Vitamin B12

CHEM 489
March 2nd, 2010

Blake Whitlow
Ruben Lopez III

Linked Disease

- **Pernicious Anemia**
  - Often seen in the elderly
  - Lack of the Intrinsic Factor in the stomach
  - Vegetarians, not true for lacto-ovo vegetarians
  - The common symptom is fatigue

History of B_{12}

- **Dr. Newcastle**
  - Fed anemic patients his regurgitated gastric juices
- **Dr. Whipple**
  - Bled dogs to induce anemia
  - Raw liver cured the fastest
- **Minot and Murphy**
  - Confirmed that the key compound was within the liver tissue
- **Karl A. Folkers and Alexander R. Todd**
  - Discovered, Isolated, and named cobalamine

Structure of Cyanocobalamin B_{12}

- Dr. Dorothy Crowfoot Hodgkin
  - 1950's
  - X-Ray Crystallography

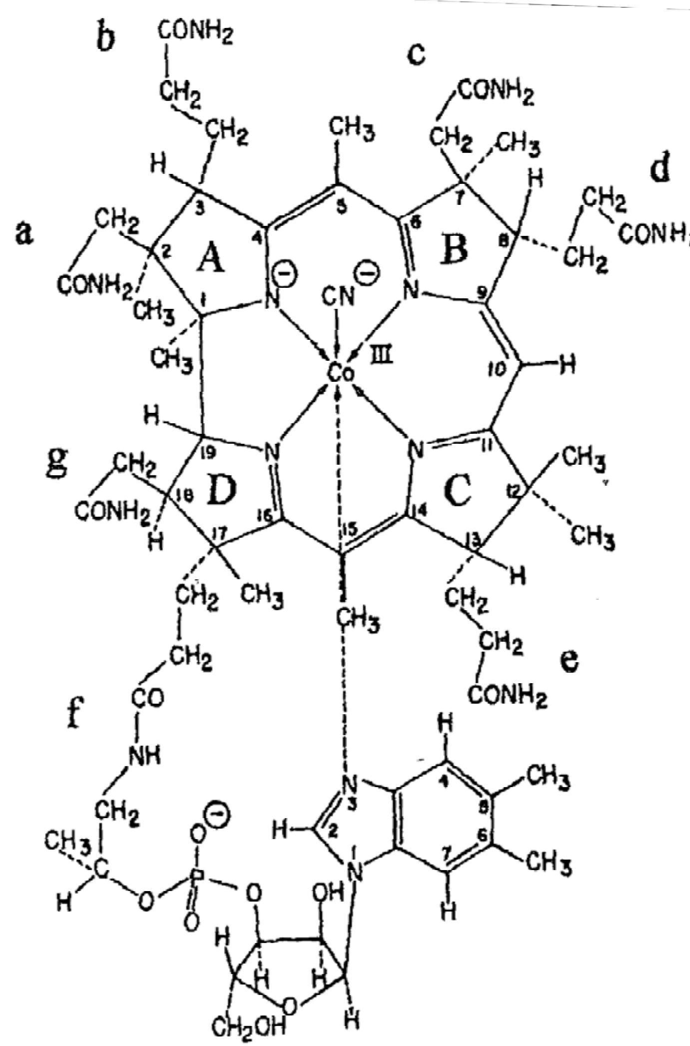
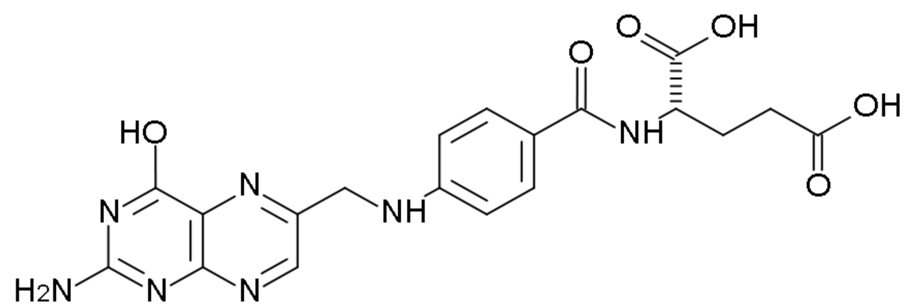


FIGURE 1 The molecular structure of B_{12} (cyanocobalamin) with rings A–D, side chains a–g, and ring carbon atoms 1–19 labeled.

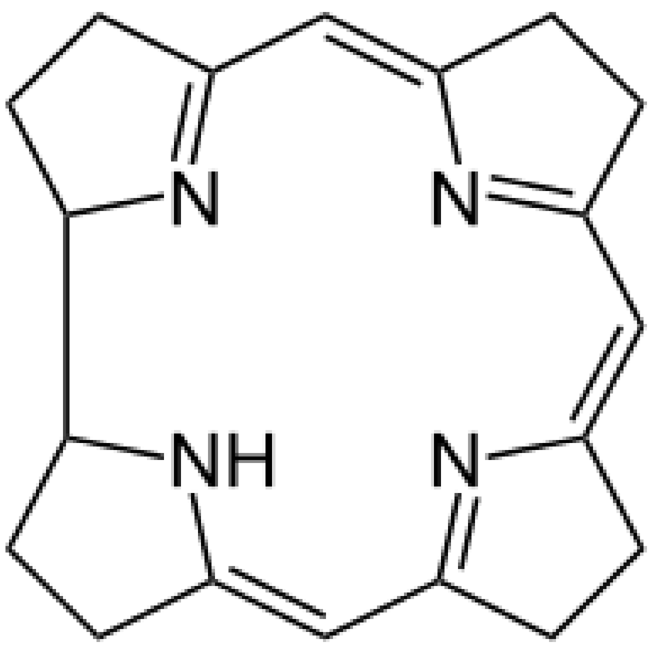
Functions of B_{12}

- Involved in the metabolism of every cell of the body
  - DNA synthesis and regulation
- Used to regenerate folate in the body
  - Most B12 deficient symptoms are actually folate deficient symptoms
  - Due to poor synthesis of DNA when the body does not have a good supply of folic acid for the creation of thymine

