CHAPTER V: ANABOLISM and PRODUCTION of BIOMASS and METABOLITES

2. Amino Acid Production

1. Introduction

Anabolic reactions aim at synthesizing cellular components. During anabolism (or biosynthesis), cells use free energy to construct more complex molecules and structures from smaller, simpler precursors.

Amino acids synthesized within the cell are largely used for protein formation, as numerous regulatory systems are present in the cell.

The backbones of amino acids are derived from acetyl-CoA, as well as intermediates of the tricarboxylic acid (TCA) cycle, glycolysis, and the pentose phosphate pathway. To make the process efficient and economical, amino acid biosynthesis precursors come from a few main amphibolic pathways.

Twenty amino acids are required for protein biosynthesis, and they are formed from the metabolic precursors listed in Table 1. This table shows that only a few compounds serve as substrates for amino acid synthesis. For example, oxaloacetate is the starting point for six amino acids, α-ketoglutarate is the precursor for four, and pyruvate for three amino acids.

Table 1: Precursors used for amino acid biosynthesis

Precursor	**Amino Acids**
Pyruvate	Alanine, Valine, Leucine
Oxaloacetate	Aspartate, Asparagine, Methionine, Lysine, Isoleucine, Threonine
2-Ketoglutarate	Glutamate, Glutamine, Arginine, Proline
3-Phosphoglycerate	Serine, Cysteine, Glycine
PEP and Erythrose-4-Phosphate	Phenylalanine, Tryptophan, Tyrosine
Ribose-5-Phosphate	Histidine

The most readily used form of nitrogen is the ammonium form, but other forms can be incorporated, including molecular N_2. Nitrates and nitrites are used in the form of ammonium thanks to the corresponding reductases. The use of molecular nitrogen is possible only in a limited number

of microorganisms (e.g., *Azotobacter, Achromobacter, Klebsiella, Bacillus, Enterobacter, Actinomyces, some Clostridium, photosynthetic bacteria, cyanobacteria*), some of which are symbiotic (*Rhizobium, Frankia*).

