Ch. 3: Vitamins, Cofactors, Coenzymes, Enzymes

* **Readings**

- P. J. Bruice, Organic Chemistry, 2nd ed., Prentice-Hall, Upper Saddle River, New Jersey, **1998,** Chapter 22.
- H. Dugas, Bioorganic Chemistry - A Chemical Approach to Enzyme Action, 3rd ed., Springer Verlag, New York, **1996,** chapter 7.
- O. Isler, G. Brubacher, Vitamins I: Fat-Soluble Vitamins, Thieme Verlag, Stuttgart, **1982**.
- O. Isler, G. Brubacher, S. Ghisla, B. Kraeutler, Vitamins II: Water-Soluble Vitamins, Georg Thieme Verlag, Stuttgart, **1988**.
- http://www.indstate.edu/thcme/mwking/vitamins.html

Glossary

- **vitamin (water soluble vitamin, fat soluble vitamin)**: a substance needed in small amounts for normal body functions that the body cannot synthesize in adequat amounts
- **enzyme**: a protein that is a catalyst
- **metalloenzyme**: an enzyme that has a tightly bound metal ion **cofactor**: an organic molecule or a metal ion that certain enzymes need in order to catalyze a reaction or a process
- **coenzyme**: an organic molecule (vitamin) as cofactor
- **prosthetic group**: a cofoctor permanently associated with the protein, often covalently bound
- **holoenzyme**: catalytically active enzyme-cofactor complex.
- **apoenzyme**: an enzyme without its cofactor (enzymatically inactive protein)

Glossary

- **metabolism:** reactions that living organism carry out
- **catabolism**: energy and simple molecules are formed from complex molecules
- **anabolism**: synthesis of complex biomolecules using energy

Problems - 1

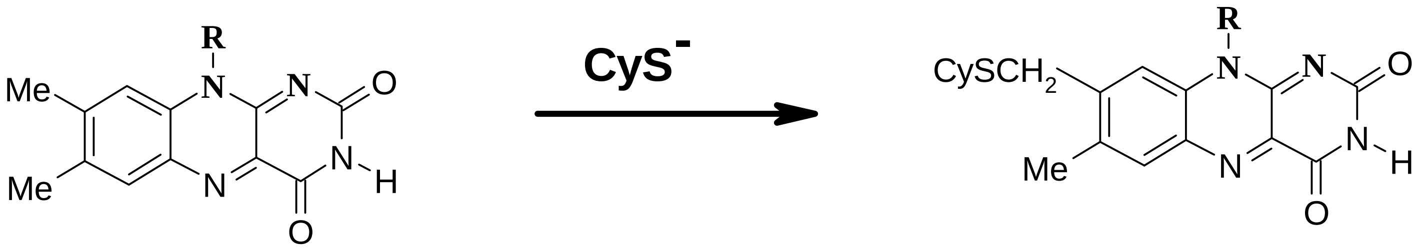
- Vitamin C and Vitamin E are both natural radical inhibitors. Explain why this is so.
- Explain why vitamin C is a „strong“ acid
- Ascorbinsäure can be methylated by diazomethane. Which functional group is most sensitive towards methylation?
- (L)-Sorbose which is an intermediate in the technical synthesis of vitamin C, forms a furanosid by reaction with aceton/H^+.
  Discuss the mechanism of formation. Are there diastereotopic methyl groups?

Problems - 2

- Apply Woodward-Hoffmann-Rules for the synthesis of Vitamin D_3. Sum up the WH-rules for the electrocyclic and sigmatropic reactions under thermal respectively photochemical conditions.
  a) Which stereoisomer is formed by irradiating (2E,4Z,6Z)-octatriene?
- Stereoselective oxidoreduction of ketones and alcohols using horse liver alcohol dehydrogenase (HLADH) needs NADH as cofactor. NADH is rather expensive and has to be recycled. Recyclization can be achieved by natrium dithionite. Formulate the reaction sequence for the keton reduction by HLADH/NADH and the subsequent recyclization of NAD^+ by dithionite.
- Assign the pro-R hydrogen and the pro-S hydrogen of NADH.
- Compare typical applications of NAD^+/NADH und FAD as oxidoreductases with regard to their involvements in the biochemical processes.

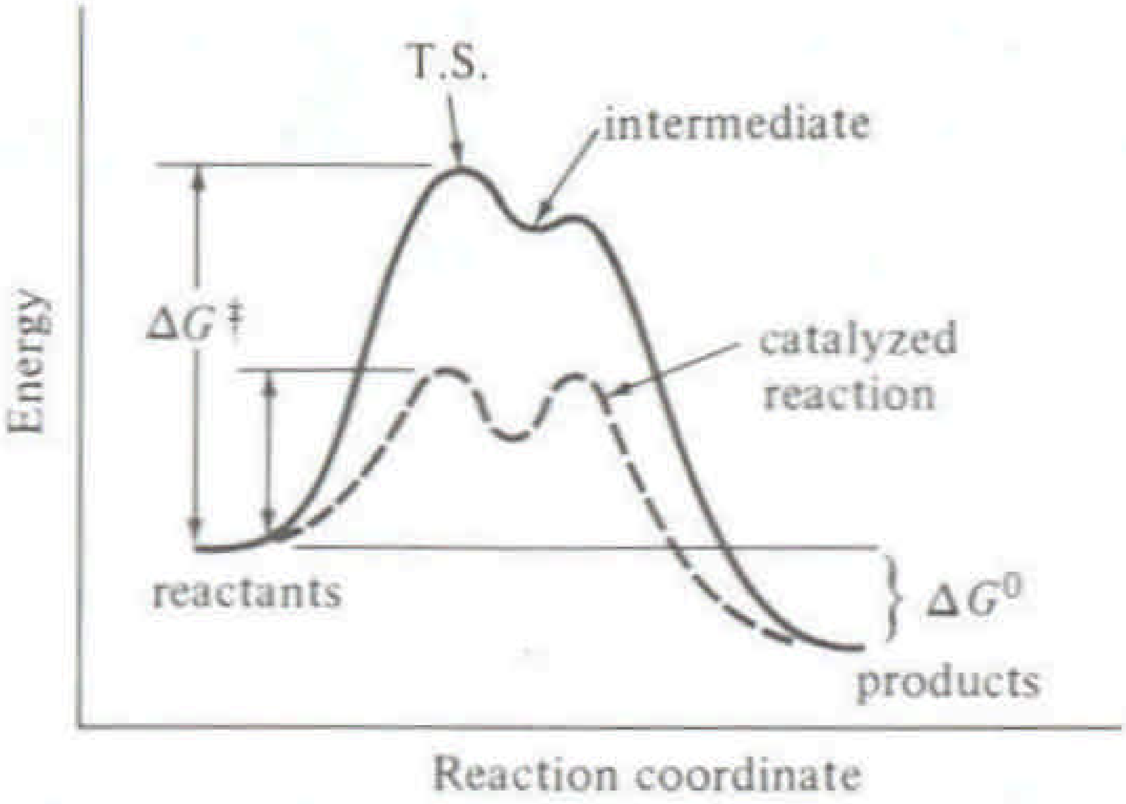
Problems - 3

- Nature exploits the reactivity of C-8 methyl group of flavins in order to link to the apoenzyme via a SH group of a cystein. The overall process is as given below. The last step is the oxidation of the cystein bound $FADH_2$ to enzyme-FAD.



- Discuus the similarity of decarboxylation of a β-keto acid and enzymatic pyruvate decarboxylation.
- The pyrimidine-amino-group in thiamin pyrophosphate contributes in the enzymatic TPP-dependent processes. How can this be explained?

Enzymatic reactions and catalysis



Vitamins, coenzymes, cofactors, enzymes

protein + coenzyme (vitamin)

↓

enzyme

↑

protein + cofactor (metal ion)

Vitamins, coenzymes, enzymes cofactors

Vitamins that are coenzyme precursors

Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin (B_2)	Flavin coenzymes	*a*
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

[a] No specific name; deficiency in humans is rare or unobserved.

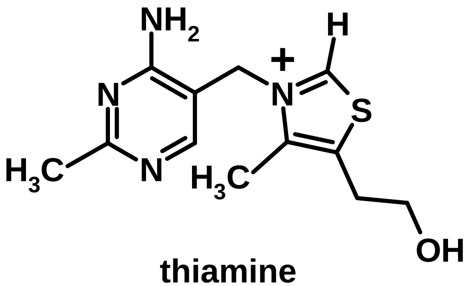
TABLE 12-1. THE COMMON COENZYMES

Coenzyme	Reaction Mediated	Section Discussed
Biotin	Carboxylation	21-1A
Cobalamin (B_{12}) coenzymes	Alkylation	23-2E
Coenzyme A	Acyl transfer	19-2A
Flavin coenzymes	Oxidation-reduction	14-4
Lipoic acid	Acyl transfer	19-2A
Nicotinamide coenzymes	Oxidation-reduction	12-2A
Pyridoxal phosphate	Amino group transfer	24-1A
Tetrahydrofolate	One-carbon group transfer	24-4D
Thiamine pyrophosphate	Aldehyde transfer	16-3B

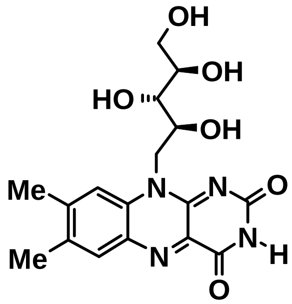
- D. Voet, J. G. Voet, *Biochemistry*, second edition ed., John Wiley & Sons, New York, **1995**.