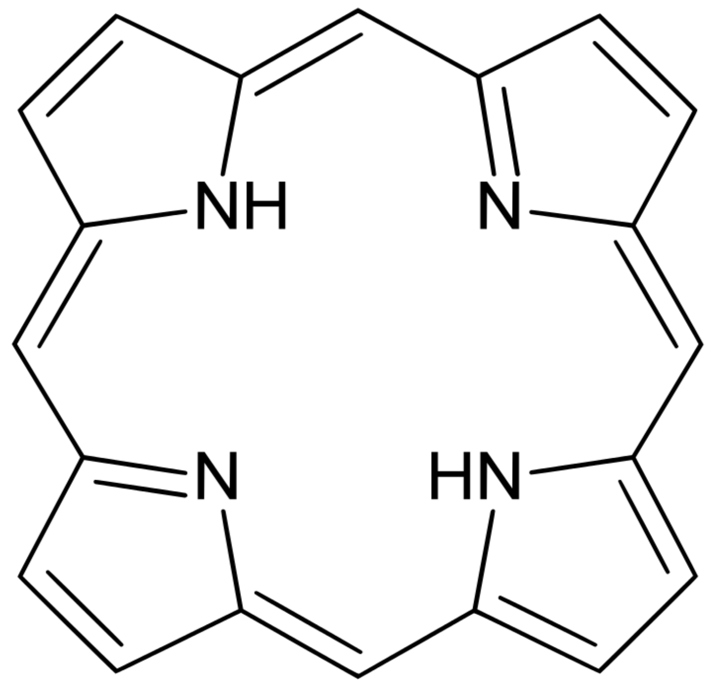


Folic Acid

Corrin vs. Porphyrin



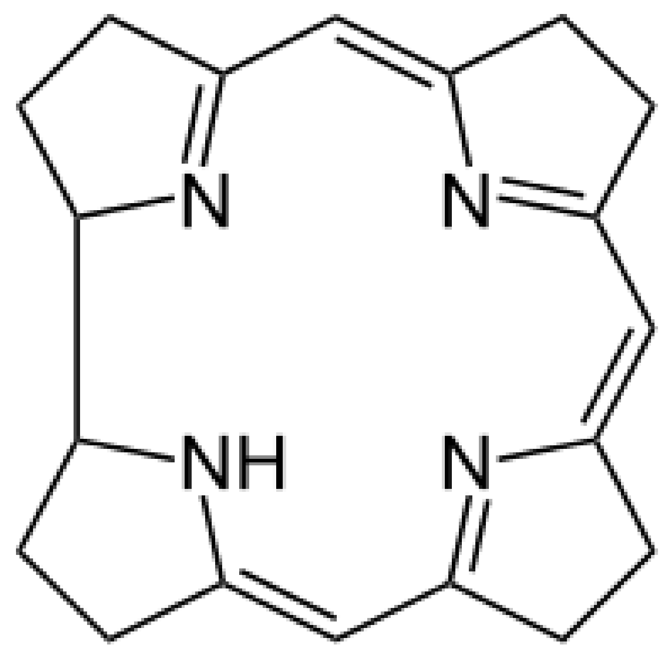
Corrin Ring



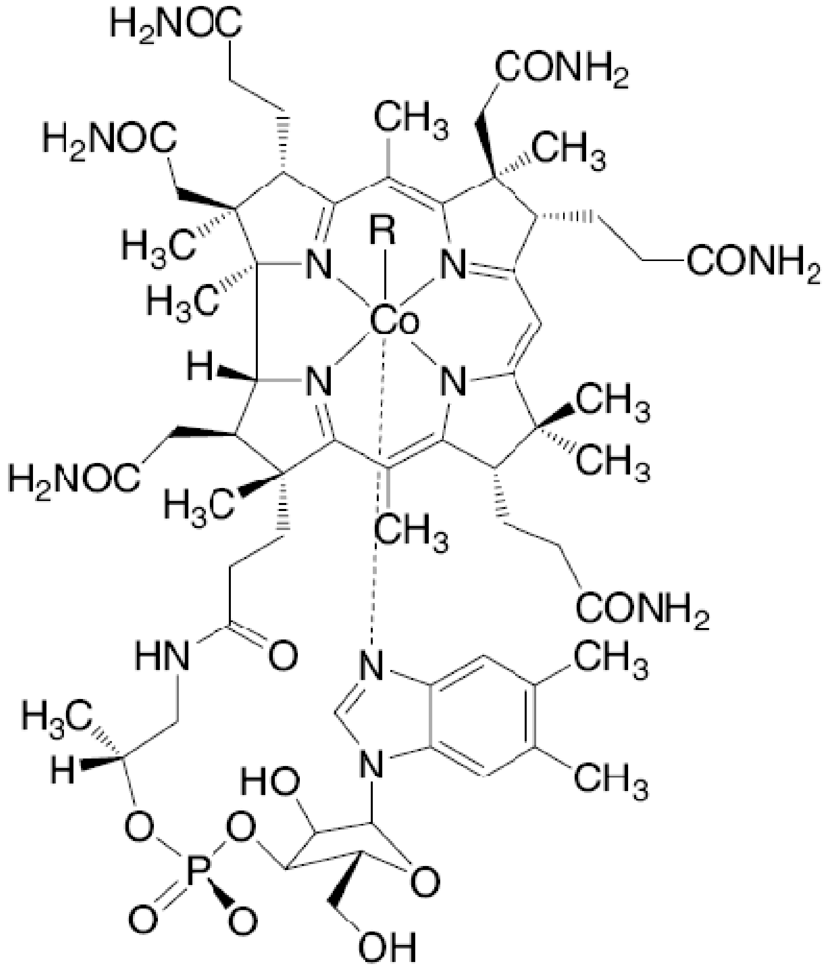
Porphyrin Ring

Corrin vs. Porphyrin

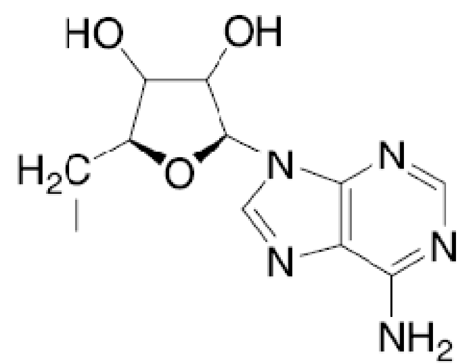
- High number of sp^3 carbon centers
- More flexible
- Not as flat as a porhpyrin ring
- Relatively high rigidity and resistance to change in electronic structure
- Two tautomeric forms



Cobalamins



R = CN (Vitamin B_{12})
R = CH_3 (Methylcobalamin)
R = 5'-Desoxyadenosyl
(Coenzyme B_{12})



5'-Desoxyadenosyl (-CH_2Ad)

Scheme 1.14 Vitamin B_{12}, methylcobalamin and coenzyme B_{12}.

Known Enzymatic Reactions

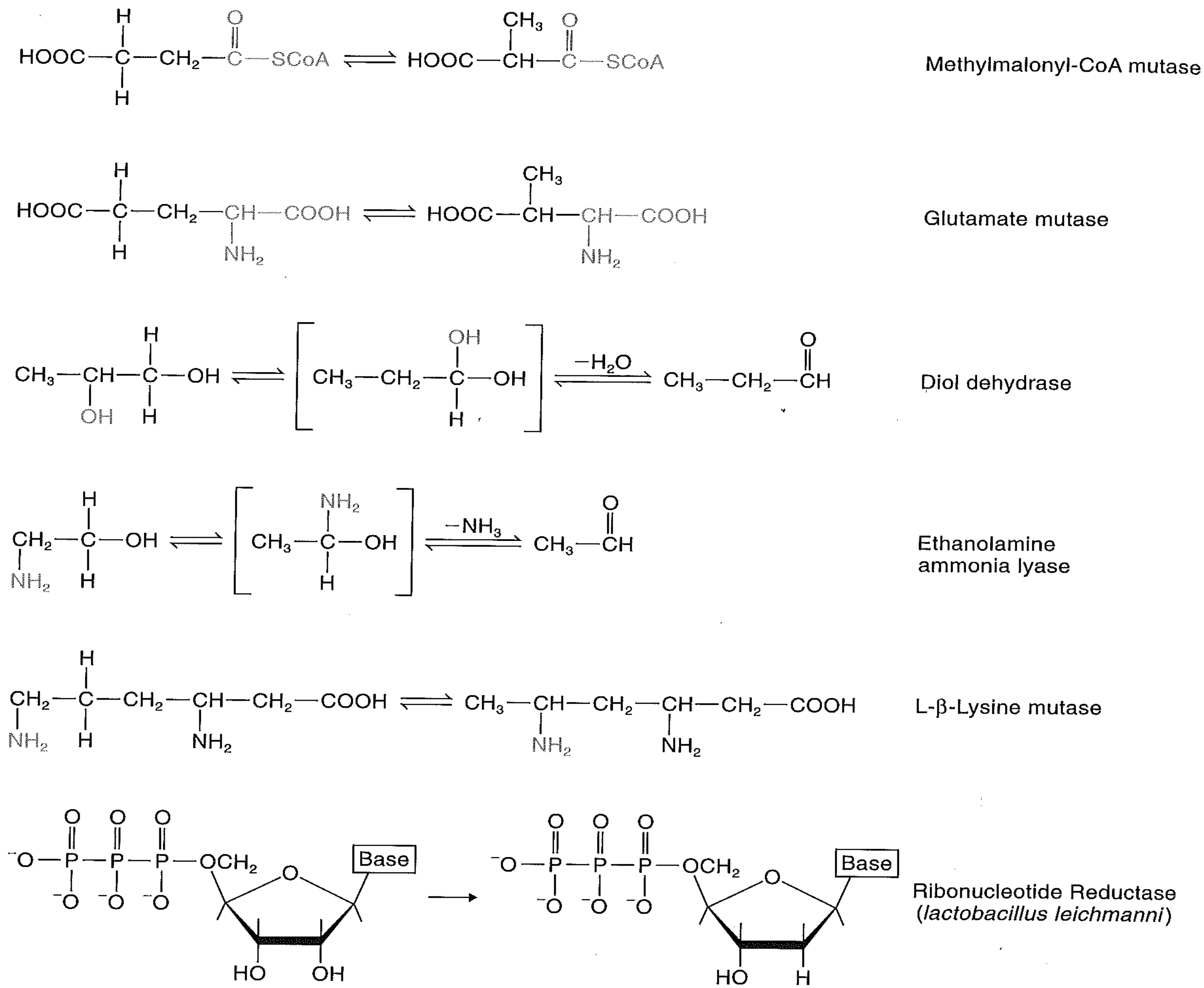


Figure 11.28
Reactions catalyzed by coenzyme B_{12}-dependent enzymes.

