Bioconjugates

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Conditions

1. 5-7 lectures plus substantial homework

2. Participation at lectures > 70 %

3. Examination: 25-30 min presentation based on literature

Website:
http://szerves.chem.elte.hu/oktatas/ea/Hudecz/index.htm#biokonjugatumok

Email: ferenc.hudecz@ttk.elte.hu

Outline: Protein bioconjugates

1. Historical background

2. Functional groups of proteins/glycoproteins
   - N-nucleophiles: -NH_2, imidazole, indole, guanidino
   - S-nucleophiles: -SH, CH_2-S-CH_3
   - O-nucleophile: -OH
   - O/C-nucleophiles: -CHO, -COOH, -$CONH_2$

3. Creation of reactive groups → Introduction, Transformation
   - Limited reactivity (eg. – OH vs. - CHO)
   - Improved selectivity (e.g. - NH_2 vs. - SH)
   - Space considerations
   - Convenient chemistry (e.g. - COOH vs. - NH_2)

4. Detection of reactive groups → Destructive, Non-destructive
   - sensitive
   - quantitative
   - quick
   - small sample

5. Conjugation
   - Chemical synthesis
   - Enzymatic synthesis (e.g. - NH_2 vs. - SH)
   - Gene technology

6. Analysis of conjugates
   - Purification
   - Structure determination

Introduction

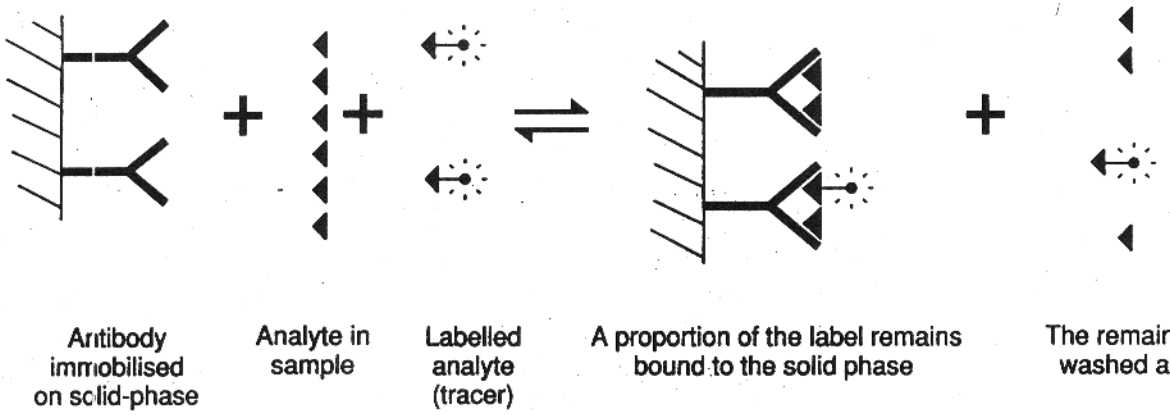
Design of bioconjugates

Why?	Synthetic antigens or drug targeting
What?	Peptide epitope, drug, reporter molecules
With What?	Protein, DNA, liposome
How?	Covalent bond

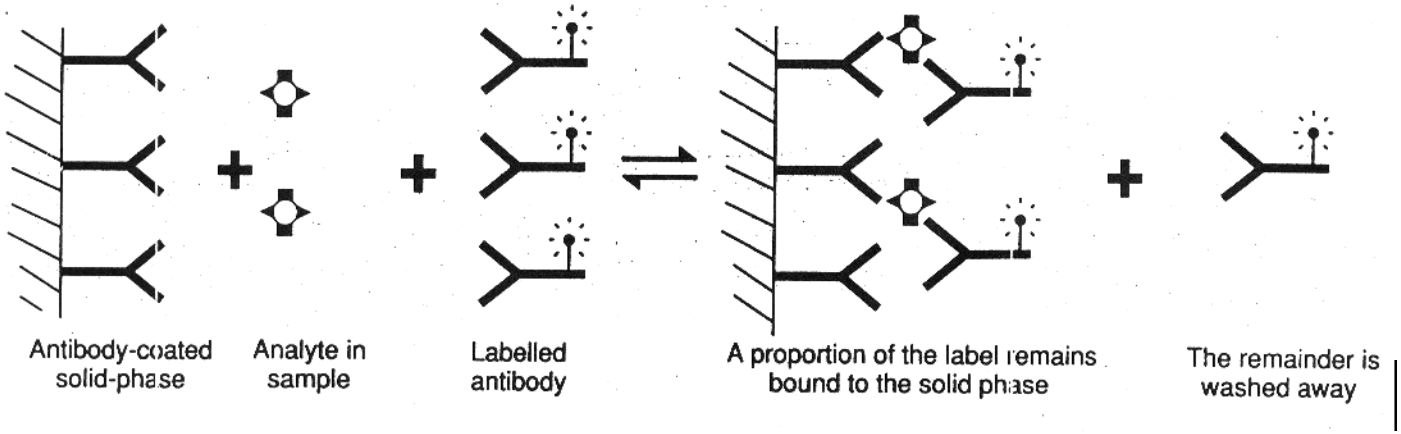
Introduction

Application of bioconjugates: immunoassays

a) competitive

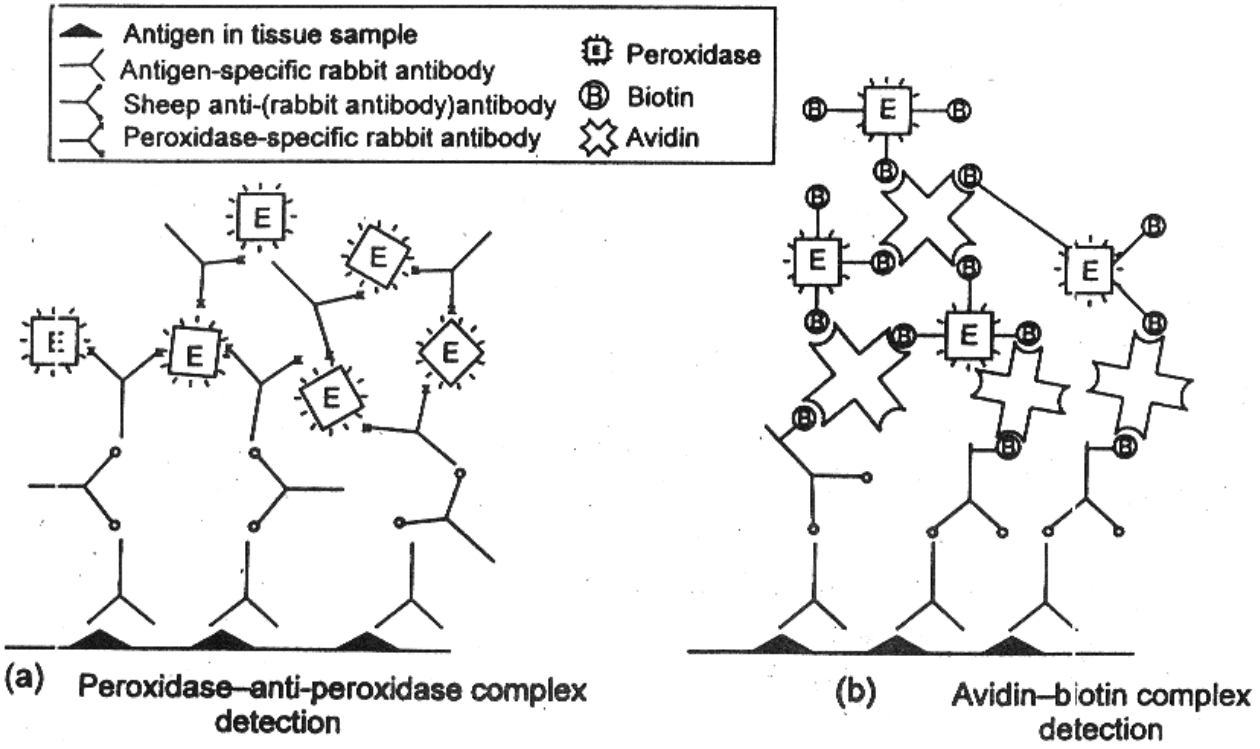


b) direct



Introduction

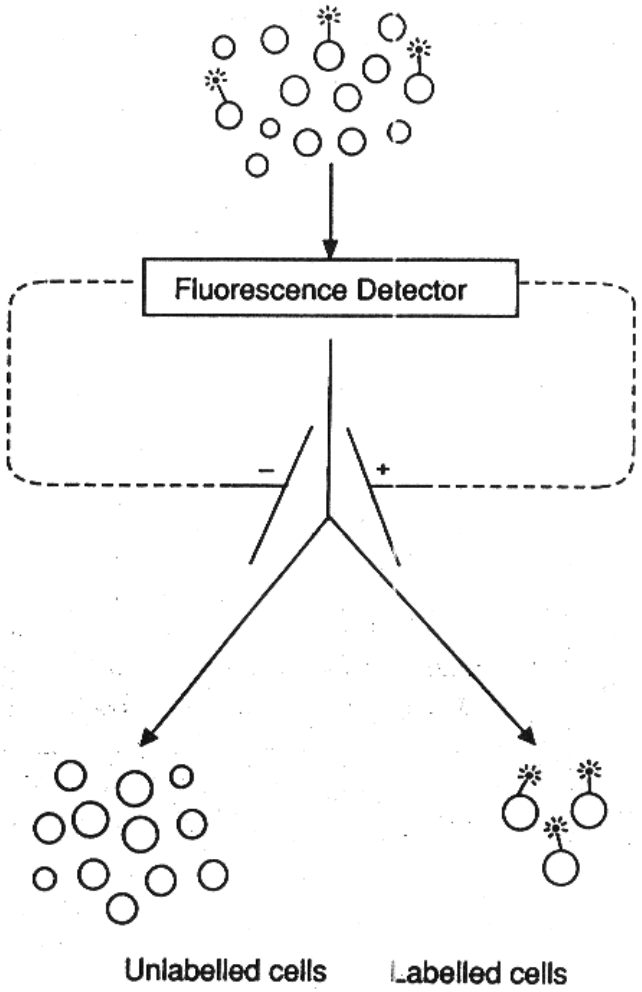
Application of bioconjugates: histo- and cytochemistry



