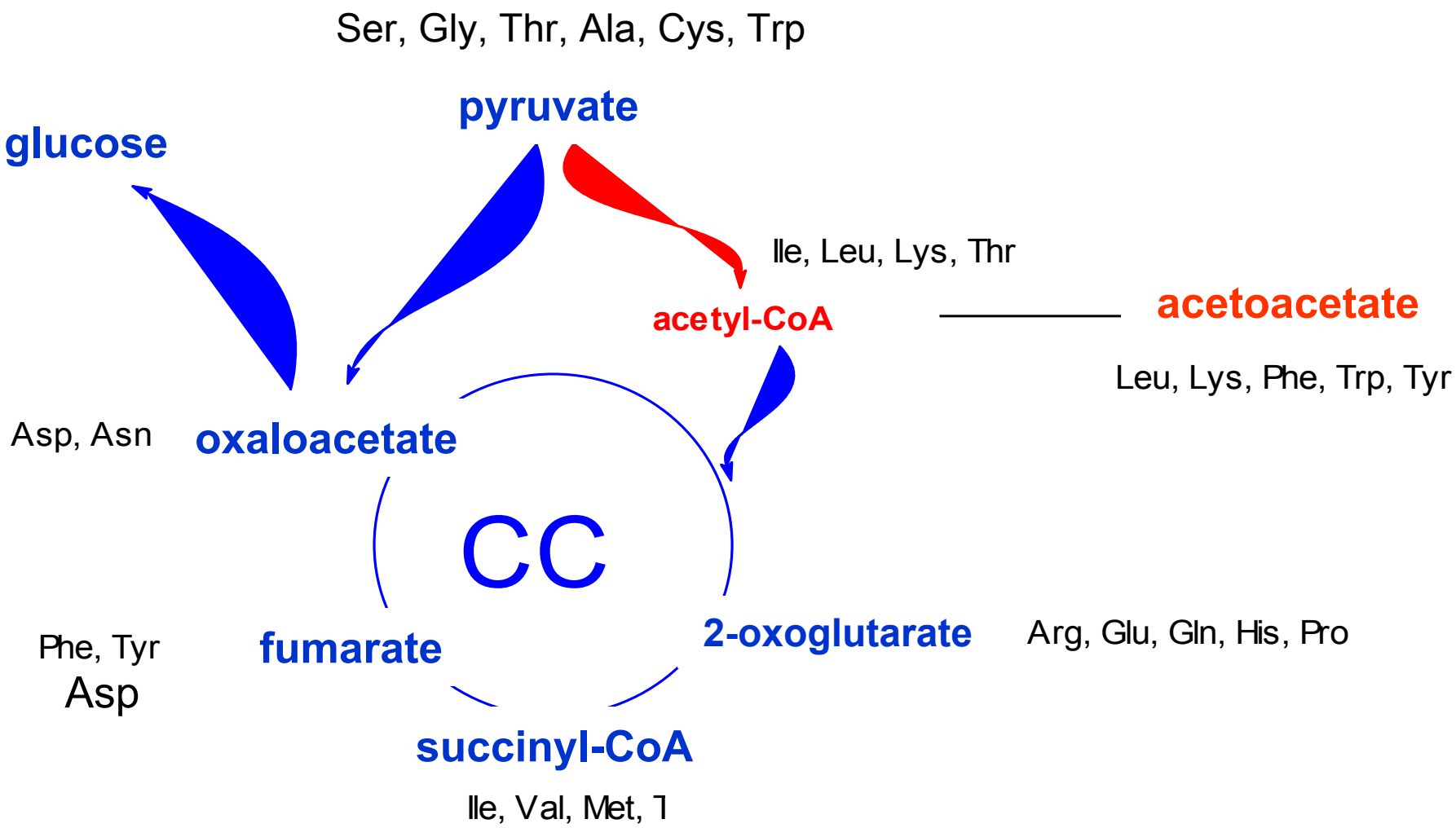
# Metabolism of amino acids

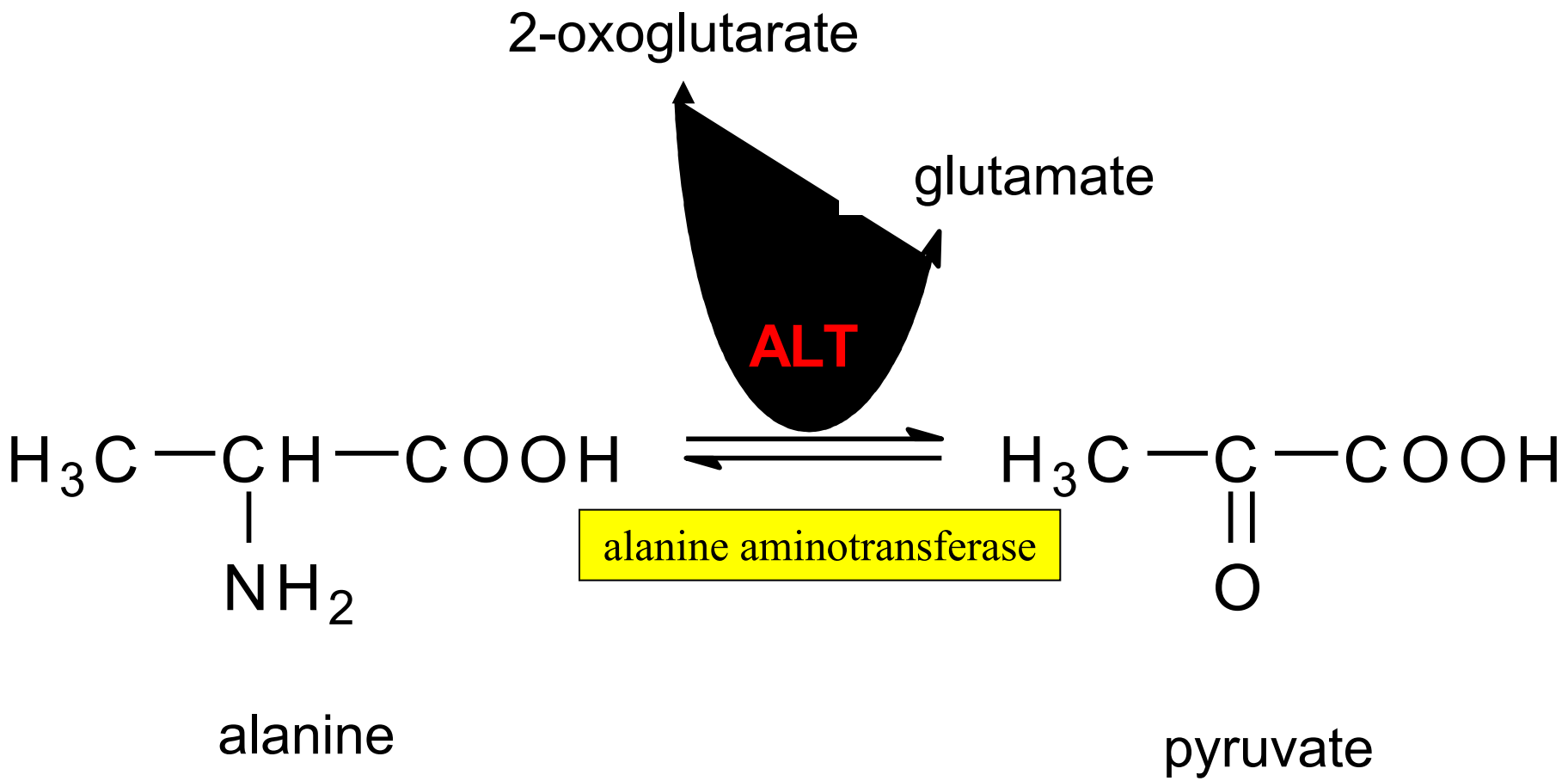
# Intermediates of amino acid catabolism

- **Glucogenic (13)** → pyruvate and/or CAC intermediates
- **Ketogenic (2)** = Leu, Lys → acetyl-CoA + acetoacetate
- **Mixed (5)** = Thr, Ile, Phe, Tyr, Trp

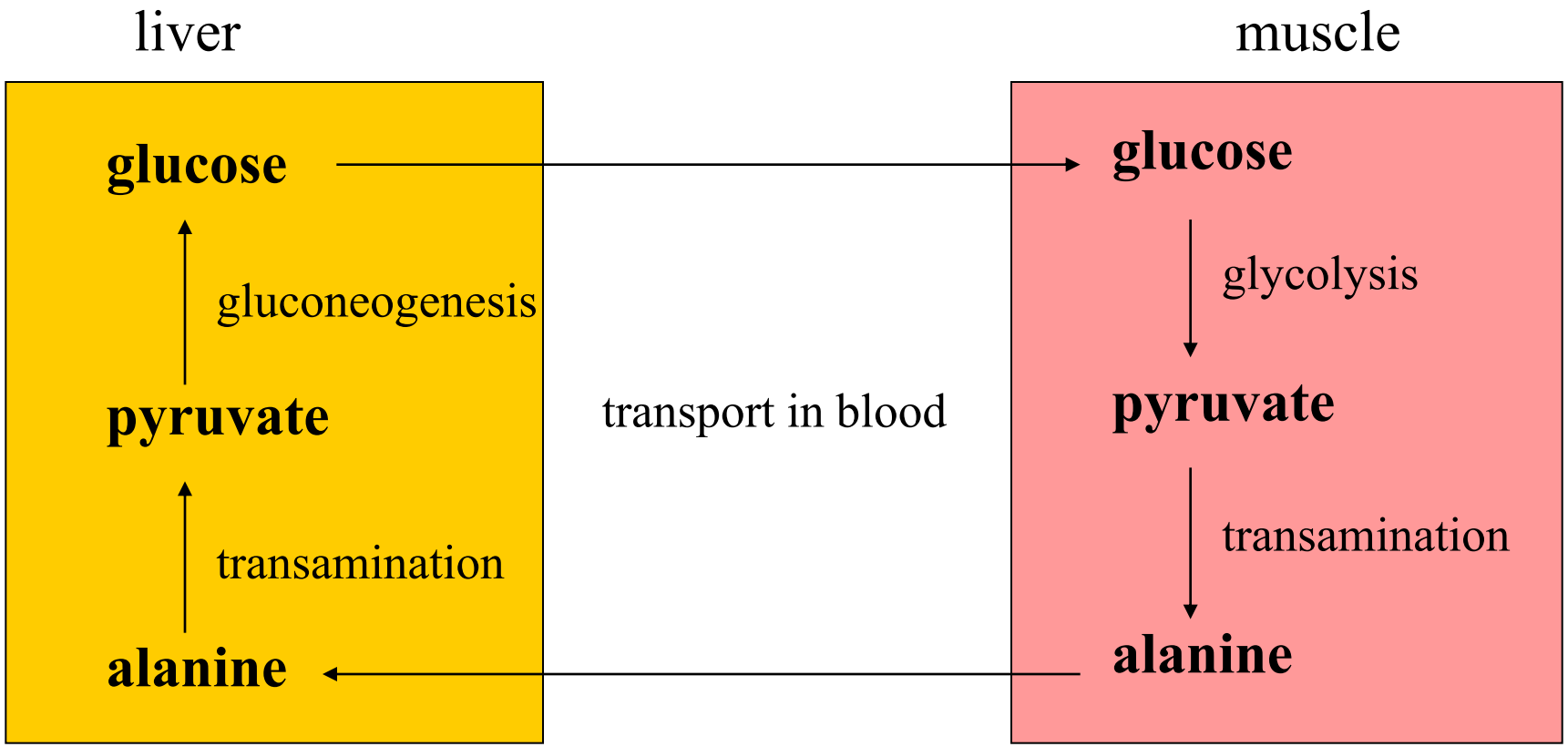
# Intermediates of amino acid catabolism



# Transamination of alanine



# Glucose-alanine cycle




- alanine is non-toxic transport of ammonia from muscles to liver
- in the liver, alanine is the substrate for gluconeogenesis

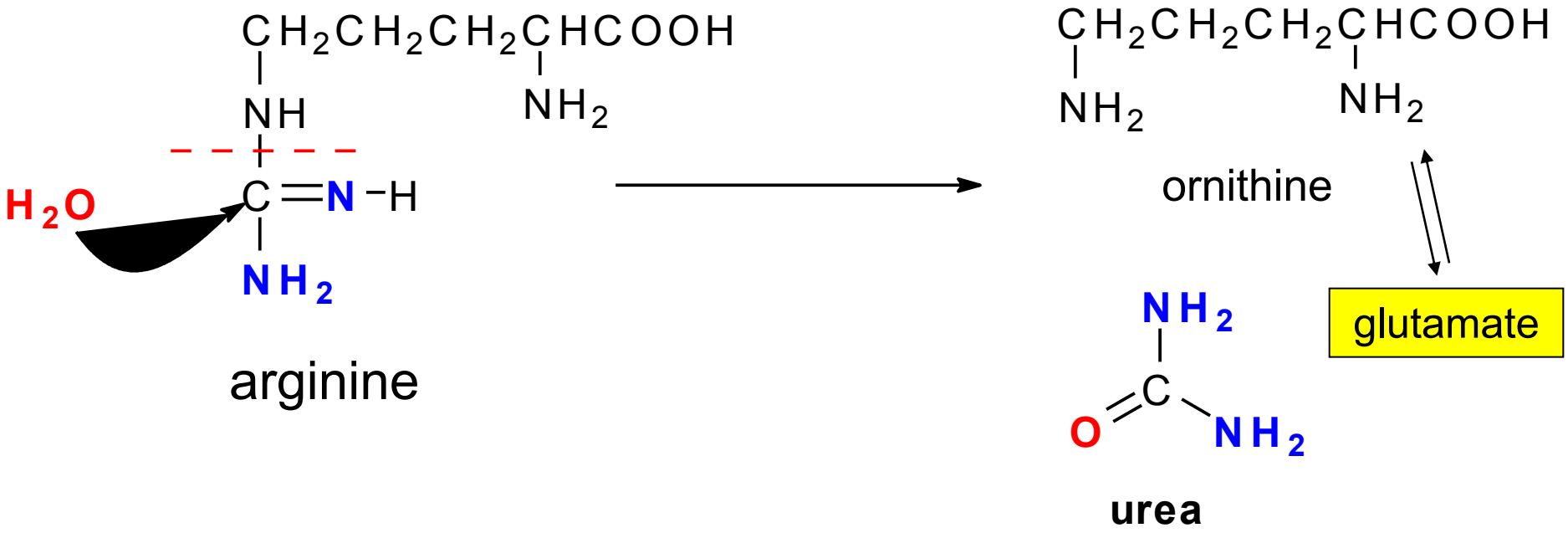
# Alanine - summary

- readily made from pyruvate (transamination)
- ALT is clinically important enzyme, mainly in liver, elevated catalytic concentration in blood serum – liver diseases
- Ala is released to blood mainly from muscles, together with Gln (postresorption phase)
- semiessential AA (in metabolic stress) – important substrate for gluconeogenesis

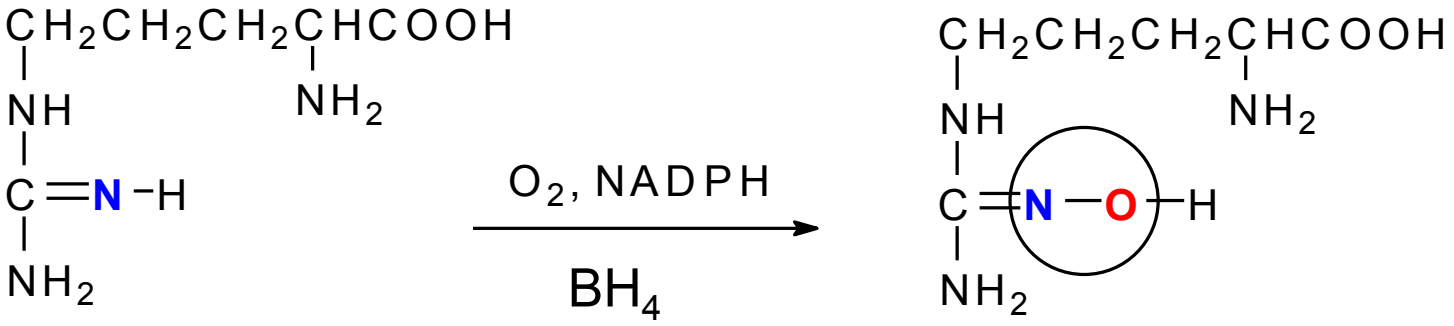
Arginine

no transamination

# Hydrolysis of arginine → urea



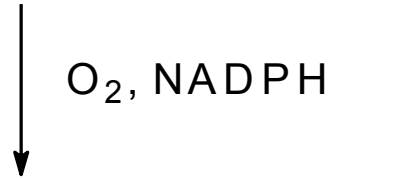
# NO is signal molecule from arginine

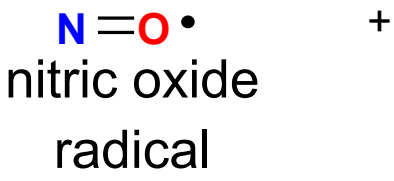



N-hydroxyarginine

**Exogenous NO sources**

- glycerol trinitrate
- isosorbide dinitrate
- amyl nitrite
- isobutyl nitrite
- sodium nitroprusside

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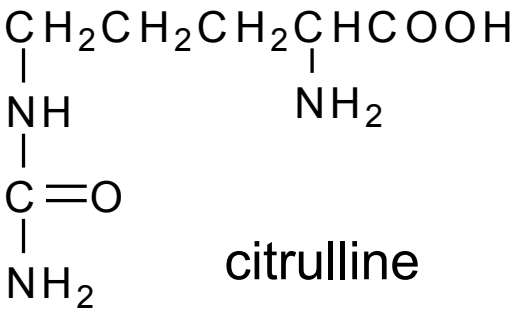
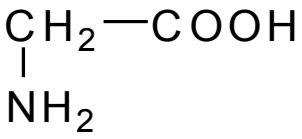


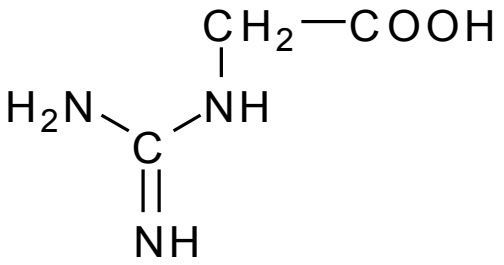

citrulline

# Synthesis of creatine (1. part)

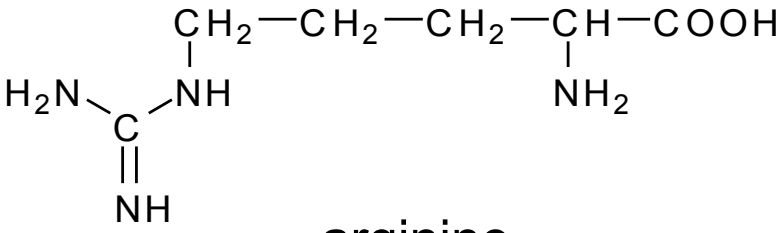
from Greek κρέας (meat)