1,2 Shift Mechanism

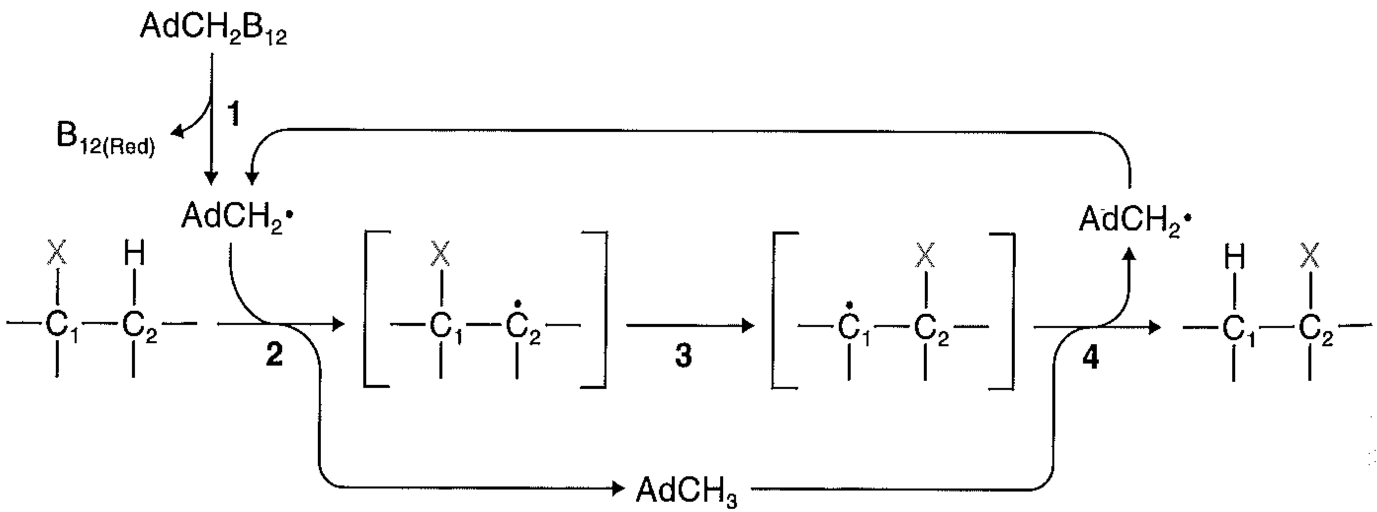


Figure 11.29
Mechanistic scheme for the 1,2-shift that occurs in many coenzyme B_{12}-activated enzymes.

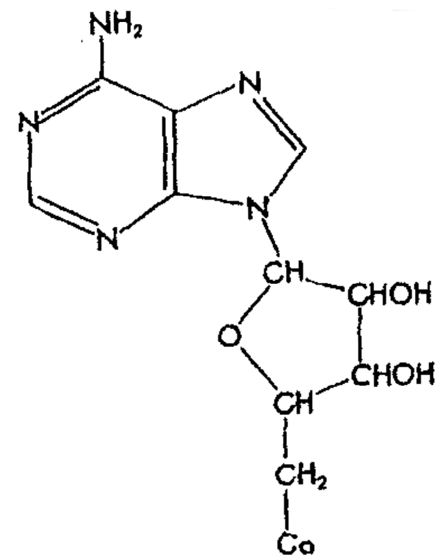
Coenzyme B_{12}-catalyzed reactions

2. Methyl ($-CH_3$) group transfers between two molecules

$$\text{Co–Ado} = \text{Co(II)} + \text{Ado}\cdot$$

$$\text{Co–Me} + B = \text{Co(I)} + B\text{Me}^+$$

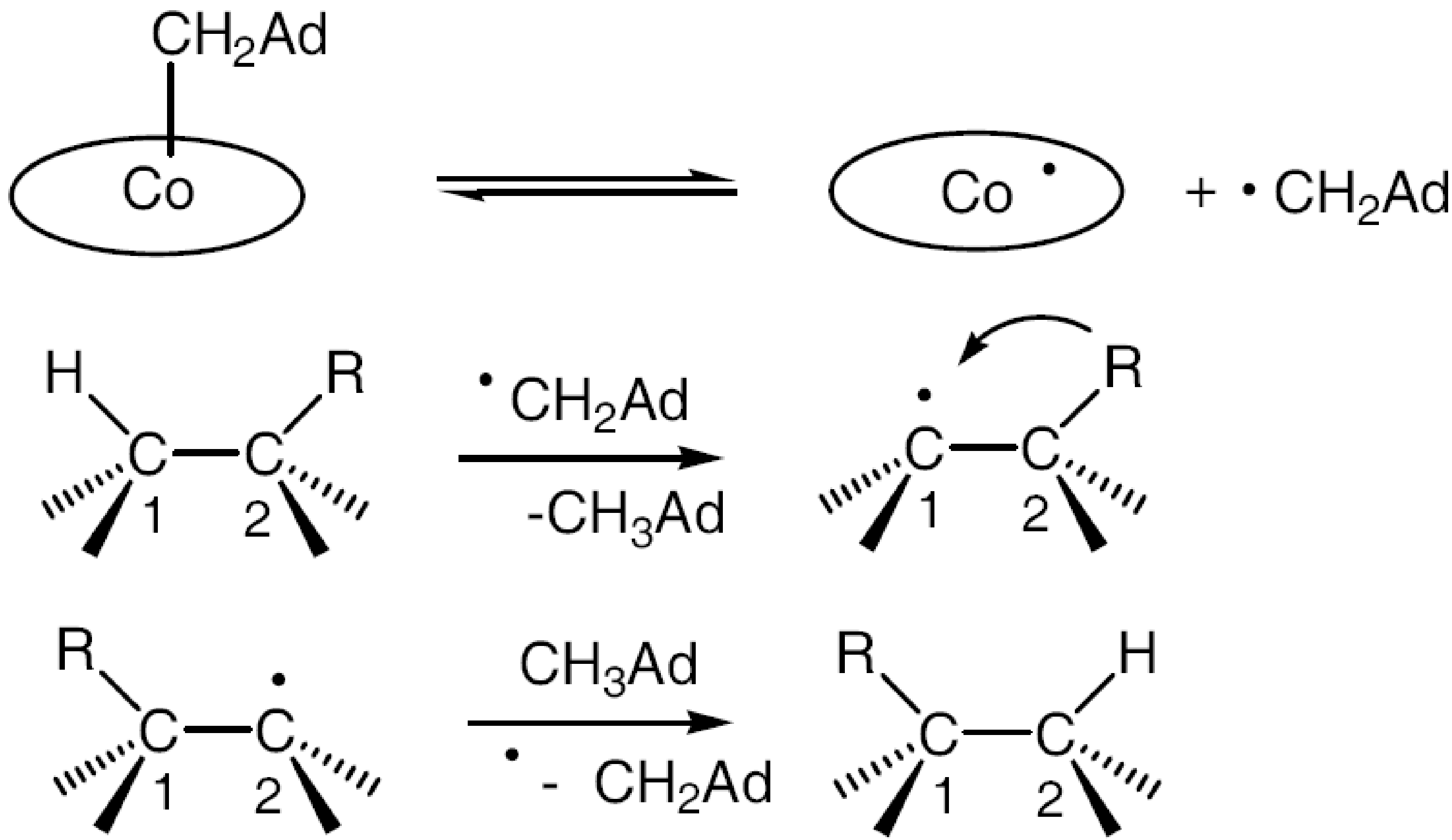
- B denotes a neutral or anionic nucleophile
- Homolytic Fission
- Heterolytic Fission



The Ado (5′-deoxyadenosyl) ligand.