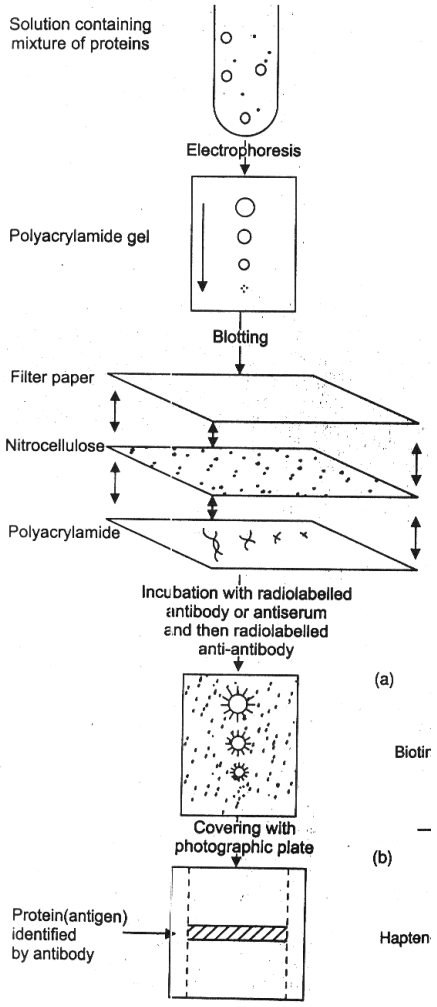
Introduction

Application of bioconjugates

FACS analysis

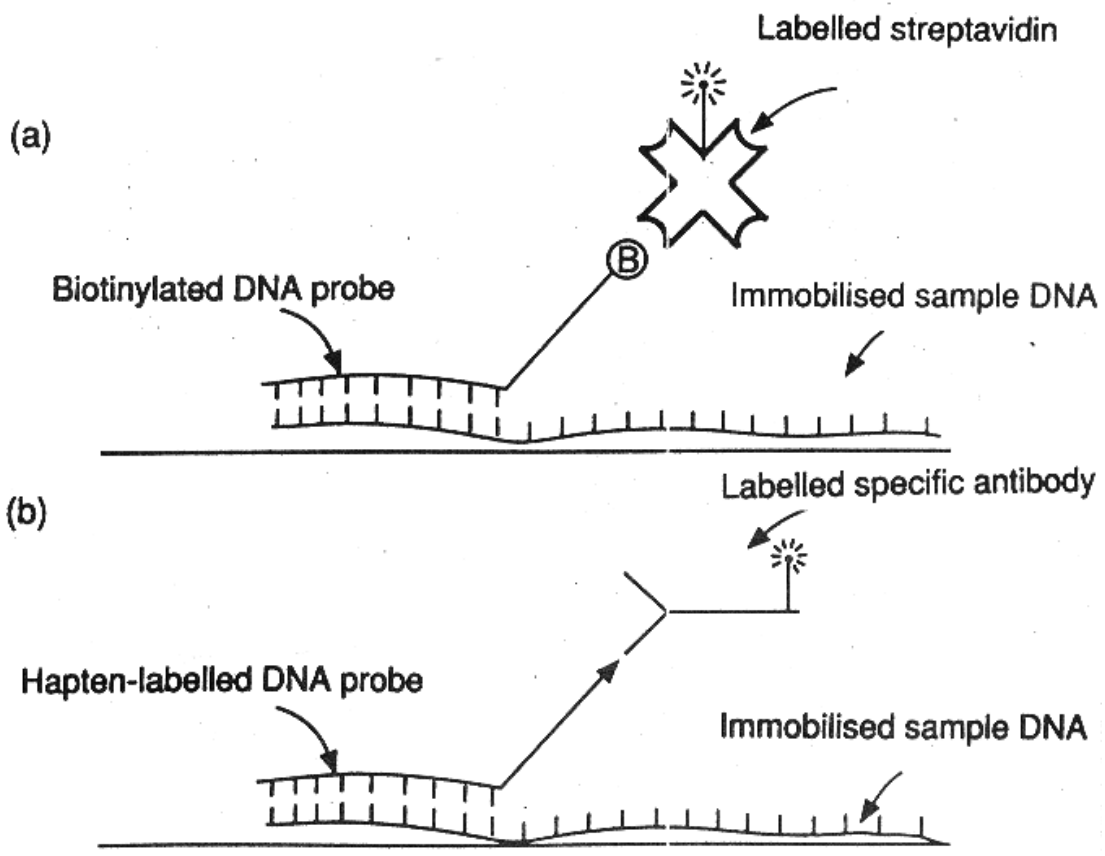


Western blotting

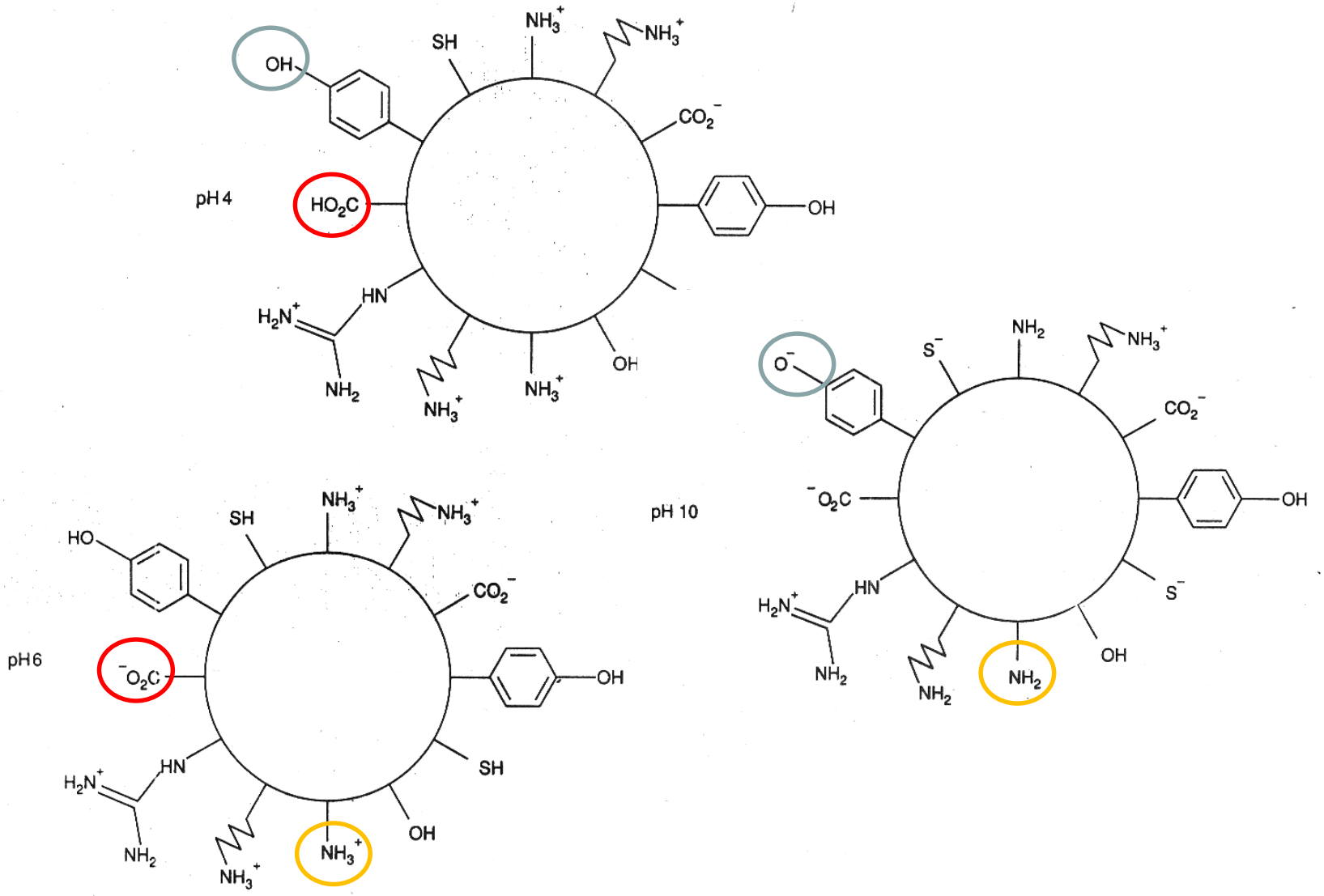


Introduction

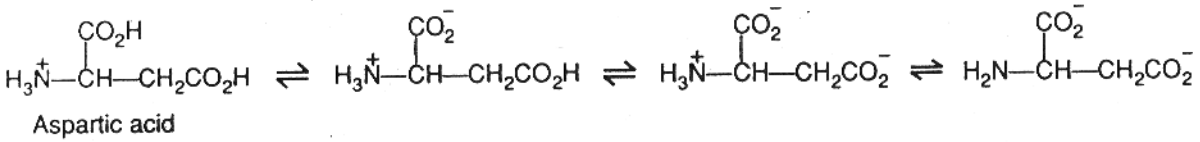
Application of bioconjugates: DNA detection