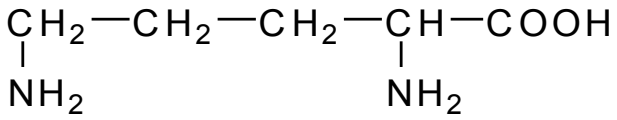
glycine



guanidinoacetate



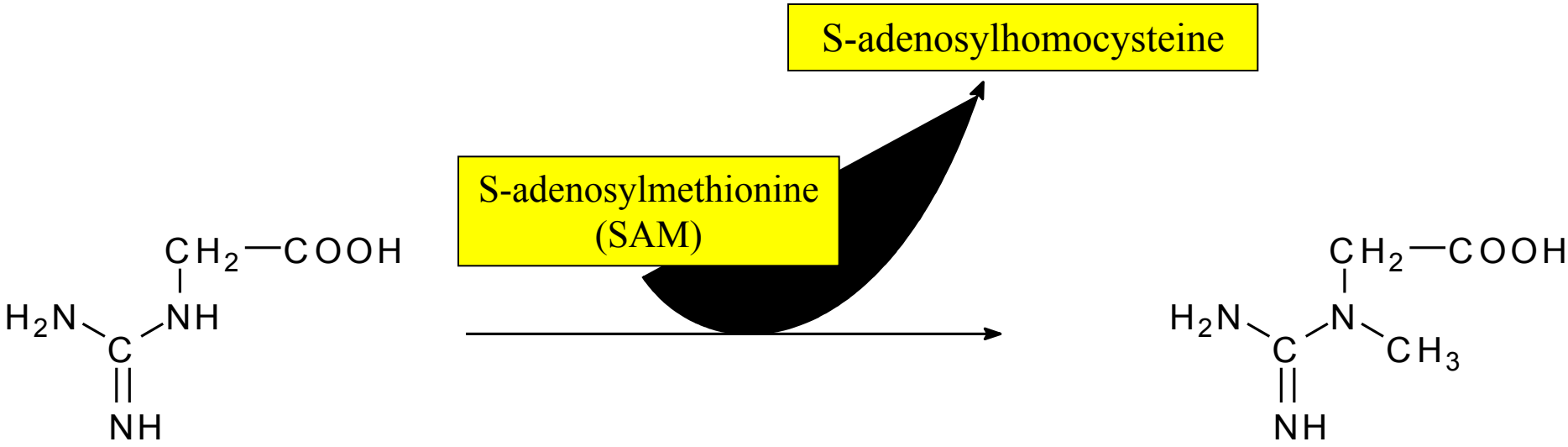
arginine



ornithine

# Synthesis of creatine (2. part)

$N^1$-methylation of guanidinoacetate

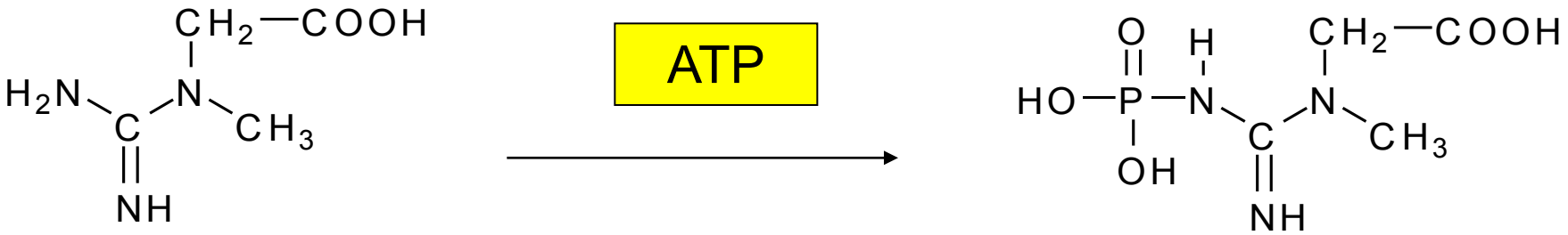



guanidinoacetate

**creatine**

*N*-methylguanidine-*N*-acetate

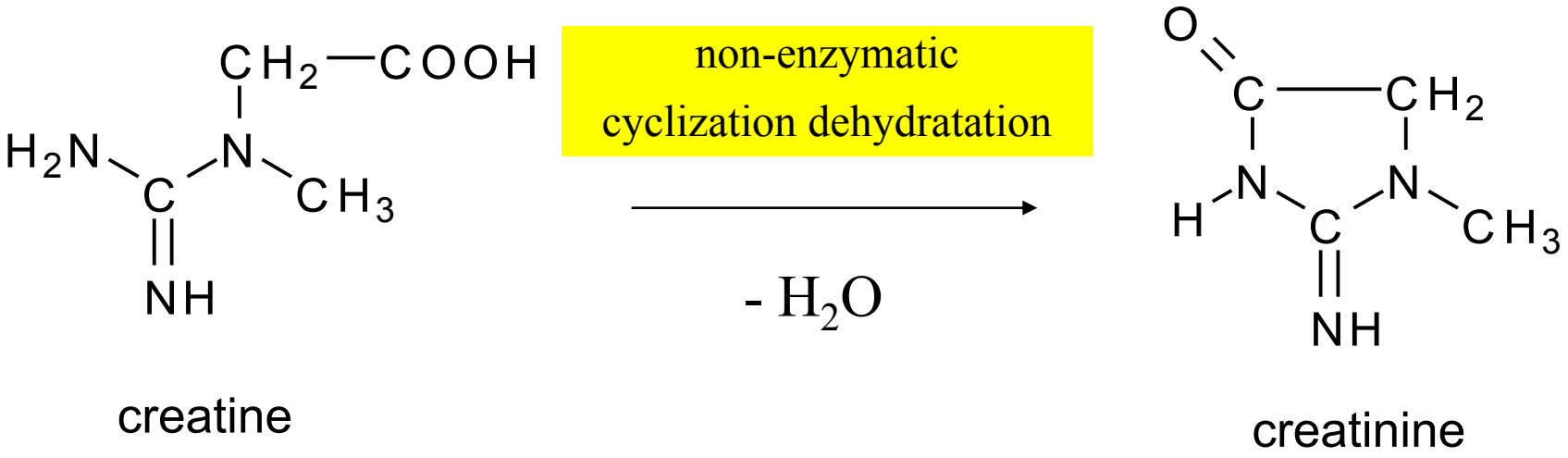
# $N^2$-Phosforylation of creatine




creatine

creatine phosphate

# Creatinine is a catabolite of creatine made in non-enzymatic reaction

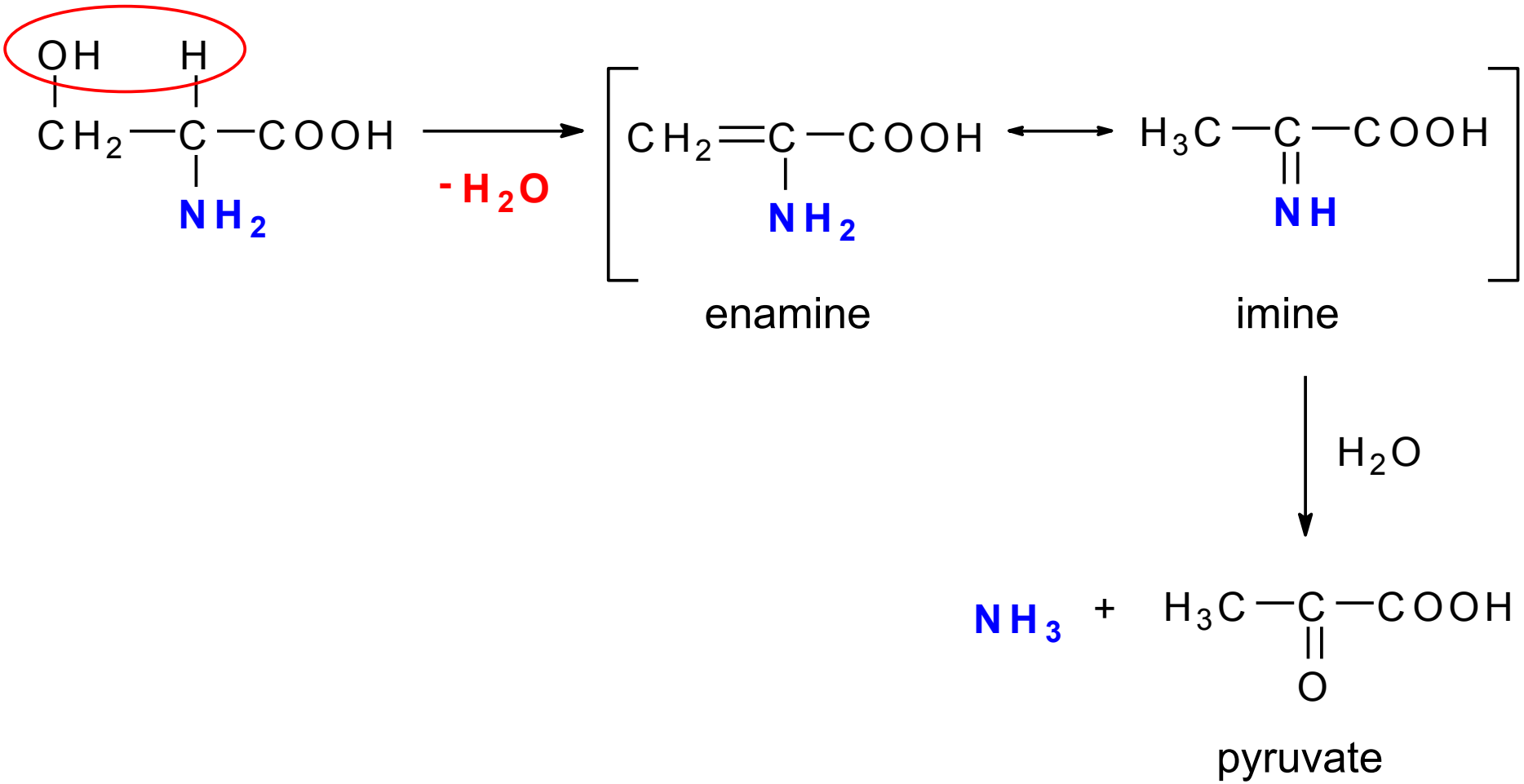


# Arginine - summery

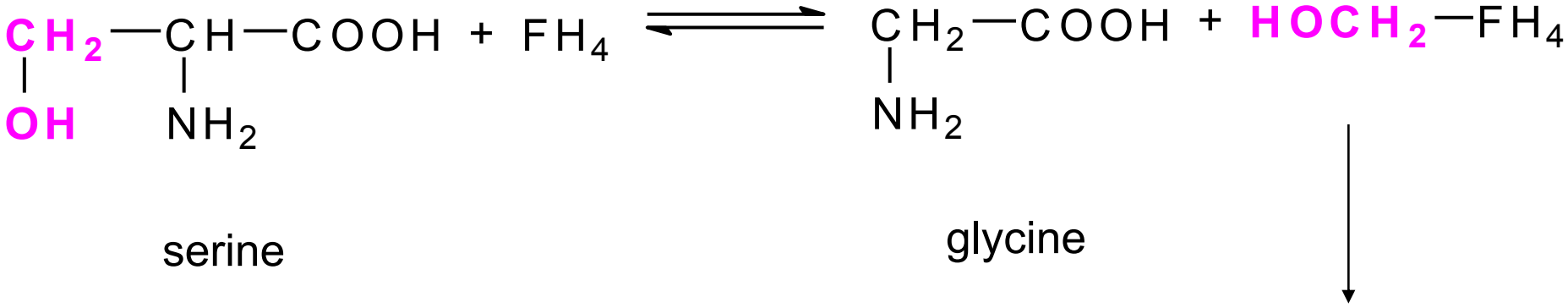
- semiessential AA (childhood)
- the most basic AA (guanidine, p$K_B$ = 0.5)
- no transamination, Arg releases ornithine + urea
- Arg + Gly + Met $\rightarrow$ creatine
- releases NO (vasodilator)
- OTC (over-the-counter) preparations in pharmacy

# Serine

## Dehydratation + deamination of serine



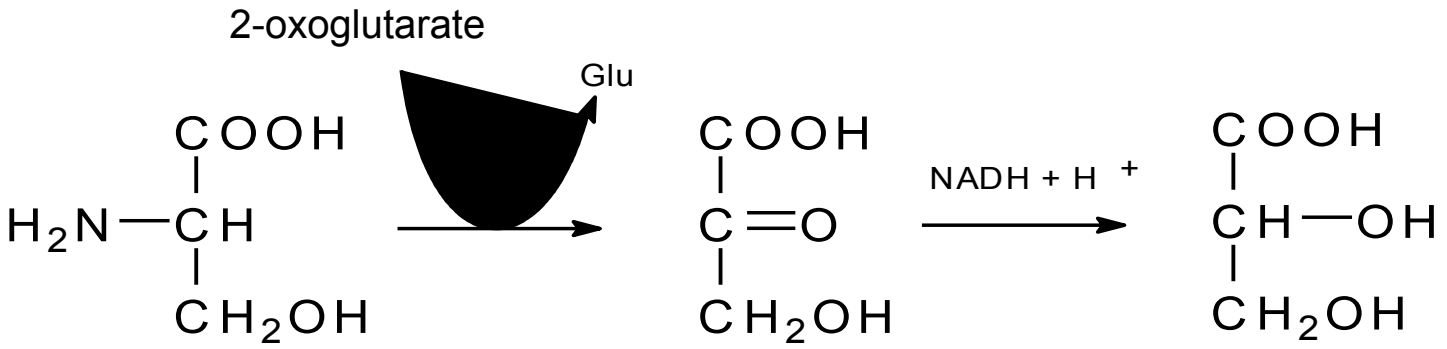
# Conversion of serine to glycine




cofactor:

tetrahydrofolate ($FH_4$)

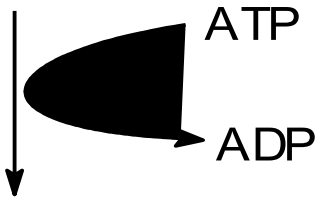
# Transamination of serine and glucose formation

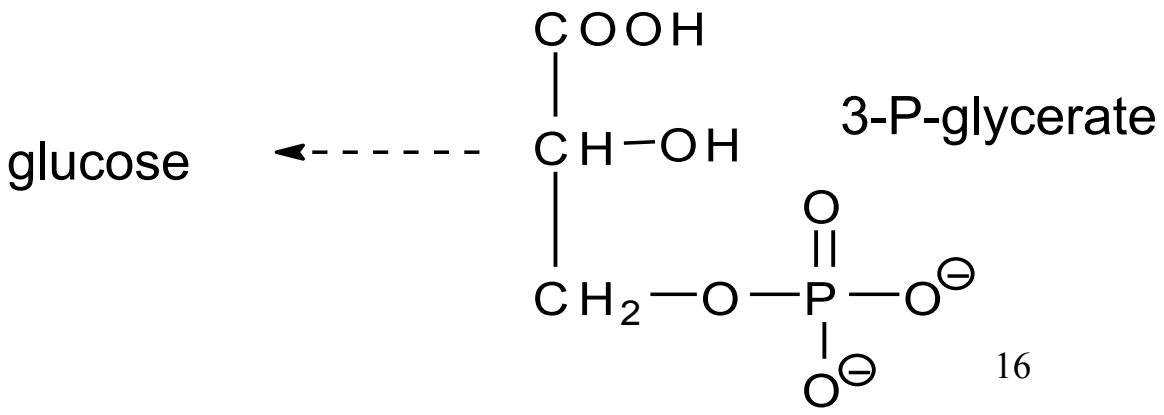



serine hydroxypyruvate glycerate

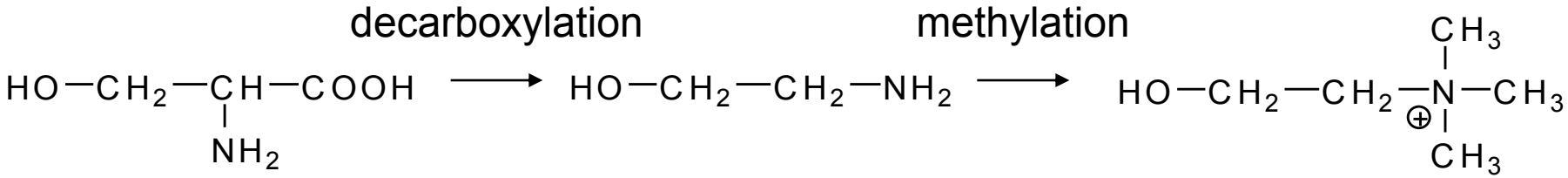
reverse reaction:
synthesis of serine

pathway is different -
through phosphoserine



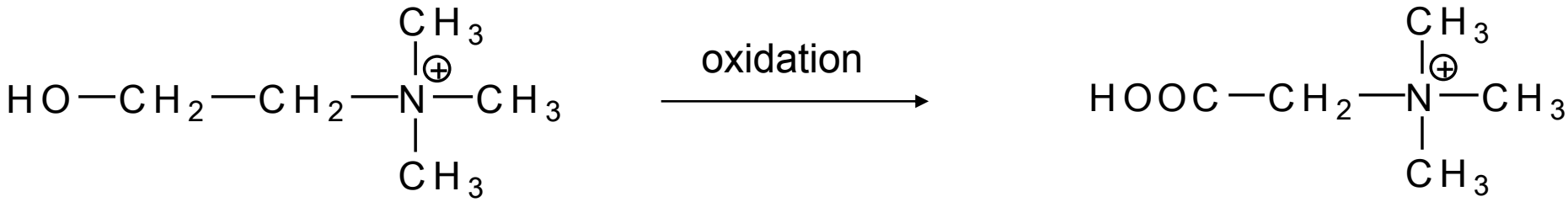



# Decarboxylation of serine gives ethanolamine. Methylation of ethanolamine leads to choline




serine ethanolamine choline

# Betaine is made by choline oxidation




choline betaine

# Serine - summary

- non-essential glucogenic AA
- source of C1 fragments (attached to tetrahydrofolate)
- component of glycerophospholipids
- decarboxylation gives ethanolamine → choline
- carbon skeleton used for selenocysteine
- **serine side chain in proteins:**

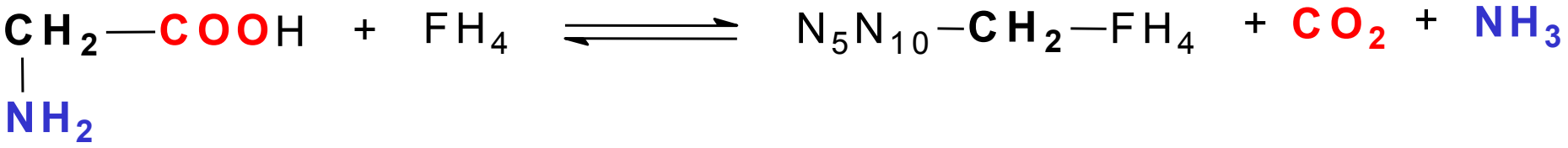
  the site of phosphorylation

  the linkage of oligosaccharides (*O*-glycoside bond)

  nucleophilic -OH group in active site of enzyme (serine proteases)

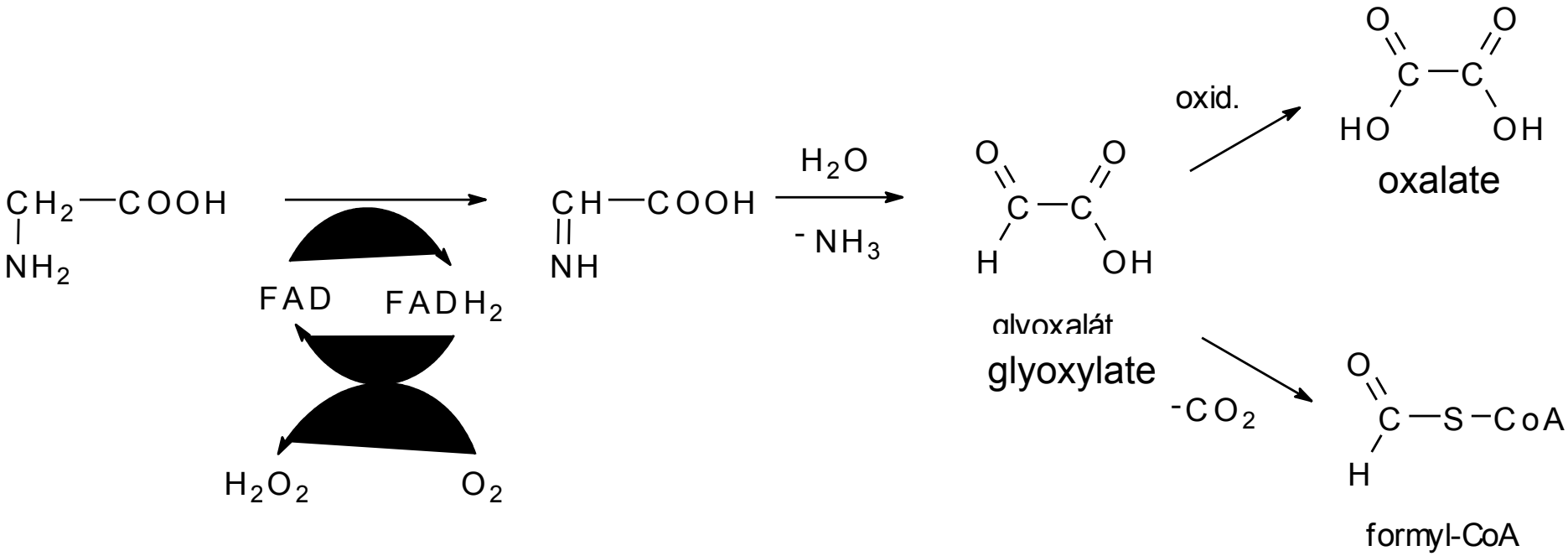
# Glycine

## The complete catabolism of glycine




C1 fragment (methylene) is transferred to tetrahydrofolate

# Oxidative deamination of glycine




60 % catabolism of glycine and ethanolamine

30 % catabolism of vitamin C

10 % food (spinach, rhubarb, mangold, tea, cocoa)

# Glycine - summary

## Catabolism

- complete oxidation to $CO_2 + NH_3$
- oxidative deamination to oxalate

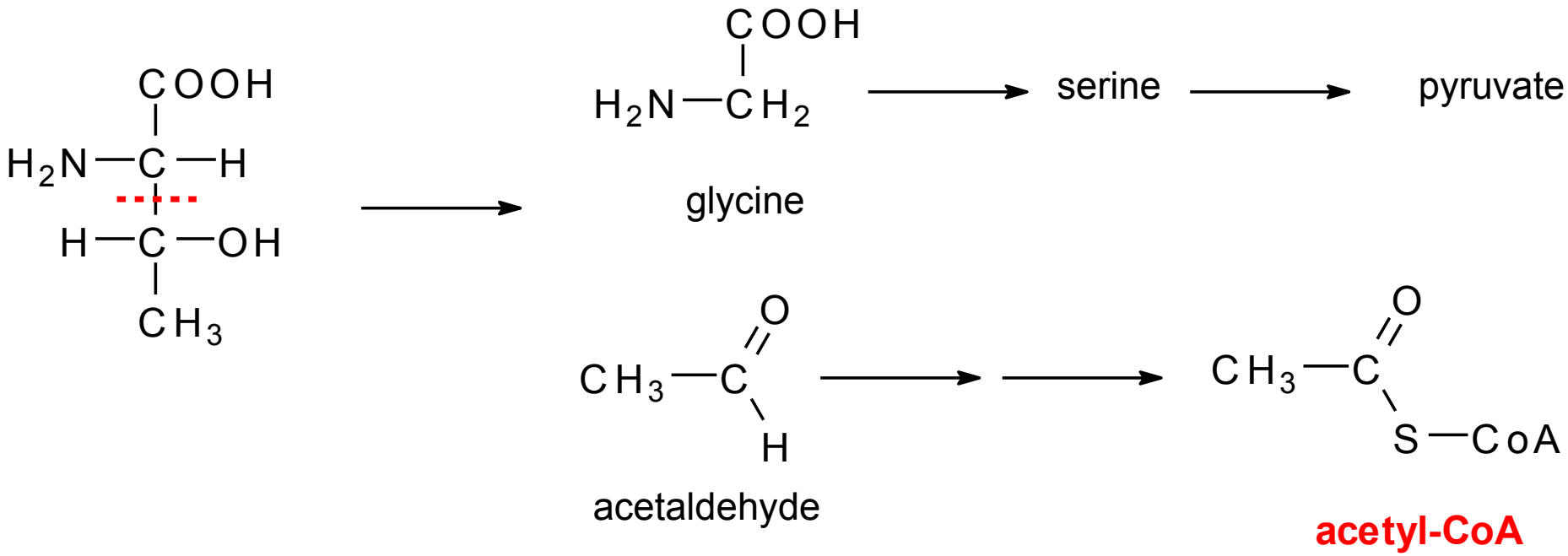
## Anabolic conversions

- donor of C1 fragment
- serine
- porphyrines
- purine bases
- creatine
- glutathione (GSH)
- conjugation agent (bile acids, xenobiotics)

# Threonine

no transamination

## Threonine (4C) is split to glycine (2C) and acetaldehyde (2C)

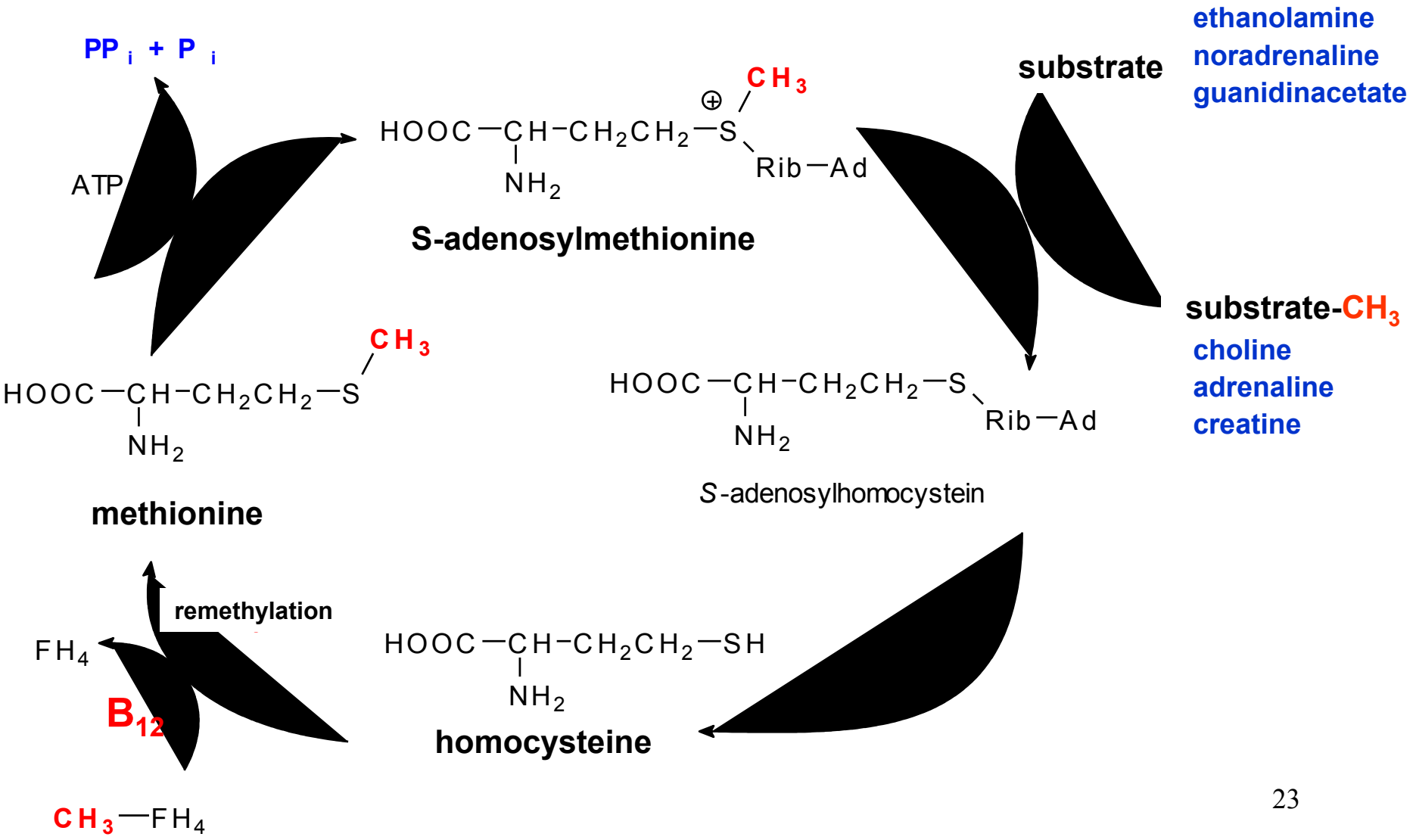



- essential AA
- two asymmetric C atoms
- the site of phosphorylation and glycosylation in proteins