Coenzyme B_{12}-catalyzed reactions

1. Rearrangements in which a hydrogen atom is directly transferred between two adjacent atoms with concomitant exchange of the second substituent, X, which may be a carbon atom with substituent, an oxygen atom of an alcohol, or an amine.



Coenzyme B_{12}-catalyzed reactions

Homolytic Cleavage:

•A reaction where a hydrogen and a group on an adjacent carbon atom exchange places.

•Takes place by way of a radical mechanism. (i.e. Deoxyadenosyl Radical)

•These rearrangements are very rare in organic chemistry.

Coenzyme B_{12}-catalyzed reactions

Deoxyadenosyl Radical was Characterization:

- Electron Paramagnetic resonance signals formed when diol dehydrase and five equivalents of coenzyme B_{12} were incubated.
- Shows the generation and persistance of deoxyadenosyl radical.

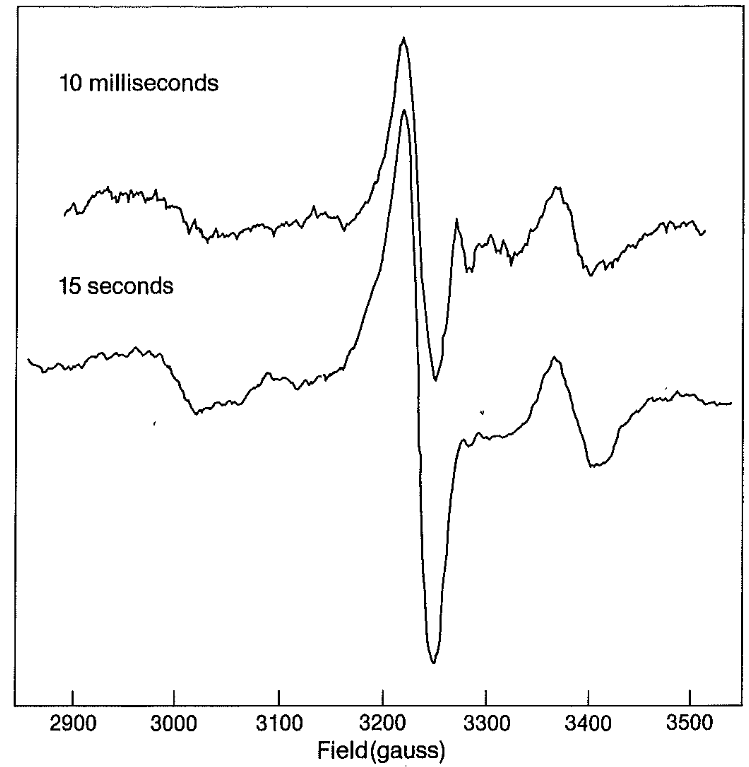
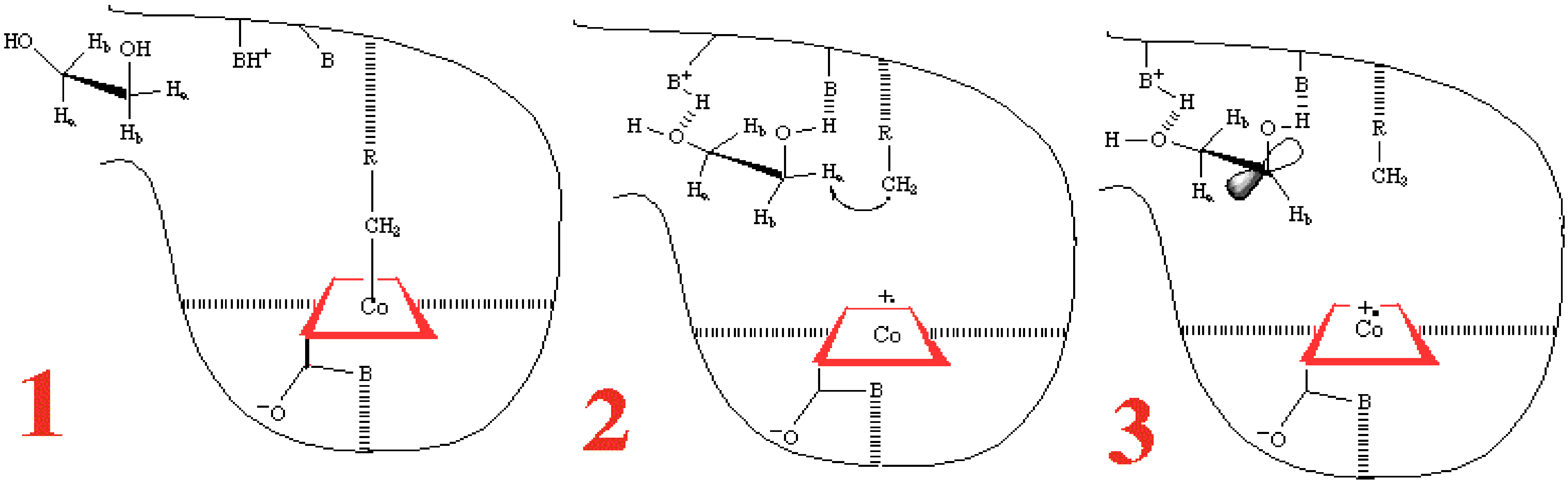


Figure 11.30
Electron paramagnetic resonance signals formed when diol dehydrase and five equivalents of coenzyme B_{12} were incubated with 1,2-propanediol, showing the generation and persistence of the deoxyadenosyl radical. (Adapted from J. E. Valinsky et al., *Journal of the American Chemical Society* **96**, 4709–4710 (1974)).

Coenzyme B_{12}-catalyzed reactions

Mechanism of Cobalt-Carbon Bonding:



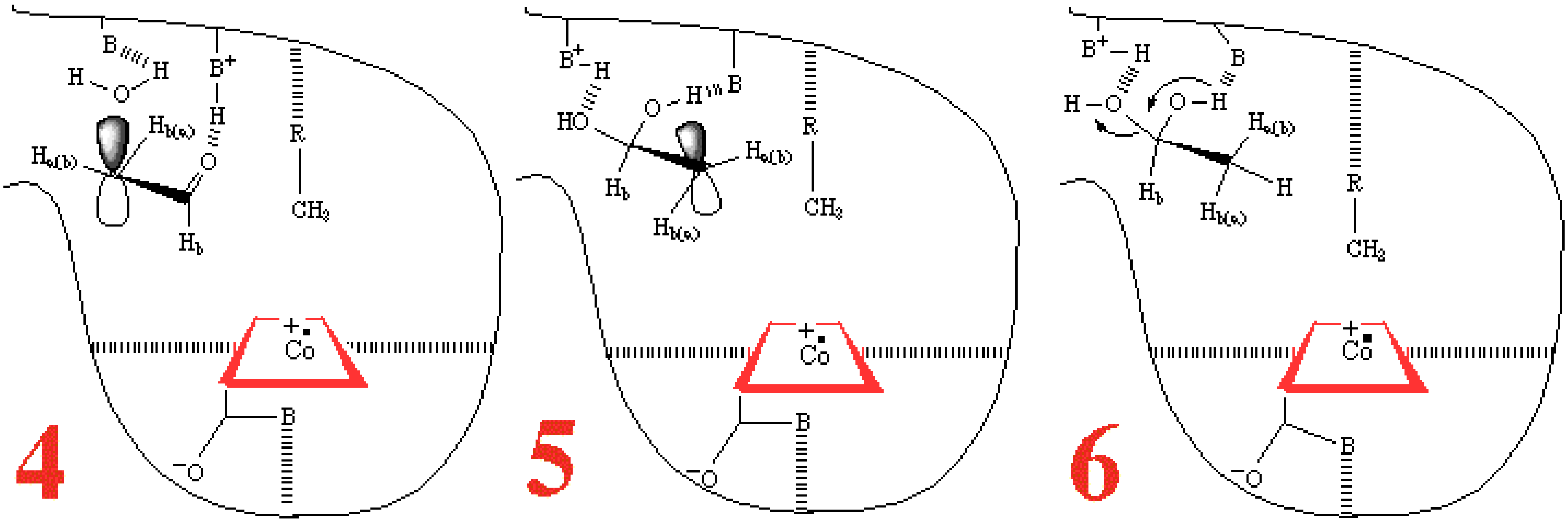
Step 1: substrate (1,2-dihydroxyethane) approaches the holoenzyme, attempting to plug the reaction 'bottle'.