Structure of water soluble vitamins

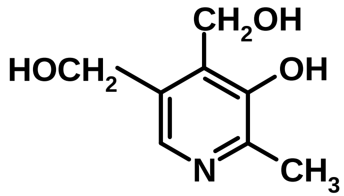
BO/BBM



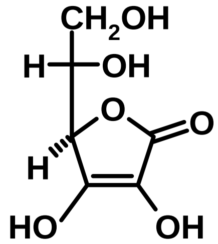
thiamine
vitamin B_1



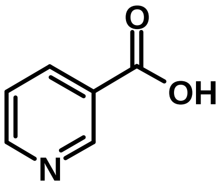
riboflavin
vitamin B_2



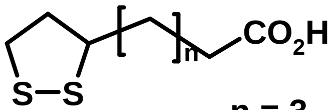
pyridoxine
vitamin B_6



vitamin C
ascorbic acid

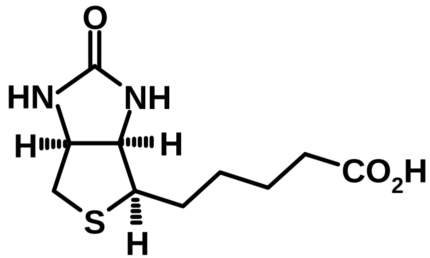


niacin
nicotinic acid

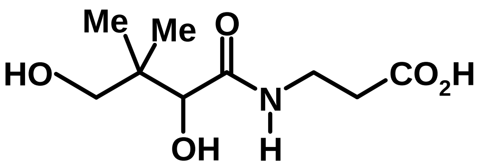


n = 3

lipoate

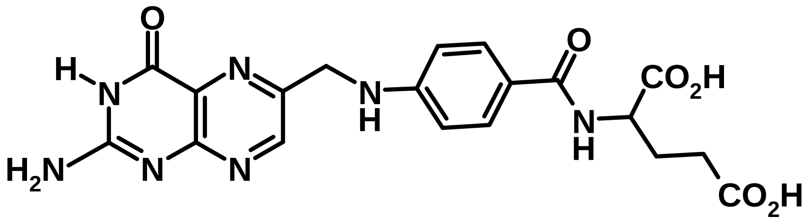


biotin
vitamin H



pantothenate

subunit of CoA-SH

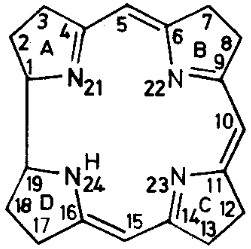


folate

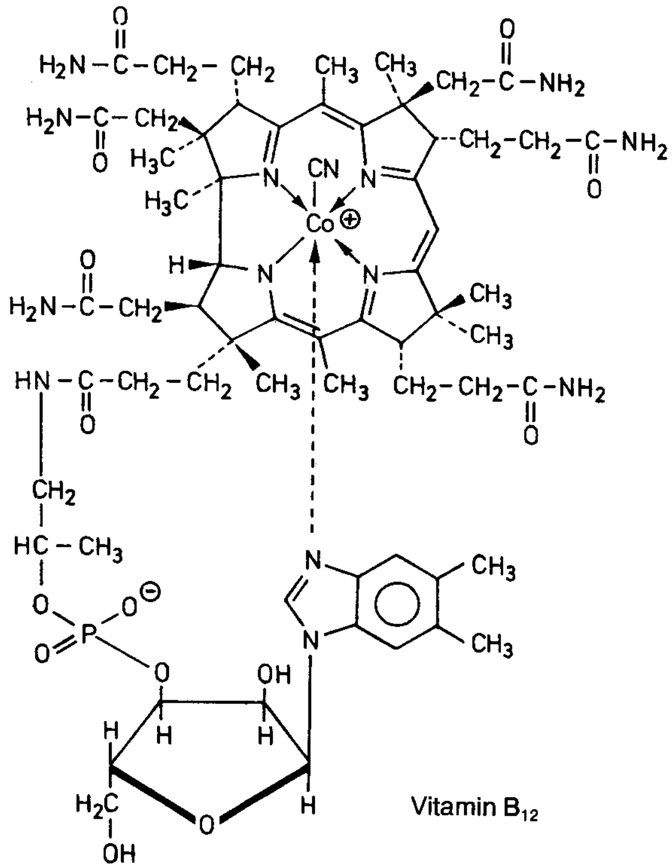
vitamin B_{12}

Vitamin B_{12}

BO/BBM/SKB



Corrin[1]

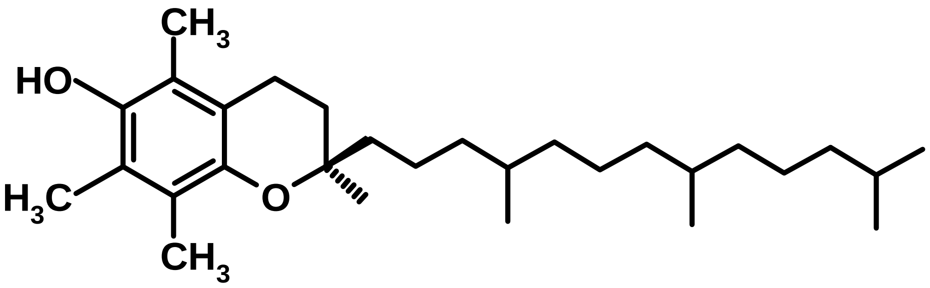


Vitamin B_{12}

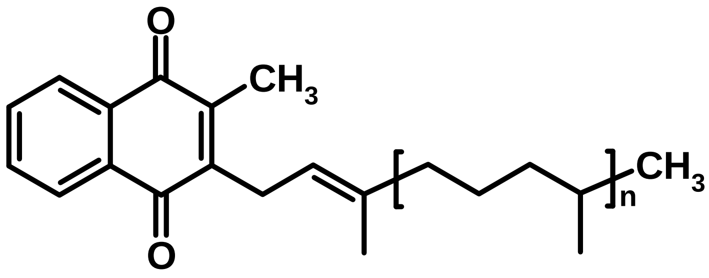
- Discovering Nature's Diverse Pathways to Vitamin B_{12}: A 35-Year Odyssey: A. I. Scott, *J. Org. Chem.* **2003**, *68*, 2529-2539..

BO/BBM

Fat soluble vitamins

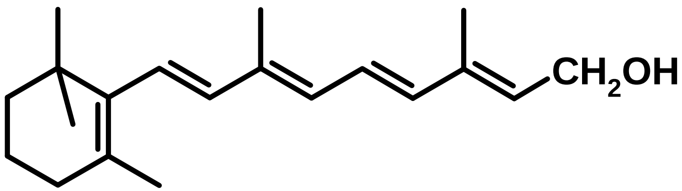


vitamin E
α-tocopherol

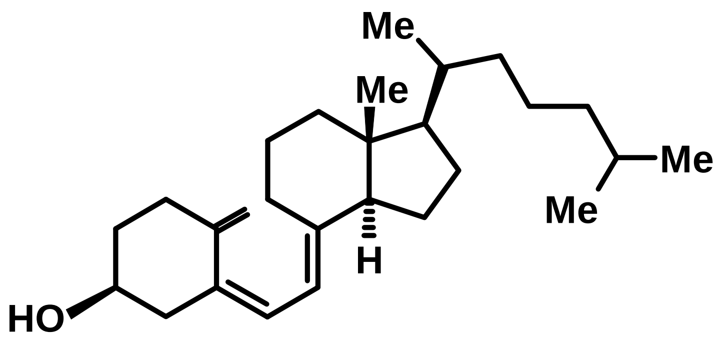


n = 3

vitamin K_1



vitamin A (all-trans)