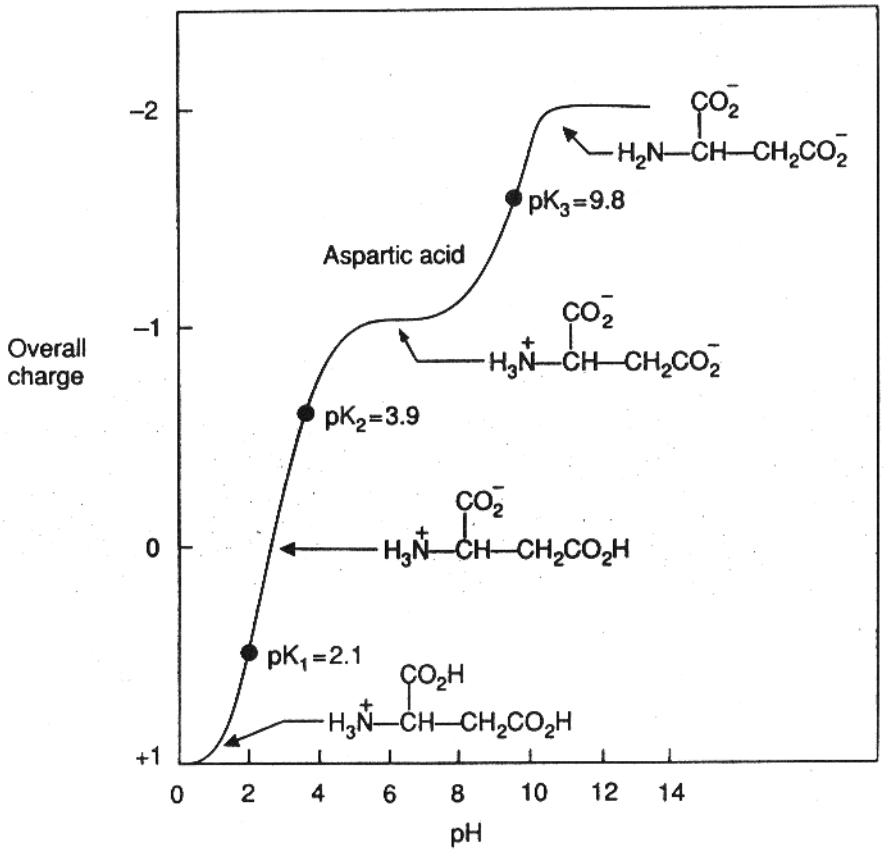


Ionisation status of proteins as function of pH

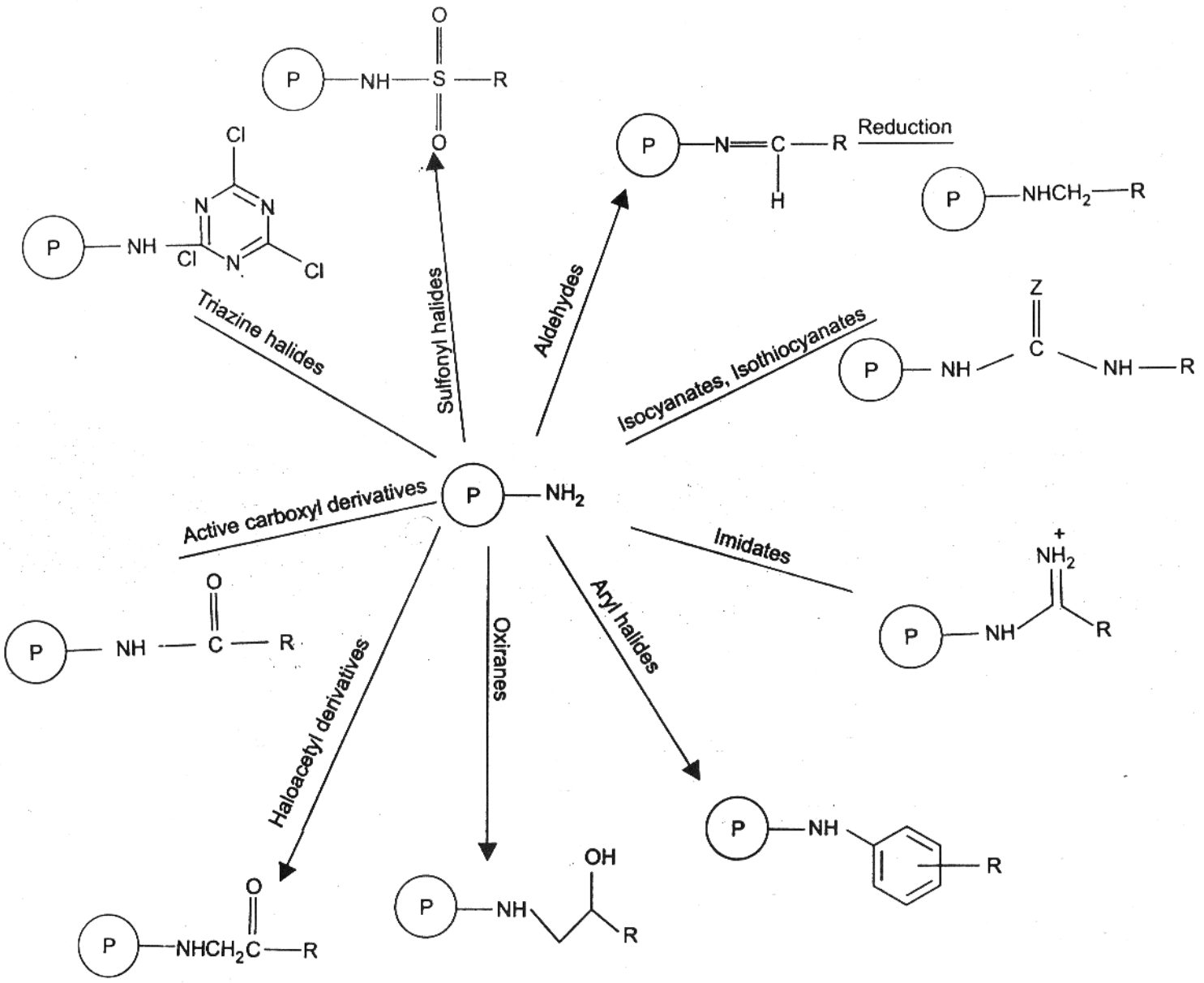


Ionisation of aspartic acid

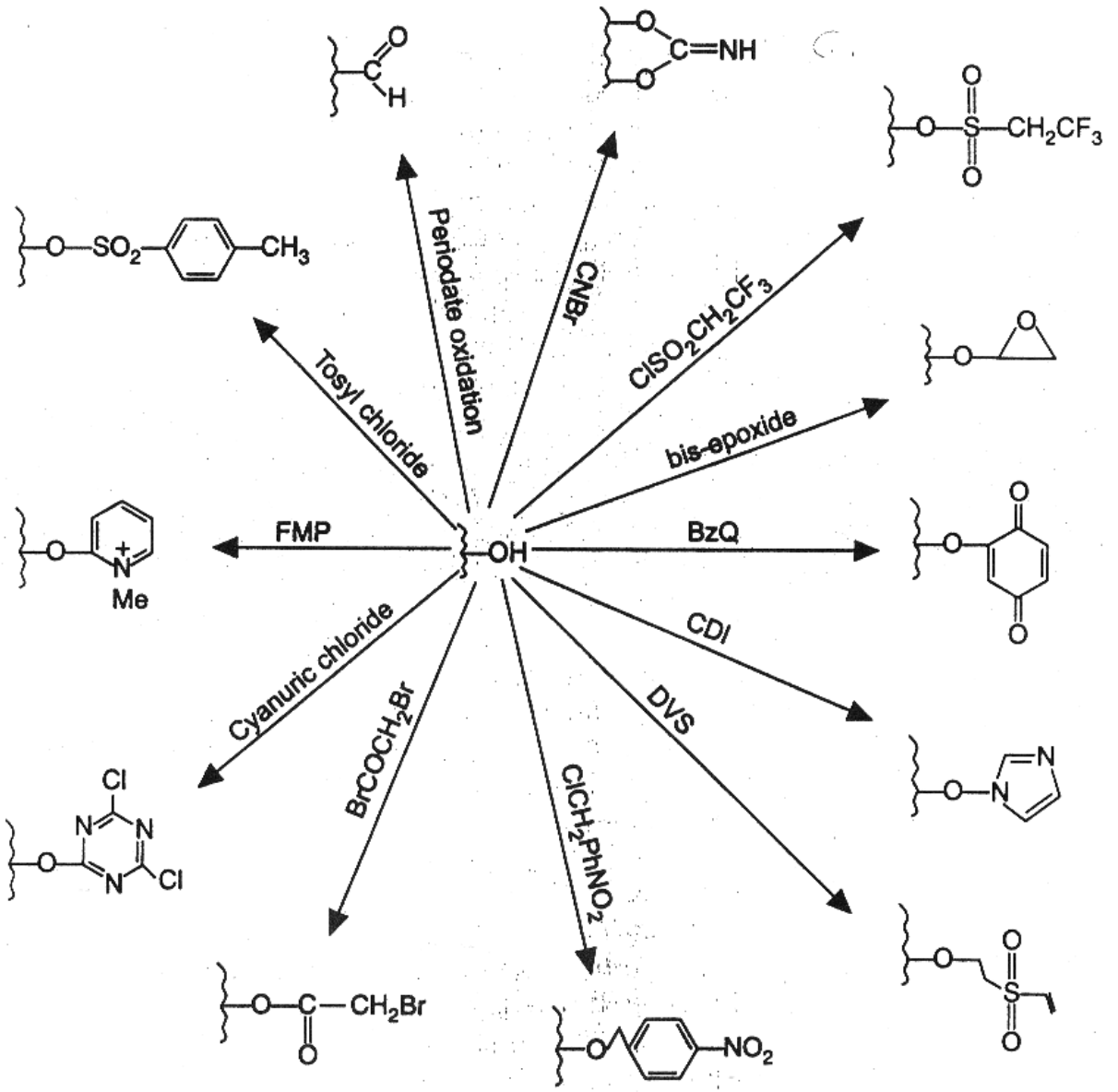




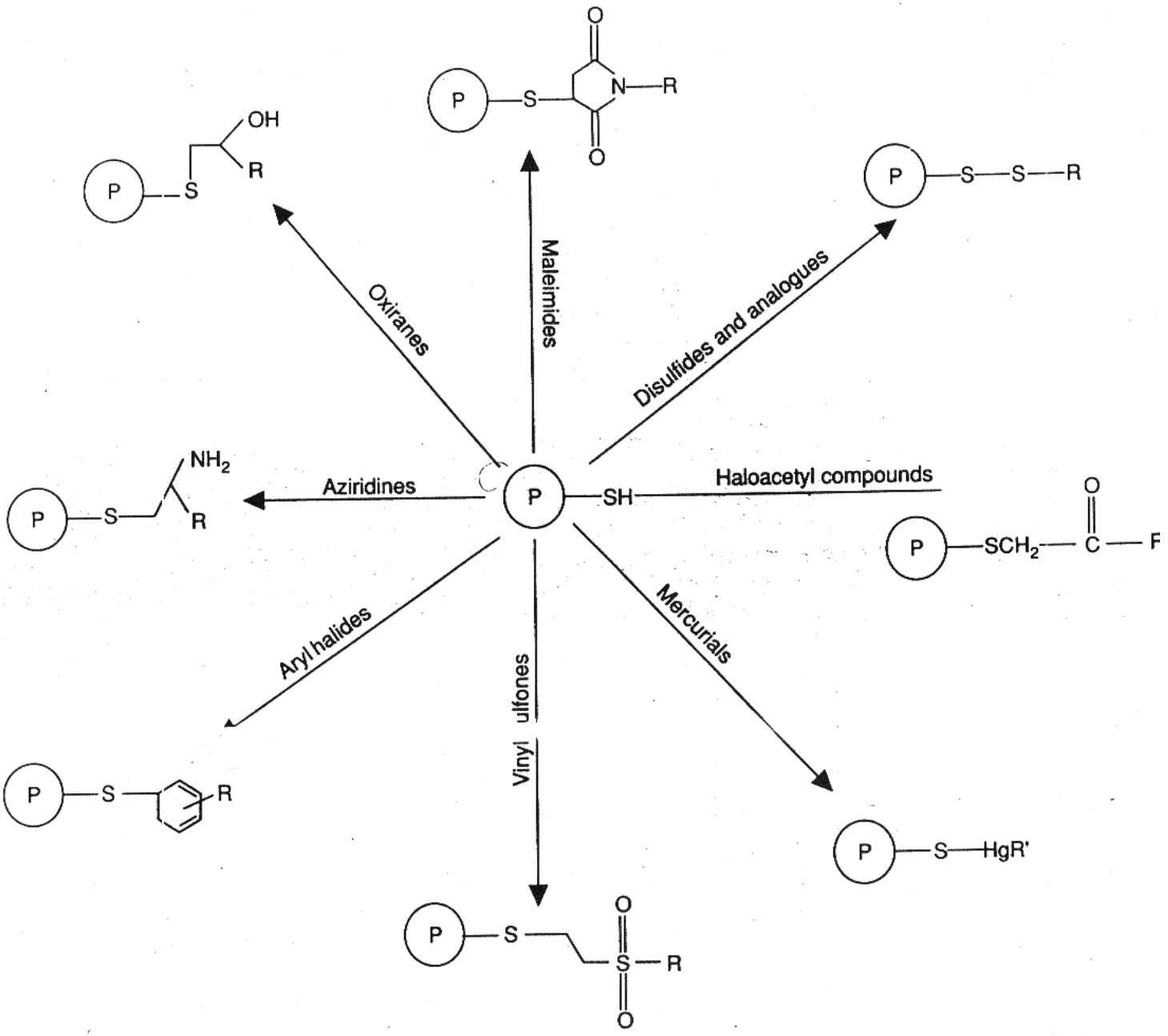
Reactivity and transformation of amino group



Reactivity and transformation of hydroxyl group



Reactivity and transformation of thiol group



Historical background

1) Establishment of protein structure – function relationship (1925 –

- Solubility

  Sumner, J.B., Graham V.A.: The nature of insoluble crease *Proc Soc Exp Biol Med* 22 504 (1925)

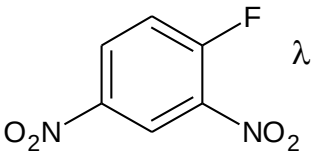
- Identification of amino acid side chain(s) necessary for the protein function