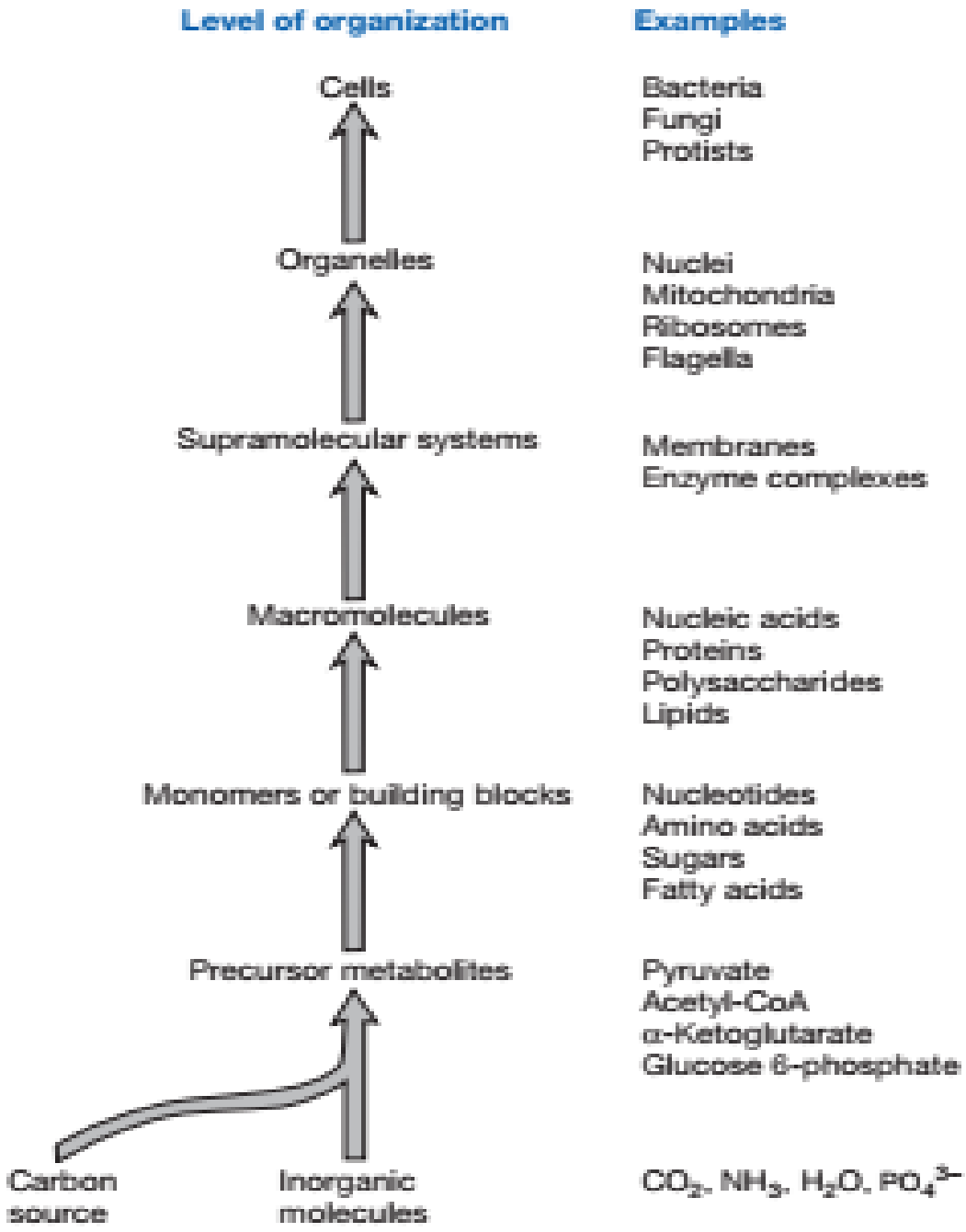

Figure 01: The Construction of Cells.

[The biosynthesis of procaryotic and eucaryotic cell constituents. Biosynthesis is organized in levels of ever greater complexity].

2. Biosynthesis of amino acids derived from α-ketoglutarate (2-oxoglutaric acid): L-glutamate, L-glutamine, L-arginine, L-proline

α-Ketoglutarate is the precursor of L-glutamate and L-glutamine, which serve not only as basic components for protein synthesis but also as important intermediates in nitrogen metabolism. Additionally, L-glutamate is the precursor of L-proline, L-arginine, and ornithine.

- **L-glutamate** is synthesized by the amination of α-ketoglutarate in a reaction catalyzed by glutamate dehydrogenase:

$$COOH–CH_2–CH_2–CO–COOH + NH_3 + NADPH,H^+ \rightarrow COOH–CH_2–CH(NH_2)–COOH + H_2O + NADP^+$$

α-Ketoglutarate Glutamate

- **Proline** is synthesized through a pathway involving only a few reactions. Glutamate from α-ketoglutarate is phosphorylated by glutamate kinase to form 5-phosphoglutamate, which is then converted by 5-phosphoglutamate dehydrogenase into glutamate semialdehyde. This spontaneously cyclizes to pyrroline-5-carboxylate, which is reduced to L-proline by Δ^1-pyrroline-5-carboxylate reductase (Fig. 2).

- **L-arginine** synthesis is more complex. Glutamate is acetylated by amino acid acetyltransferase to form N-acetylglutamate, then converted to semialdehyde via N-acetylglutamate kinase and N-acetylglutamate semialdehyde dehydrogenase. Acetylation of the NH_2 group prevents spontaneous cyclization, leaving the aldehyde available for transamination. Removal of the acetyl group produces L-ornithine, addition of a carbonyl group (C=O) gives L-citrulline, and finally, replacement of the oxo group (=O) with an imine group (>C=N–) yields L-arginine.