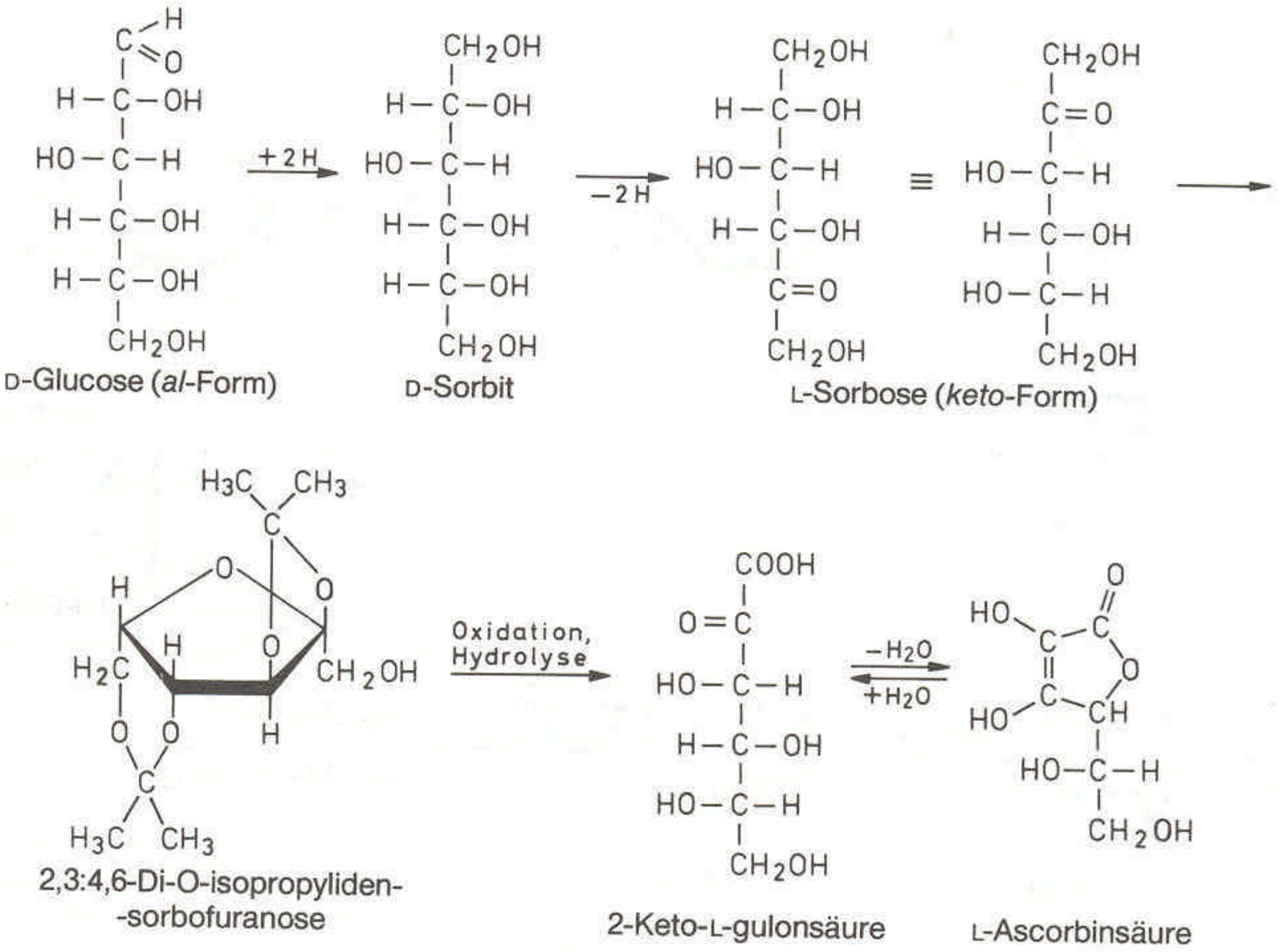


vitamin D_3

Vitamin C

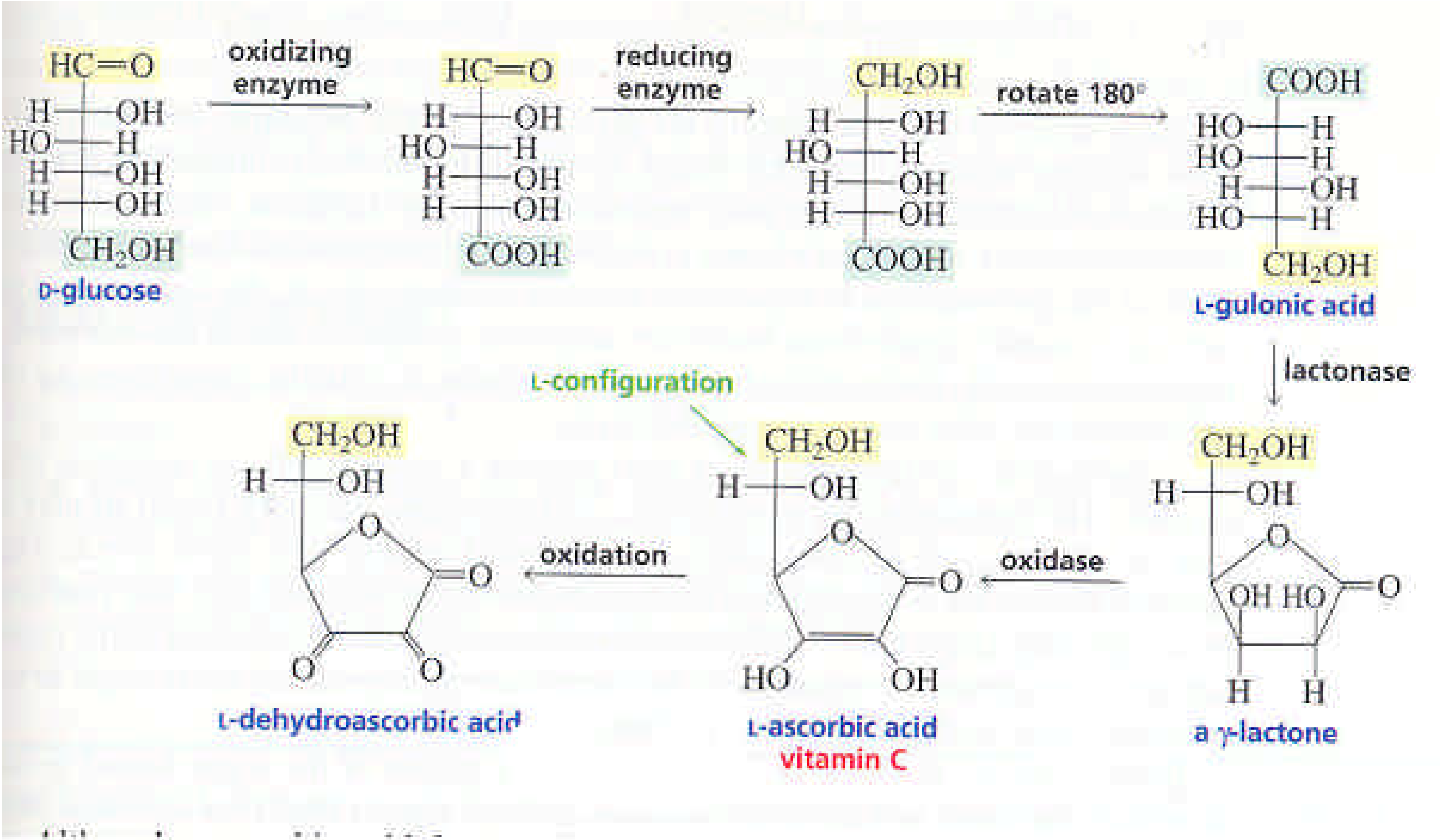
- mp 190-192°C
- $pK_1 = 4.17$; $pK_2 = 11.57$
- anti-scorbutically factor (essential for the synthesis of collagen)
- reducing agent in hydroxylation reactions
- radical scavenger (inhibitor)

Industrial synthesis of vitamin C (Reichstein, 1934)



- H. Beyer, W. Walter, *Lehrbuch der Organischen Chemie*, **1998**.

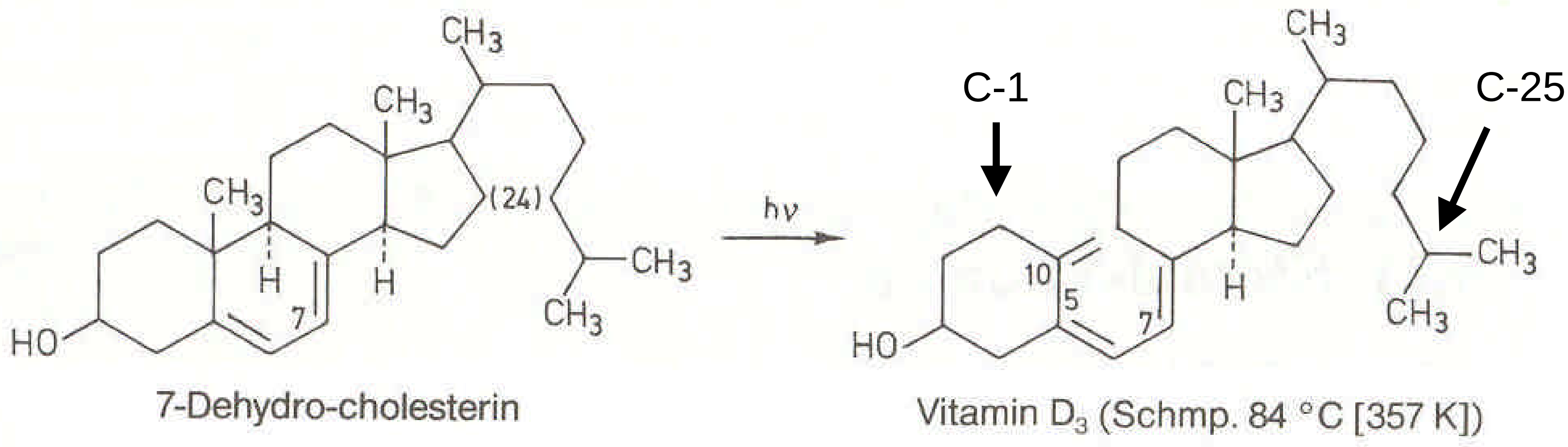
Enzymatic synthesis of vitamin C



Vitamin D

- Metabolism of calcium and phosphorous
- Deficiency impairs bone formation (rickets)
- Excess causes calcification of soft tissues

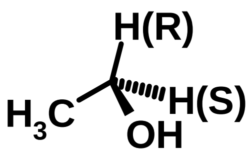
Photochemical formation of vitamin D_3



* **two step reaction**
  - first step: conrotatory photochemical ring opening
  - second step: [1,7] sigmatropic antarafacial hydrogen shift (thermal)
* **Woodward-Hoffmann rules**
* Active form **1,25-dihydroxyvitamin D_3**

Oxidoreduction: Pyridine nucleotide coenzymes (NAD^+/$NADP^+$) (Vitamin B_3, Niacin)

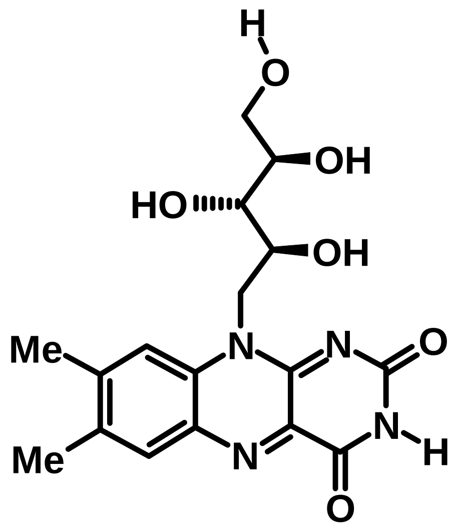
* NAD^+/NADH; $NADP^+$/NADPH
  - Structure
  - Oxidation reactions
  - Reduction reactions (Pyruvate → lactate; lactate d**ehydrogenase)**
  - Comparison with $LiAlH_4$
  - Mechanism and stereochemistry (Prochirality)



H(R) and H(S) enantiotopic hydrogens

Bioactive flavins

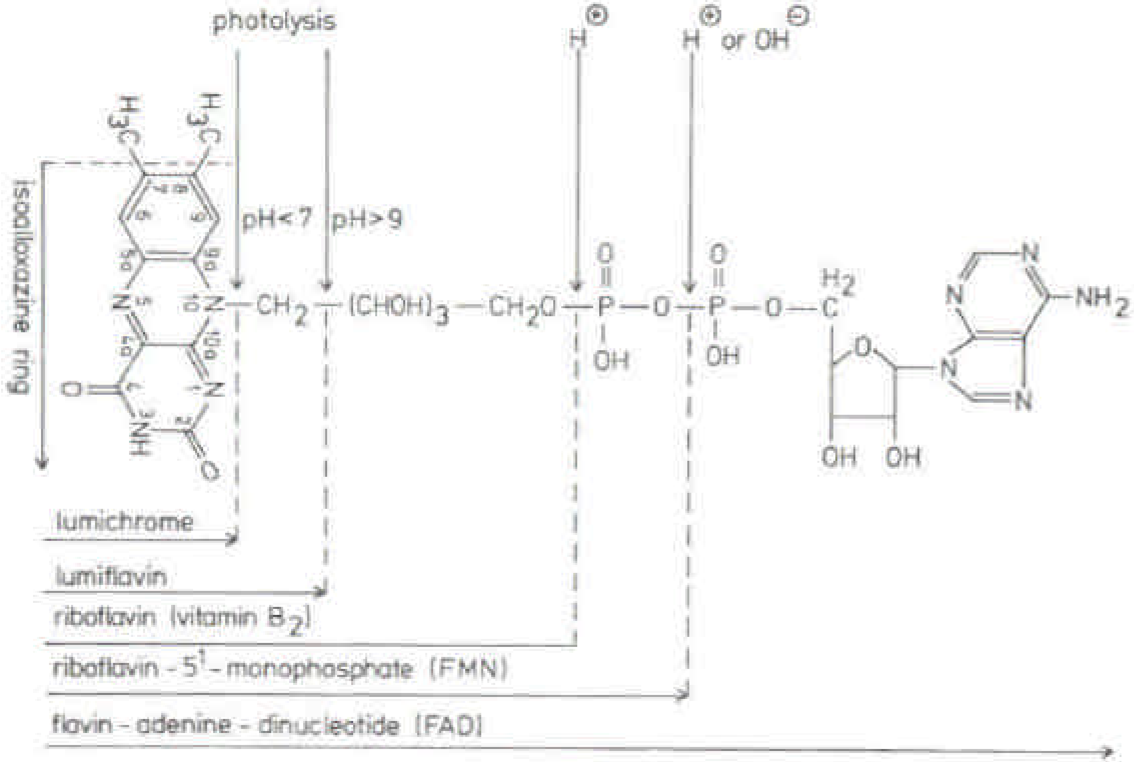
* vitamin B_2 – riboflavin
* flavin mononucleotide (FMN)
* flavin adenine dinucleotide (FAD)



