S–S–)
Ribonucleotide reductase
Deoxyuridine diphosphate (dUDP)
dUMP
N^5, N^{10}-Methylene THF
THF
Thymidylate synthetase
dTMP
Kinase
ATP
ADP
UTP
Glutamine + ATP + H_2O
Glutamate + ADP + Pi
CTP synthetase
CTP

Salvage pathway

The pyrimidines can also serve as precursors in the salvage pathway to be converted to the respective nucleotides.

This reaction is catalysed by pyrimidine phosphoribosyltransferase which utilizes PRPP as the source of ribose 5-phosphate.