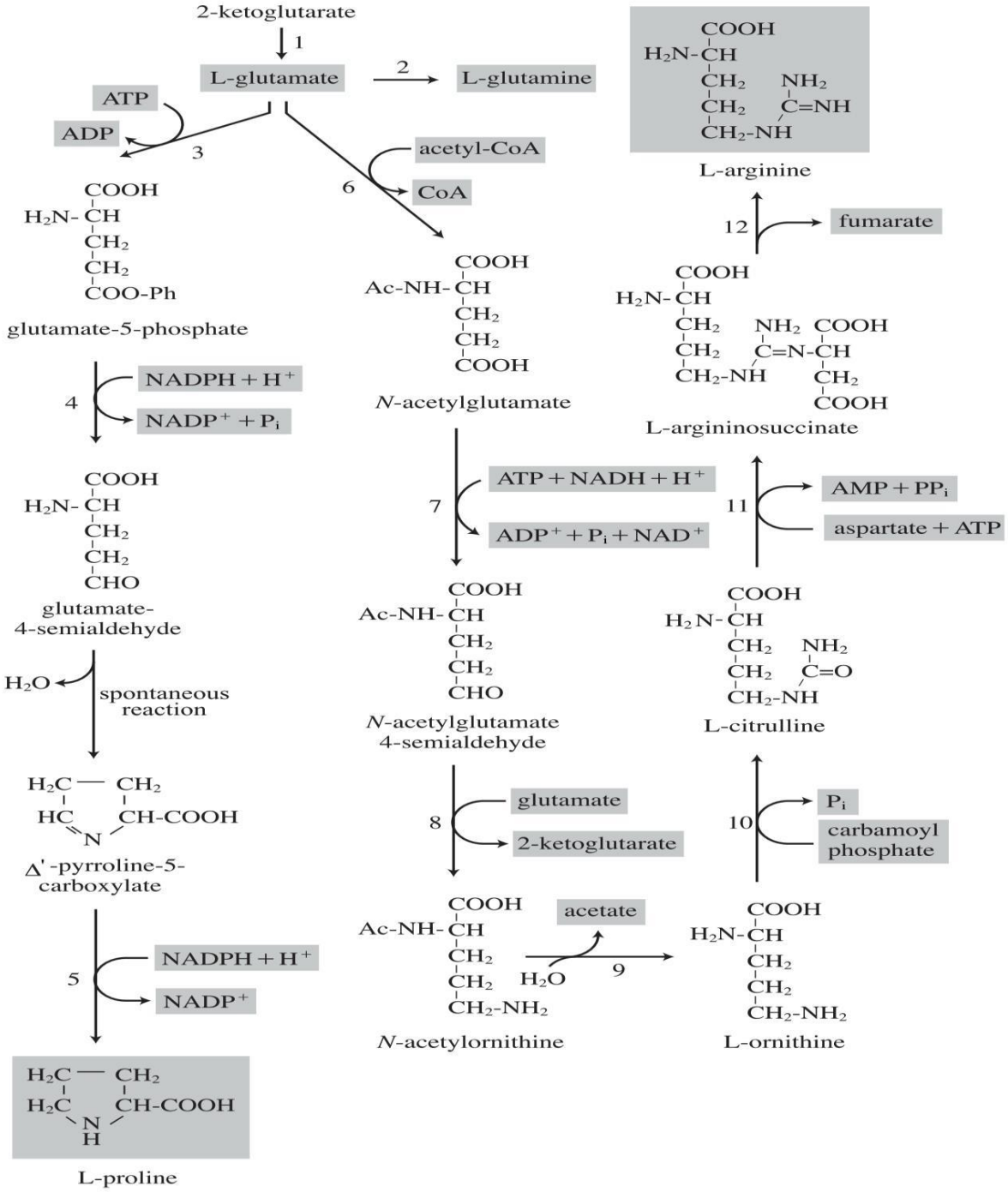

Figure 2: Biosynthesis of glutamate, glutamine, proline, and arginine from α-ketoglutarate.

1. Glutamate dehydrogenase or glutamate synthase	7. N-acetylglutamate kinase and N-acetylglutamate semialdehyde dehydrogenase
2. Glutamate synthetase	8. N-acetylornithine transaminase
3. Glutamate kinase	9. N-acetylornithine deacetylase
4. Glutamate semialdehyde dehydrogenase	10. Ornithine transcarbamoylase
5. Δ^1-pyrroline-5-carboxylate reductase	11. Argininosuccinate synthetase
6. Amino acid acetyltransferase	12. Argininosuccinate lyase

3. Biosynthesis of amino acids derived from oxaloacetate and pyruvate

The amino acids are: L-alanine, L-valine, L-leucine, L-aspartate, L-asparagine, L-methionine, L-lysine, L-threonine, L-isoleucine

Pyruvate and oxaloacetate are converted into **alanine** and **aspartate** by transaminase-catalyzed reactions, using glutamate as the $–NH_2$ donor.