Step 2: -cobalt-carbon bond breaks homolyticallly.
-is now weakened by binding of the metalloenzyme to the large coenzyme.

Step 3: hydrogen atom taken from substrate by the 5'-deoxyadenosyl radical creating a methyl.

Coenzyme B_{12}-catalyzed reactions

Mechanism of Cobalt-Carbon Bonding:



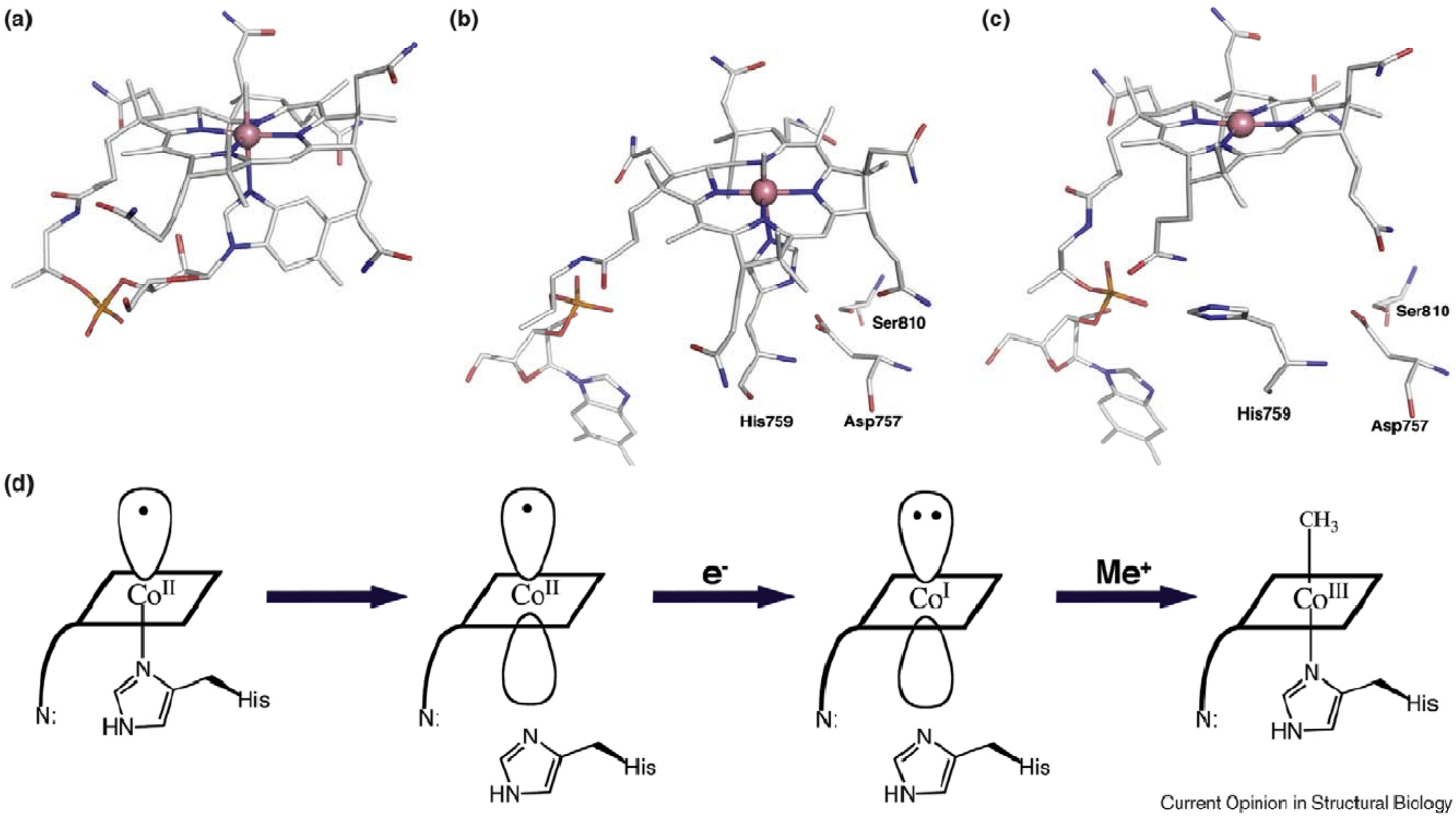
Step 4: enzyme base (B) and the acid (BH+) cause assisted beta-hydroxy fragmentation. (i.e. loss of water molecule)

Step 5: re-addition of a water molecule, resulting in a substrate reorientation of C2 towards the 5'deoxyadenosyl methyl.

Step 6: substrate dehydration and release of ethanal and a water molecule.

Coenzyme B_{12}-catalyzed reactions

Methionine synthase:

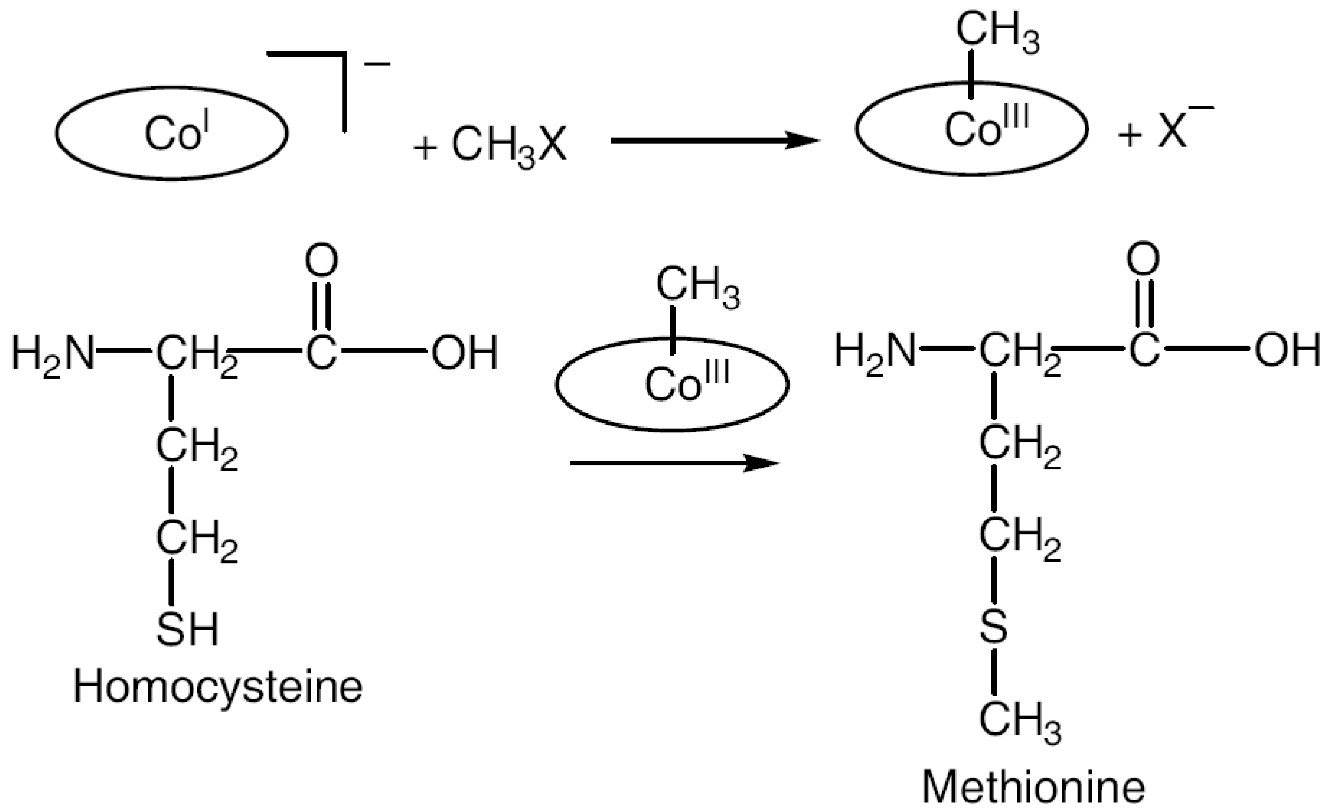


Coenzyme B_{12}-catalyzed reactions

2. Methyl ($-CH_3$) group transfers between two molecules

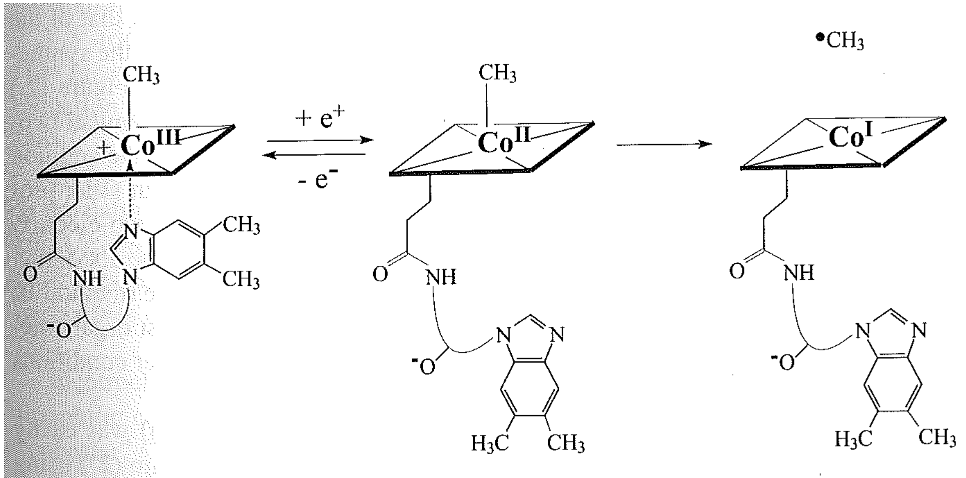
-Ability to suppress the protonation of cobalt I.

-Ability to change the equilibrium constant for methyl transfer.



Coenzyme B_{12}-catalyzed reactions

Heterolytic Cleavage:



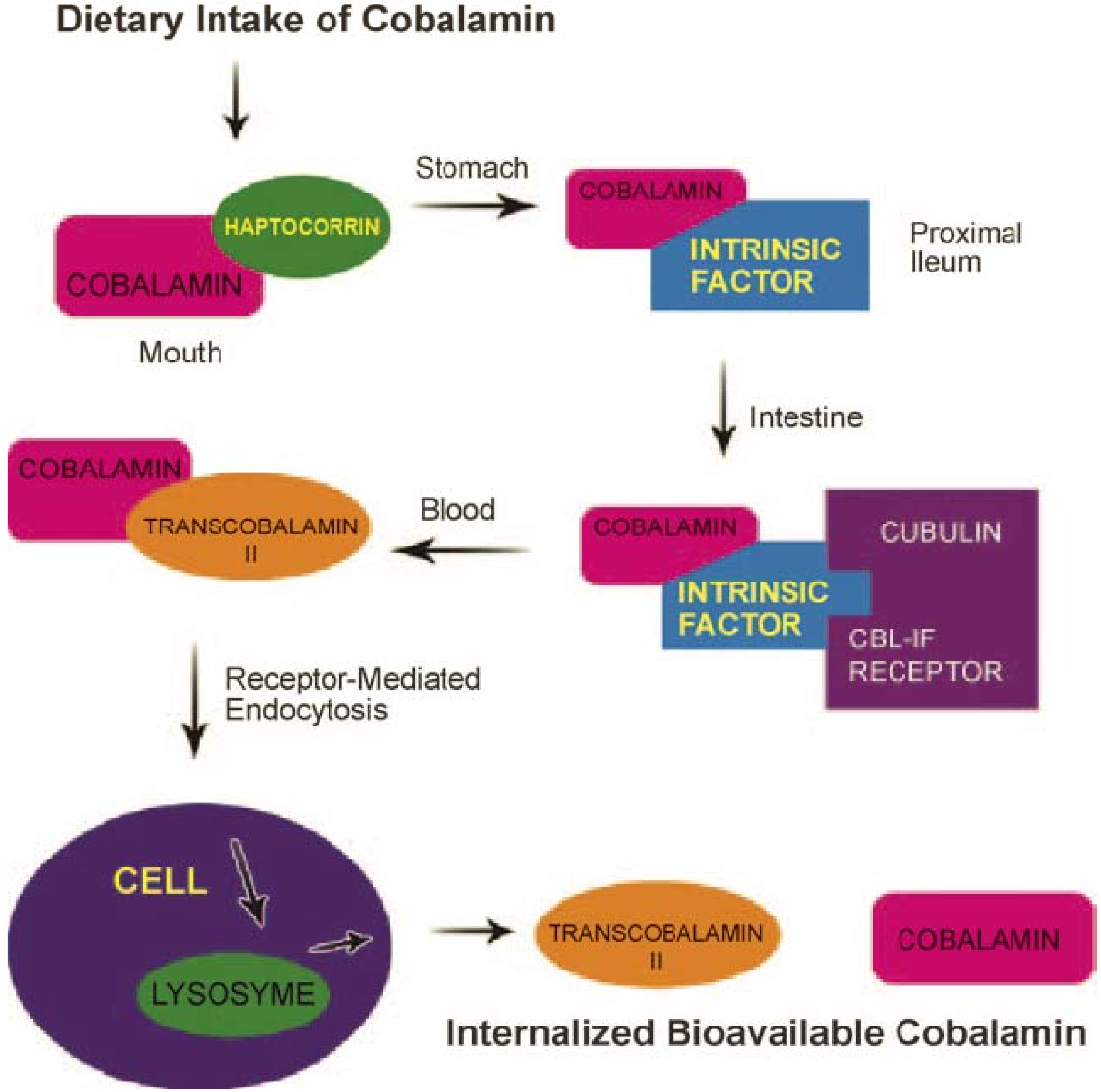
4 9⁻ 6⁻

FIGURE 11 The one-electron reduction of methylcob(III)alamin (**4**) gives methylcob(II)alamin anion (9^-) and occurs with decoordination of the nucleotide base; methylcob(II)alamin anion (9^-) rapidly decomposes into cob(I)alamin (6^-) and a methyl radical.

- For Methyl transfer reactions involving CH3-B12, Co(I) is involved. Reaction catalysed by methionine synthase. This involves two methyl group transfers:
- Me-cob(III)alamin + homocysteine ---> cob(I)alamin + methionine
- cob(I)alamin + methyltetrahydrofolate ---> Me-cob(III)alamin + tetrahydrofolate