Flavin Biochemistry

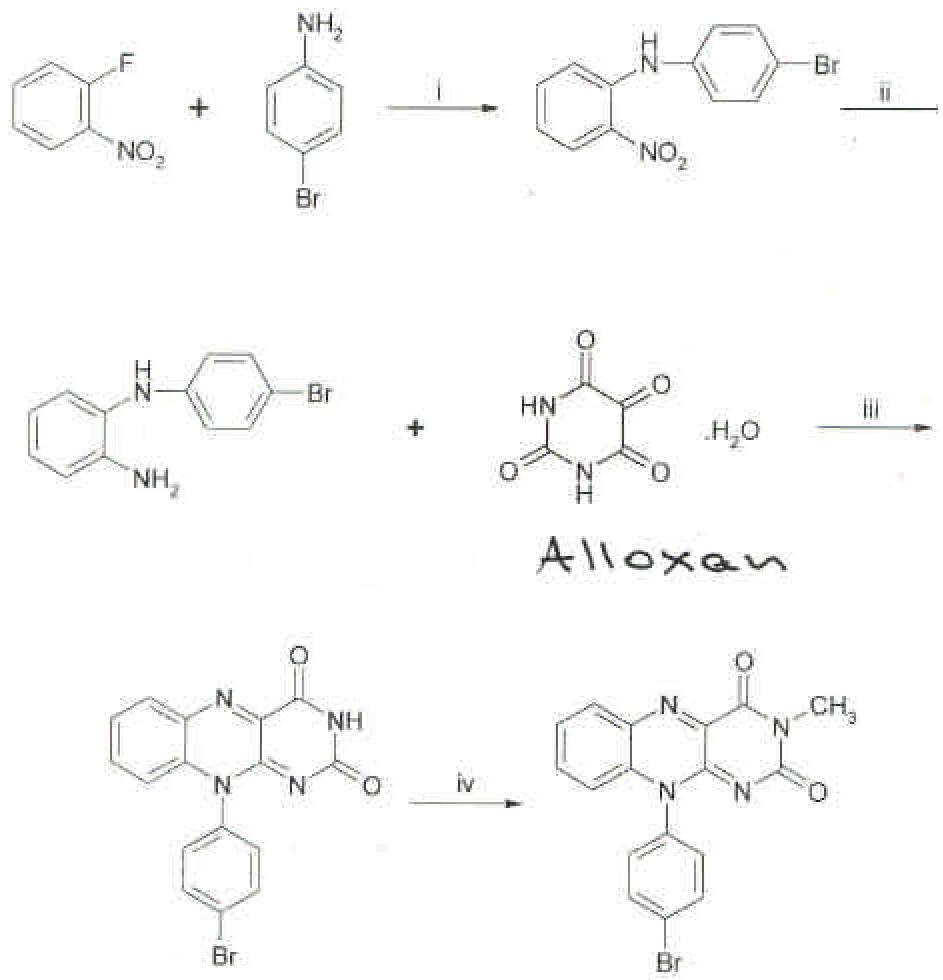
* Dehydrogenation
* Activation of oxygen, hydroxylation, oxygenation
* Electron transfer, electron transport, respiration chain, etc.
* Light emission, bacterial luciferase (bioluminescence)
* Photobiology: Photolyase, phototropism, photomorphogenesis, circadian processes (Cryptochrome, Phototropin, blue-light photo receptors)

Flavin structure



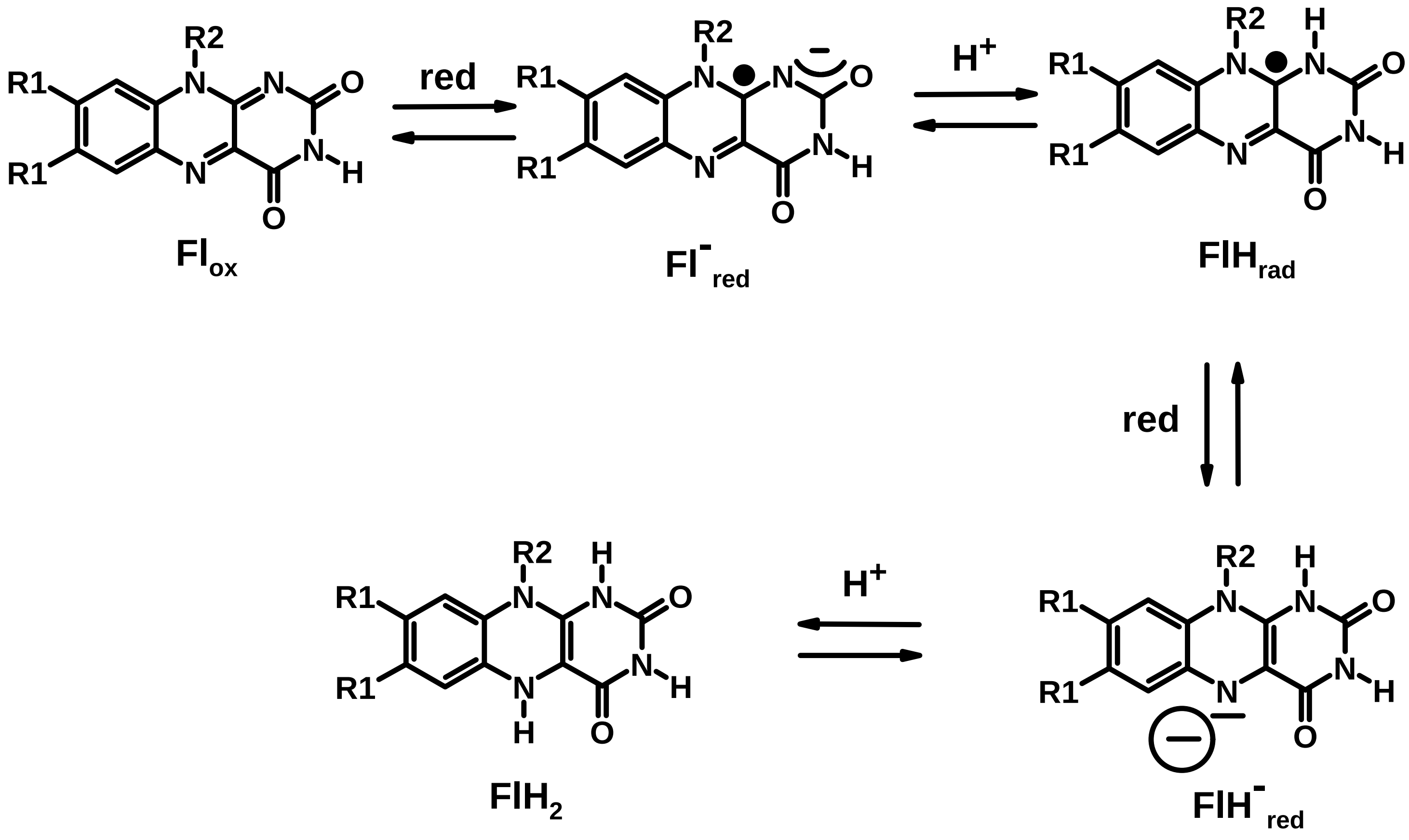
- F. Müller, *Chemistry and Biochemistry of Flavoenzymes, Vol. I*, CRC, Boca Raton, **1991**.

Isoalloxazine synthesis



i) KOH, DMSO; ii) Zn-powder, AcOH; iii) AcOH, H_3BO_3; iv) K_2CO_3, CH_3I, DMF

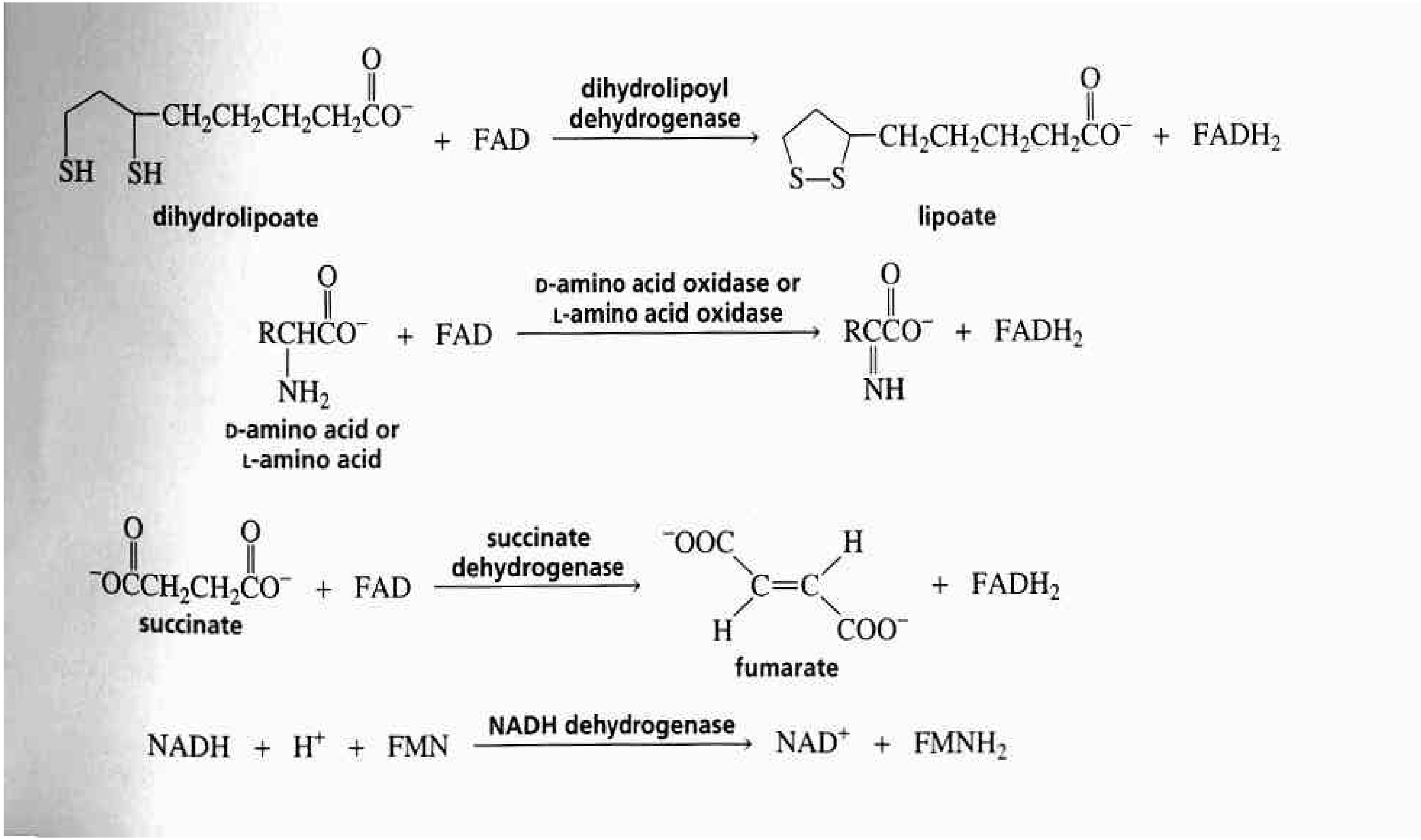
Redox processes of flavins (electron transfer/protonation)