  Olcott, H.S., Fraenkel-Conrat, H.:
  Specific group reagents for proteins *Chem Rev* 41 151 (1947)
  Herriot, R.M.:
  Reactions of native proteins with chemical reagents
  Adv Prot Chem 3 161 (1947)

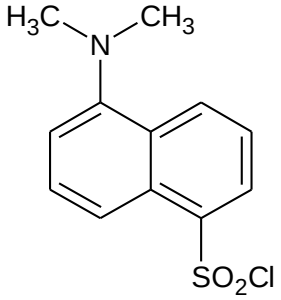
2) Structural studies

Proteins

- Determination of N-terminal amino acid



λ = 254 nm

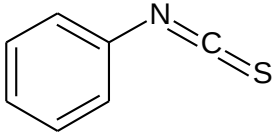


λ_s = 360 nm

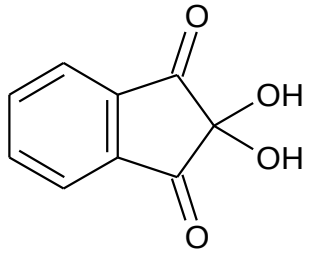
λ_e = 480 nm

- Sequencing (1956)

Edman, P., Begg, G.: A protein sequenator *Eur J Biochem* 1 80 (1967)

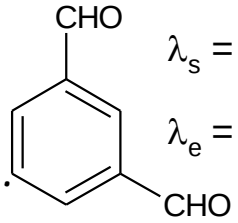


op.: -21 °C



- Determination of amino acid composition (1960)

Moore, S., Stein, W.H.:
Chromatographic determination of amino acids ...
Methods in Enzymol 6 819 (1963)