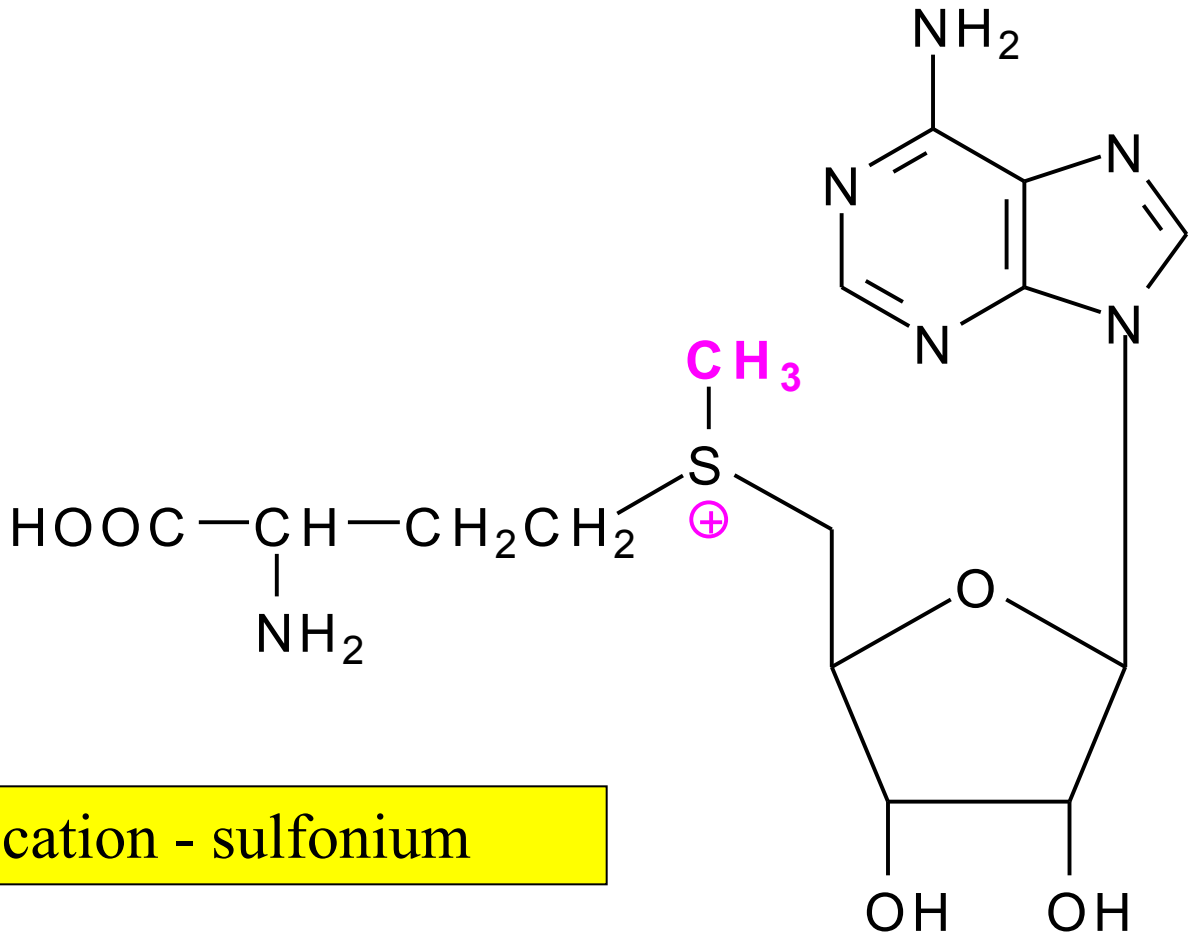
Methionine

no transamination

# Methionine is methylation agent (homocysteine side product)

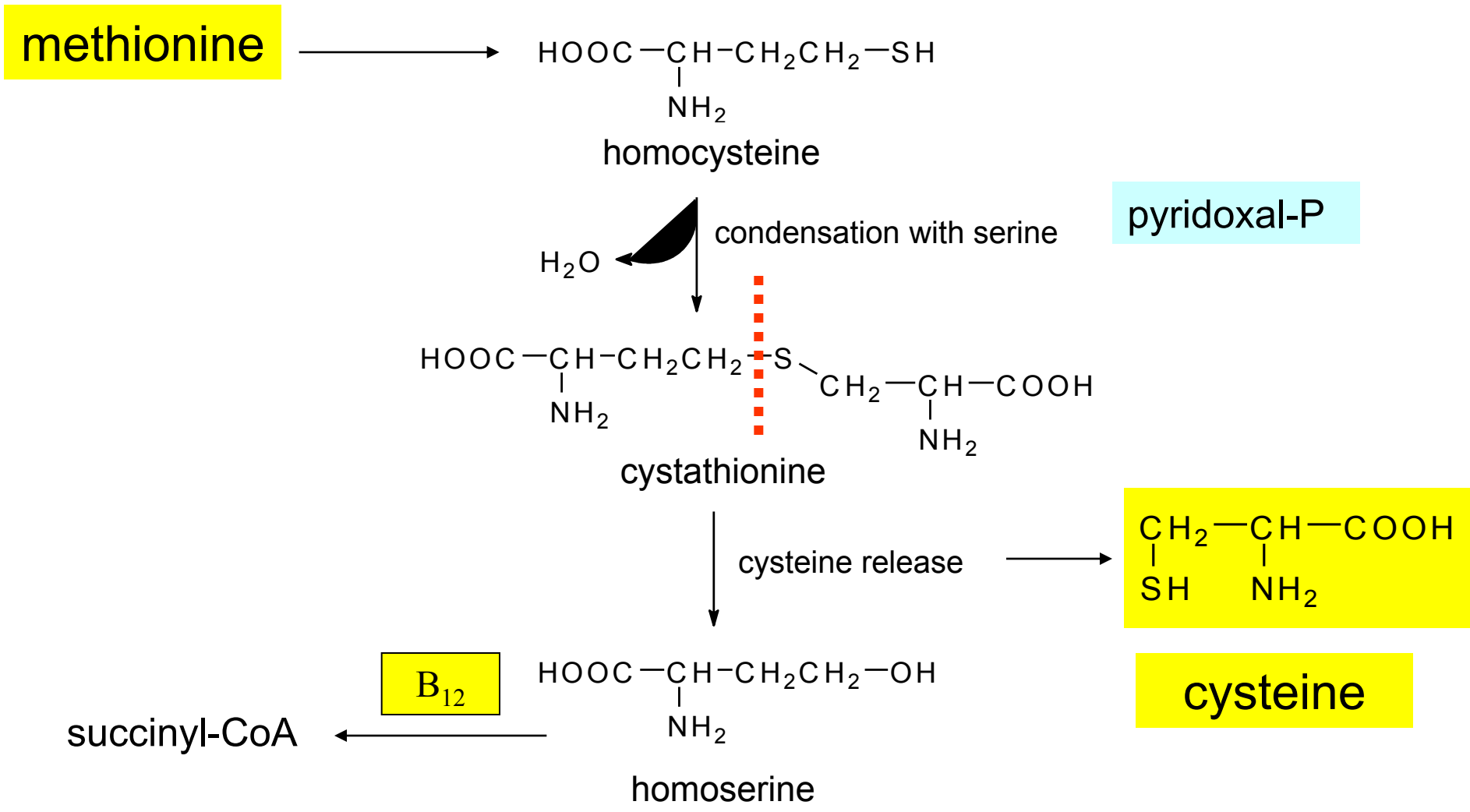


# *S*-Adenosylmethionine (SAM) contains trivalent positively charged sulfur atom




cation - sulfonium

# Cysteine is made from methionine



# Methionine - summery

- essential AA, rather rare in foodstufs
- S-adenosylmethionine (SAM) is methylation agent
- metabolized to cysteine $\Rightarrow$ Cys is non-essential AA
- C-skeleton of cysteine comes from serine, sulfur atom from methionine
- final catabolite is succinyl-CoA (glucogenic)

# Homocysteine is harmful

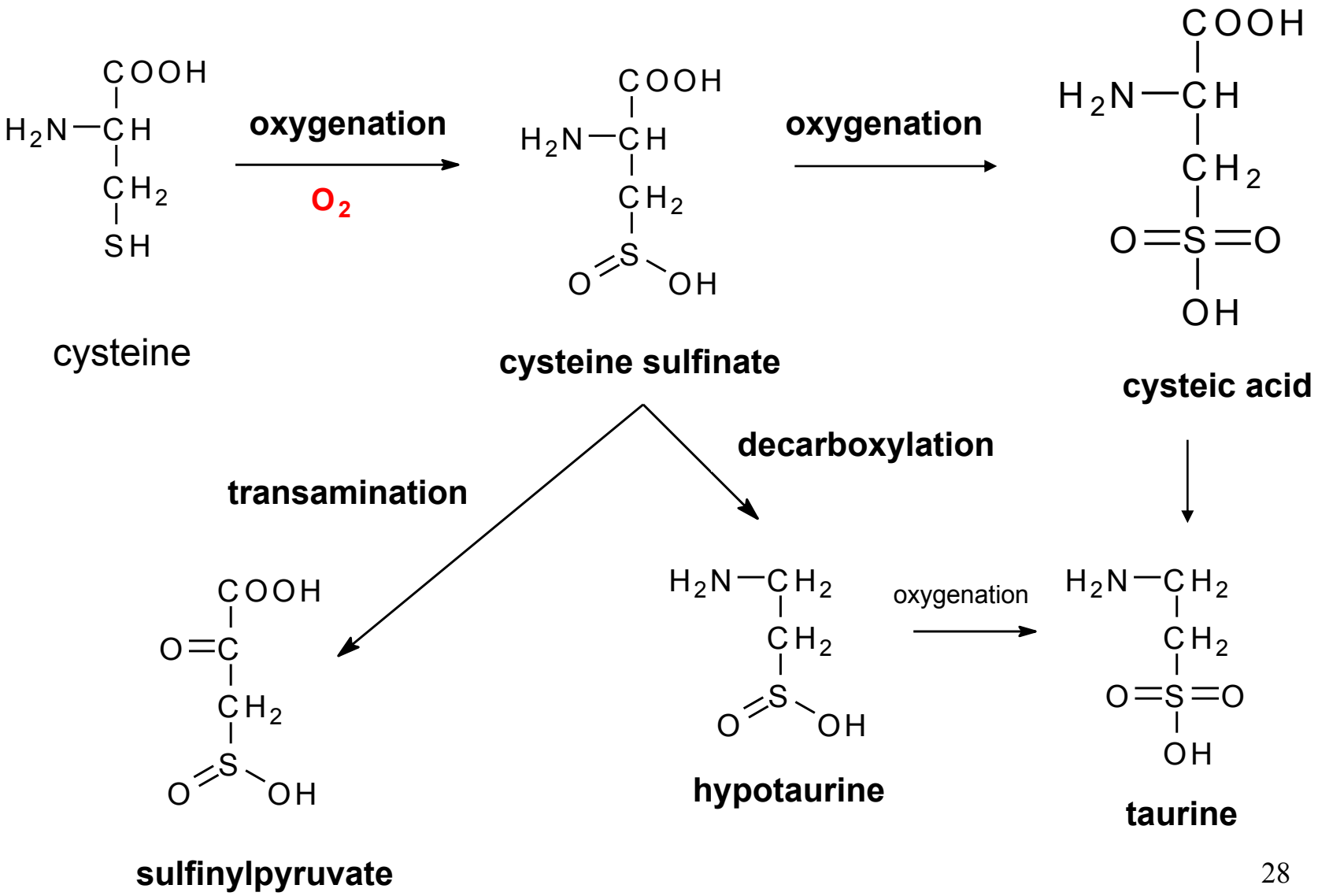
- mechanism of its action is not yet understood
- direct action on blood vessel epithelium
- decreases thrombocyte life and fibrinolysis
- supports formation of oxygen radicals – damage of vessel wall
- increases LDL lipoperoxidation
- elevated blood level of homocysteine is risk factor of cardiovascular diseases

to eliminate homocysteine - three vitamins are needed:

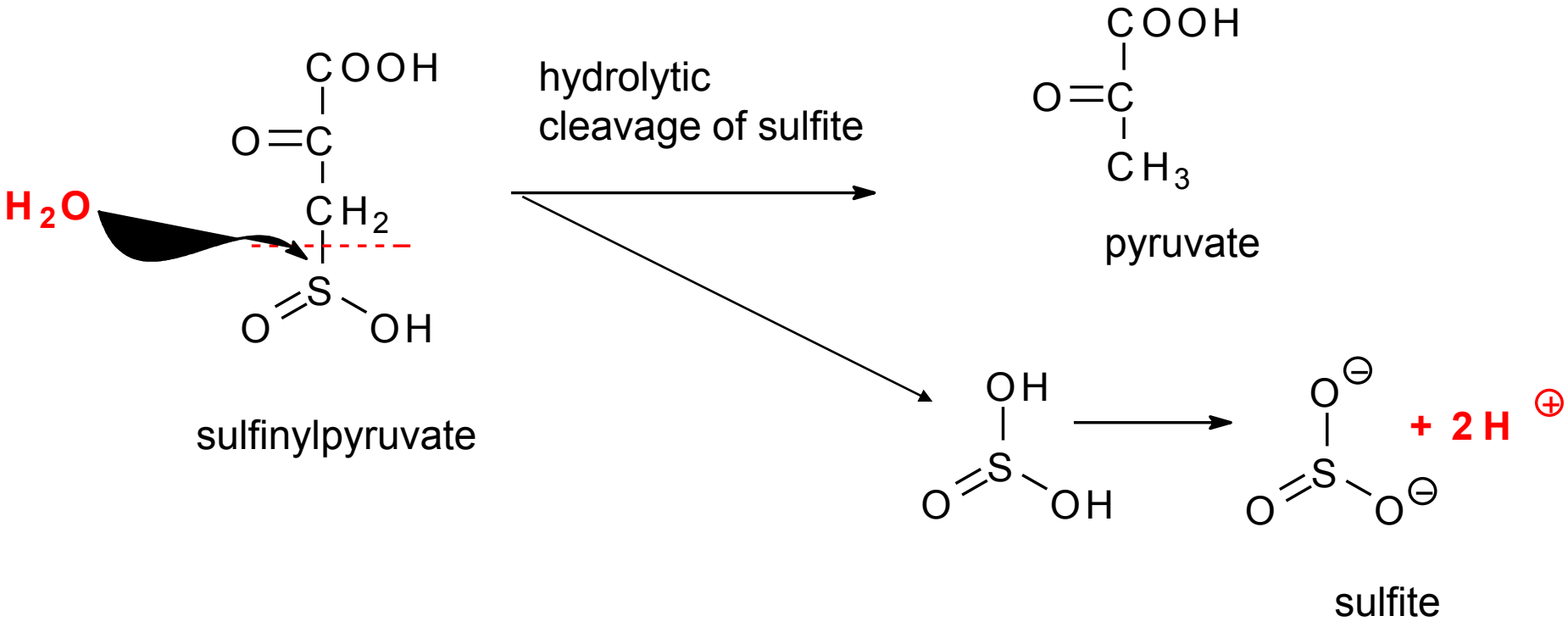
**folate, cobalamine, pyridoxin**

# Cysteine

## Cysteine catabolism: oxygenation of -SH group



# The formation of sulfite

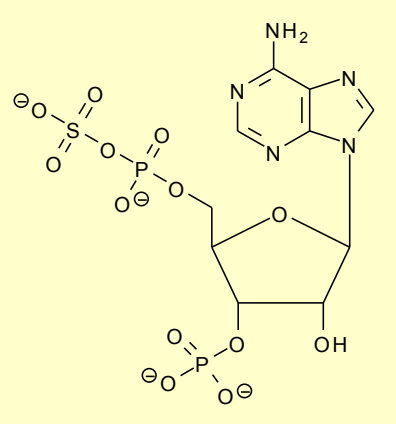



under physiol. pH – dissociation only to $HSO_3^-$

# Sulfite oxidase catalyzes sulfate formation

cysteine

PAPS



$$HSO_3^- + H_2O \rightarrow SO_4^{2-} + 3H^+ + 2e^-$$

blood plasma
(0.5 mmol/l)

urine

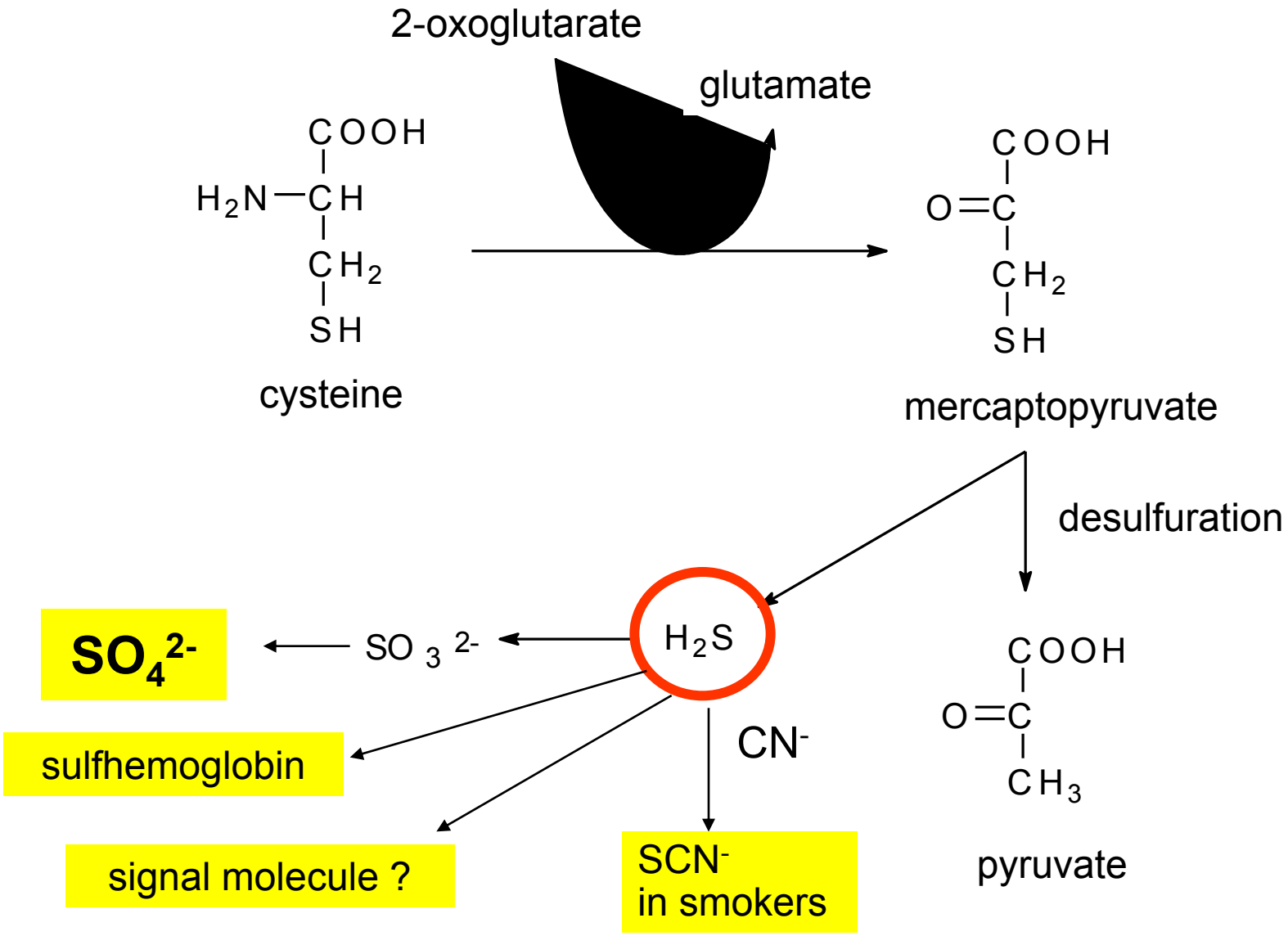
acidify
body fluids

reduce
molybdopterine

# Distinguish

| | |
|---|---|
| **Sulfite** | anion $SO_3^{2-}$ |
| **Sulfide** inorganic | anion $S^{2-}$ (e.g. ZnS zinc sulfide) |
| **Sulfide** organic | R-S-R dialkylsulfide |
| **Sulfate** | anion $SO_4^{2-}$ |

# Transamination of cysteine and sulfane production



# Cysteine - summary

- both pathways go to pyruvate (glucogenic)
- main catabolism: sulfur oxygenation → sulfite → sulfate
- high protein diet leads to physiologic acidosis
- cystein provids taurine – conjugation agent (e.g. bile acids)
- taurine is semiessential AA in metabolic stress
- taurine is a component of „energy drinks“
- cysteine – part of glutathione (GSH) - antioxidant
- decarboxylation of Cys – cysteamine, in CoA-SH
- in proteins – disulfide bonds (tertiary structure)
- cysteine proteases: active site contains –SH group

# Six amino acids provide pyruvate

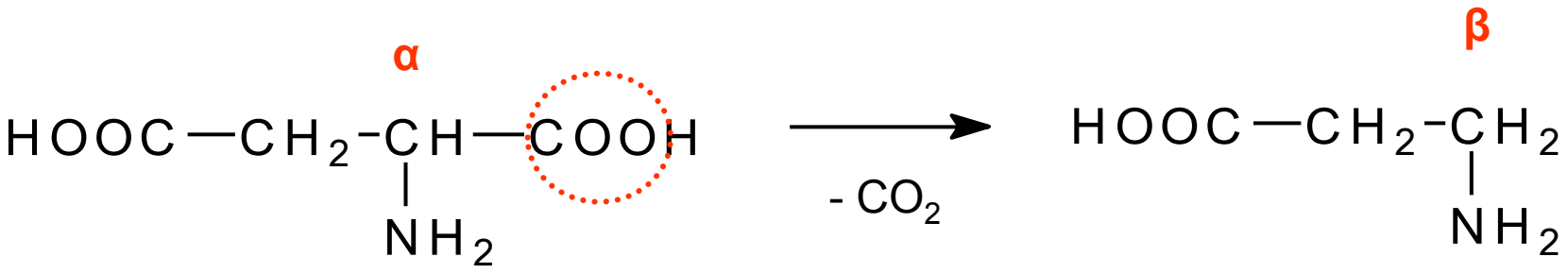
1. Serine – dehydratation + deamination
2. Glycine – *via* serine
3. Threonine – *via* glycine
4. Alanine – transamination (ALT)
5. Cysteine – both catabolic pathways
6. Tryptophan – *via* alanine (see later)

# Aspartate

- Transamination of Asp → oxaloacetate (CAC)
- AST (aspartate aminotransferase) – clinically important enzyme
- in urea cycle, Asp donates one nitrogen into urea and releases fumarate
- decarboxylation of Asp → β-alanine (part of coenzyme A)
- donor of nitrogen in purine synthesis (fumarate released)
- whole structure given for pyrimidine bases synthesis
- aspartam (sweetener)
- condensation with ammonia → asparagine