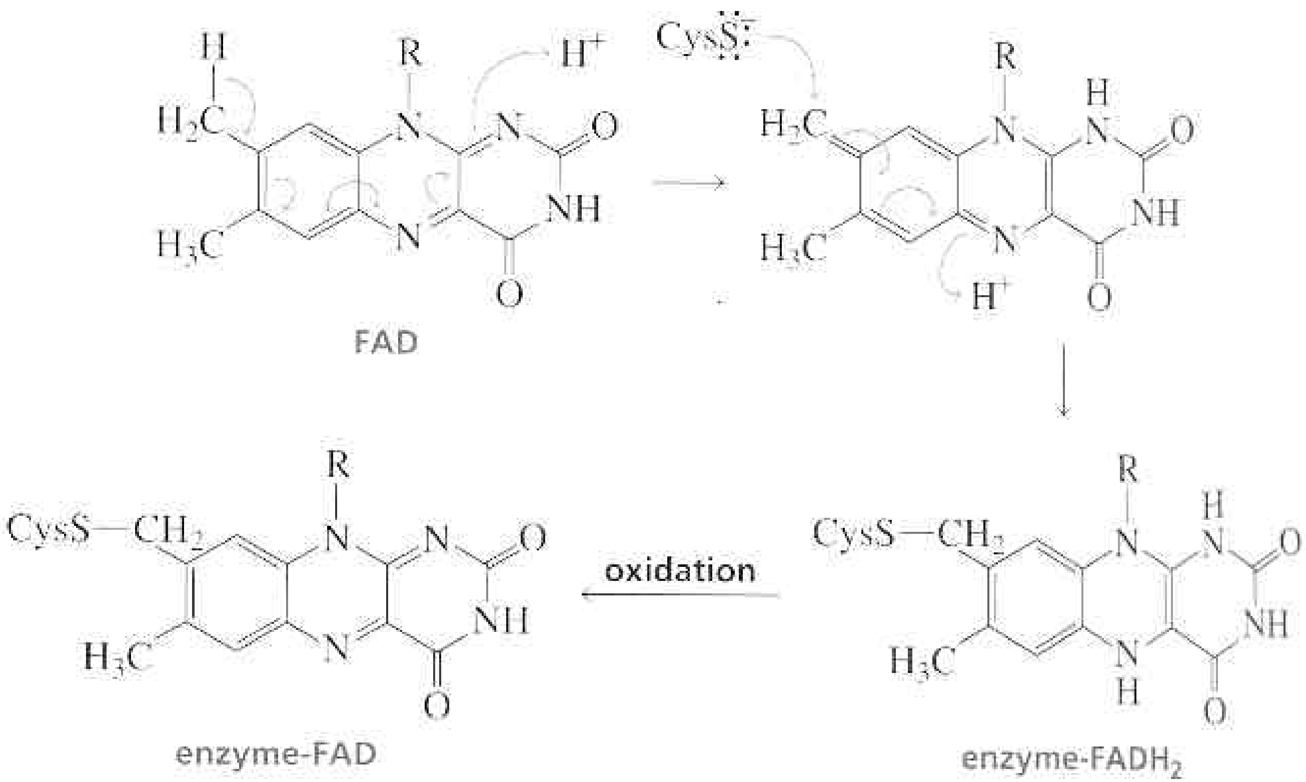
Some enzymatic processes of flavin nucleotides

- dihydrolipoyl dehydrogenase
- L-amino acid oxidase
- succinate dehydrogenase
- NADH dehydrogenase
- luciferase catalyzed bioluminescence

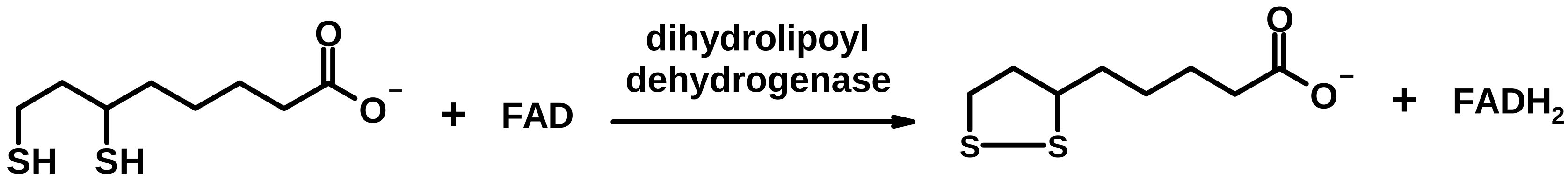
Enzymatic processes of flavin nucleotides

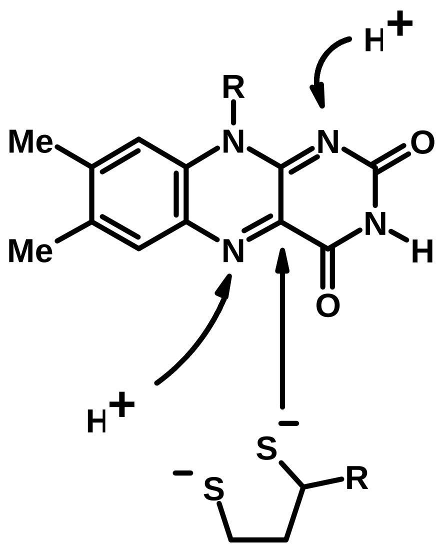


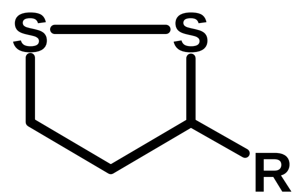
8a-S-Cysteinylflavin: Binding of flavins at the protein



FAD-dehydrogenase – oxidation of a dithiol



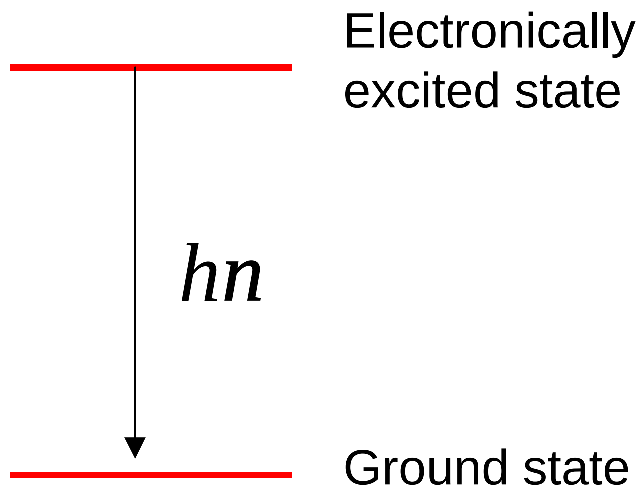




Bioluminescence

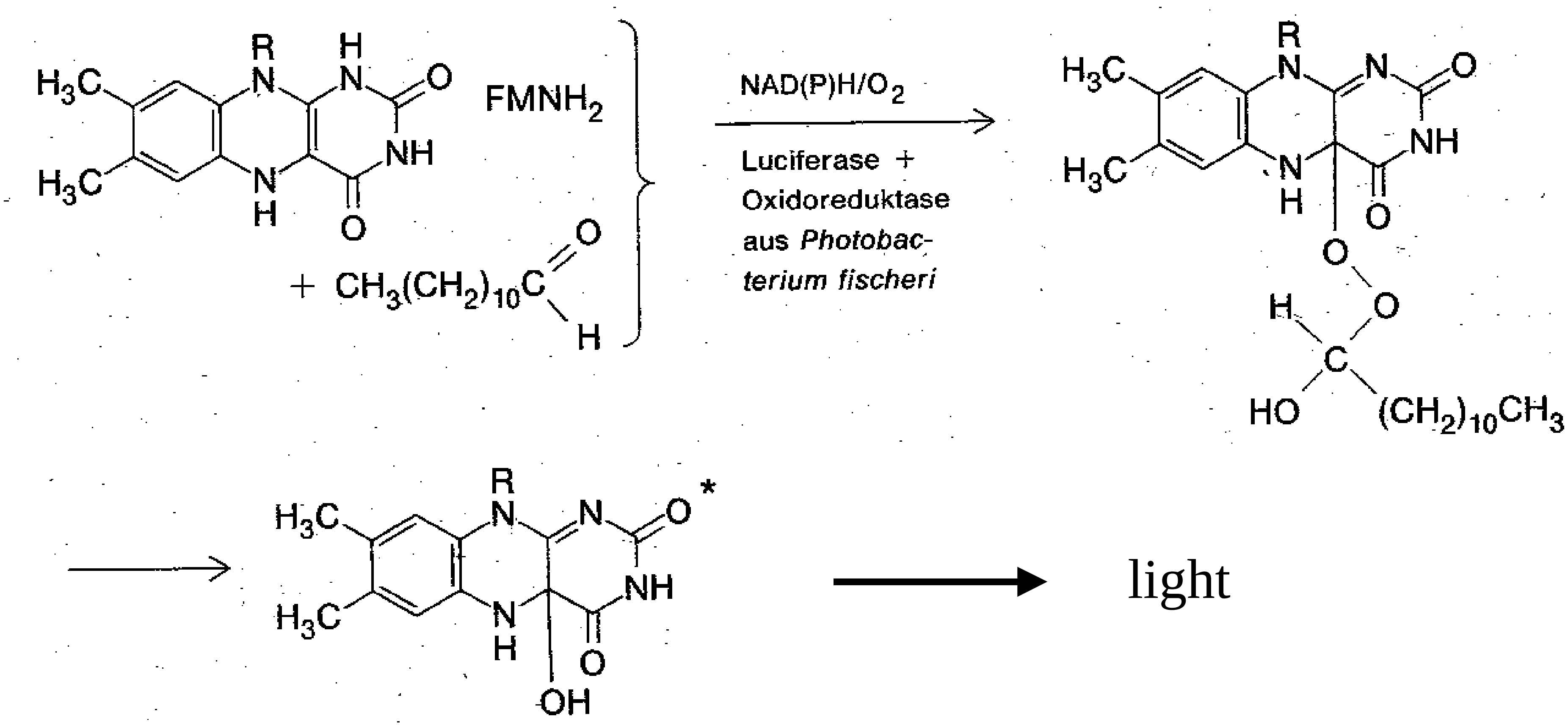
- Photoluminescence
- Chemoluminescence
- Bioluminescence
- Triboluminescence
- Electroluminescence
- Lyoluminescence
- Aquoluminescence
- Sonoluminescence
- Galvanoluminescence
- …
- …
- XYZ-Luminescence