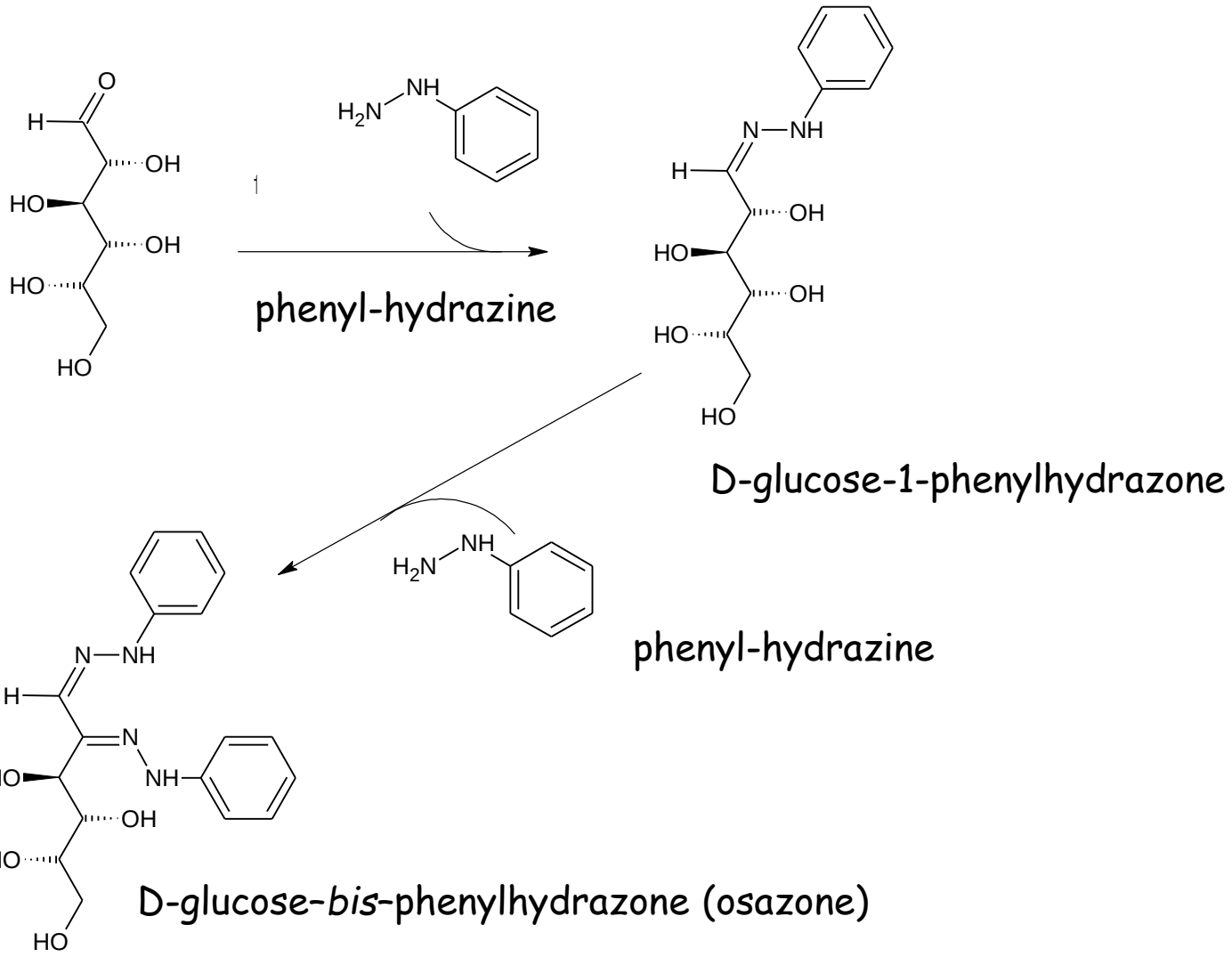


λ_s = 340 nm

λ_e = 455 nm

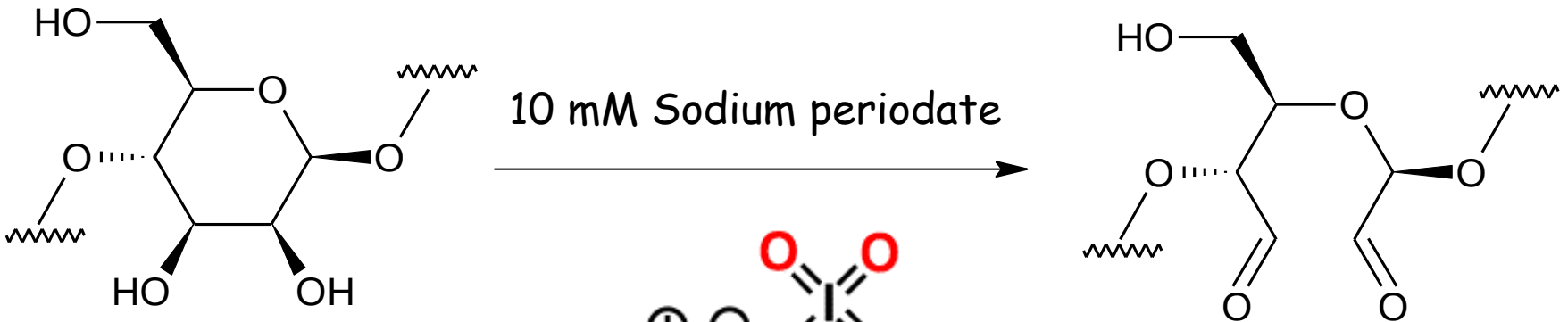
Carbohydrates

- E. Fischer (1884) Reducing monosaccharides



Carbohydrates

- Periodate oxidation to form oxo-function



β-D-mannosyl unit
of a polysaccaride chain

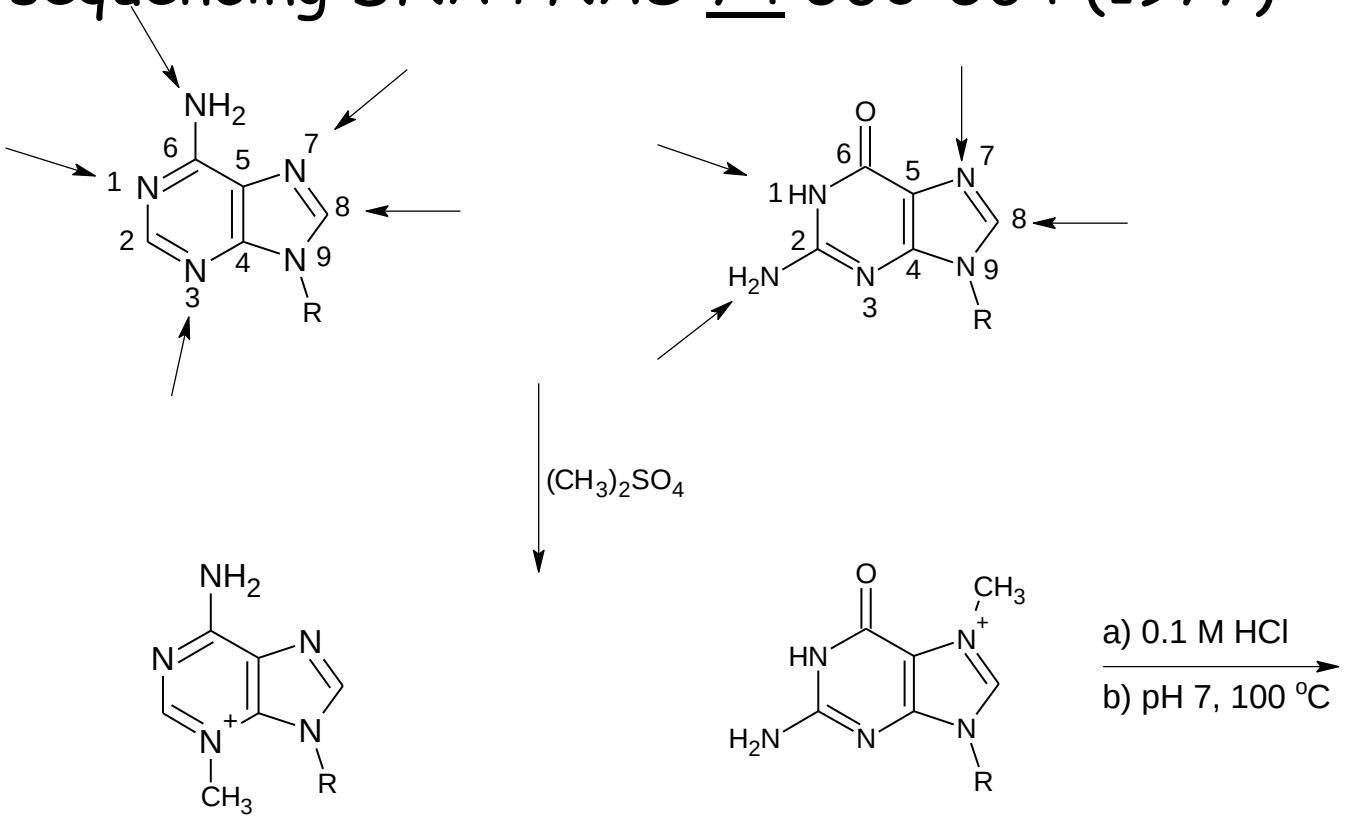
$NaIO_4$

C – C bond cleavage and
oxidation of the *OH* groups

oxo-group from (secondary) hydroxyl-function

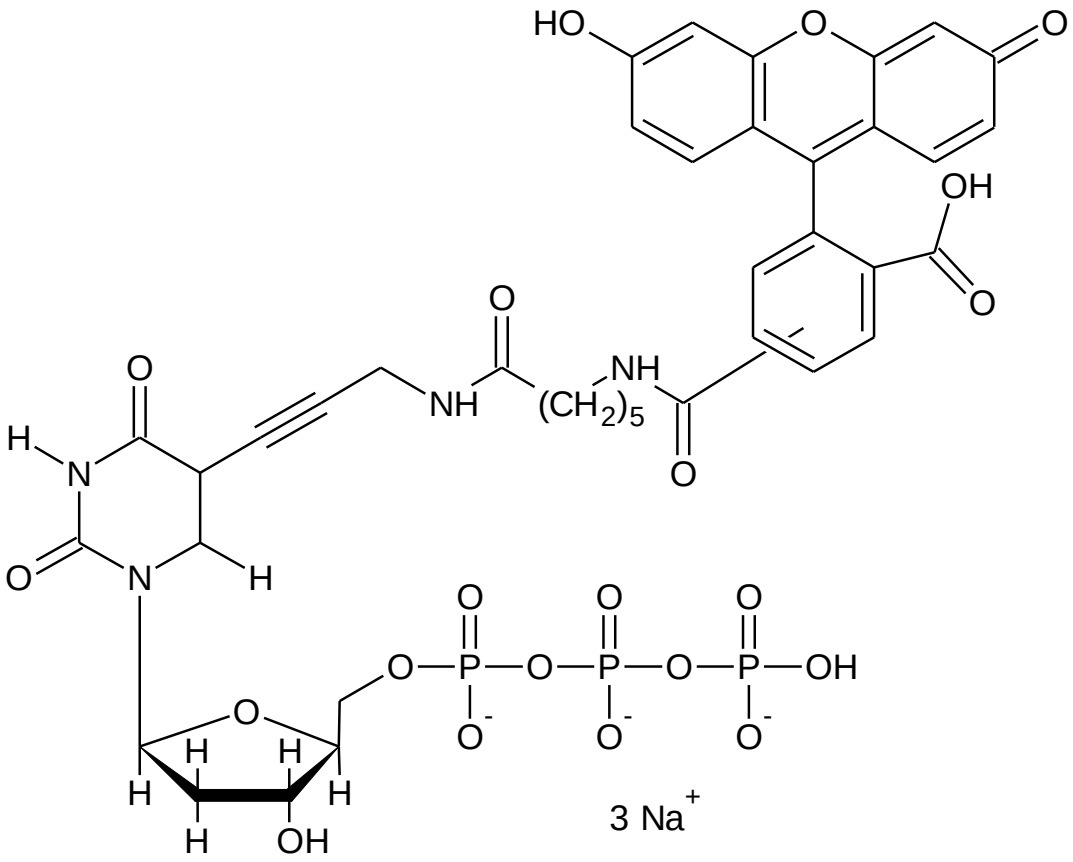
Nucleic acids

- Maxam, A.M., Gilbert, W … et al. A new method for sequencing DNA *PNAS* 74 560-564 (1977)