Asparagine synthetase synthesizes **asparagine** from aspartate and ammonia, consuming ATP in a reaction similar to that catalyzed by glutamine synthetase (Fig. 03).

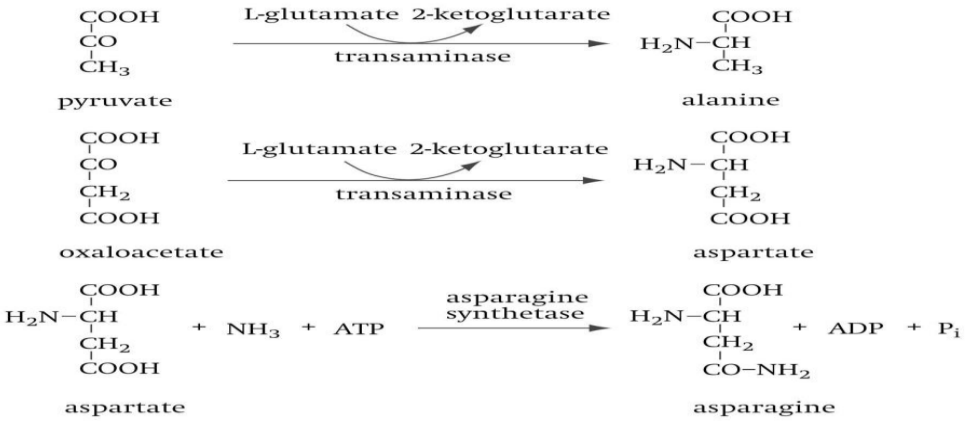

Figure 3: Biosynthesis of alanine, aspartate, and asparagine

Aspartate semialdehyde, synthesized in two steps from L-aspartate, is a central intermediate for **L-methionine, L-threonine, L-isoleucine**, and **L-lysine**. It is reduced to homoserine by homoserine dehydrogenase.

Via a phosphorylated intermediate (homoserine-P) and threonine synthase, the hydroxyl group of homoserine is shifted from carbon 4 to carbon 3, forming **L-threonine**.

Another enzyme acting on homoserine is homoserine acyltransferase, producing succinylhomoserine. The succinyl group is then replaced by a cysteinyl residue, and elimination of ammonia and pyruvate gives homocysteine. The final step for **L-methionine** synthesis is methylation, with N^5-methyltetrahydrofolate as the methyl group donor.

For **lysine** synthesis, aspartate semialdehyde condenses with pyruvate to produce 2,3-dihydrodipicolinate (ring form). Reduction and ring opening via succinylation give diaminopimelate. The L,L form is converted to the *meso* form and finally decarboxylated to L-lysine.

Accumulation of lysine can be achieved in mutants auxotrophic for methionine and threonine. Threonine can be accumulated in *E. coli* mutants auxotrophic for lysine and methionine.