Population of excited state by some process XYZ

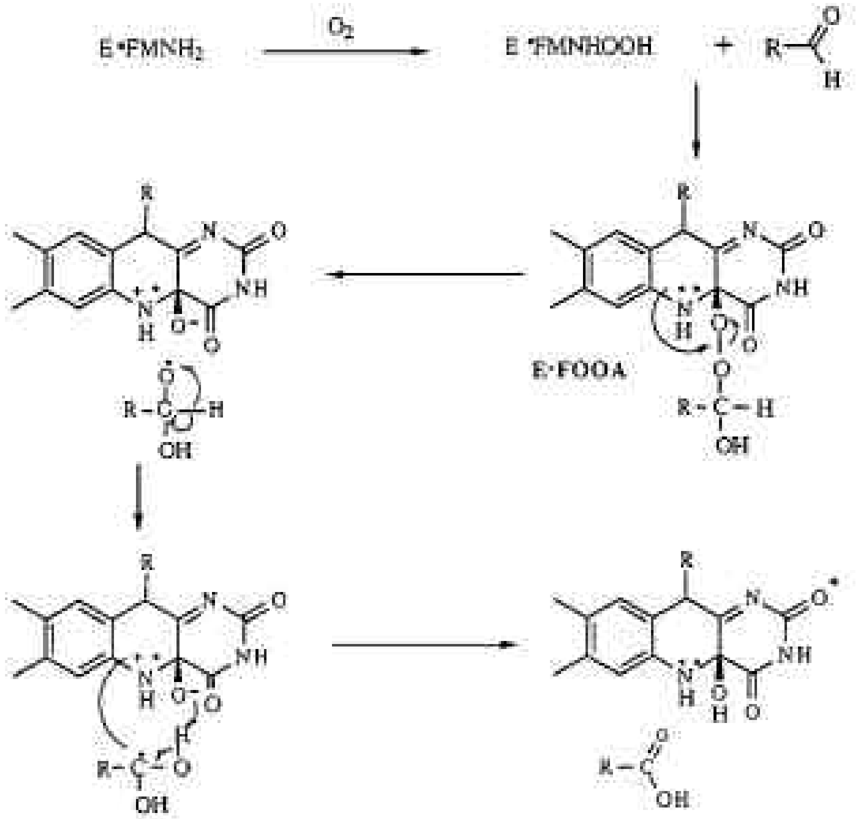


$$hn = E_e - E_g$$

Bioluminescence



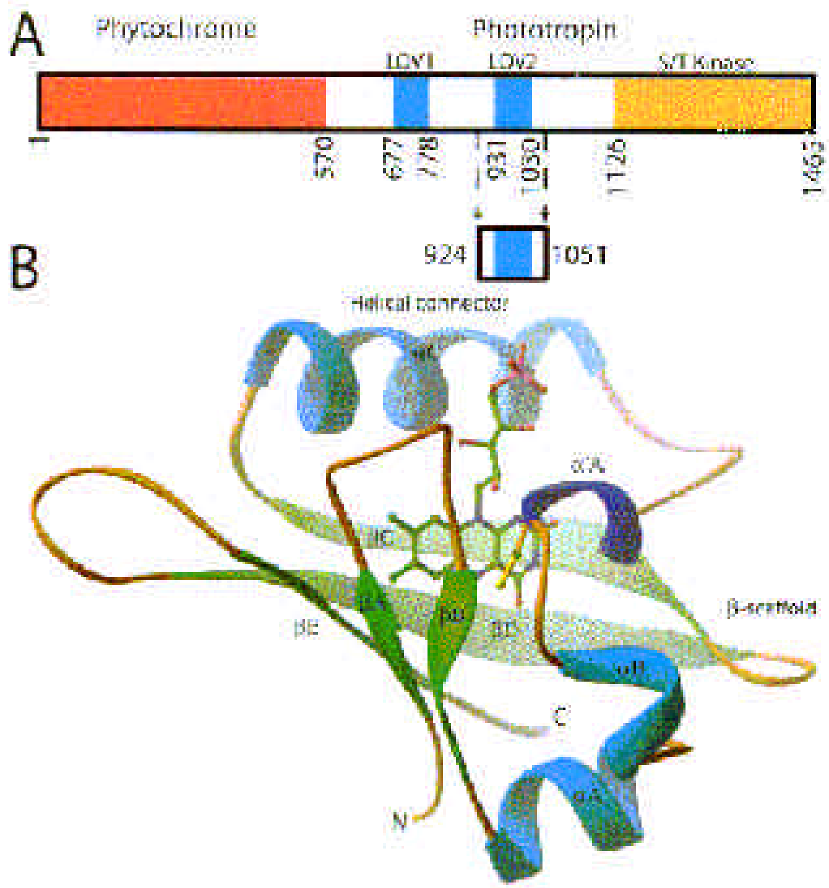
Bacterial bioluminescence



- enzyme catalyzed reaction: luciferase
- reaction cascade ultimately leads to the formation of the excited state (S_1) of 4a-hydroxyflavin

LOV domain of Phototropin

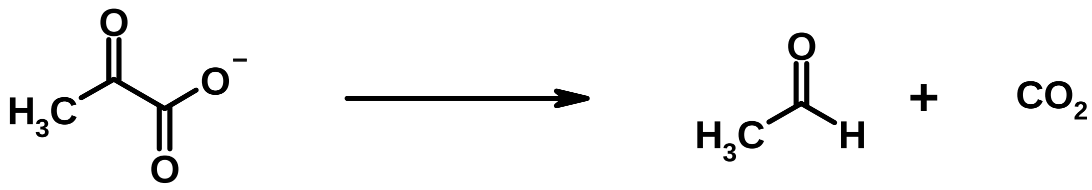
- Genetic information translated into protein structure
- Enzymatic synthesis of FMN
- Molecular self-assembly
- Input light, output morphogenesis

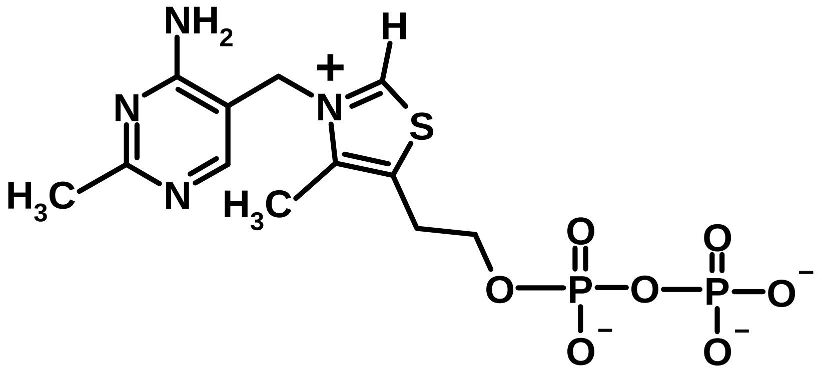


Δ S. Crosson, K. Moffat, **2001**.

Δ See also: http://www.uni-regensburg.de/Fakultaeten/nat_Fak_III/GK/SP/download/Kottke_Phototropins.pdf

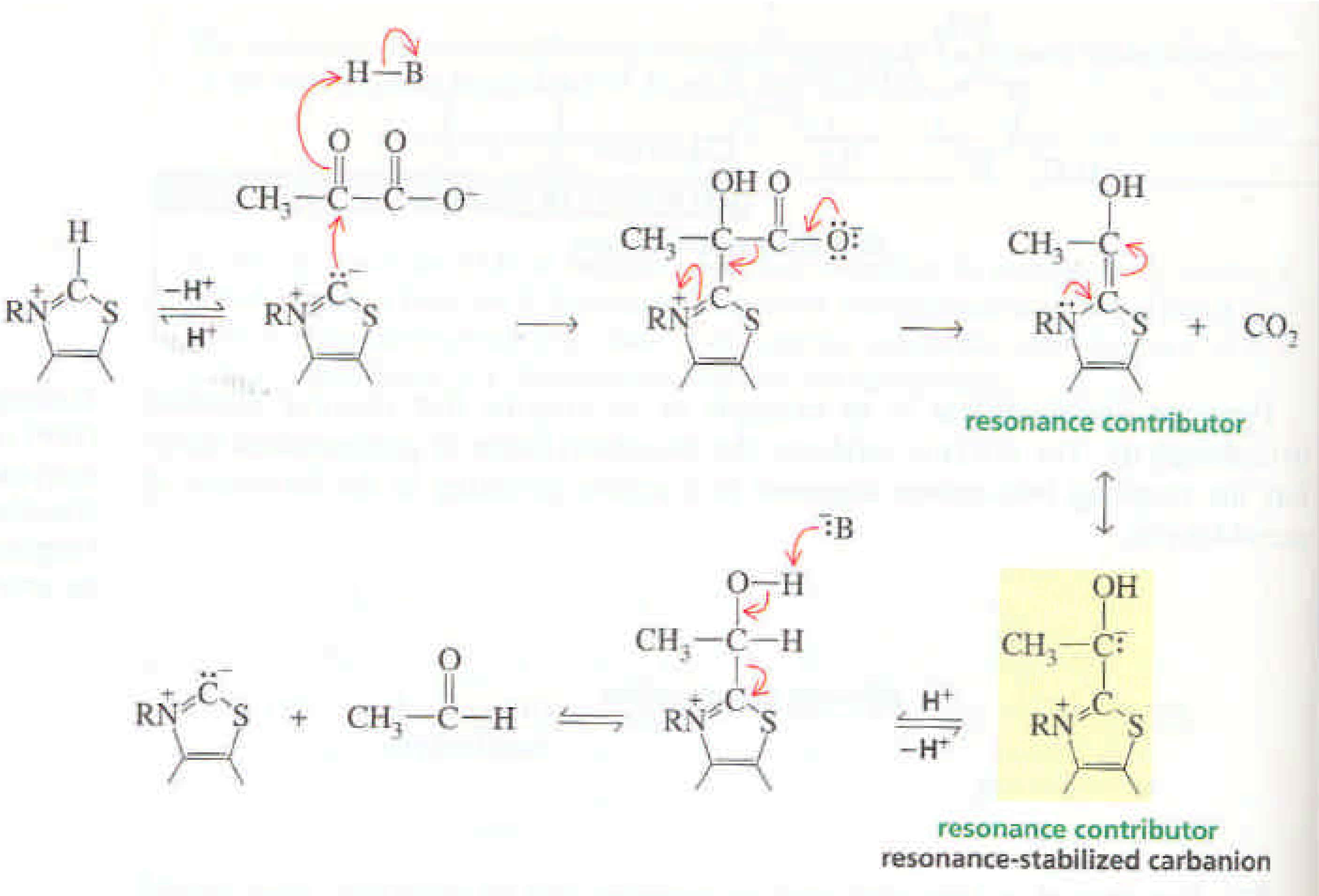
Pyruvate decarboxylase – cofactor thiamine pyrophosphate (TPP)