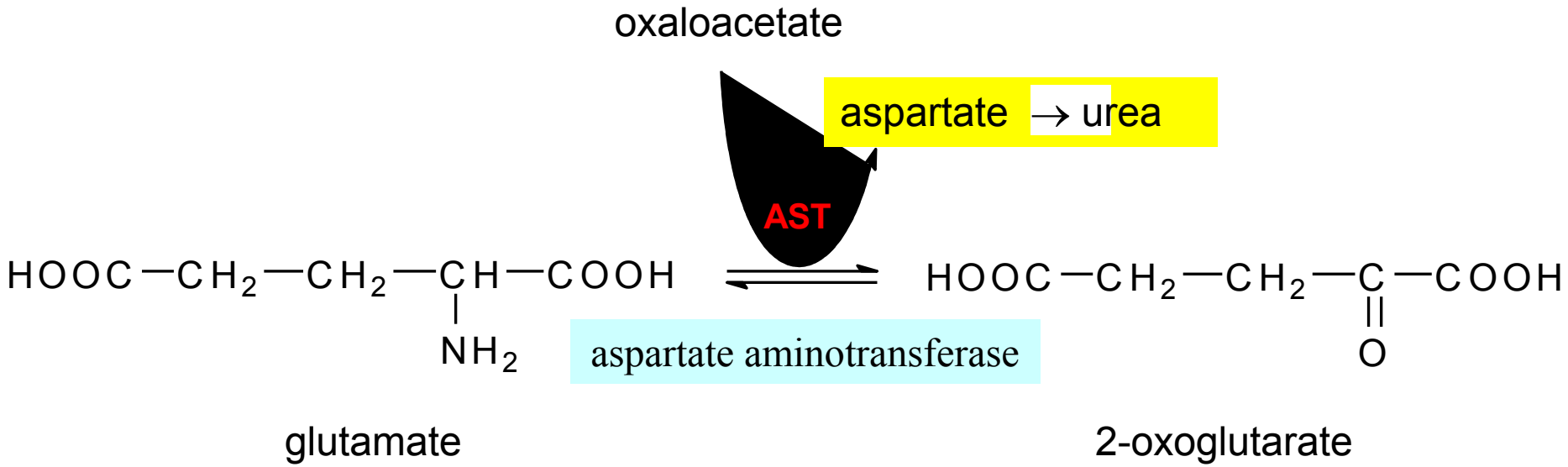
  (for cell utilization, not as detoxication of ammonia)

# β-Alanine is made by the decarboxylation of aspartate



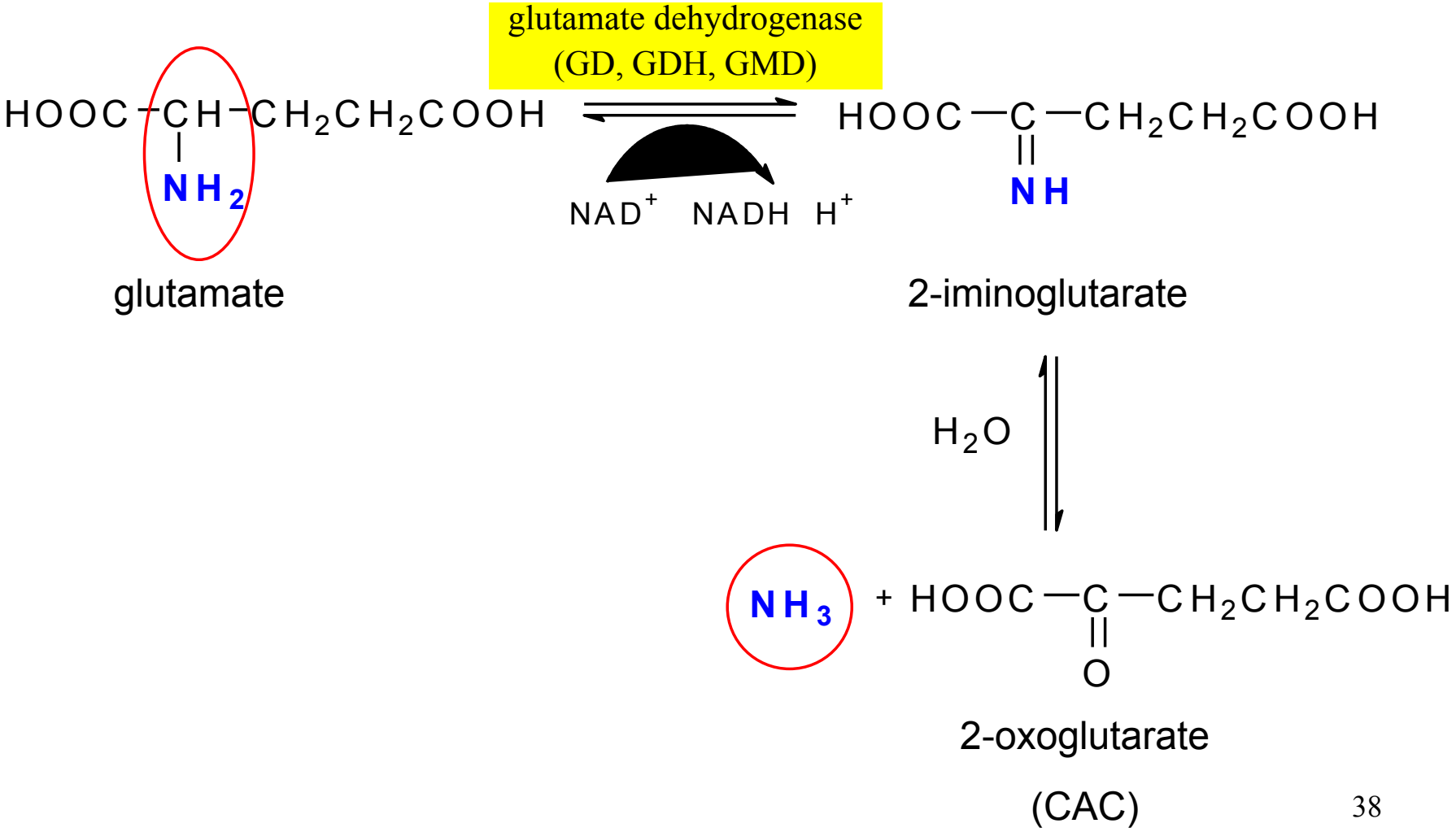

in the structure of CoA-SH

# Glutamate with oxaloacetate afford aspartate (transamination)

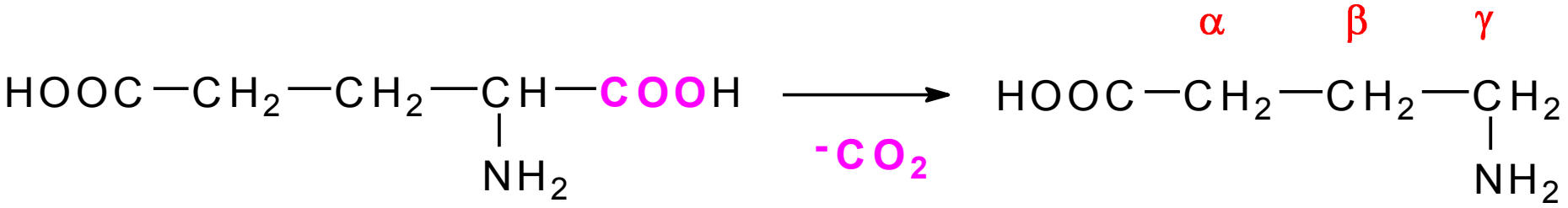



AST reaction produces aspartate for urea synthesis

# Dehydrogenative deamination of glutamate is the main producer of ammonia in tissues



# Decarboxylation of glutamate




glutamate

**GABA**

**gama-aminobutyric acid**

# Glutamate - summary

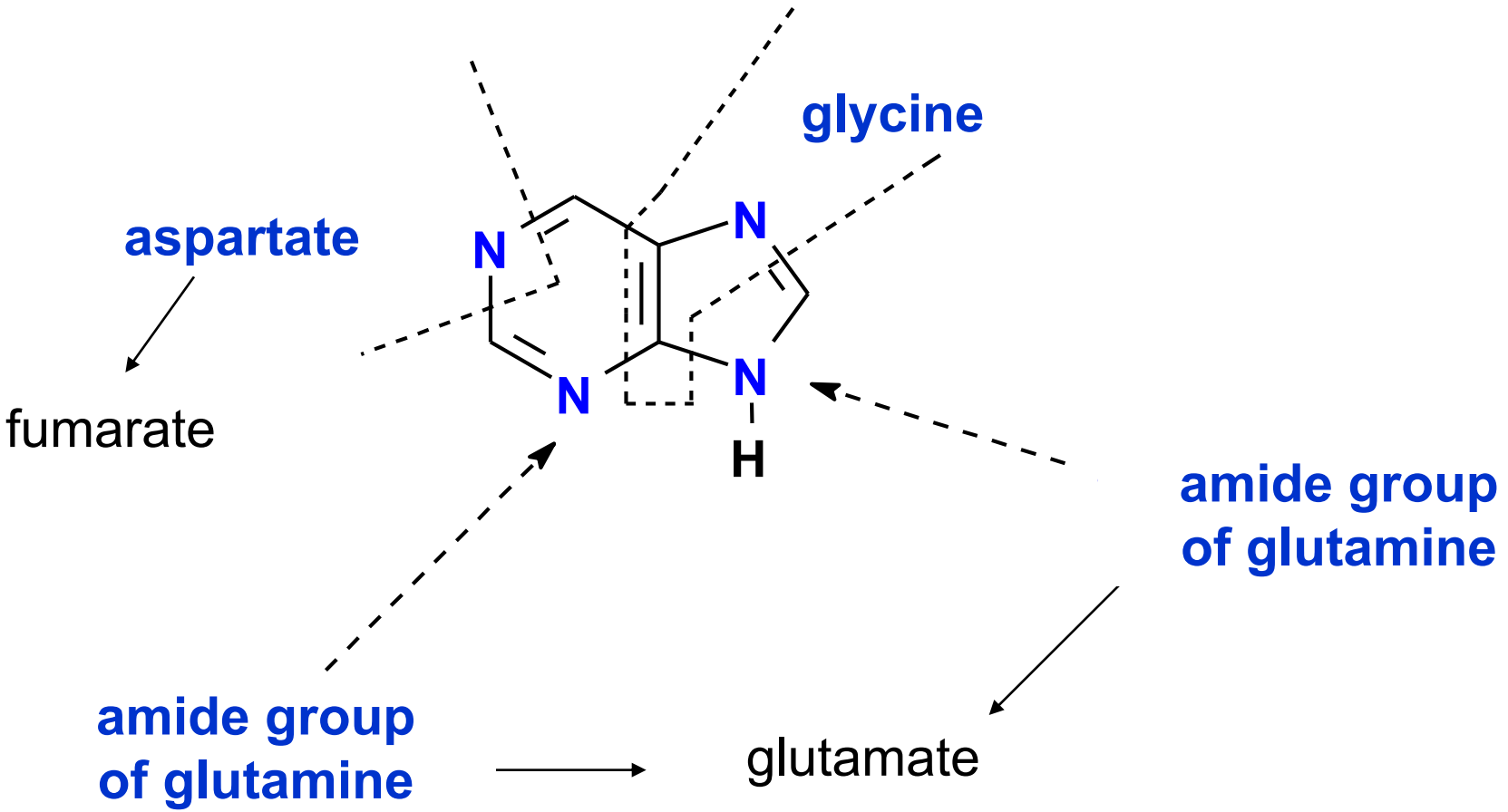
- produced in the transaminations of most AA
- glutamate dehydrogenase reaction produces most ammonia in body
- **transaminations are reversible**, so glutamate can be converted to 2-oxoglutarate (glucogenic)
- Glu + $NH_3$ ⇆ Gln (ammonia detoxification)
- glutamate is readily made from glutamine, histidine, proline, ornithine
- pure monosodium glutamate (MSG, E621), flavour enhancer, can cause health problems (chinese restaurant syndrome)

# Glutamine

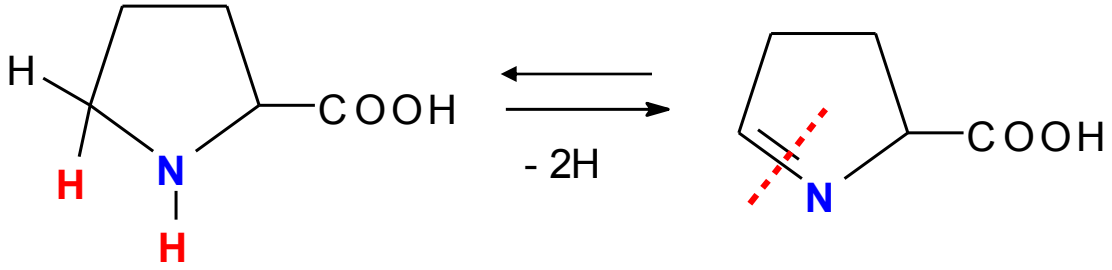
## See also the previous lecture (AA-1)

- glutamine synthesis is the way of ammonia detoxification in tissues including liver
- in kidneys, glutamine releases ammonia (deamidation)
- metabolic fuel for some tissues (enterocytes, fibroblasts, lymphocytes, macrophages)
- donor of nitrogen for syntheses (glucosamine, purines)

# Three amino acids donate four N atoms in purine bases synthesis



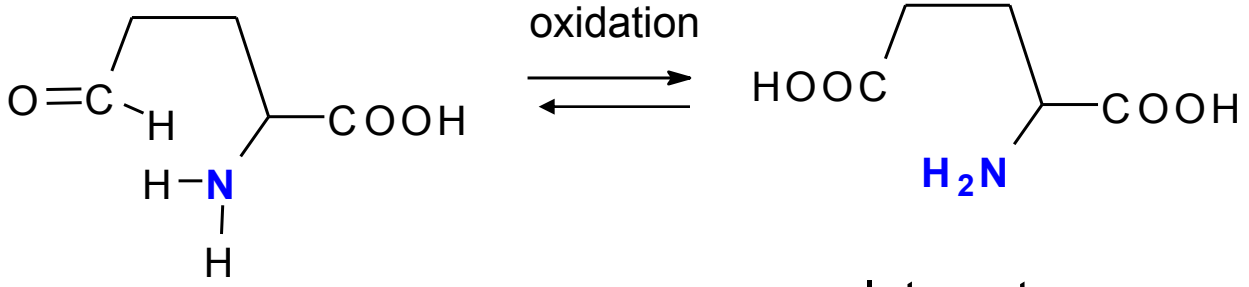
# Proline is converted to glutamate (and *vice versa*)




proline

pyrroline-5-carboxylate

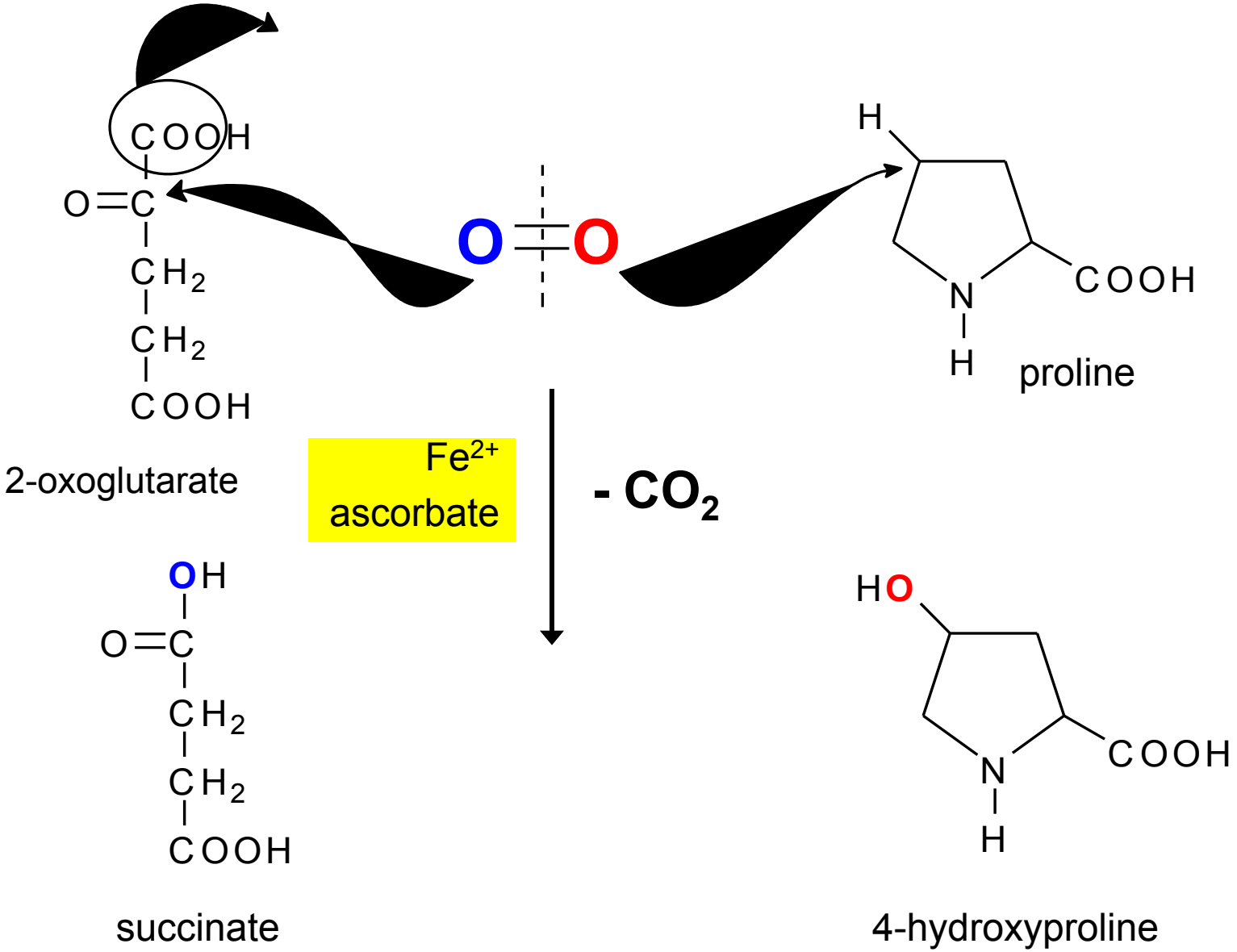
addition of $H_2O$
ring opening




glutamate 5-semialdehyde

glutamate

# Hydroxylation of proline with 2-oxoglutarate as reductant



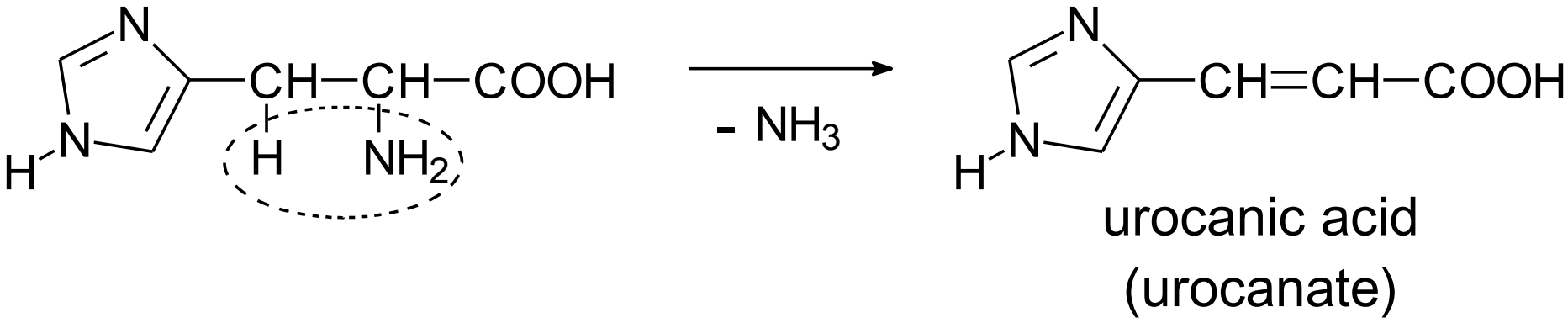
# Proline - summary

- non-essential AA, can be formed from glutamate
- converted to glutamate (glucogenic)
- hydroxylation of proline in collagen is post-translation modification, requires ascorbate (vitamin C), $Fe^{2+}$, and 2-oxoglutarate (unusual co-reductant)
- 4-hydroxyproline is catabolized to pyruvate (see Harper)

Histidine

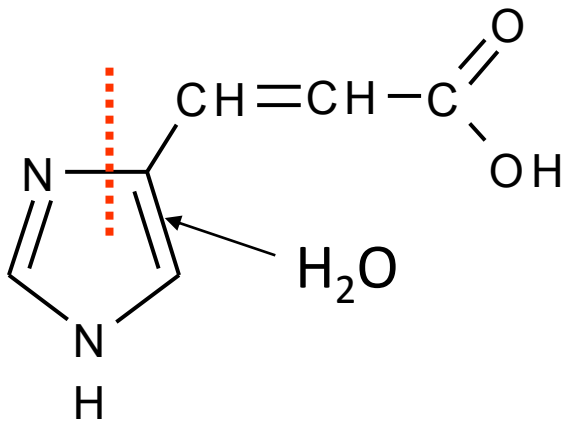
no transamination

## Catabolism of histidine starts with desaturation and deamination




urocanic acid
(urocanate)

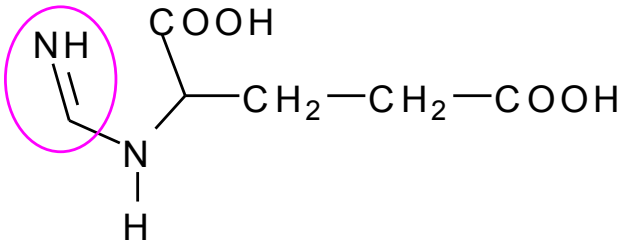
# Urocanate cleavage affords $C_1$ fragment



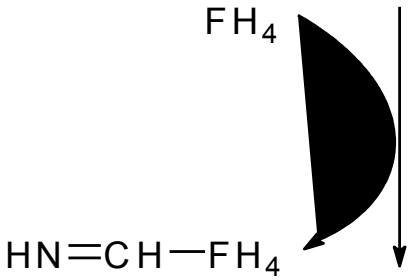

urocanate

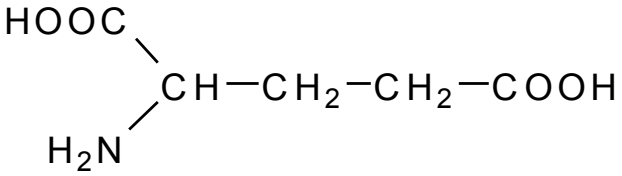
addition of water

oxidative ring splitting



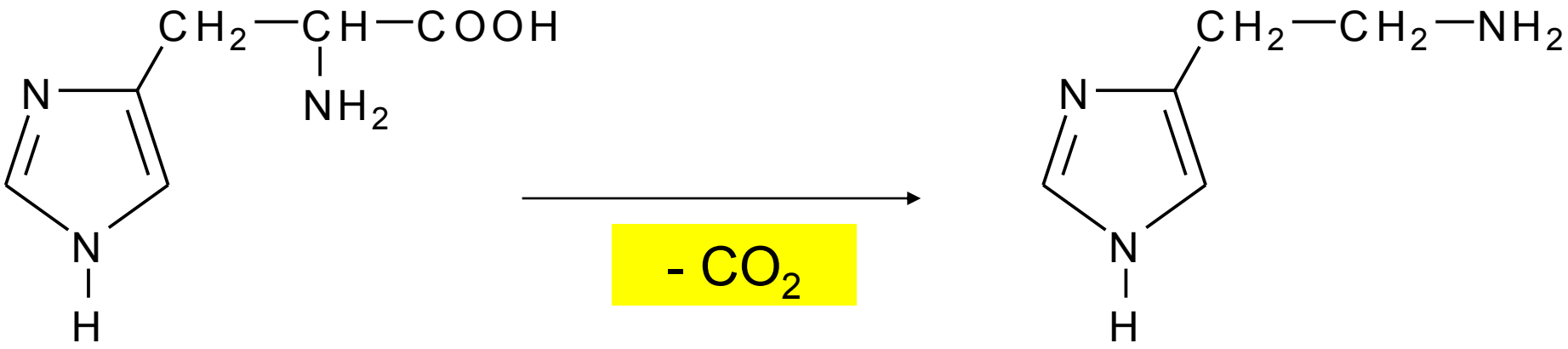

*N*-formiminoglutamate (Figlu)




glutamate

# Decarboxylation of histidine → histamine

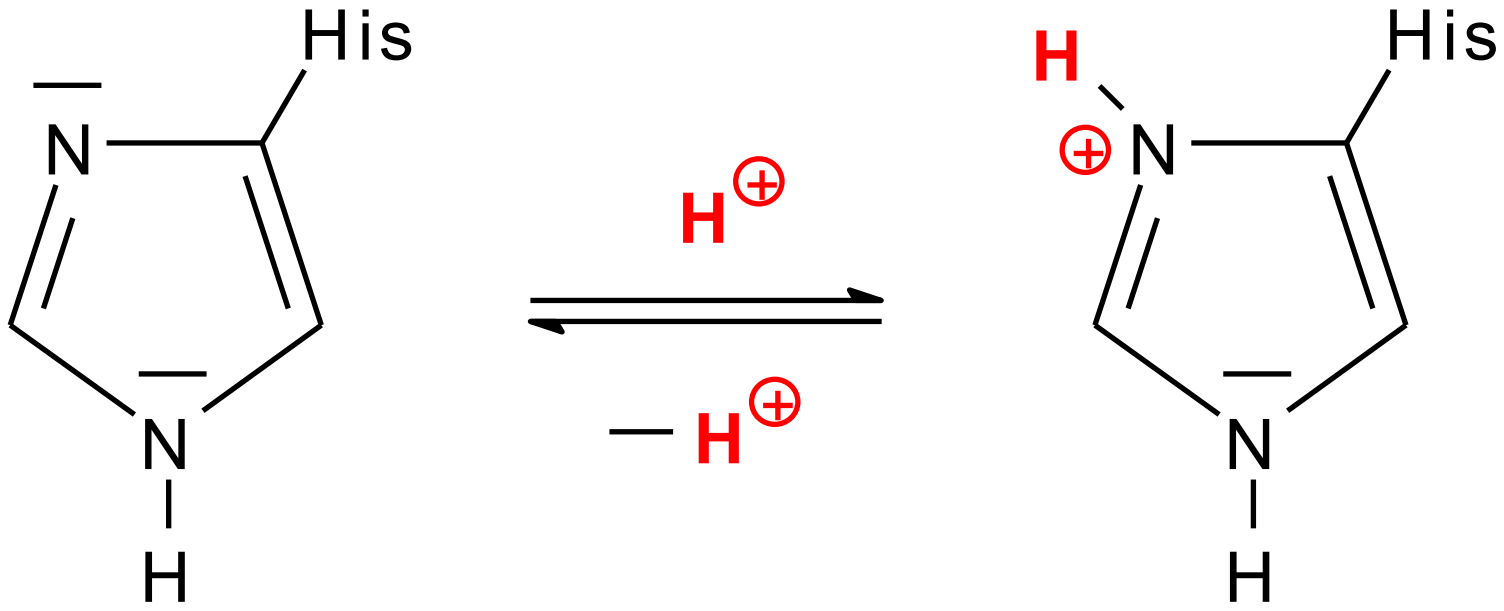



histidine

histamine

- histidine decarboxylase occurs in mast cells and basophils
- histamine stimulates HCl production in stomach
- is released in allergic reactions
- triggers inflammatory response
- antihistaminics are drugs blocking the action of histamine