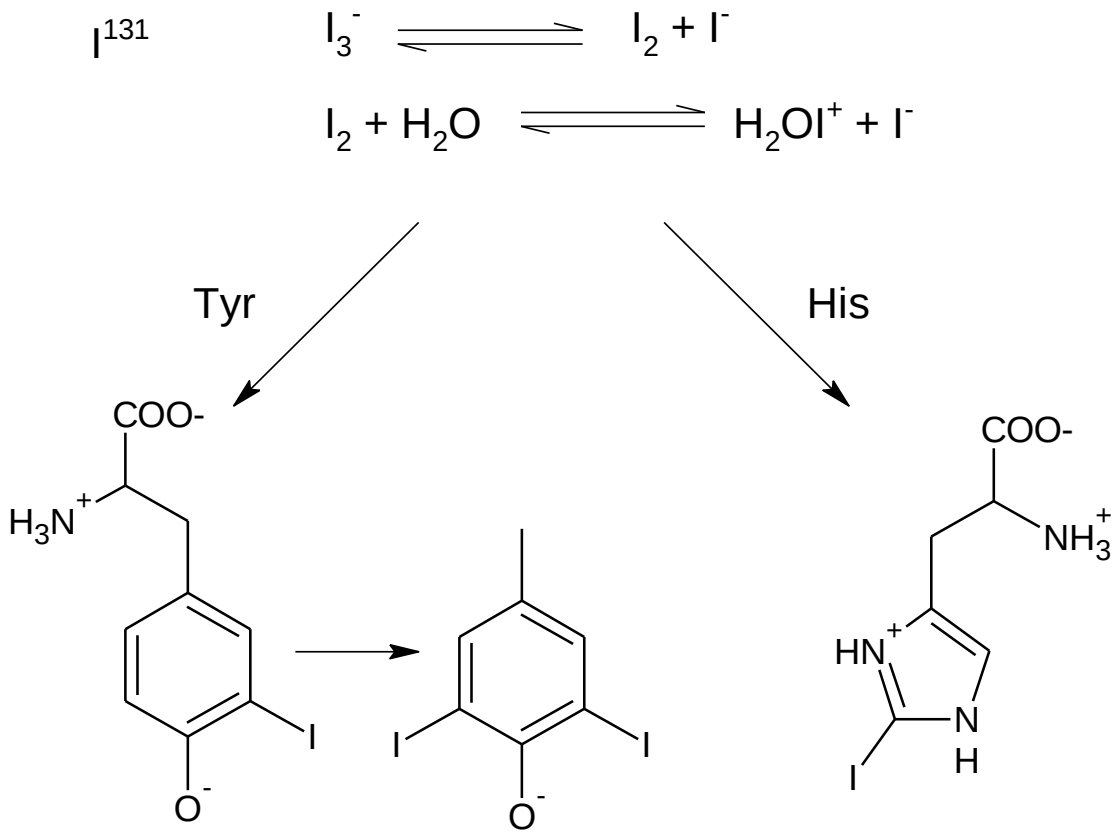
- Sanger, F. et al. *PNAS* 74 5463 (1977)
- Smith, L.M. et al. *Nature* 321 674 (1986)

- Structure of ChromaTide fluorescein-12-dUTP (C-7604)



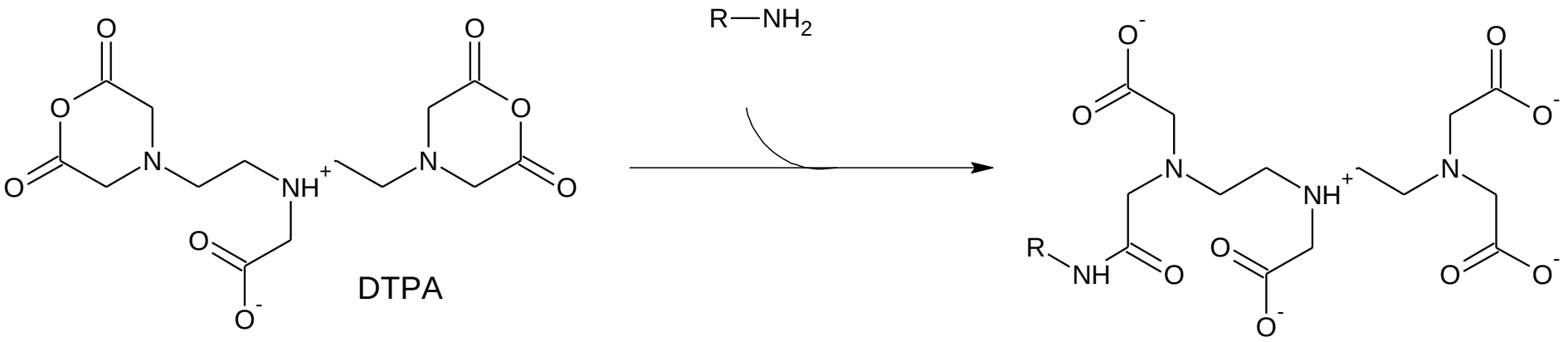
2) Detection of biopolymers in cell (tissue)

- Labelling proteins with radioactive isotope
  - Li, C.H.: Iodination of tyrosine groups in serum albumin and pepsin *JACS* 67 1065 (1945)



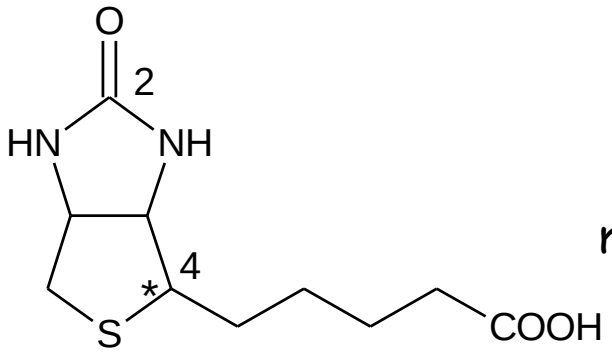
- Hnatowich, D.J. et al.:

The preparation and labeling of DTPA-coupled albumin *Int J Appl Radiat Isot* 33 327-332 (1982)



Labelling of proteins with biotin

- Bayer, E.A., Wilehek, M.: The use of avidin-biotin complex *Methods Biochem Anal* 26 1 (1980)
- Chaiet, L., Wolf, F.J.: The properties of streptavidin, a biotin-binding protein produced by streptomyces *Arch Biochem Biophys* 106 1 (1964)
- Green, N.M.: Avidin *Adv Protein Chem* 29 85 (1975)



$K_a = 10^{15}\ M^{-1}$

op.: 232-233 °C

m.p.

D-Biotin

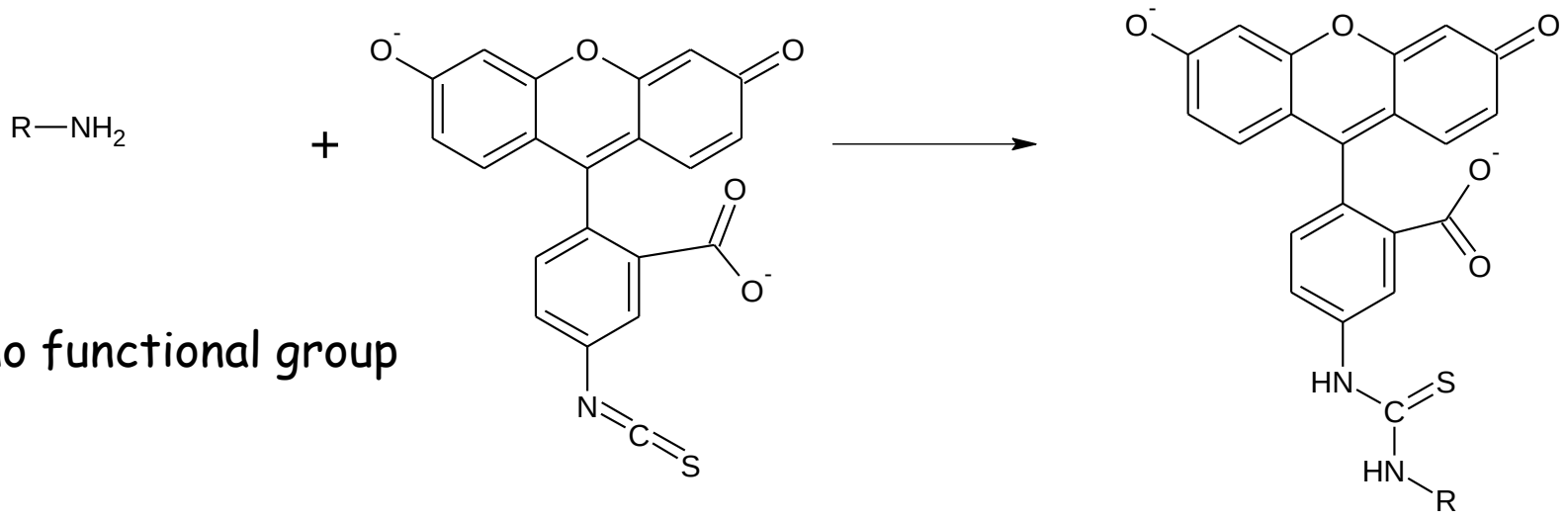
[Hexahidro-2-oxo-1H-tieno[3,4-d]imidazol-4-pentánsav]

Hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-pentanoic acid; (+)-Biotin

Labelling of biopolymers with fluorophore

McKinney, R. et al.