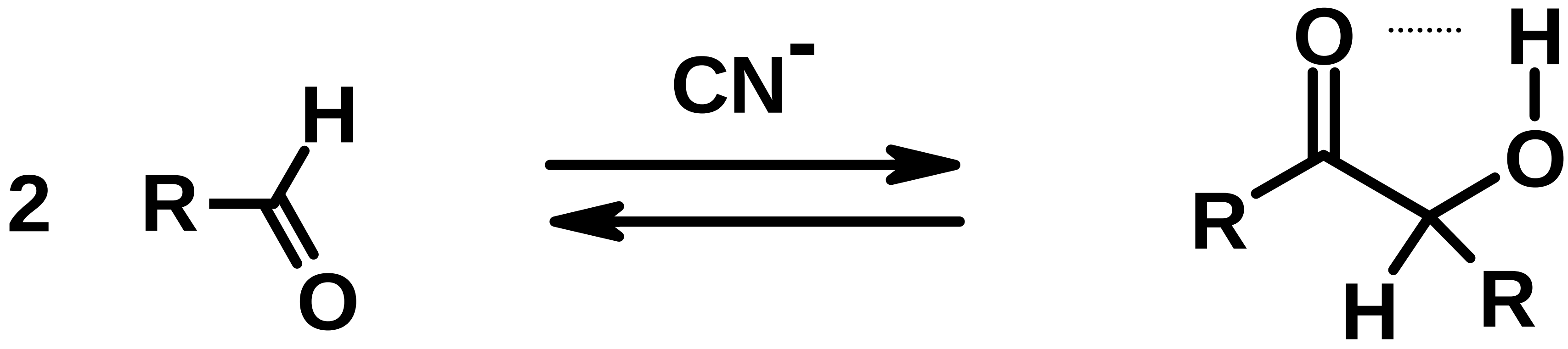


thiamine pyrophosphate
TPP

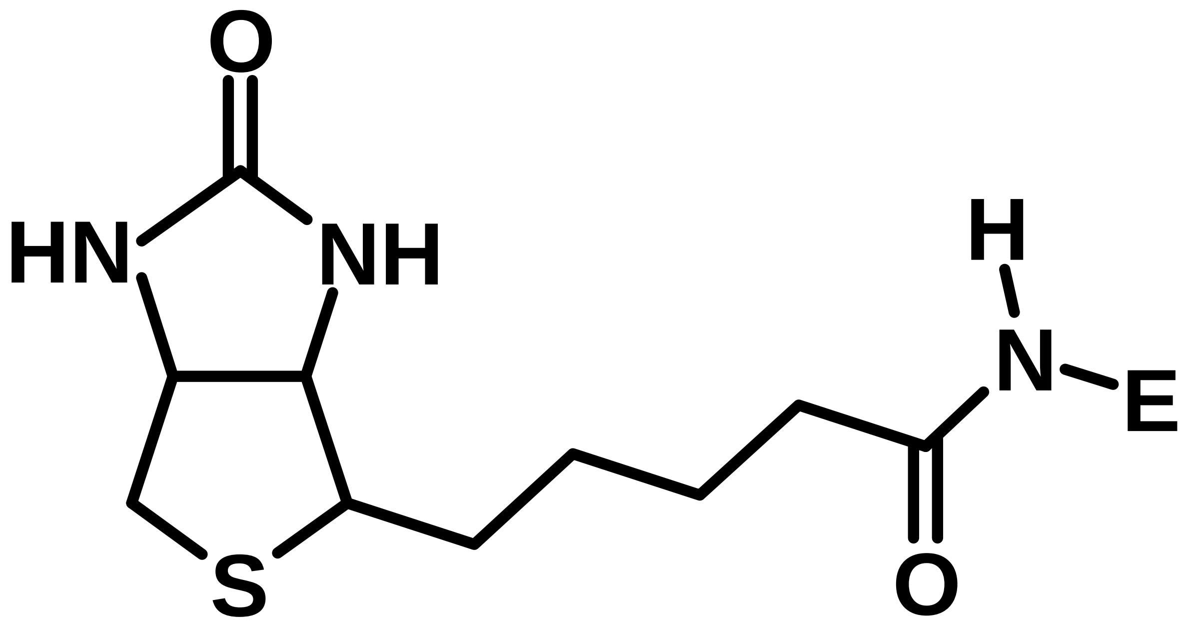
Pyruvate decarboxylase



Benzoin-type condensation – compare its mechanism to TPP catalyzed processes (transketolase)



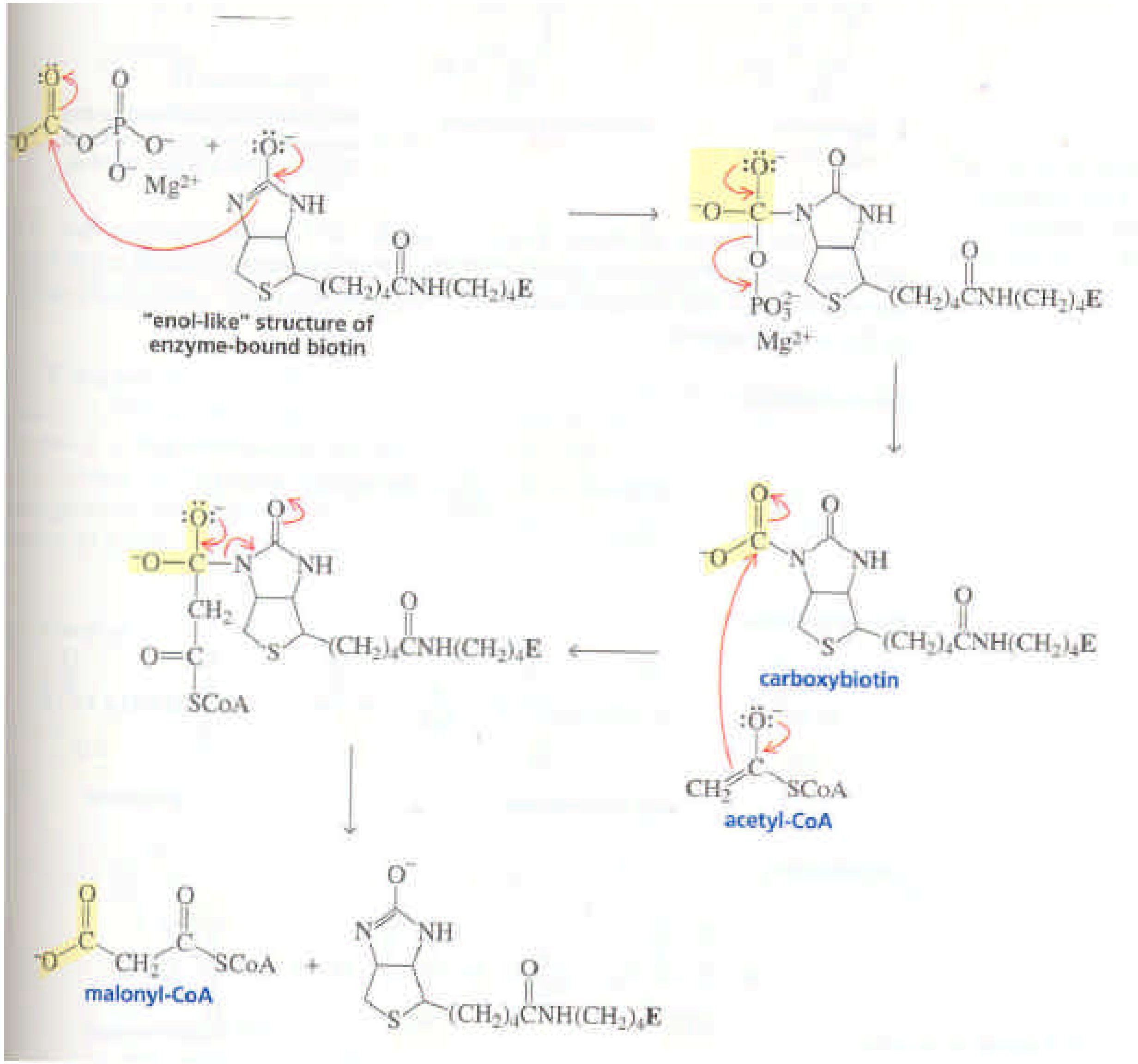
Enzyme bound biotin



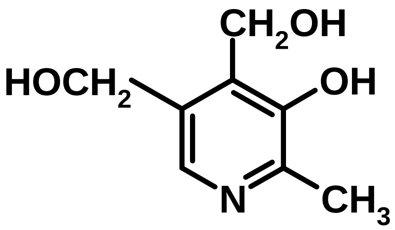
E: enzyme

Biotin – catalyzes carboxylation as a CO_2 carrier

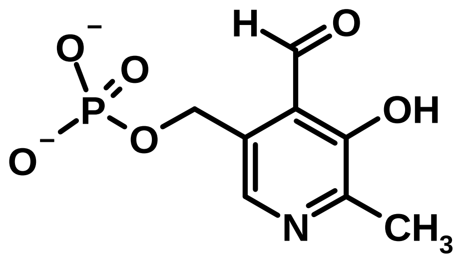
* carboxylation of activated carbon



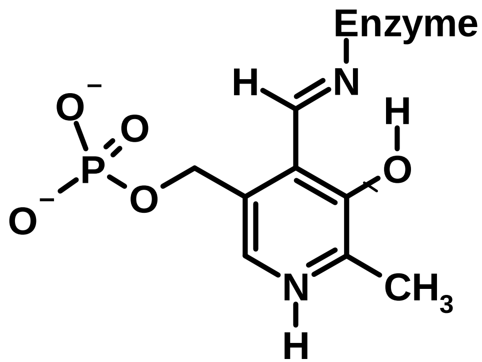
Pyridoxal phosphate (PLP)



pyridoxine
vitamin B_6



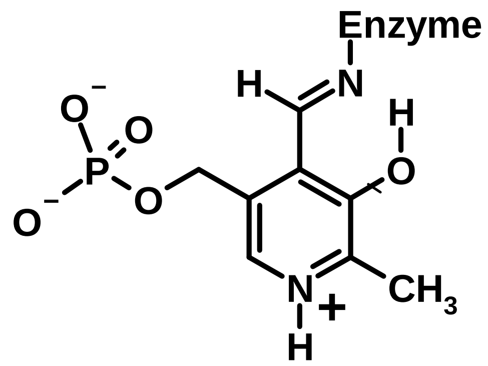
pyridoxal phosphate
PLP



enzyme covalently
bound to the
cofactor

PLP-catalyzed processes of amino acids in Nature

* transimination
* decarboxylation
* transamination
* racemization
* C_α-C_β bond cleavage („β-elimination“)
* α,β-elimination