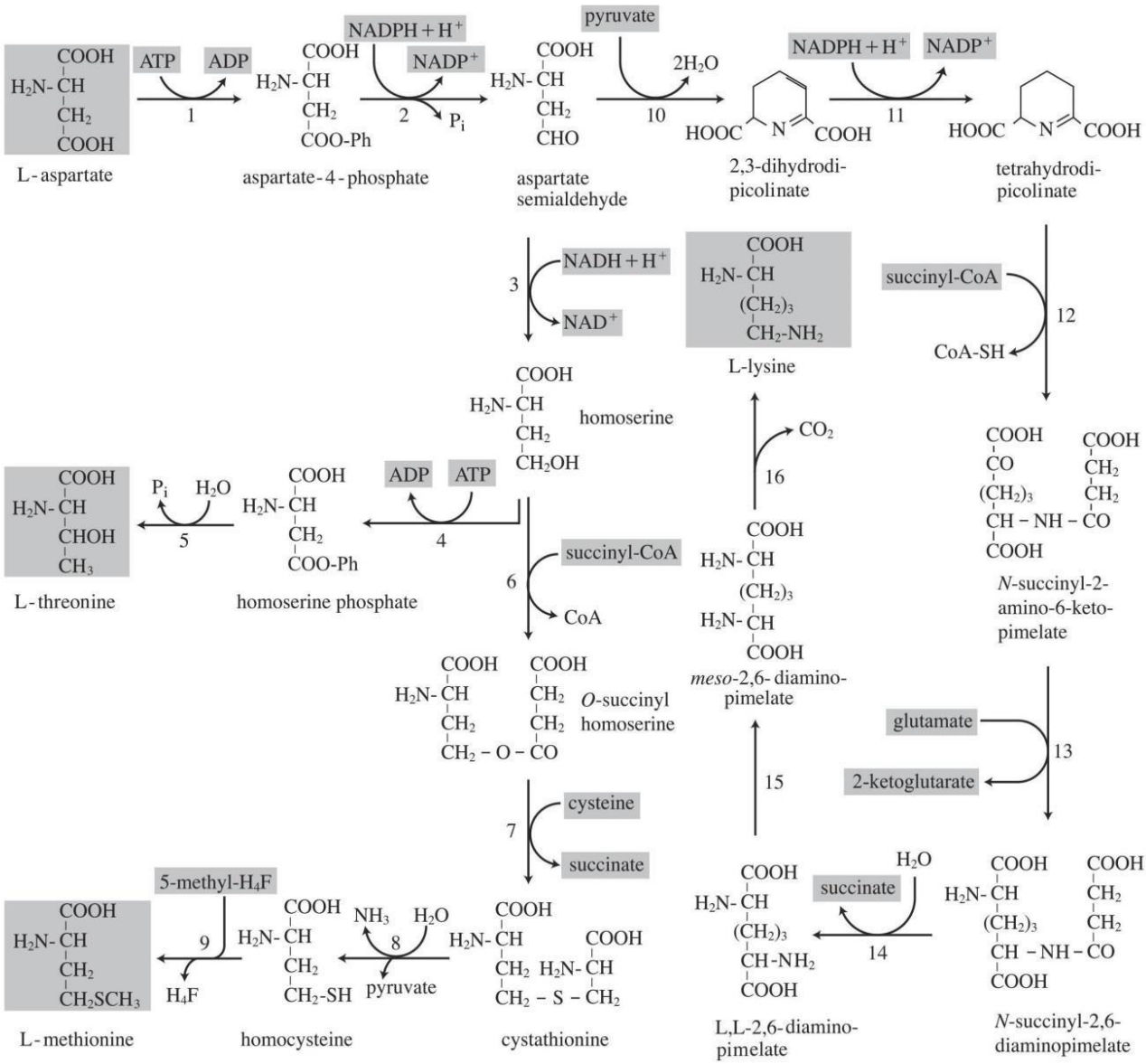

Figure 04: Detailed biosynthesis of threonine, methionine, and lysine from the common precursor aspartate.

1. aspartate kinase	9, homocysteine: 5-methyltetrahydrofolate methyltransferase
2, aspartate semialdehyde dehydrogenase	10, dihydrodipicolinate synthase
3, homoserine dehydrogenase	11, dihydrodipicolinate reductase
4, homoserine kinase	12, tetrahydrodipicolinate succinylase
5, threonine synthase	13, glutamate: succinyl-diaminopimelate aminotransferase
6, homoserine acyltransferase	14, succinyl-diaminopimelate desuccinylase
7, cystathionine synthase	15, diaminopimelate epimerase
8, cystathionine lyase	16, diaminopimelate decarboxylase. H4F: tetrahydrofolate

The biosynthesis of **leucine**, **isoleucine**, and **valine** begins with pyruvate and α-oxobutyrate. The latter is formed from threonine by threonine deaminase. It is noteworthy that both compounds (α-oxobutyrate and pyruvate) are converted by the same set of four enzymes into L-isoleucine and L-valine, respectively.

α-Oxoisovalerate, the precursor of L-valine, is also the starting point for the L-leucine pathway. The addition of acetyl-CoA yields isopropylmalate, which is isomerized to β-isopropylmalate. Dehydrogenation and amination steps lead to L-leucine.

Valine can be accumulated by mutants of certain *Aerobacter* species or *Micrococcus glutamicus* auxotrophic for isoleucine and leucine.

Isoleucine (L-isoleucine is one of the most expensive amino acids) can be produced from threonine-rich media by *Streptomyces rimosus* or *Serratia*, and from media containing α-aminobutyric acid (α-ABA) by strains of *Bacillus subtilis*, *Pseudomonas*, *E. coli*, etc.