Factors affecting the rate of reaction of fluorescein isothiocyanat with serum proteins *J Immunol* 93 232 (1964)



amino functional group

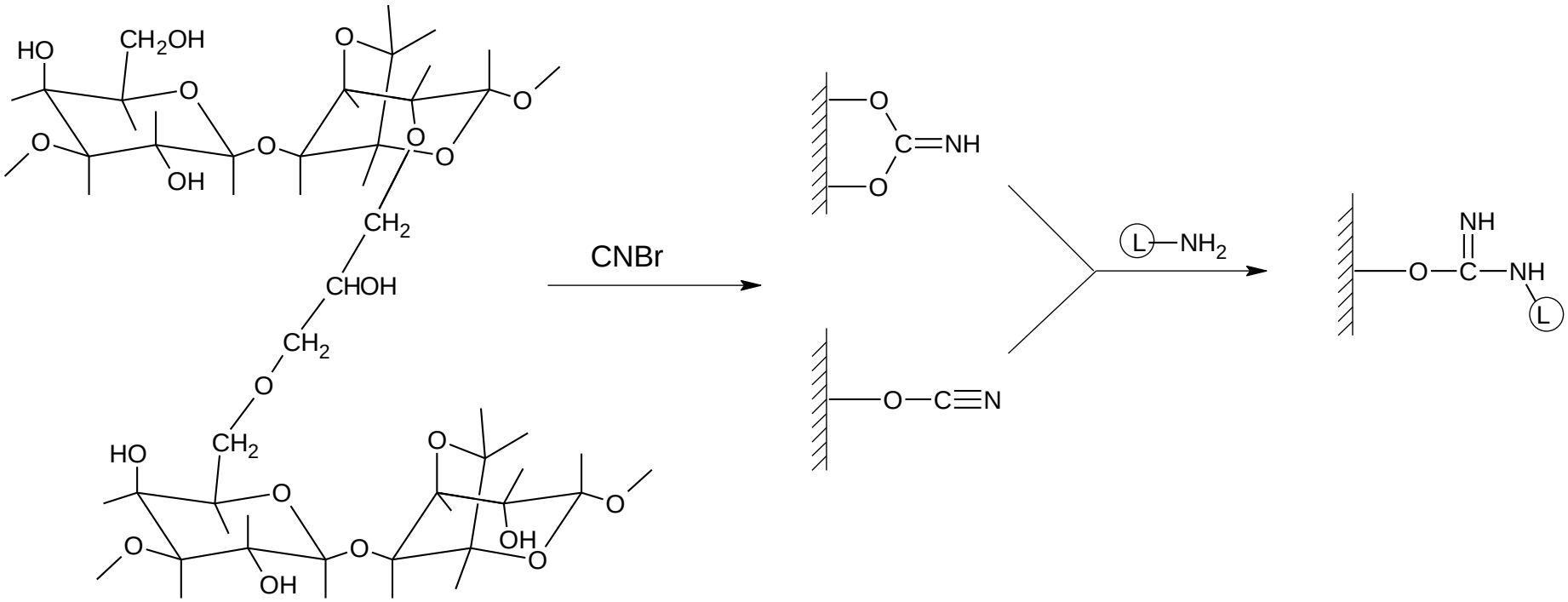
fluorescein isothiocyanate

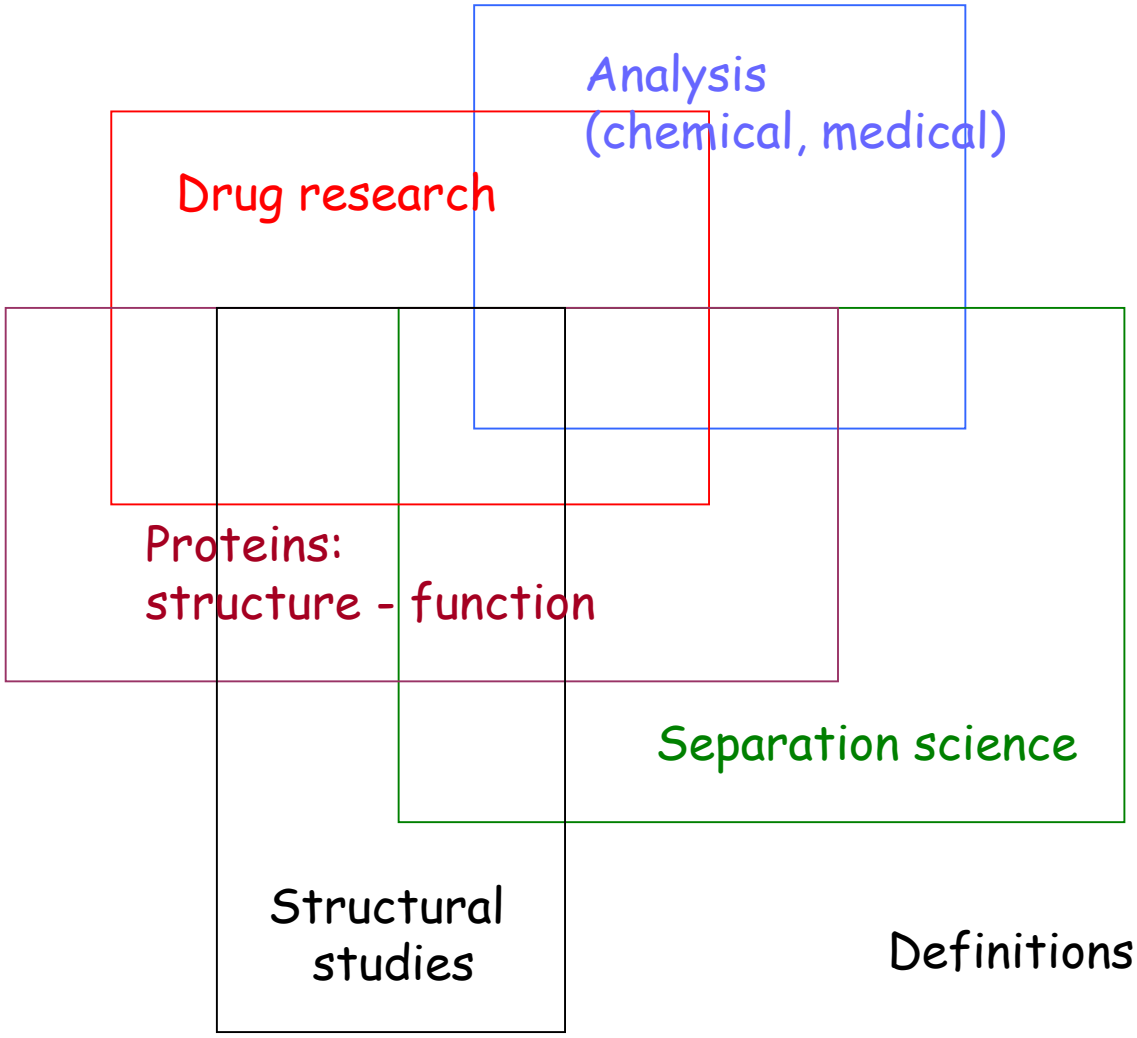
thiourea linkage

2) Affinity chromatography

- Bethell, G.S. et al. A novel method of activation of cross-linked agarose with 1,1'-carbonyldiimidazol which gives a matrix for affinity chromatography

J Biol Chem 254 2572 (1979)

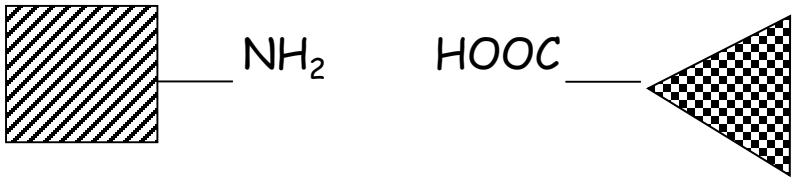




Definitions

1. Two or more active components
2. Covalent linkage

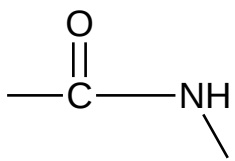
- Approaches
  1. Size of the partners
     - Small – small
     - Small – big
     - Big – big
  2. Type of linkage
     - Direct



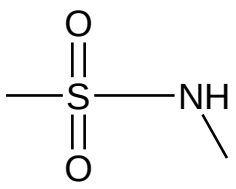
     - Indirect

3. Type of linkage

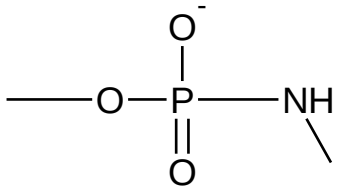
- Acid amide



carboxylic acid

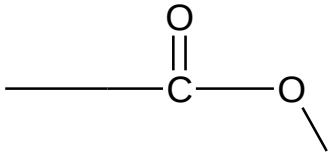


sulphonic acid

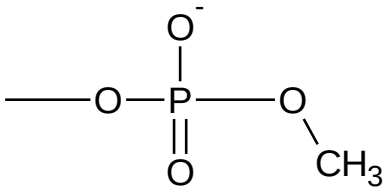


phosphoric acid amid

- Acid ester

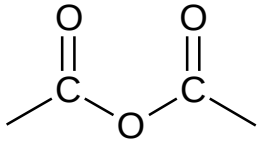


carboxylic acid

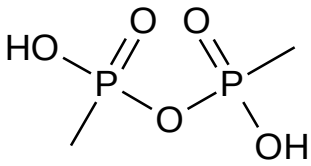


phosphoric acid

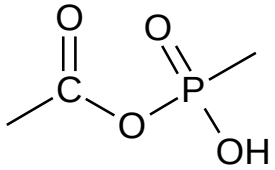
- Acid anhydride



carboxylic acid

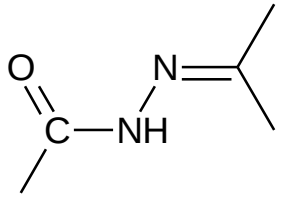


phosphoric acid



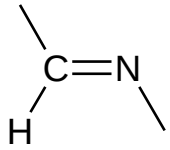
mixed

- Hydrazone



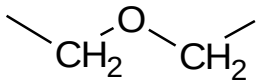
Aldehyde + hydrazine

- Schiff base



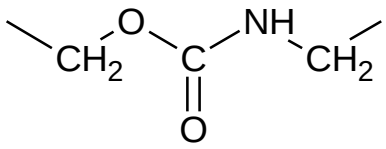
Aldehyde + amine

- Ether



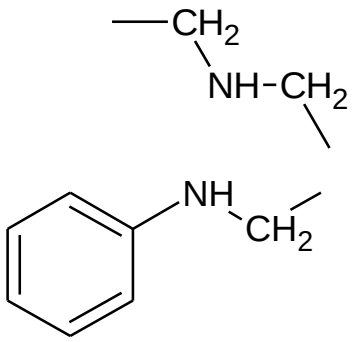
Alcohol

- Carbamate



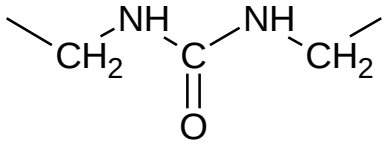
Alcohol

- Secunder amine
(N-glycoside)