# Histidine is responsible for buffering actions of proteins




$pK_B = 8$

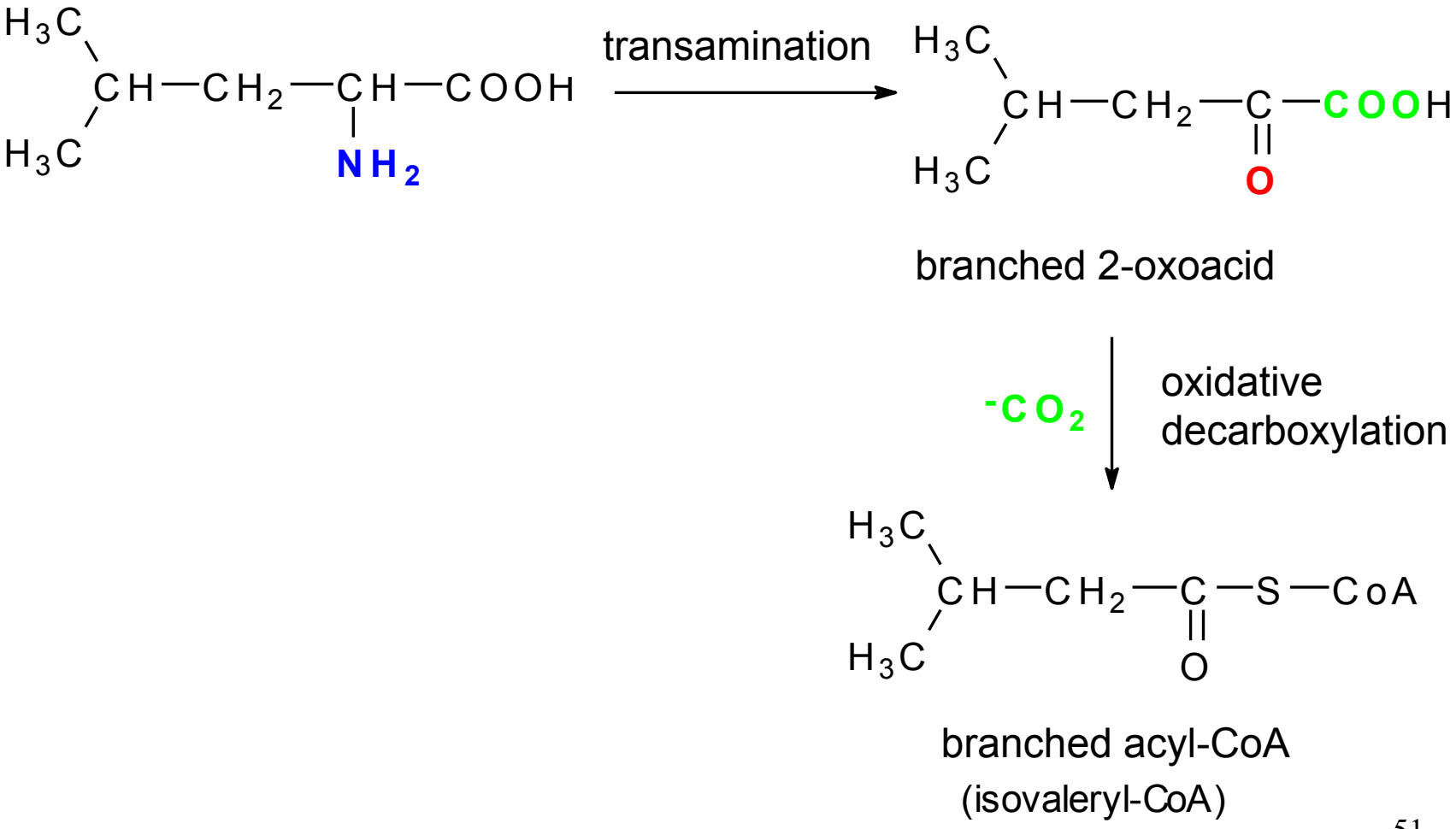
$pK_A$ (His) = 6

$pK_A$ (His in proteins) = 6-8

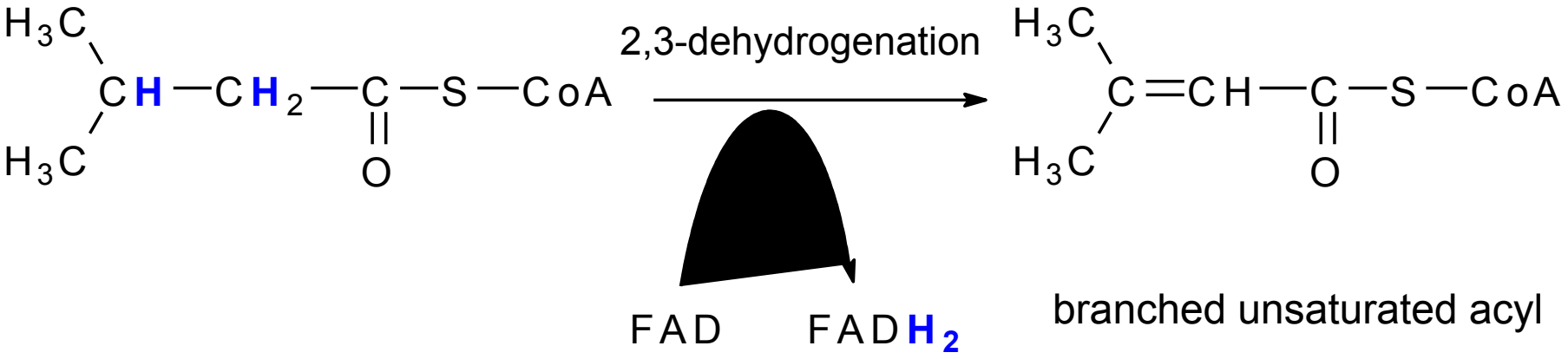
# Histidine - summary

- semiessential AA
- no transamination, catabolism begins with desaturation and deamination
- the source of 1C groups (formimino)
- converted to glutamate (glucogenic)
- histidine is abundant in hemoglobin – buffer system
- post-translation modification: methylation of His in actine/myosine → 3-methylhistidine – its urine excretion is the indicator of muscle proteolysis and nutrition status

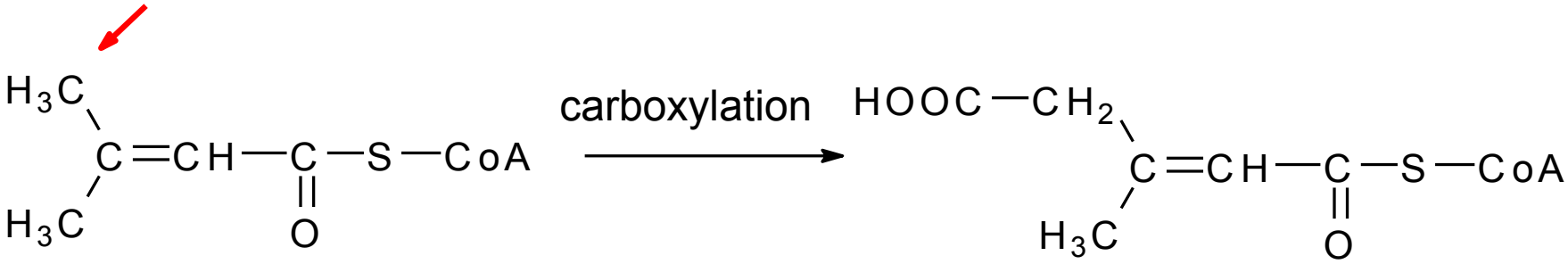
# Leucine (1) - transamination + decarboxylation



# Leucine (2) - dehydrogenation



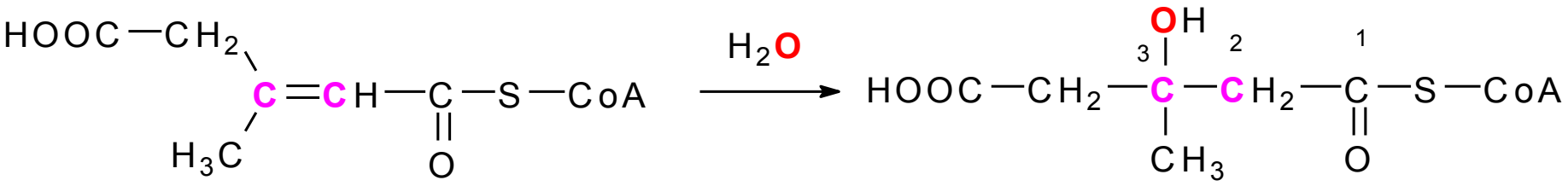
# Leucine (3) – carboxylation at C4




acyl of dicarboxylic

branched unsaturated acid

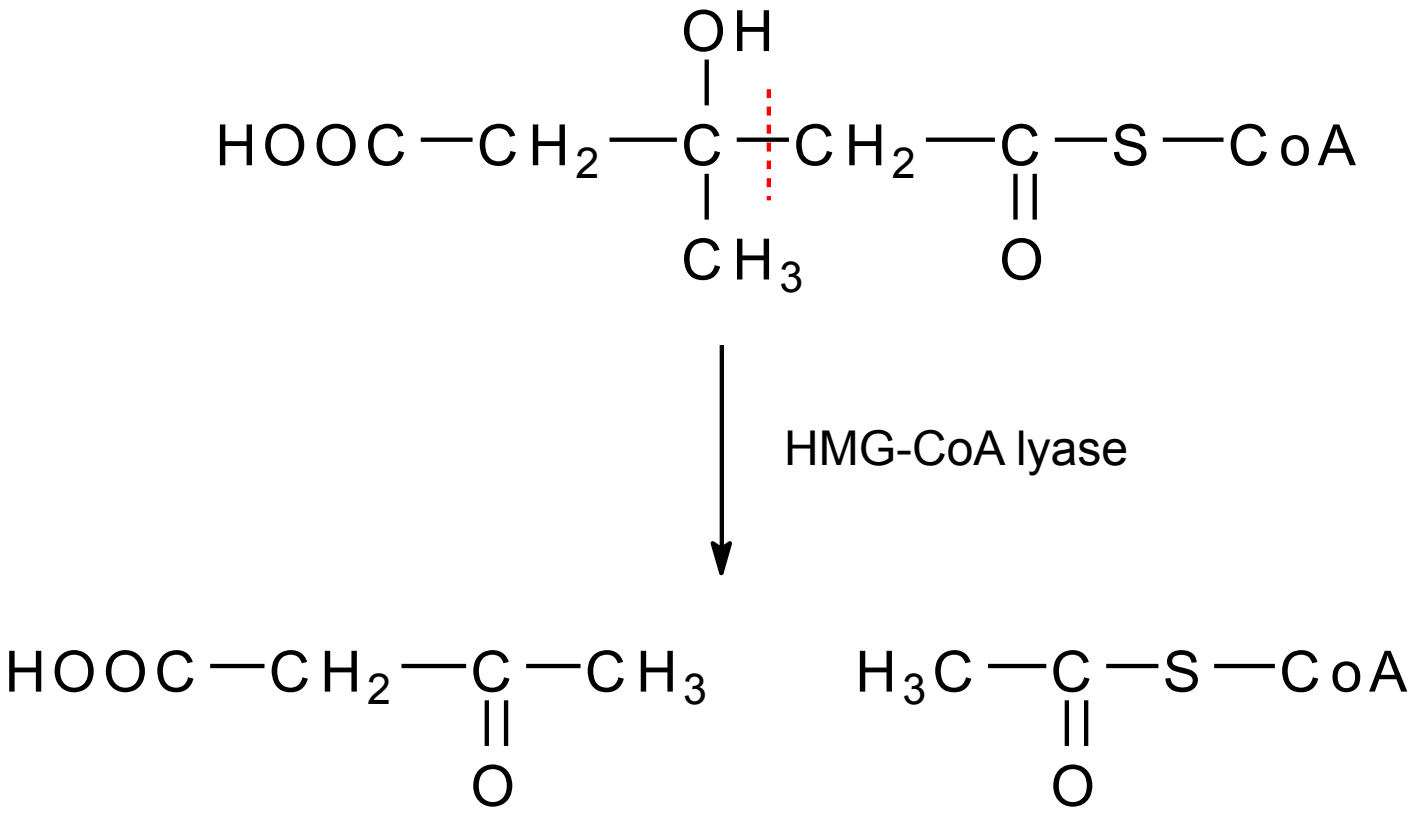
# Leucine (4) – hydratation of double bond




3-hydroxy-3-methylglutaryl-CoA

**(HMG-CoA)**

# Leucine (5) – splitting the C-C bond in HMG-CoA




**acetoacetate** **acetyl-CoA**

# Compare the final products of BCAA

| | | |
|---|---|---|
| **Leucine** | acetyl-CoA + acetoacetate | ketogenic |
| $B_{12}$ **Isoleucine** | acetyl-CoA + succinyl-CoA | ketogenic<br>glucogenic |
| $B_{12}$ **Valine** | succinyl-CoA | glucogenic |

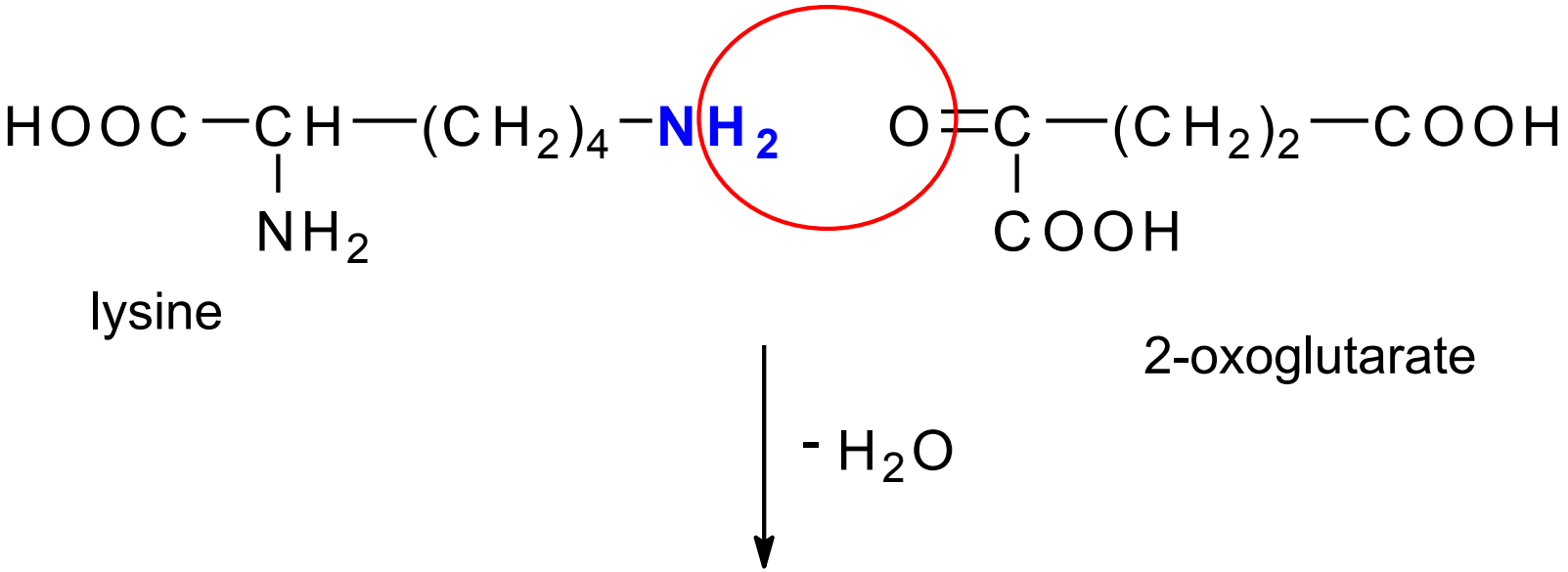
# BCAA - summery

- all BCAA are essential
- the first three reactions are the same (transamination, oxid. decarboxylation, dehydrogenation), final products are different
- leucine – ketogenic, valine – glucogenic, isoleucine – mixed
- after meal, BCAA make about 70 % of AA in blood, because the liver does not utilize them (lack of aminotransferases)
- **BCAA are most utilized in muscles and brain**
- BCAA infusion are applied in severe catabolic conditions

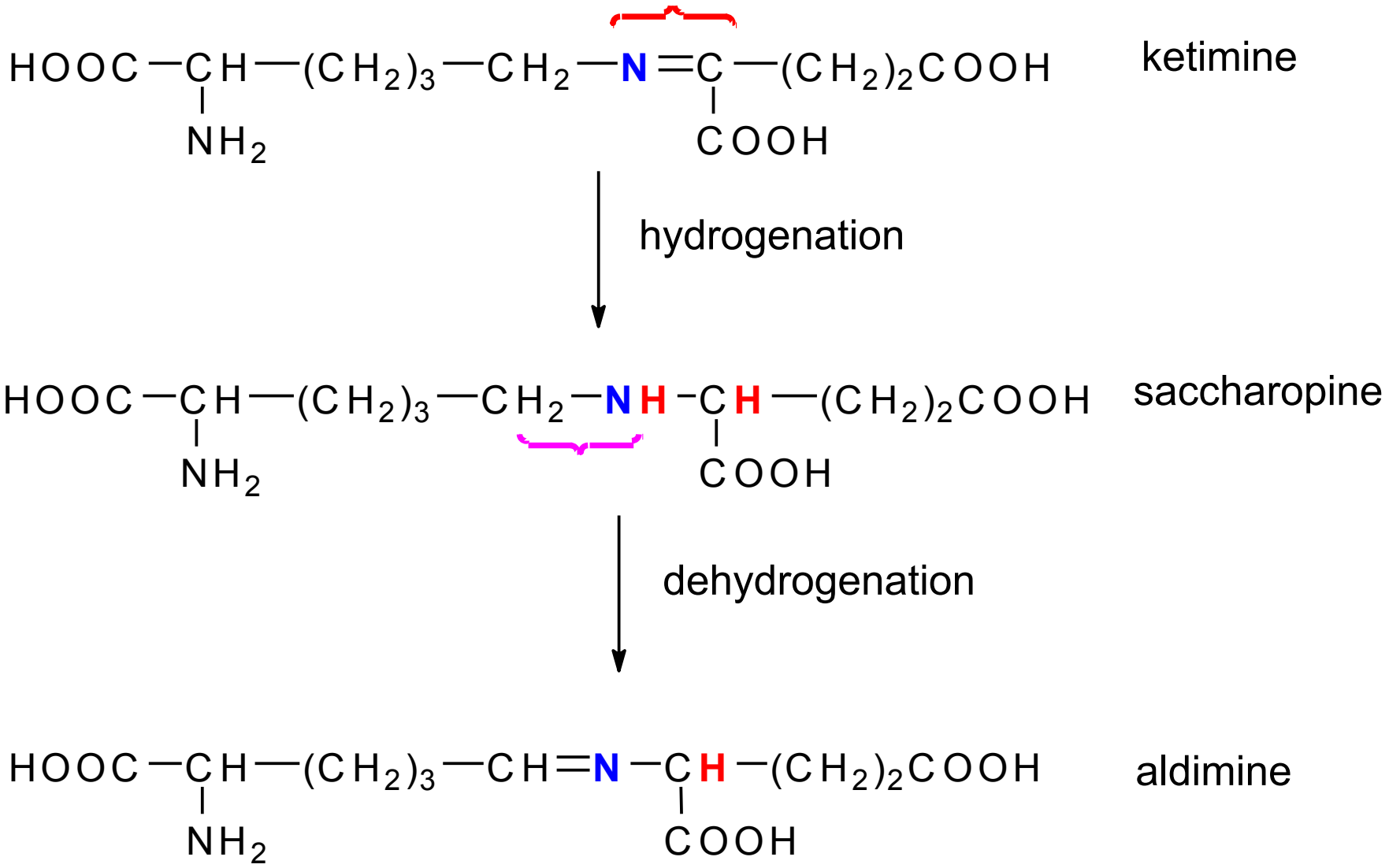
Lysine

no transamination

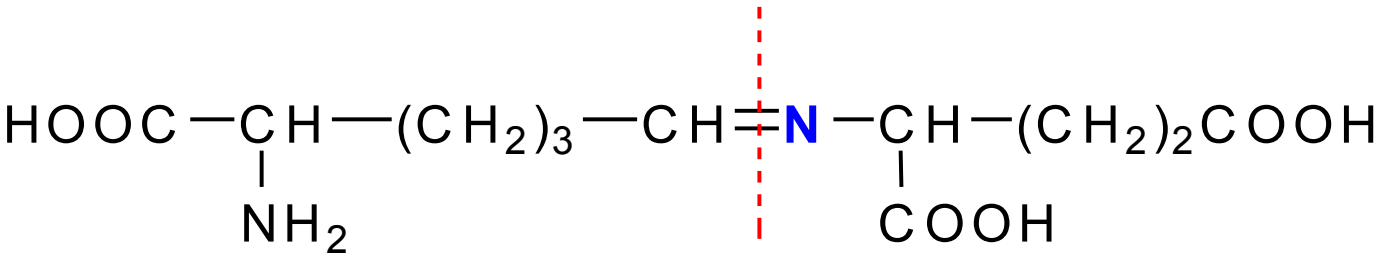
# Lysine catabolism (1)