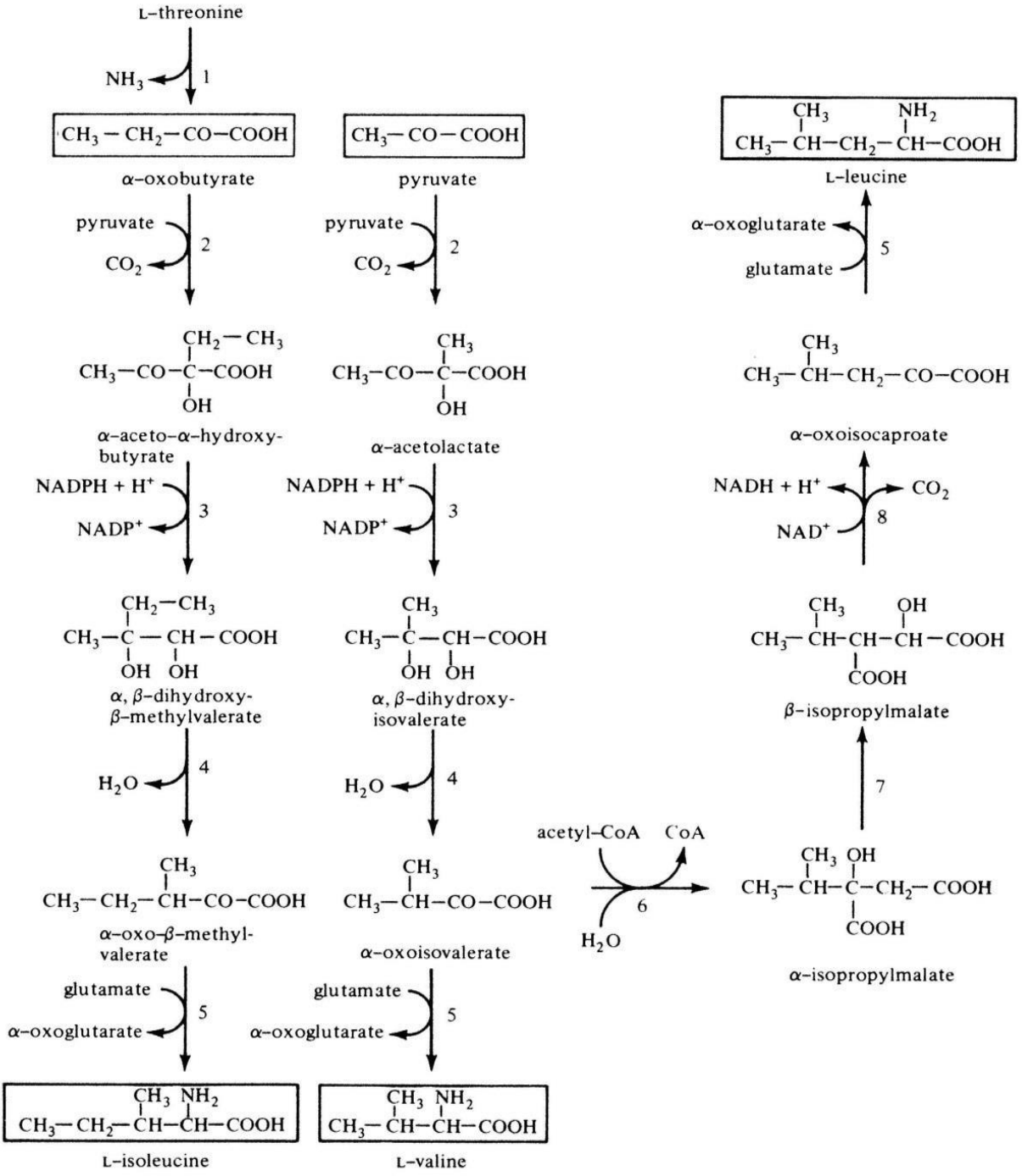

Figure 05: Detailed biosynthesis of L-isoleucine, L-leucine, and L-valine (amino acids derived from oxaloacetate and pyruvate).

1. threonine deaminase	5. transaminase C
2. acetohydroxy acid synthase	6. α-isopropylmalate synthase
3. acetohydroxy acid isomeroreductase	7. isopropylmalate isomerase
4. dihydroxy acid dehydratase	8. β-isopropylmalate dehydrogenase