Dietary Uptake of B_{12}

- Intrinsic Factor
- Haptocorrin
- Transcobalamin II

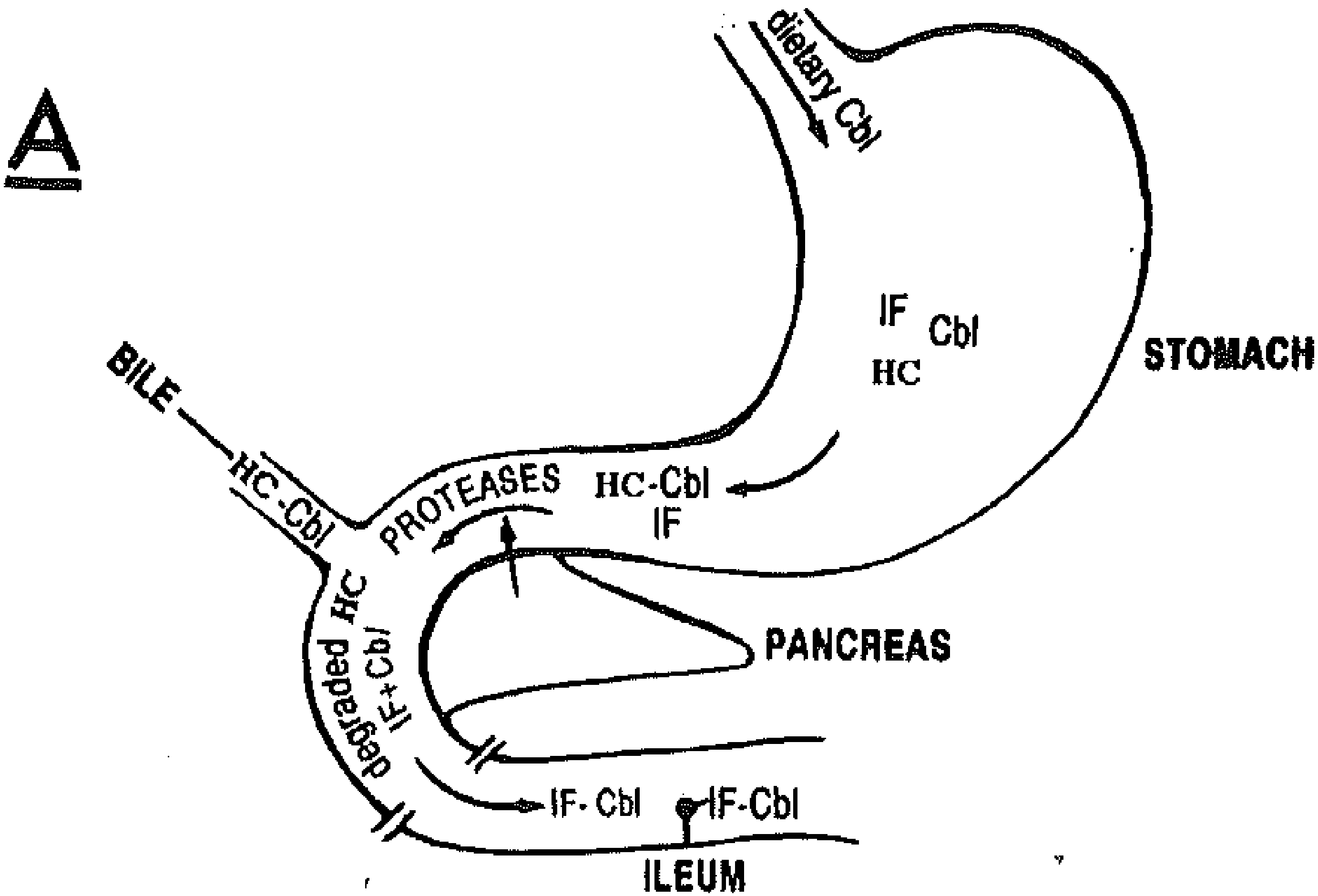


Dietary Uptake of B_{12}

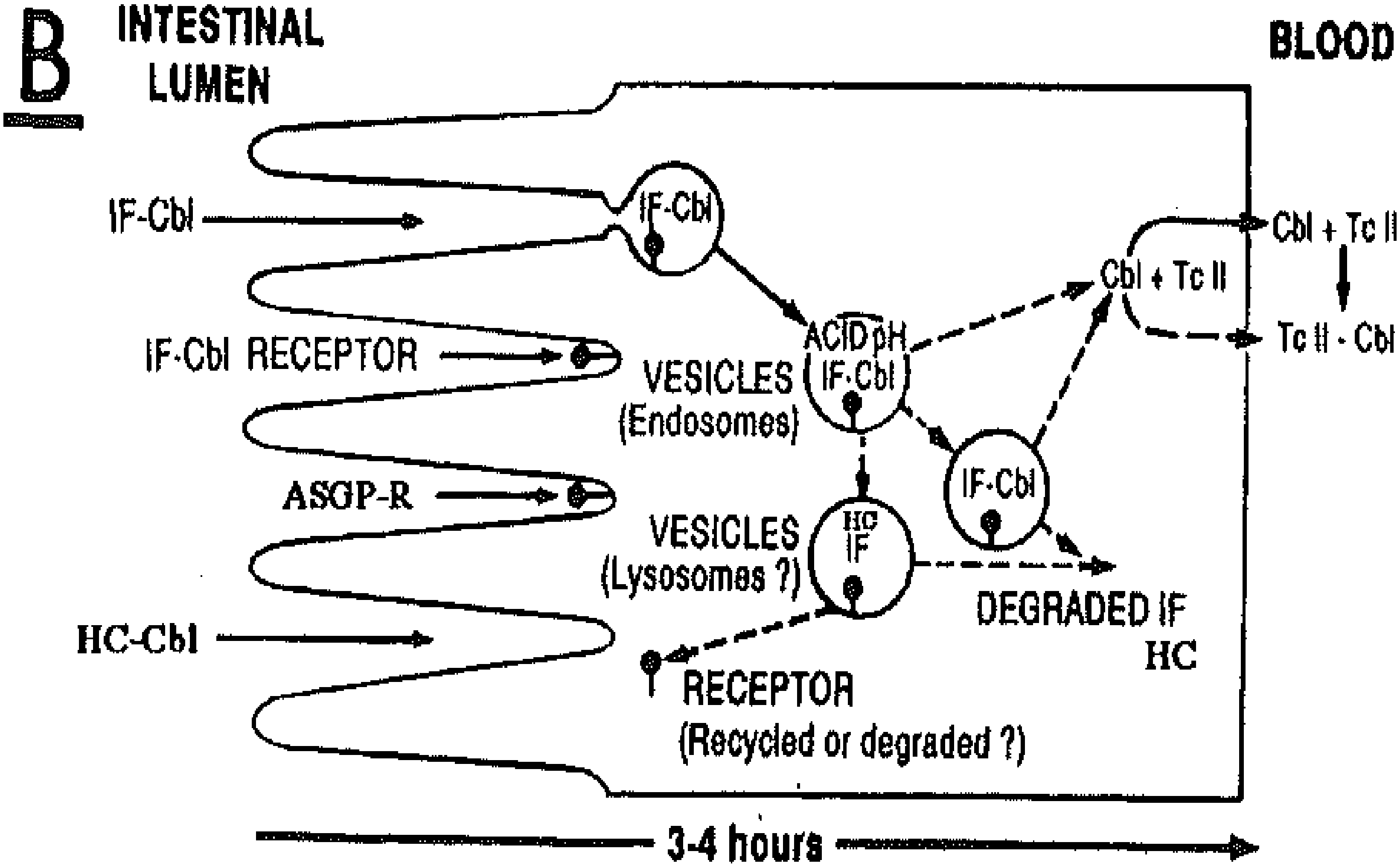
TABLE 1 Properties of Cbl-Binding Proteins

Property	IF	Hc	TC-II
Production	Gastric body Medium-sized ducts (salivary, pancreatic)	Granulocytes, yolk sac, mammary glands, salivary acini, and ducts	Most tissues
Carbohydrate	15%	33–40%	0
M_r	45 kDa (human)[a] 55 kDa (hog)[d]	60 kDa (rabbit)[b] 58 kDa (human)[e]	44 kDa (human)[c]
# subunits	1	1	1
# Cbl binding sites	1	1	1
K_a for Cbl*	1–4 nM[a]	0.1 nM[e]	0.02 nM[f]
pI	4.7–5.8[g]	3.5–4.3[g]	6.4[c]