# Lysine catabolism (2)

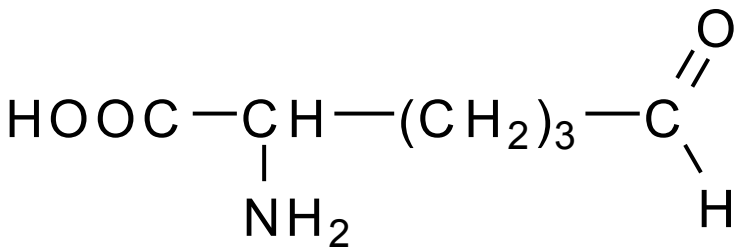


# Lysine catabolism (3)

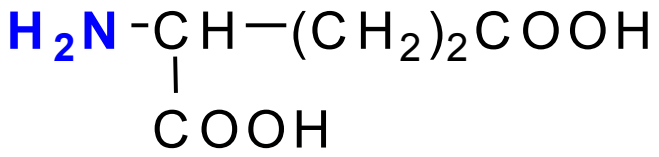



aldimine

hydrolysis

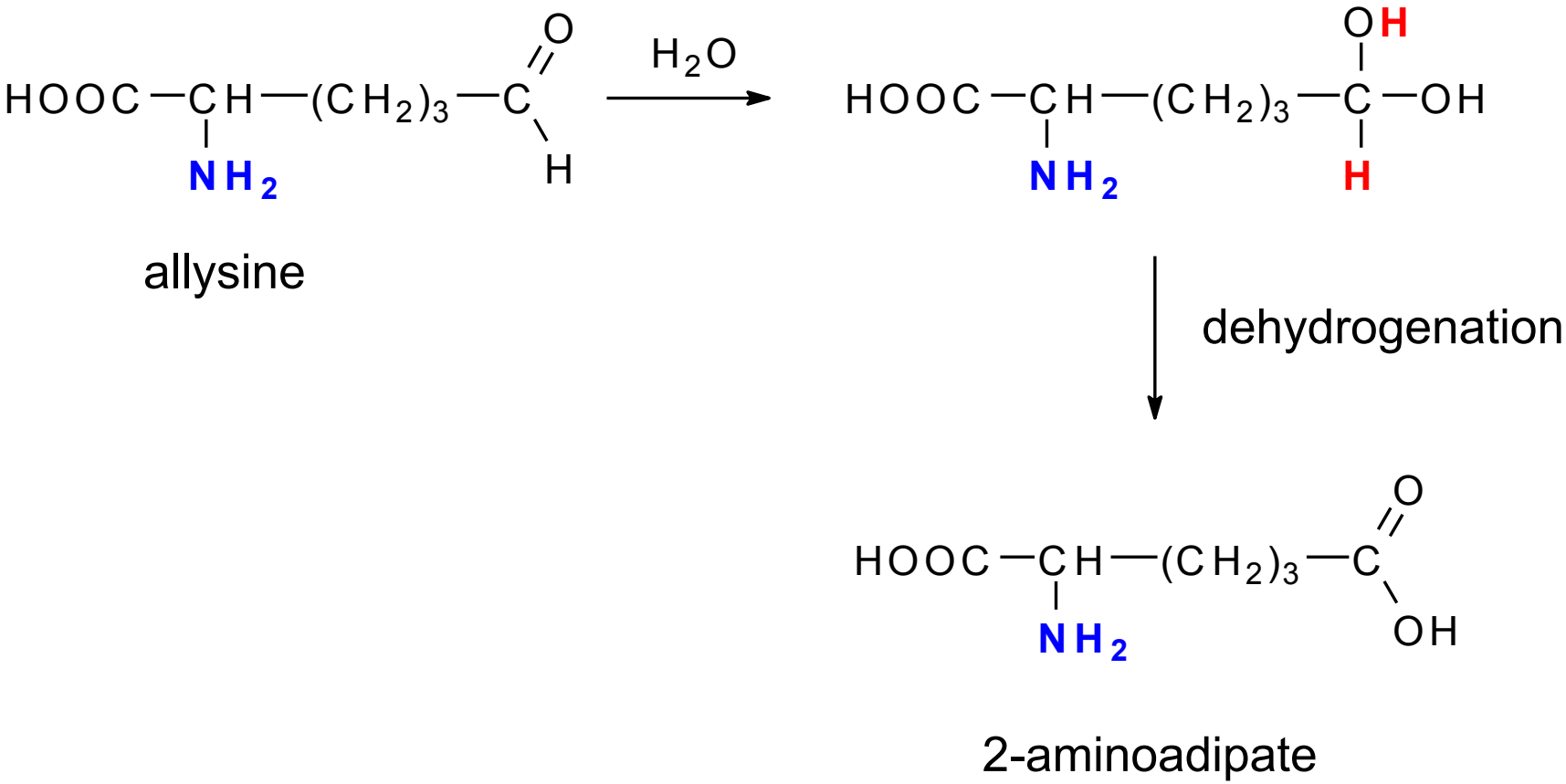



allysine




glutamate

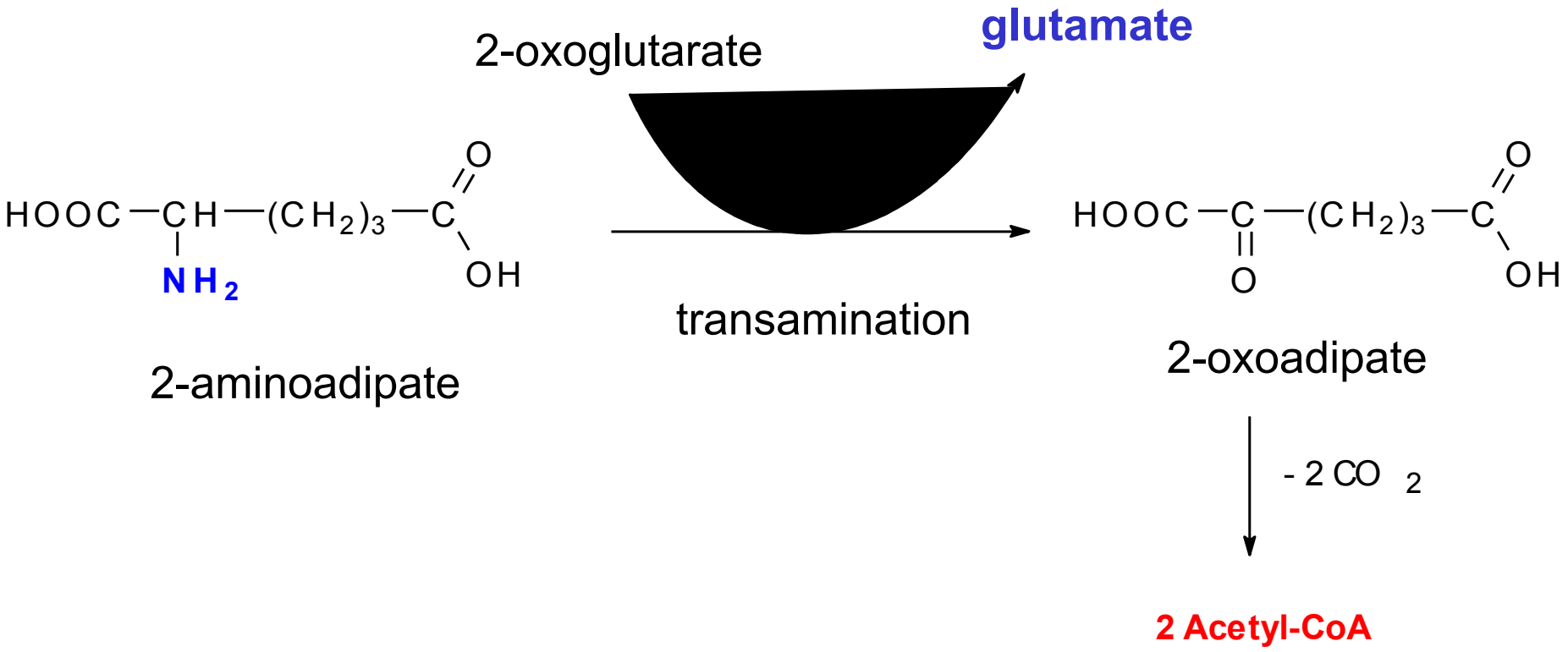
# Lysine catabolism (4)



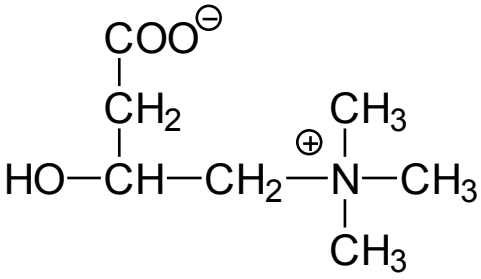

allysine

2-aminoadipate

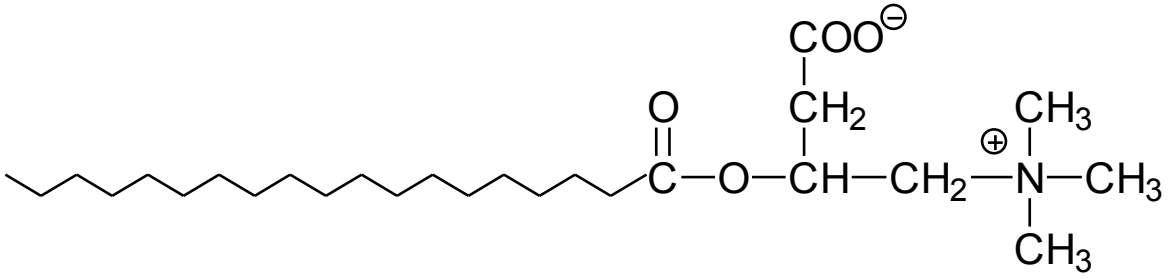
# Lysine catabolism (5)



# Lysine is the substrate for carnitine (the transfer of FA from cytosol to mitochondria)



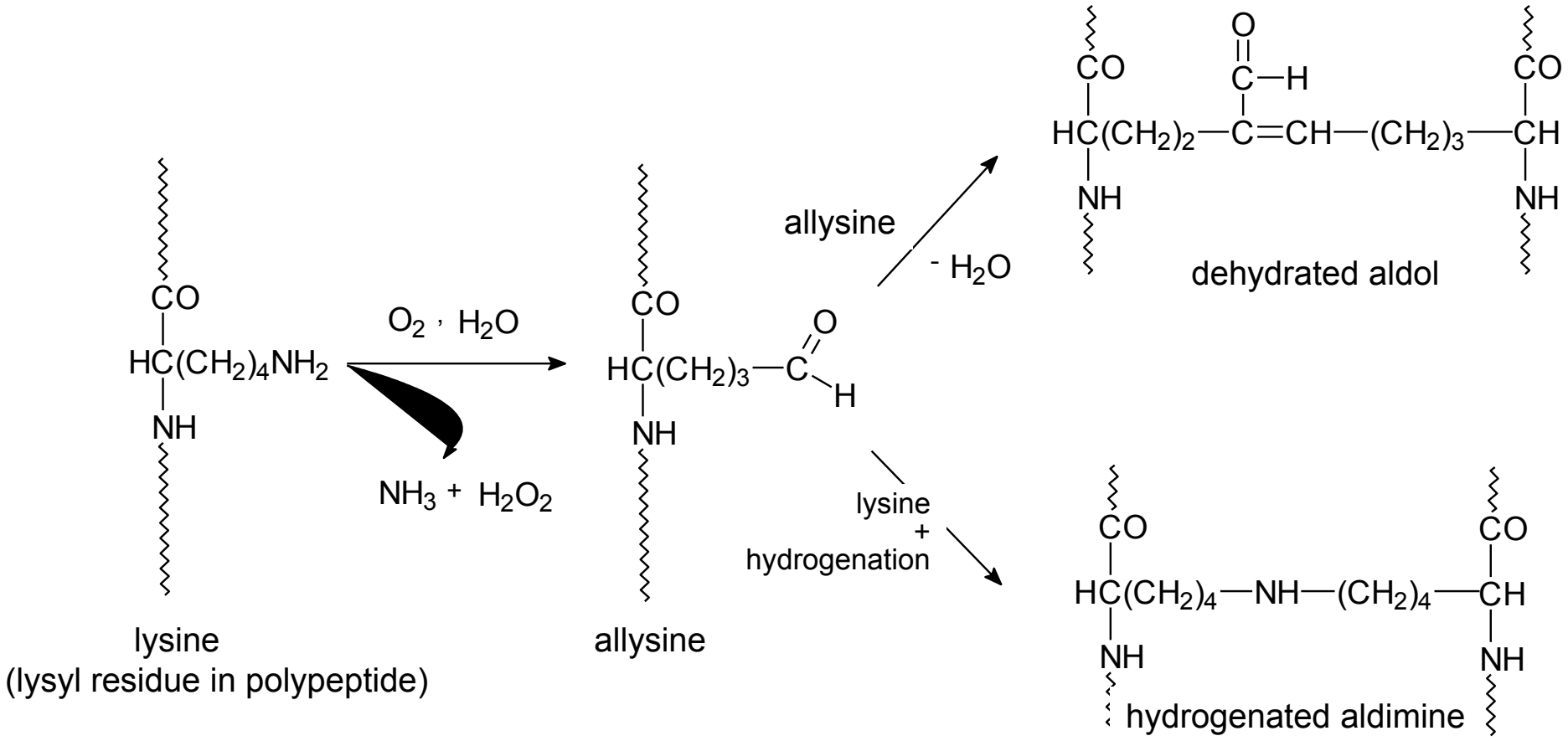

carnitine



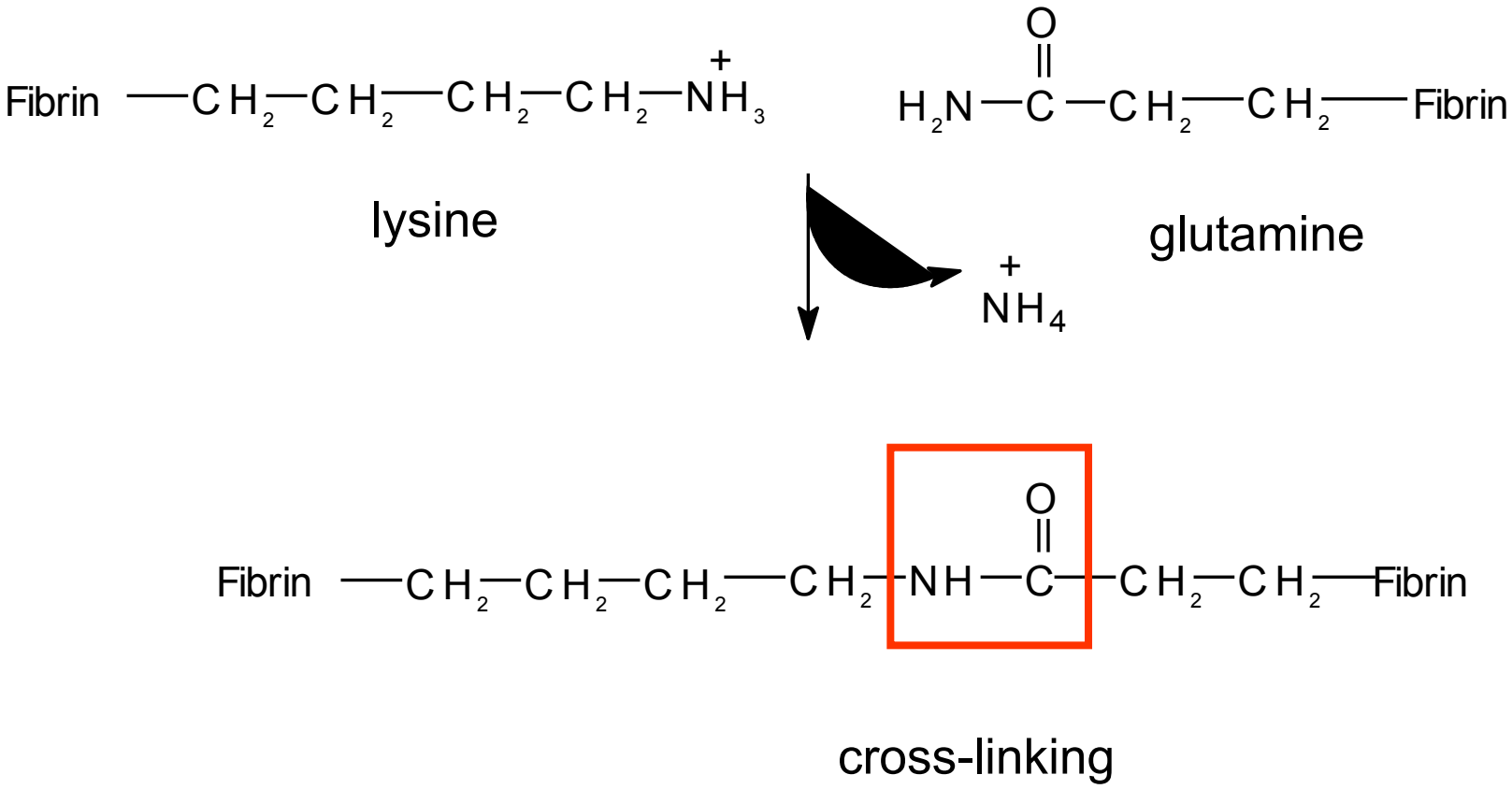

acylcarnitine

# Cross-links in collagen

products of reaction between the amino groups in side chains of **lysine** with the modified lysine side chains comprising the aldehyde group (the result of oxidation of lysine to **allysine**) – aldol type or aldimine type of cross-links.



# Formation of fibrin clot during blood coagulation (cross-linking of fibrin)



# Lysine - summary

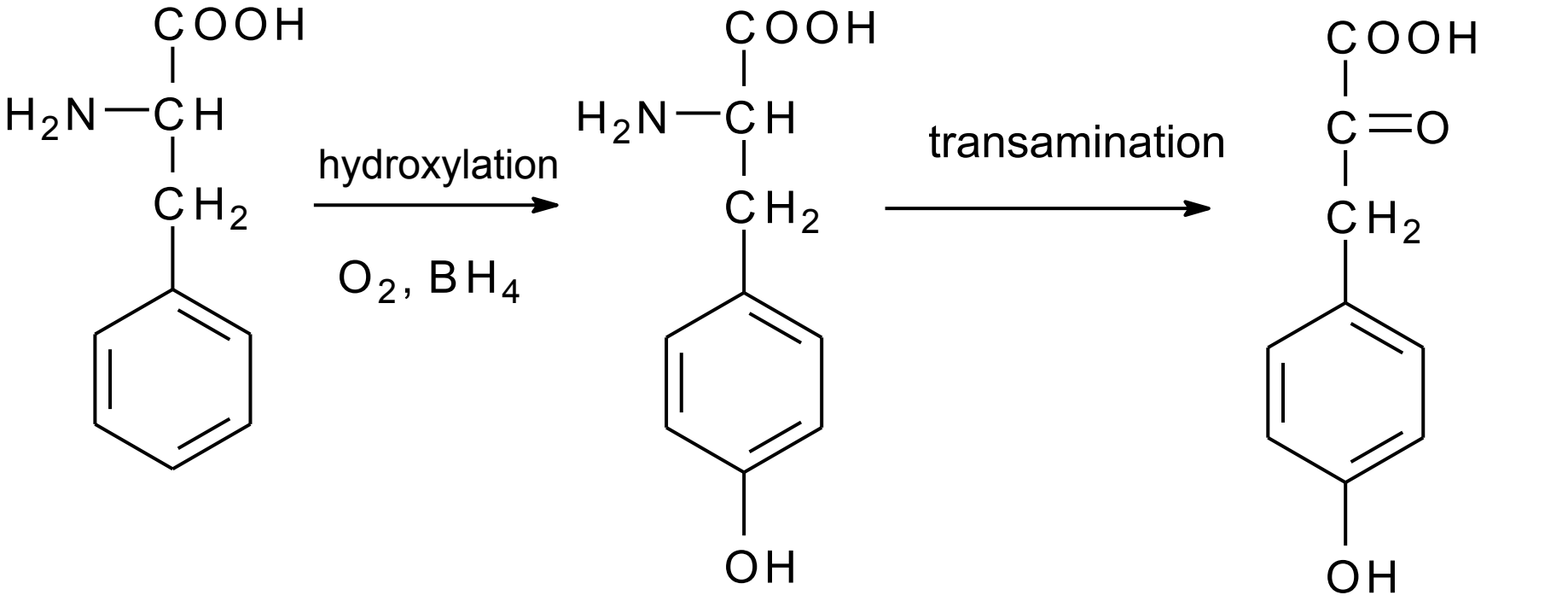
- essential AA, no transamination
- ε-amino group is removed as glutamate
- α-amino group is removed from aminoadipate by transamination
- final product acetyl-CoA (ketogenic)

**Other conversions:**

- lysine in many proteins binds ubiquitin (targeting for proteasome)
- carnitine (transport system for FA to mitochondria)
- decarboxylation → cadaverine
- in collagen: cross bridges, hydroxylation → hydroxylysine
- in fibrin: cross linking during blood coagulation