enzyme covalently bound to the PLP cofactor

Decarboxylation of an amino acid

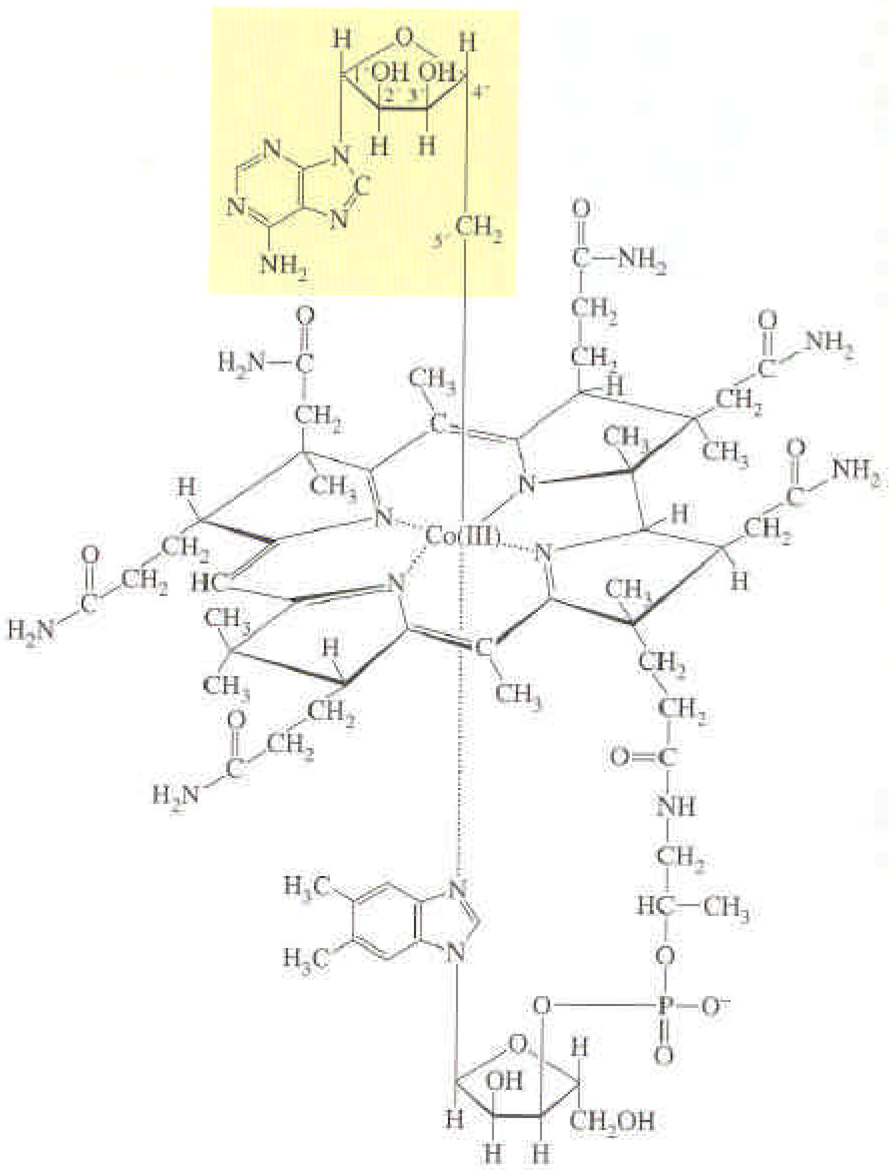
* First step: Synthesis of amino acid-bound PLP from enzyme-bound PLP
* Second step: Decarboxylation
* Third step: Transimination leading to enzyme-bound PLP and „free“ decarboxylated amino acid

Vitamin B_{12}

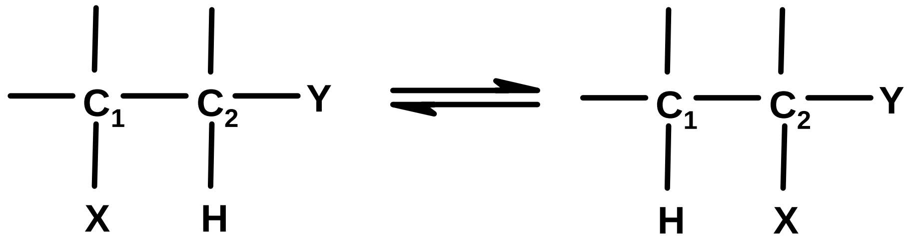
- Discovering Nature's Diverse Pathways to Vitamin B_{12}: A 35-Year Odyssey, A. I. Scott, J. Org. Chem. **2003**, 68, 2529-2539.
- Total synthesis of cobyric acid: historical development and recent synthetic innovations, D. Riether, J. Mulzer, European Journal of Organic Chemistry **2003**, 30-45.
- W. Kaim, B. Schwederski, Bioanorganische Chemie, Teubner, Stuttgart, **1991**.

Coenzym B_{12}

- vitamin B_{12} coenzyme (adenosylcobalamin)



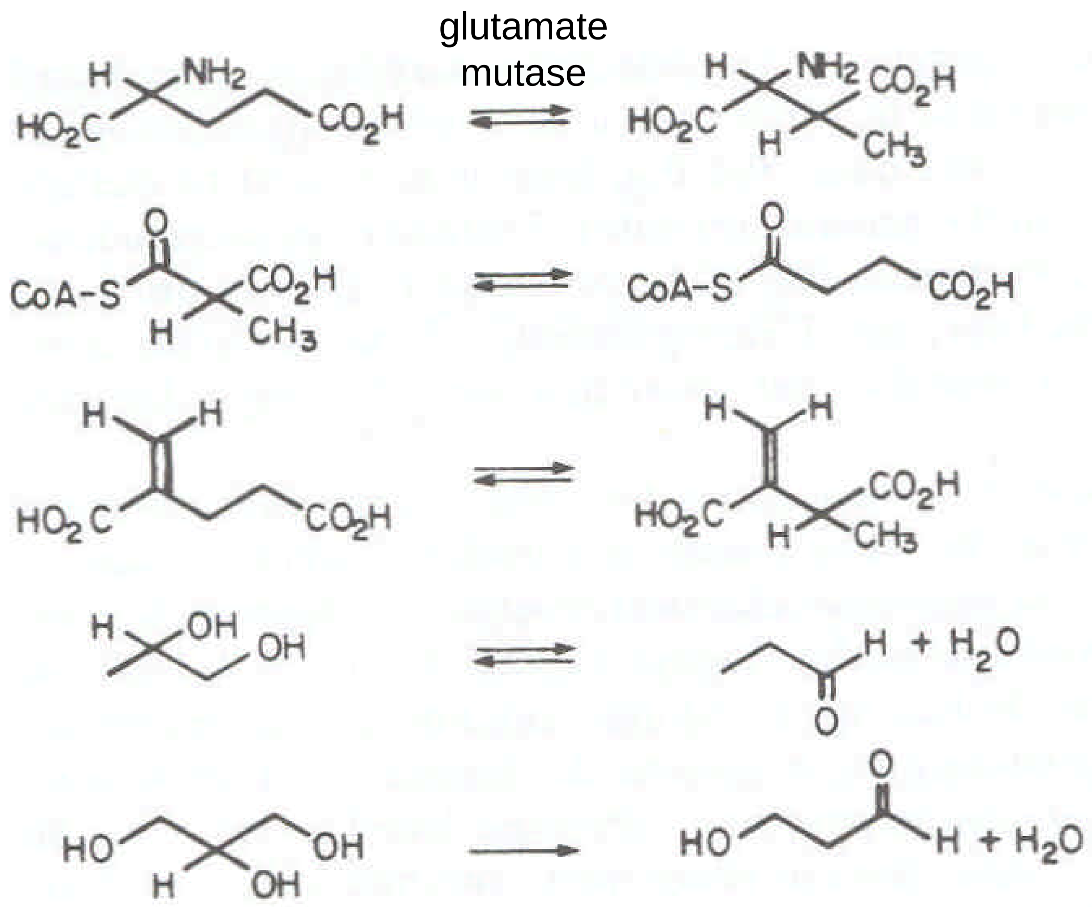
General scheme for the structure interconversion catalyzed by B_{12}



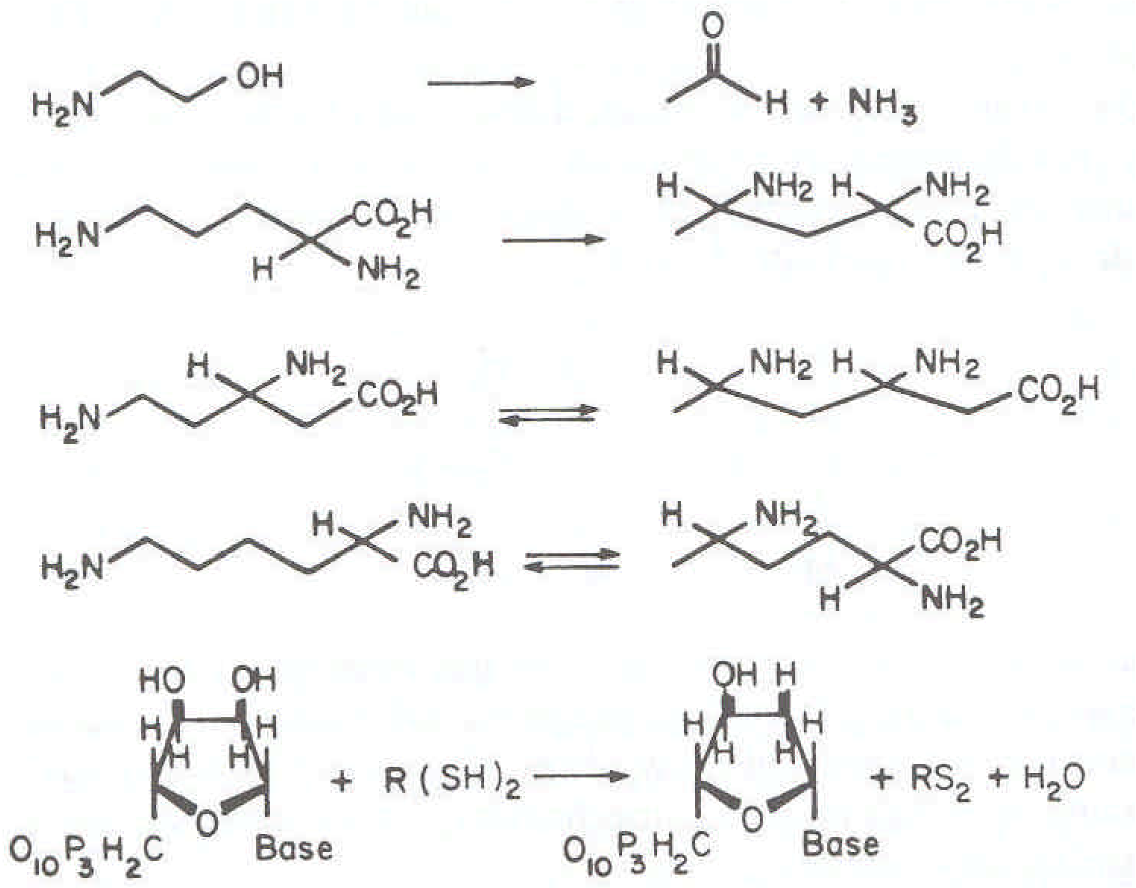
1,2 shift process

- radical mechanism

Processes requiring coenzym B_{12} - 1



Processes requiring coenzym B_{12} - 2



- H. Dugas, *Bioorganic Chemistry - A Chemical Approach to Enzyme Action*, 3rd ed., Springer Verlag, New York, **1996,** chapter 7.