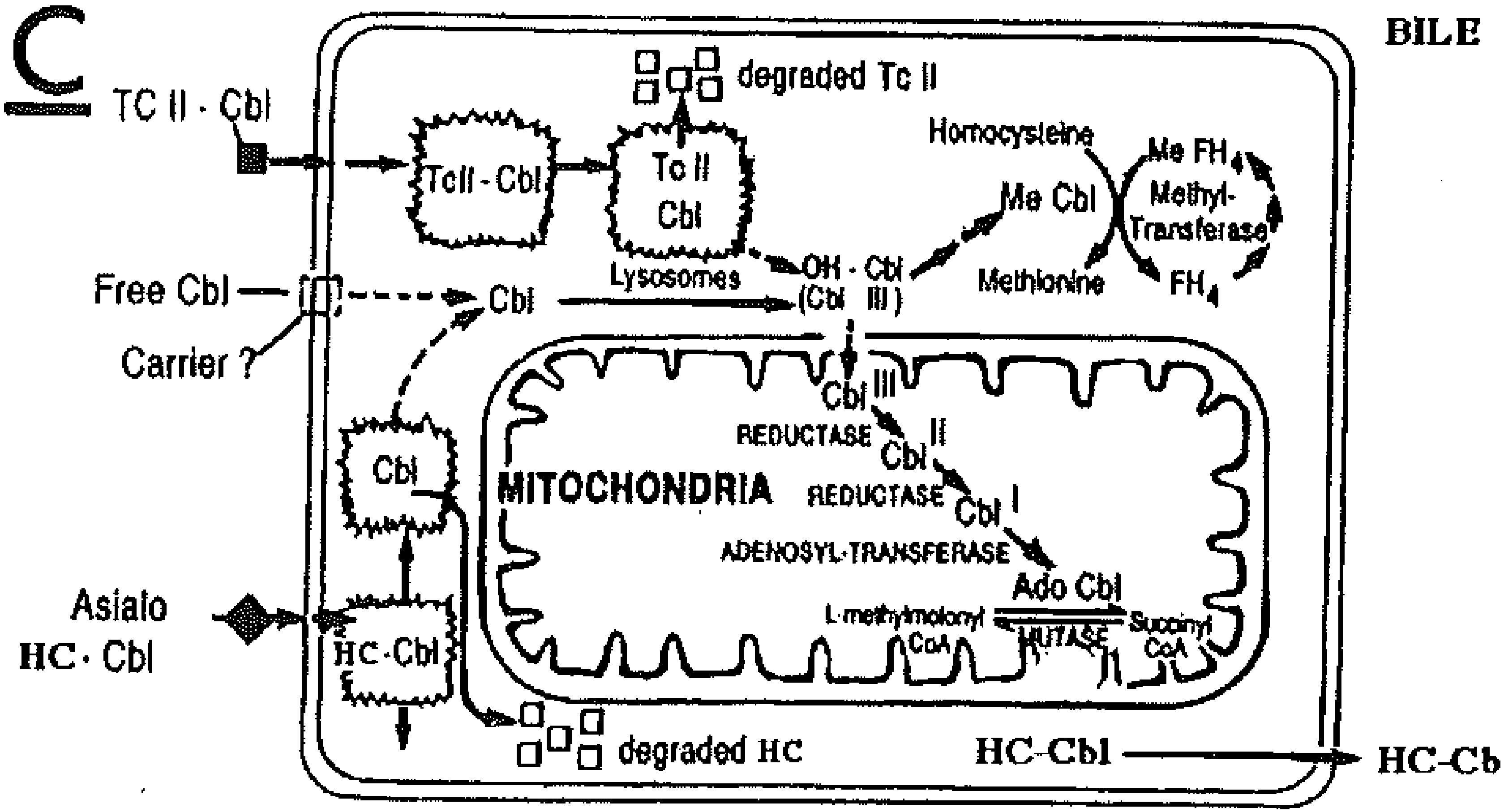
Dietary Uptake of B_{12}



Dietary Uptake of B_{12}



Dietary Uptake of B_{12}



B_{12} as a Drug Transport Vehicle

In order to take advantage of the pathway shown in slide 20:

- Need to couple B_{12} with the peptide/protein such that neither molecule obstructs the other

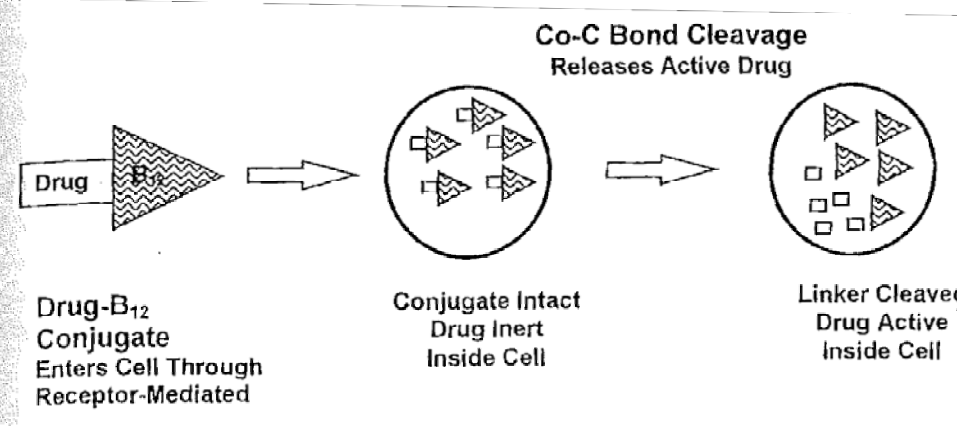


FIGURE 6 The inactive drug–cobalamin bioconjugate enters cancer cells through receptor-mediated endocytosis. Once the biconjugate is inside the cell, the linker between cobalamin and the drug is cleaved through a process that may be enzymatic or may depend upon the redox state or pH of the intracellular environment.

- Used to deliver therapeutic peptides
- Potential to aid absorption from the gastrointestinal tract increase plasma-residency time
- Limited by the quantity of B_{12} that may be absorbed from the intestinal lumen with a given dose

References:

1.) Lenhert , P.G.; Hodgkin, D.C. *Nature* **1961** *192*, 937
2.) Lenhert, P.G. *Proc.Roy.Soc.* **1968** A303, 45
3.) Mancia, F.; Keep, N.H.; Nakagawa, A.; Leadlay, P.F.; McSweeney, S.; Rasmussen, B.; Bosecke, P.; Diat , O.; Evans, P.R. *Structure* **1996**, *4*, *229*
4.) Briat, B.; Djerassi, C. *Soc. Chim. France* **1969** *135*
5.) Johansen, H.; Ingraham, L. L.; *J. Theor. Biol.* **1969** *23*, 191
6.) C. Brink, D. C. Hodgkins, J. Lindsey, J. Pickworth, J. H. Robertson, J. G. White, *Nature* **1954** *174*, 1169
7.) J. M. Pratt, *Inorganic Chemistry of Vitamin B_{12}*, Academic Press, London 1972
8.) Gaudemer, A., Zylber, J., Zylber, N., Baran-Maraszac, M., Hull, W. E., Fountoulakis, M., Konig, A., Wolfle, K., Retey, J. 1981 *Eur. J. Biochem. 199, 279-285*
9.) Leutherner, B., Leutwein, C., Shulz, H., Horth, P. Haehel, W., Schiltz, E., Schagger, H., Heilder, J. 1998, Mol. Microbiol. *28, 615-628*
10.) Lu. W.-P., Harder, S. R., Ragsdale, S. W. **1990**, *J. Biol. Chem.* 265, *3124*-3133
11.) Jarret, J. T. Choi, C. Y., Matthews, R. G. **1997**, *Biochemistry* **1926***, 15739-15748*
12.) Rusling, J. F., Miaw, C.-L., Couture, E. C. **1990***, Inorg. Chem.* **1929**, *2025*-2027
13.) B. Briat, C. Djerassi, *Bull. Soc. Chim. France* **1969** 135
14.) Jerrett, J. T., Choi, C. Y., Matthews, R. G. **1997**, *Biochemistry 36, 15739-15748*
*15.) Lexa, D., Saveant, Soufflet, J. P. **1979**,* J. Electroanal. Chem. *100, 159-172*
16.) Schefford, R., Rytz, G., Walder, L. **1983***,* Modern Synthetic Methods. Transition Metals in Organic Synthesis pp. 335-440
17.) Rusling, J. F., Miaw, C.-L., Couture, E. C. **1990**, *Inorganic. Chem.* 29, 2025-2027
18.) Connors, T. F., Arena, j. V., Rusling, J. F. **1988**, *J. Phys. Chem.* 92, 2810-2816
19.) Scheffold, R. 1988, *Nachr. Chem. Tech. Lab.* **1936**, 261-267
20.) Auer, L., Weymuth, C., Scheffold, R. **1993**, *Helv. Chim. Acta.* **1976**, 810-818
21.) Busato, S., Tinembart, O., Zhang, Z.-d., Scheffold, R. **1990**, *tetrahedron* 46, 3155-3166
22.) Busato, S., Scheffold, R. **1994**, *Helv. Chim. Acta.* 77, 92-99
23.) Steiger, B., Walder, L., Scheffold, R. **1986**, *Chimia* 40, 93
24.) Howard, W. A., Bayomi, A., Natarajan, E., Aziza, M. A., El-Ahmady, O., Grissom, C. B., West, F. G. 1997, *Bioconj. Chem.* 8, 498-502
25.) Ramanujam, K. S., Seetharam, S., Seetharam, B. **1992**, *Biochem. Biophys. Res. Comm. 182,* 439-446