# Phenylalanine, Tyrosine

## Catabolism (1)

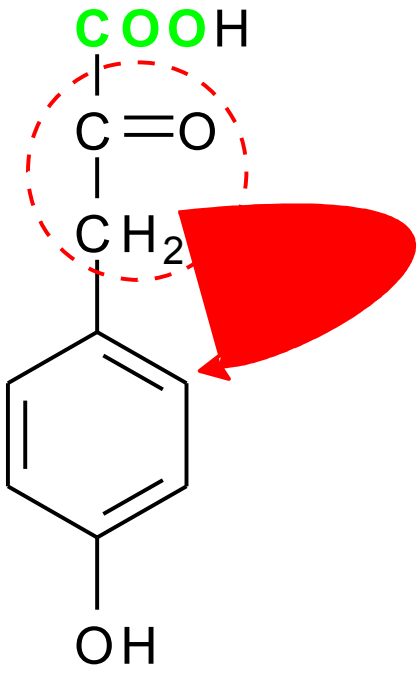



phenylalanine

tyrosine

p-hydroxyphenylpyruvate

# Catabolism (2)



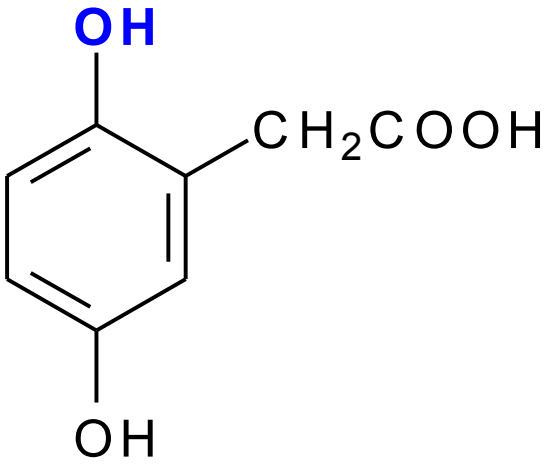

p-hydroxyphenylpyruvate

oxidative decarboxylation

rearrangement

hydroxylation

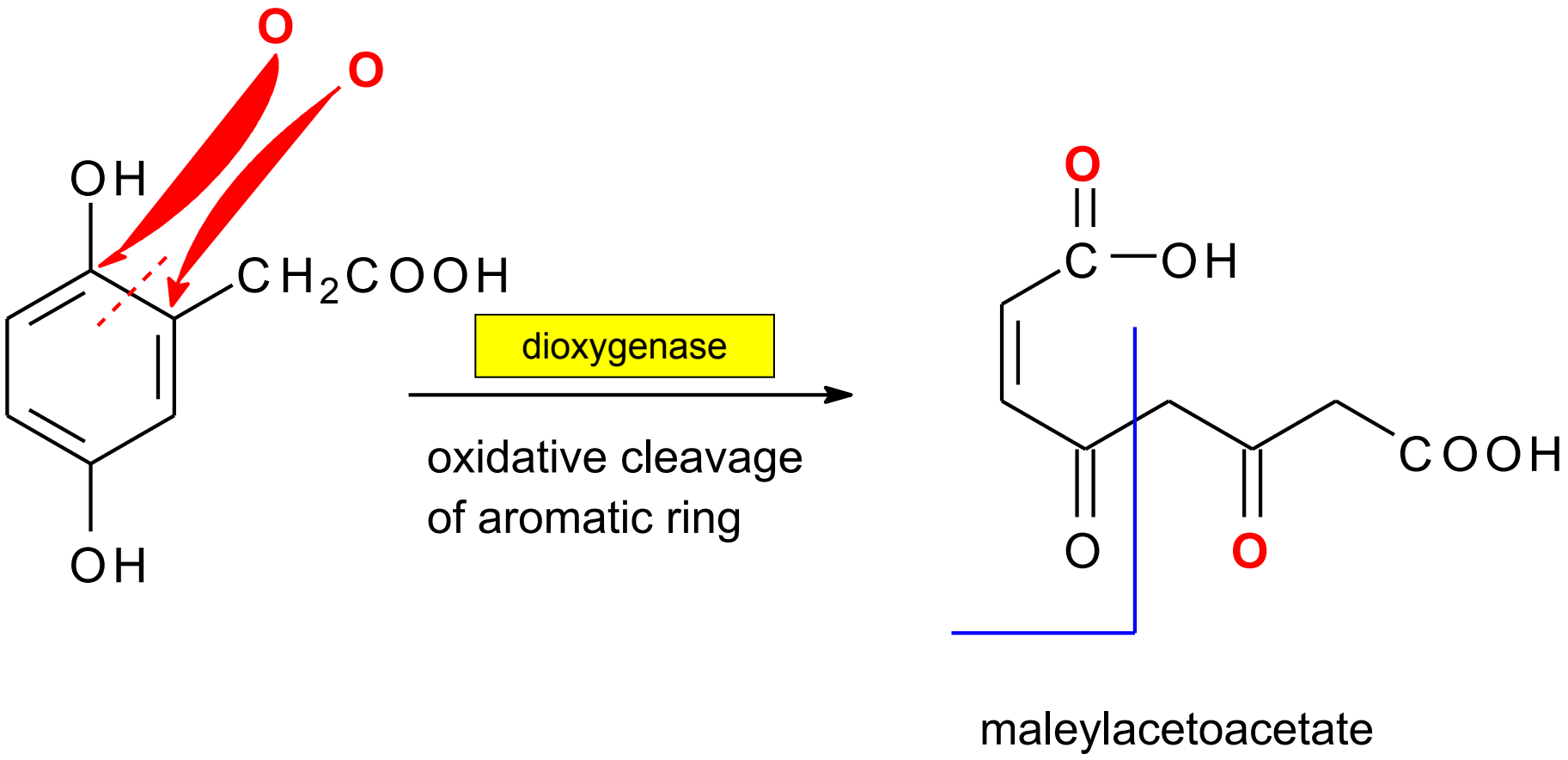
$-CO_2$



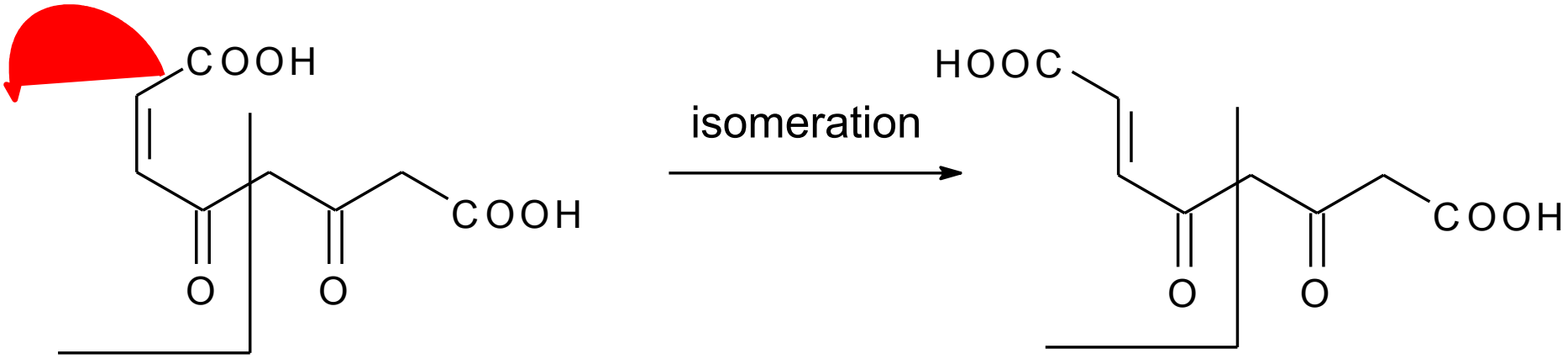

homogentisate

(2,5-dihydroxyphenylacetate)

# Catabolism (3)



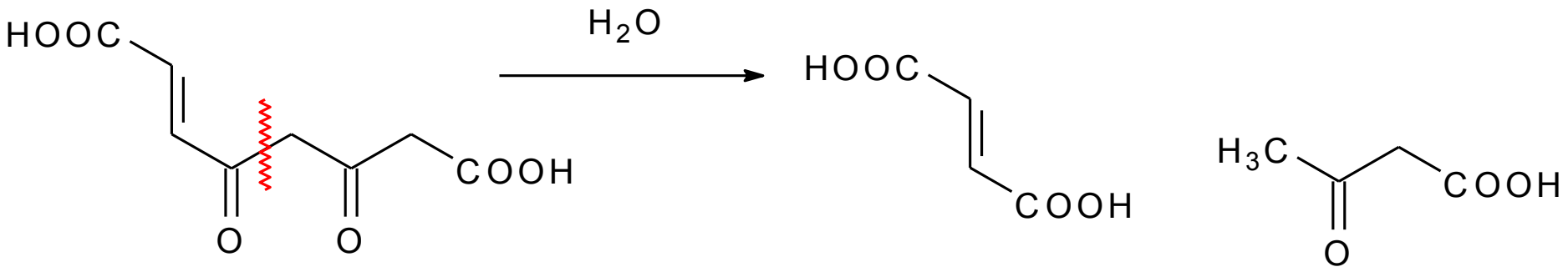
# Catabolism (4)




maleylacetoacetate

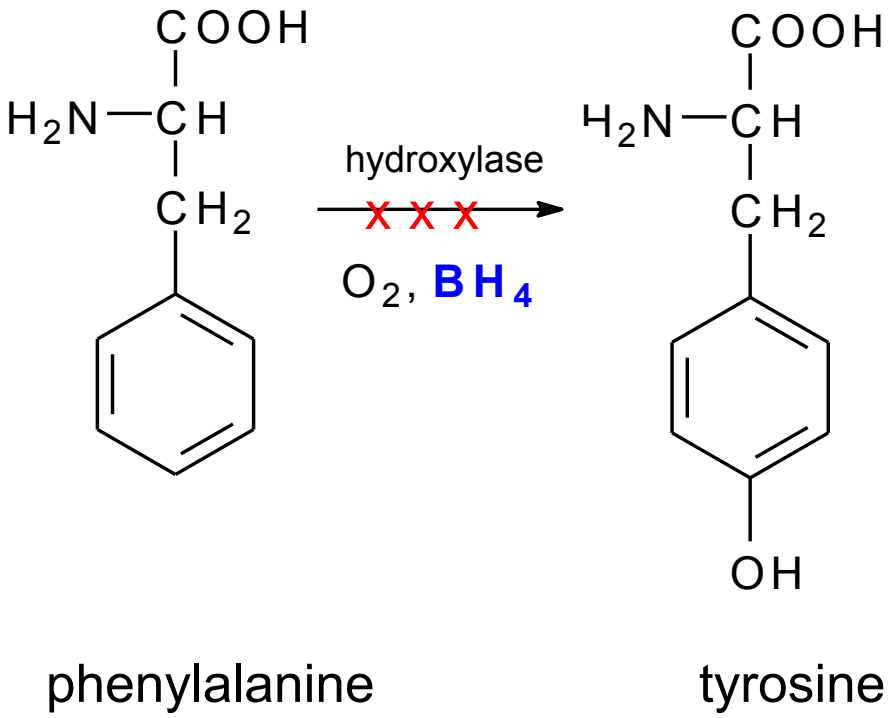
fumarylacetoacetate

# Catabolism (5)




fumarylacetoacetate fumarate acetoacetate

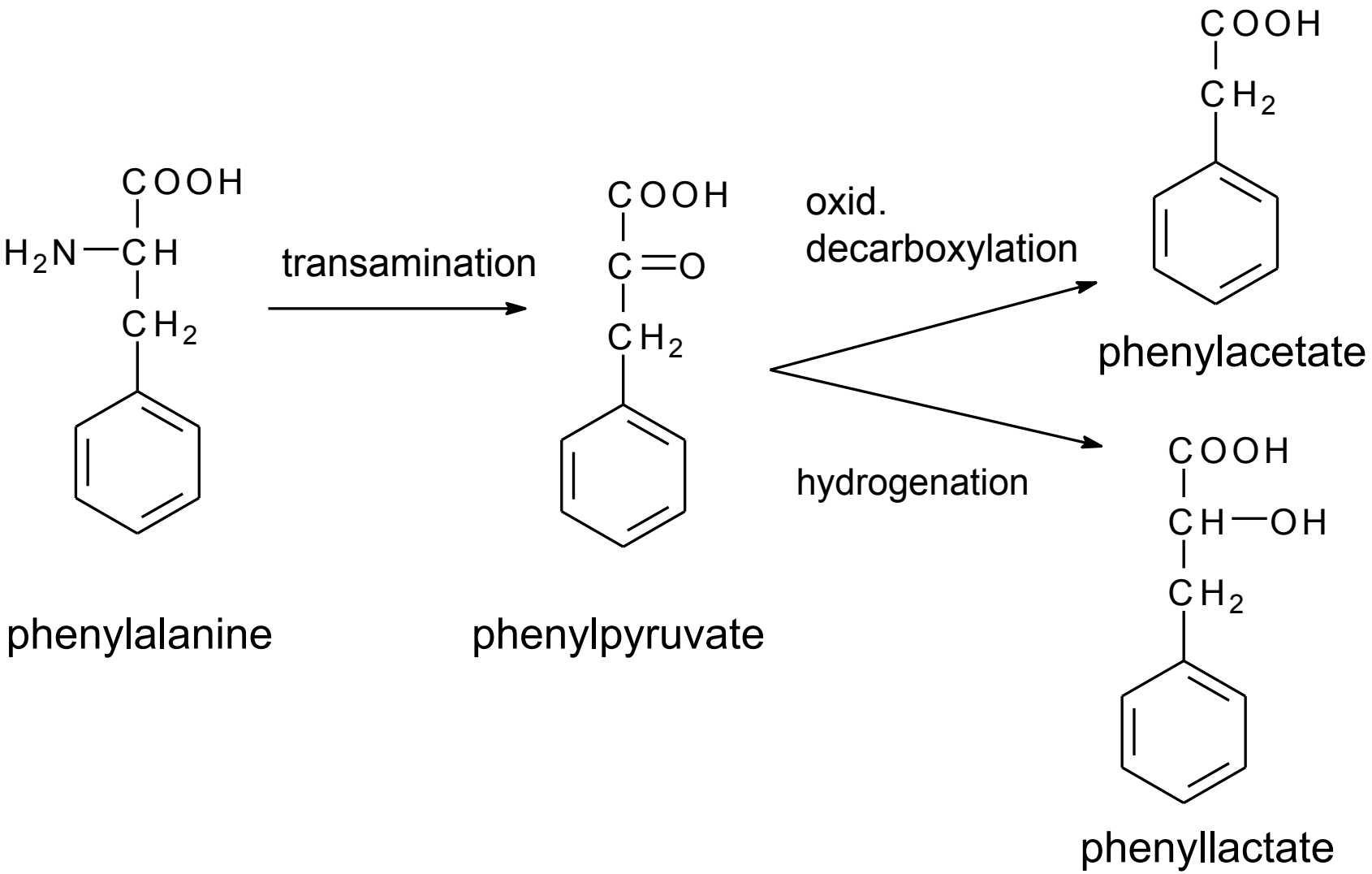
# Hyperphenylalaninemia and Phenylketonuria




phenylalanine tyrosine

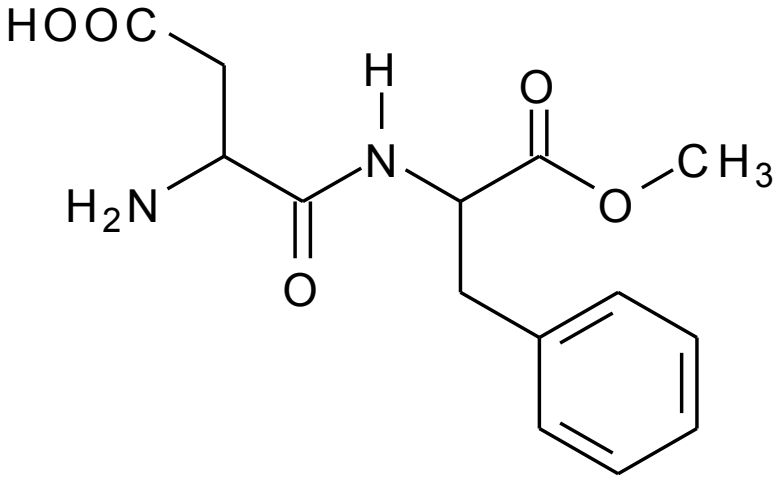
- deficit of hydroxylase or $BH_4$
- elevated blood Phe and its metabolites
- excretion of phenylpyruvate by urine

# Metabolites of phenylalanine

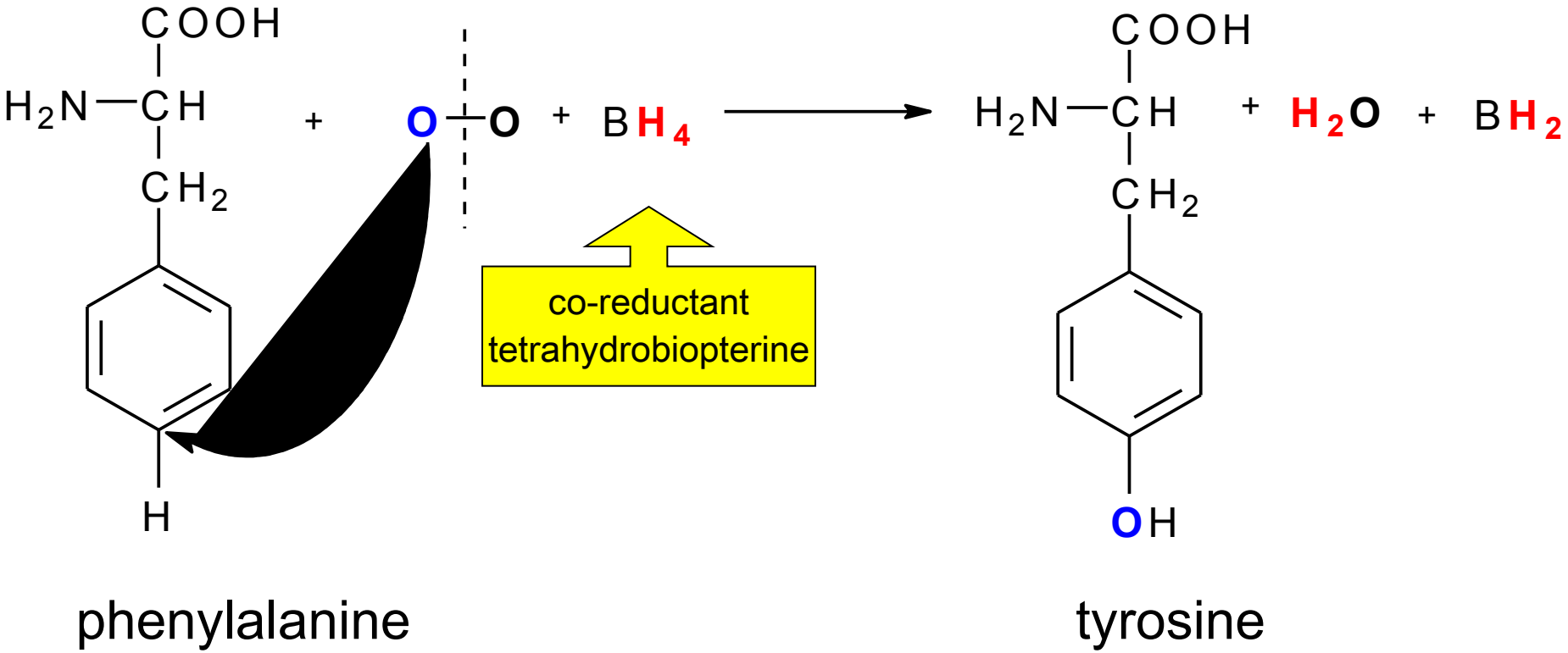


# Hyperphenylalaninemia and Phenylketonuria

- if not treated properly – mental retardation and other problems
- treatment – low phenylalanine diet
- products containing sweetener **aspartam** must be avoided
- L-aspartyl-L-phenylalanine methyl ester - phenylalanine is released by hydrolysis:



# Hydroxylation of phenylalanine gives tyrosine

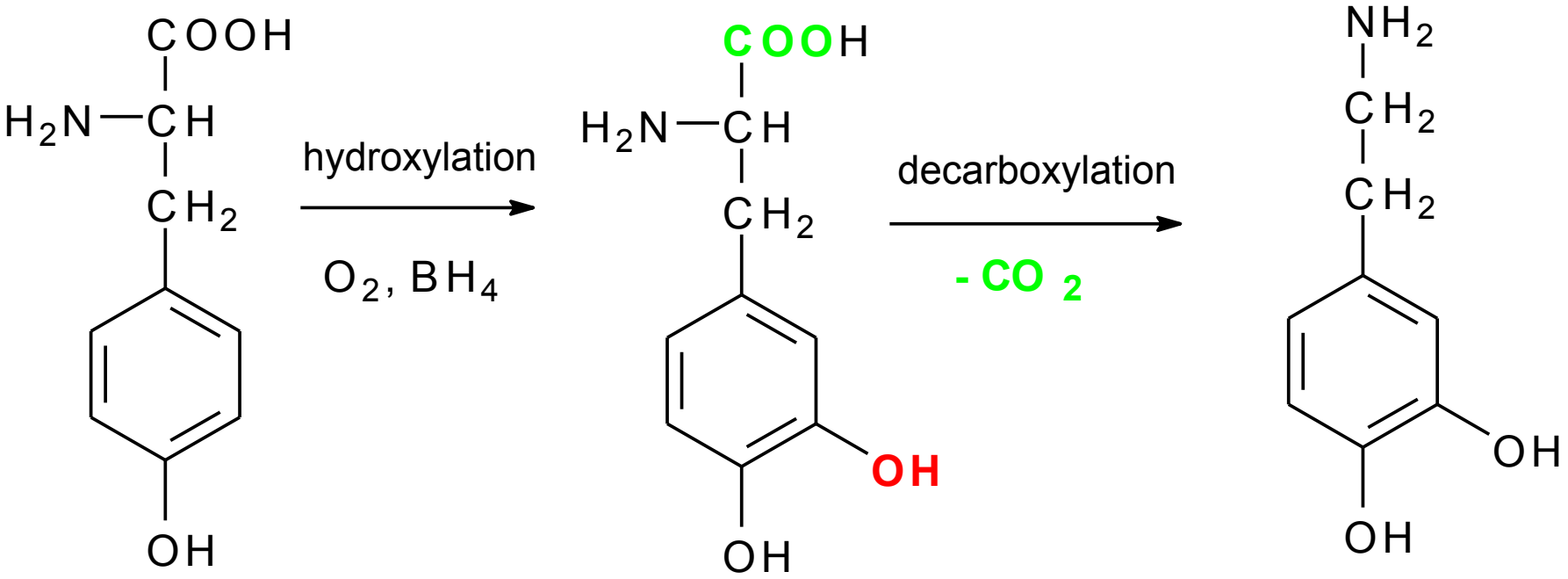



phenylalanine

tyrosine

Tyrosine

# DOPA and dopamine from tyrosine




tyrosine

**DOPA**
(3,4-dihydroxyphenylalanine)

dopamine
(catecholamine)

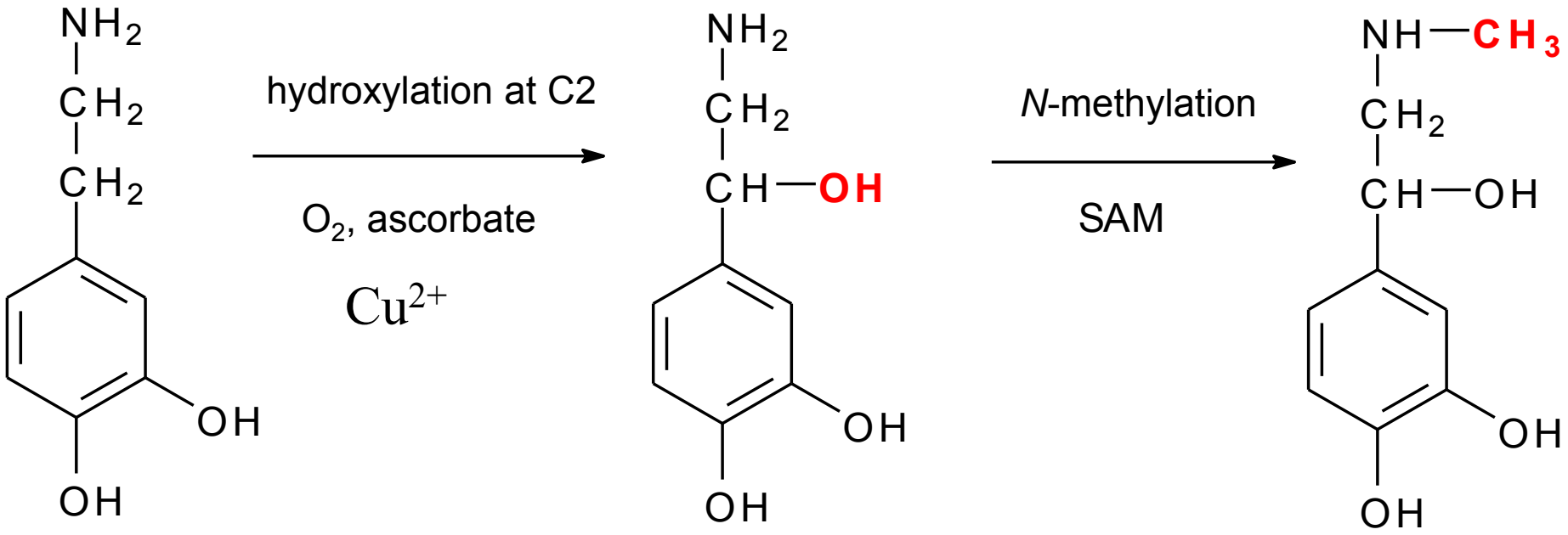
# Linguistic note

- abbreviation **DOPA** comes from older English nomenclature
- oxo group and hydroxyl group were not distinguished properly:

  DOPA = **d**i**o**xo**p**heny**l**alanine

- correct chemical name is: 3-(3,4-dihydroxyphenyl)alanine

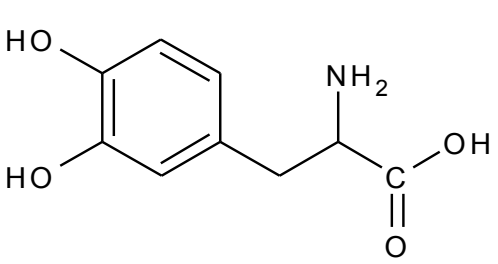
# Two more catecholamines from dopamine




dopamine noradrenaline adrenaline

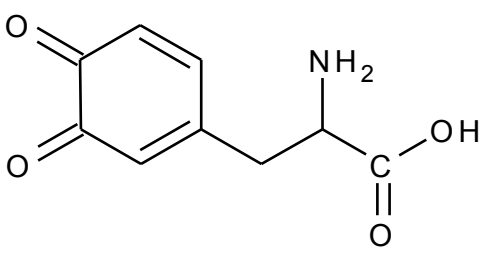
*nor-* = *N*-demethyl

# Conversion of tyrosine to melanin, a dark pigment of skin, hair, fur




DOPA

- 2H

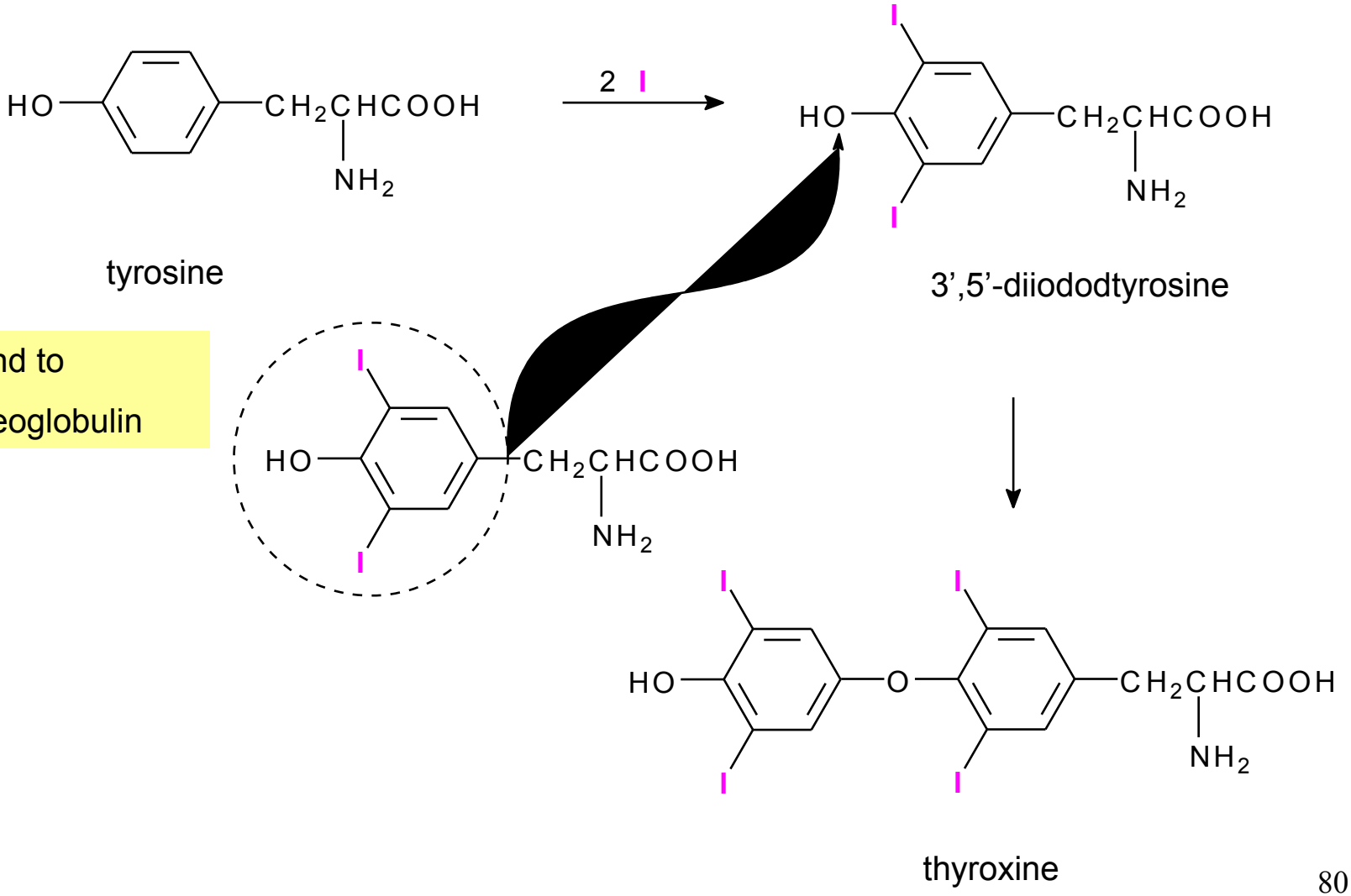



dopaquinone

condenzation

→ → → melanin

# Conversion of tyrosine to thyroxine



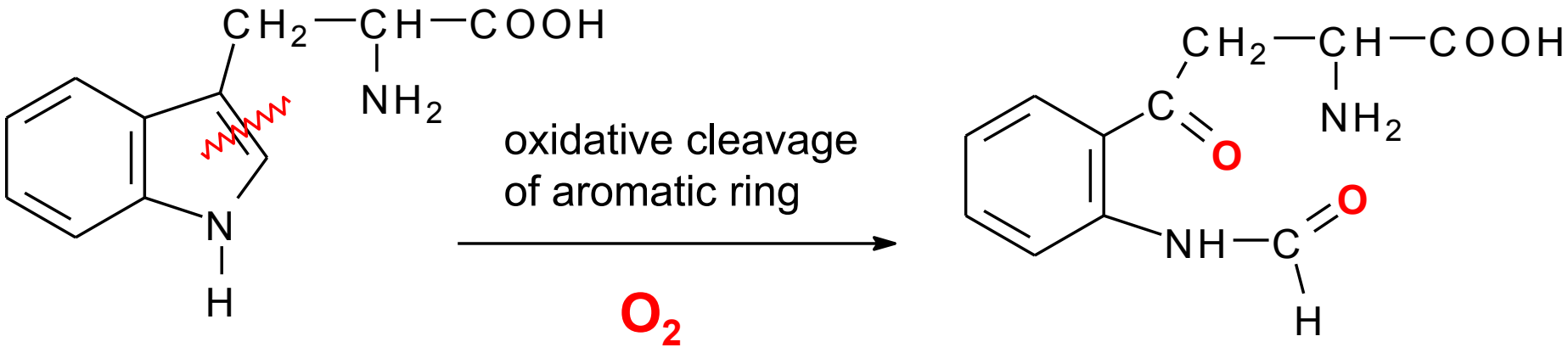
# Phenylalanine, tyrosine - summary

- Phe is essential amino acid, Tyr not
- Tyr is made by Phe hydroxylation (tetrahydrobiopterine cofactor)
- catabolism is the same for both AA (mixed AA)
- provide fumarate for CAC (glucogenic)
- acetoacetate (ketone body)
- tyrosine is converted to hormones (catecholamines, thyronines) and dark skin pigment melanin

Tryptophan

no transamination

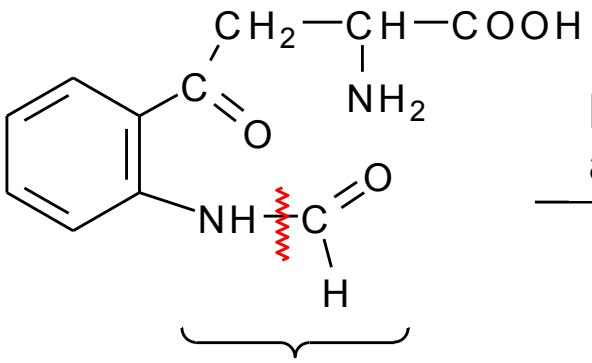
# Catabolism (1)




tryptophan

*N*-formylkynurenine

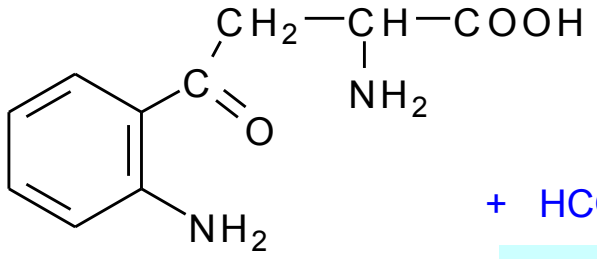
# Catabolism (2)




formamide

hydrolysis of amide group

$H_2O$

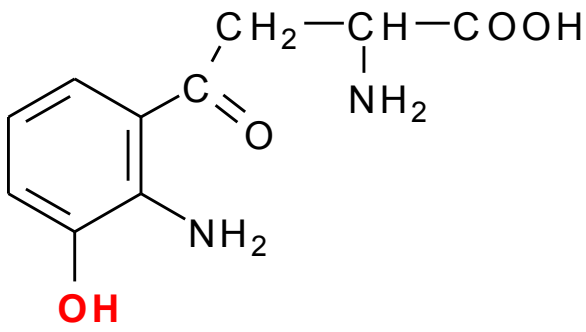



kynurenin

+ HCOOH

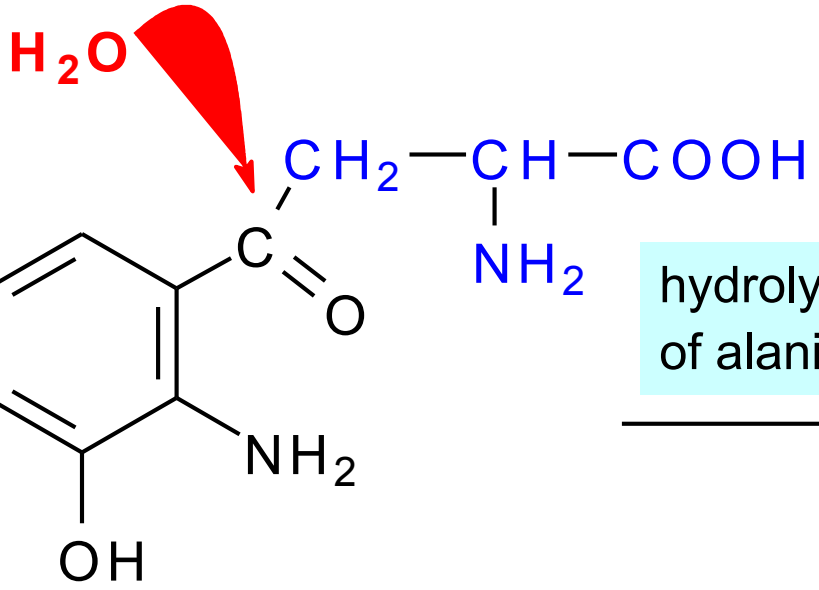
formate

hydroxylation



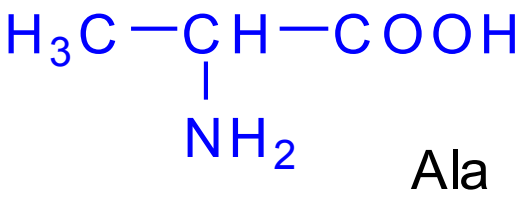

3-hydroxykynurenin

# Catabolism (3)

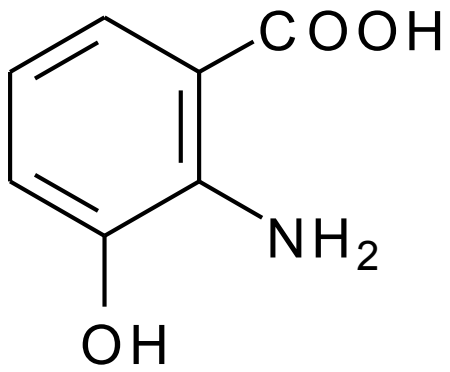



3-hydroxykynurenin

hydrolytic cleavage of alanine

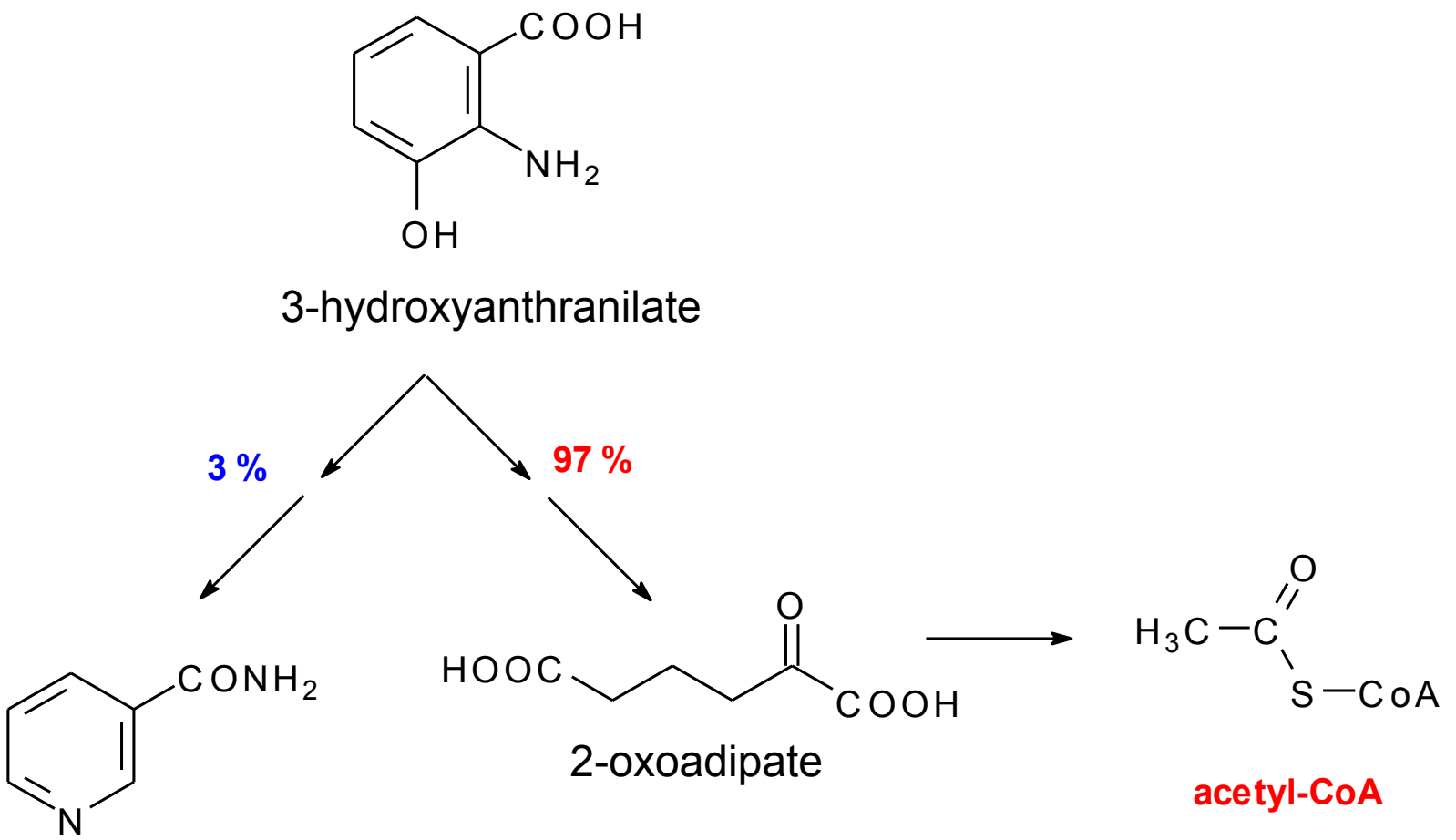



Ala



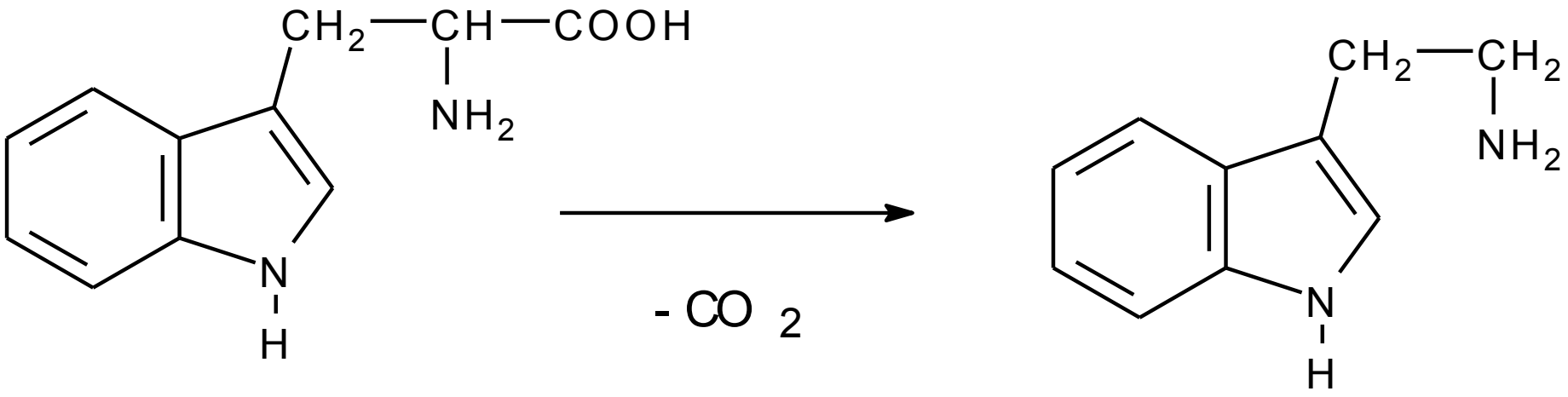

3-hydroxyanthranilate

# Catabolism (4)




nicotinamide

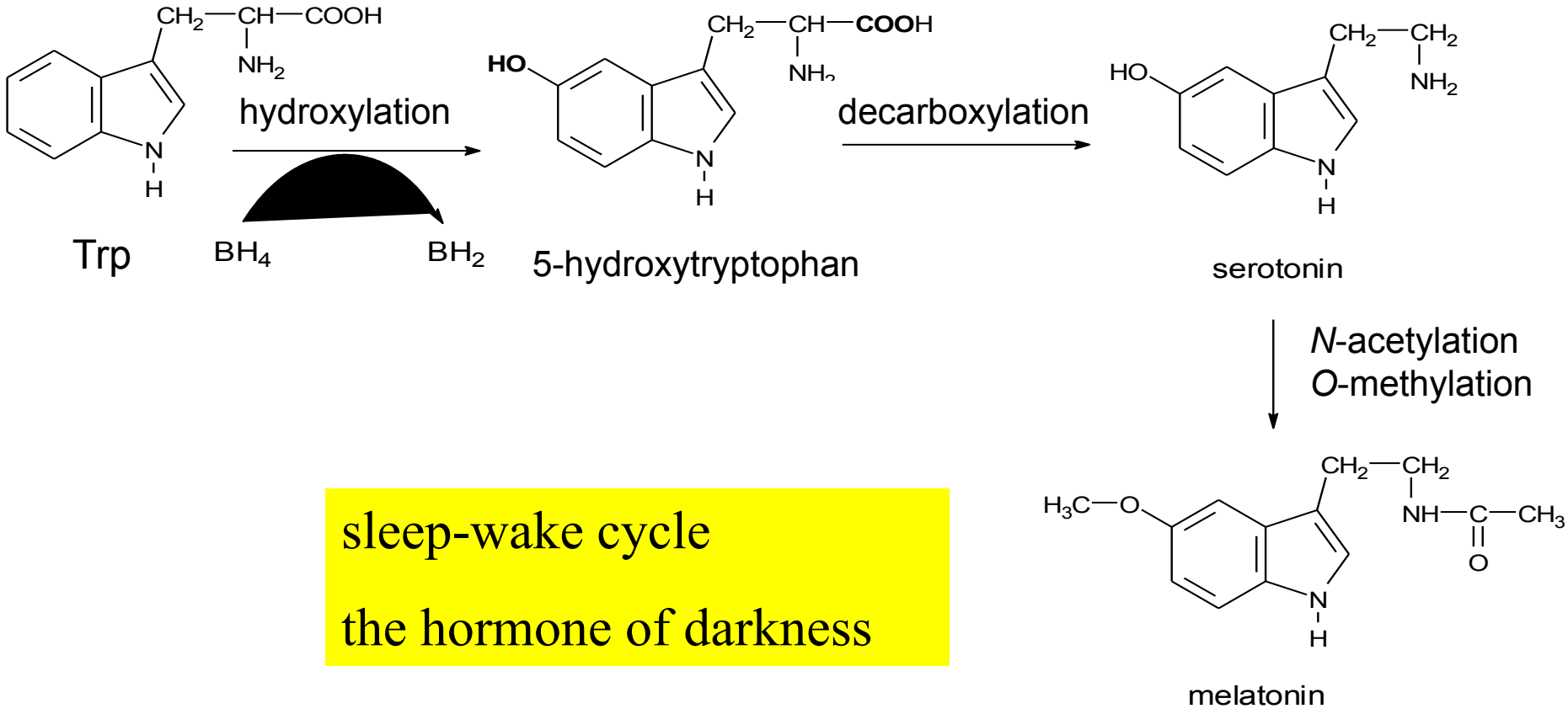
# Decarboxylation of tryptophan




tryptophan

tryptamine

# Conversion of tryptophan to melatonin




sleep-wake cycle

the hormone of darkness

# Tryptophan - summary

- essential AA
- complicated catabolism
- donor of 1C fragment (formic acid - formate)
- no transamination, amino group leaves as alanine (glucogenic)
- final product acetyl-CoA (ketogenic)
- source of nicotinamide and $NAD^+$
- bacterial decomposition in large intestine $\rightarrow$ indole and skatole (3-methylindole) – exhibit strong fecal odor

# Five vitamins are formed in the body, only four are utilized

| Vitamin | Where and how produced |
|---|---|
| Niacin | in tissues, from tryptophan |
| Biotin | large intestine (bacteria) |
| Phylloquinone | large intestine (bacteria) |
| Calciol | skin, from cholesterol (UV radiation) |
| Cobalamine | large intestine (bacteria) – **not absorbed!** |

# Seven amino acids do not undergo transamination

| Amino acid | α-$NH_2$ group is removed as |
|---|---|
| Arginine | ornithine |
| Lysine | 2-aminoadipate |
| Methionine | homoserine |
| Threonine | glycine |
| Tryptophan | alanine |
| Proline | glutamate |
| Histidine | $NH_3$ (desaturation deamination) |

| AA | Biochemically relevant product |
|---|---|
| Ala | pyruvate → glucose |
| Arg | urea, NO, creatine |
| Ser | ethanolamine → choline → betaine; donor of 1C fragment, selenocysteine |
| Gly | heme, creatine, GSH, conjugation reagent (e.g. glycocholate) |
| Met | donor of methyl, creatine, homocysteine, cysteine |
| Cys | glutathione (GSH), taurine, $SO_4^{2-}$, PAPS, cysteamine (CoA) |
| Asp | donor of -$NH_2$ (urea, pyrimidines), oxaloacetate, fumarate, β-alanine (CoA) |
| Glu | $NH_4^+$, 2-oxoglutarate, GABA, ornithine |
| Gln | $NH_4^+$, donor of -$NH_2$ (synthesis of glucosamine, purines) |
| Pro | glutamate, hydroxyproline |
| His | glutamate, histamine, donor of 1C fragment |
| Lys | glutamate, allysine (collagen), carnitine, cadaverine |
| Tyr | fumarate, catecholamines, thyroxine, melanins |
| Trp | nicotinamide, serotonin, melatonin, donor of 1C fragment, indole, skatole |

# Overview: decarboxylation of amino acids

| AA | Product | Comments |
|---|---|---|
| Ser | ethanolamine | part of phospholipids, precursor of choline |
| Cys | cysteamine | part of coenzyme A (CoA-SH) |
| Phe | phenethylamine | structural part of stimulants (amphetamine, ephedrine etc.) |
| Tyr | tyramine | occurs in some foods, may cause migraine |
| Asp | β-alanine | part of pantothenic acid, CoA-SH, carnosine |
| Glu | GABA | gama-aminobutyric acid, inhibition neurotransmiter |
| Lys | cadaverine | product of putrefaction (decay of proteins) |
| Arg | agmatine | signal molecule in CNS |
| His | histamine | triggers allergic reactions |
| Trp | tryptamine | precursor of serotonine and melatonine |
| DOPA | dopamine | catecholamine, precursor of noradrenaline/adrenaline |
| Ornithine | putrescine | putrefaction product; precursor of spermidine/spermine |