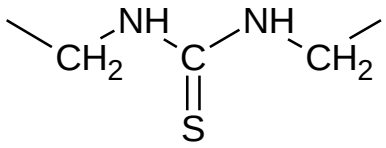
Amine
+ aldehyde
+ aryl-halogenide

- Isourea



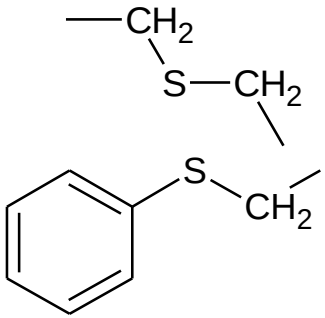
Amine

- Isothiourea



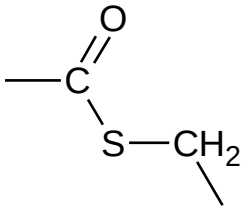
Amine

- Thioether



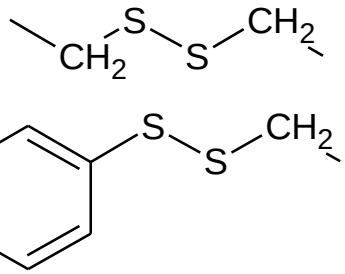
Thiol

- Thioester



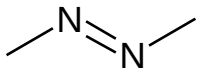
Thiol

- Disulphide



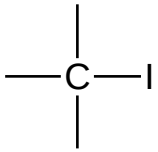
Thiol

- Diazo



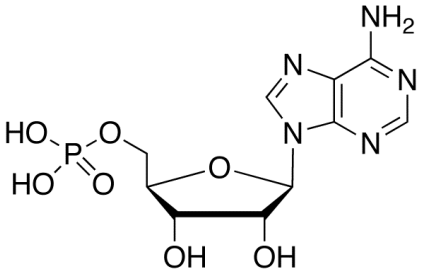
Azide

- C - X

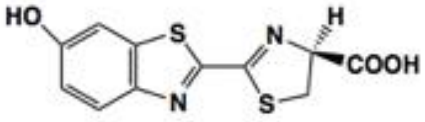


1. Example: small–small, direct, anhydride bond

„Firefly" luminescence



adenylic acid +

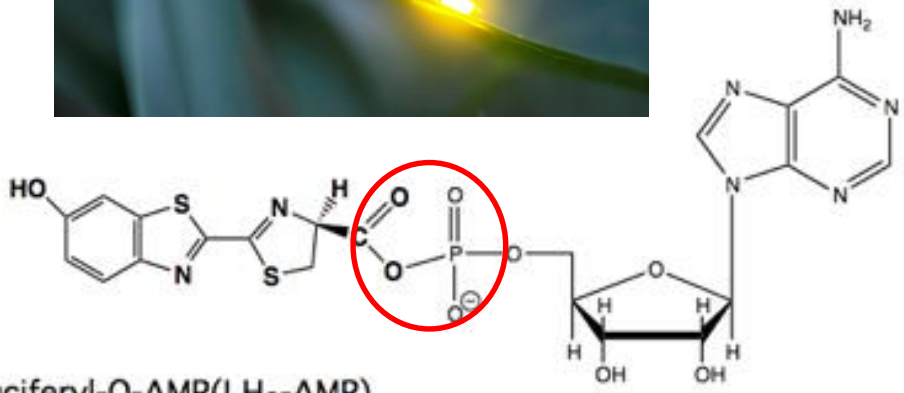


D-Firefly Luciferin (LH_2)

Beetle Luciferin

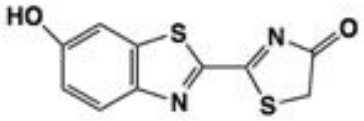
„carboxylic acid"

©nationalgeographic.com, Radim Schreiber

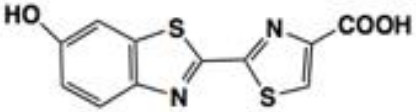


D-Luciferyl-O-AMP(LH_2-AMP)

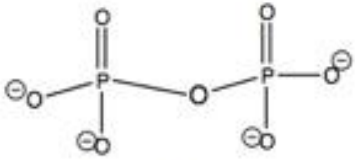
mixed anhydride



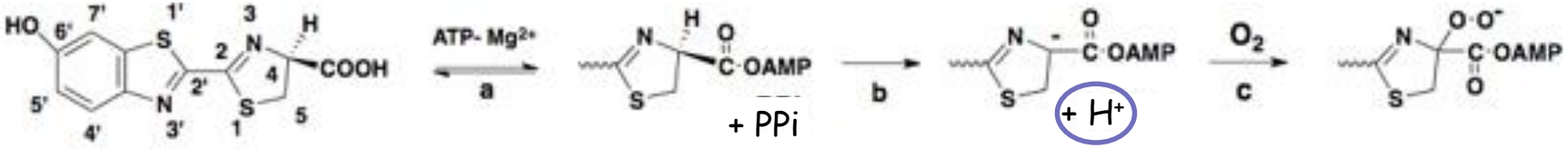
Oxyluciferin



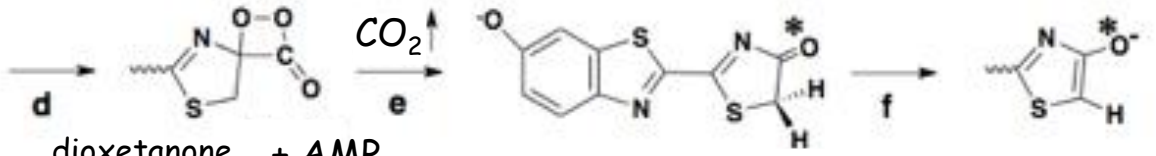
Dehydroluciferin (L)



Inorganic Pyrophosphate (PPi)



D-Luciferin (LH_2) Luciferyl-AMP)



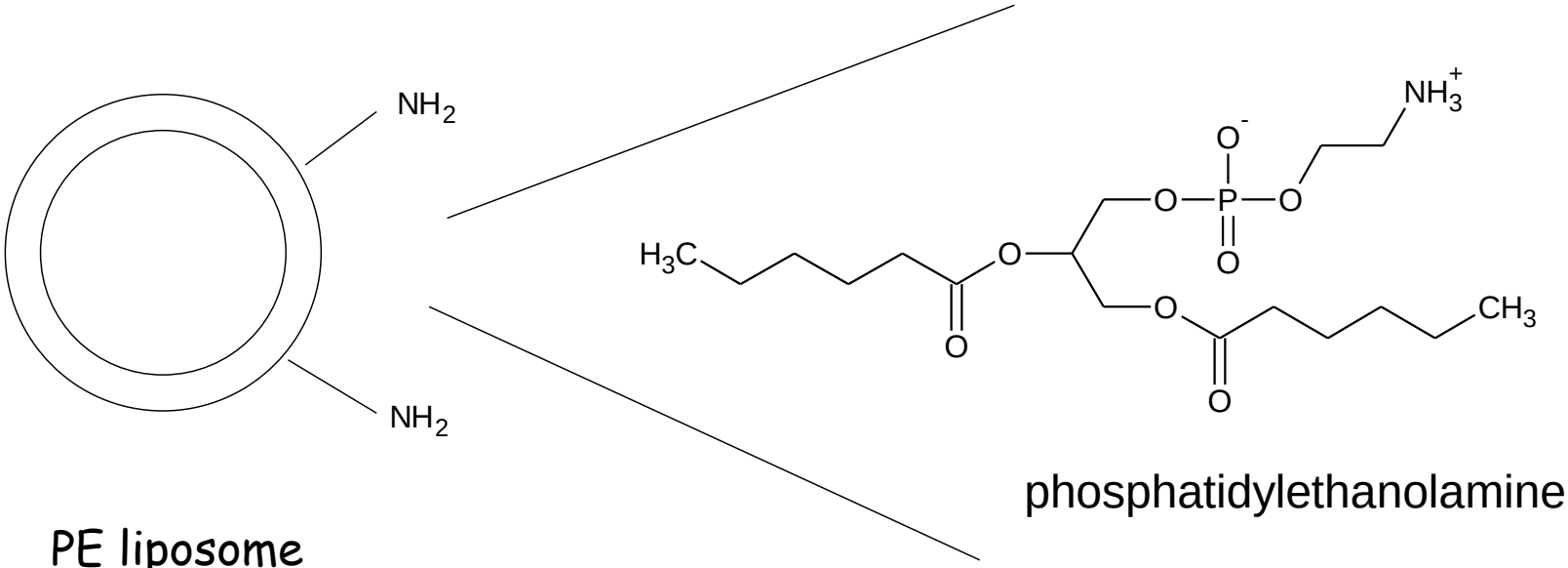
Green to red light Red light Yellow-Green light

Step a: formation of the enzyme-bound luciferyl adenylate;
Step b: a proton is abstracted from the C-4 carbon of the adenylate by a basic side chain amino acid residue of luciferase;
Step c: molecular oxygen adds to the newly formed anion;
Step d: a highly reactive dioxetanone intermediate is formed;
Step e: an electronically excited state oxyluciferin molecule, CO_2 and red light emission (λ_{max} = 615 nm) are produced (at pH 6);
Step f: the enolate dianion formed by tautomerization from the exited form of oxyluciferin and yellow-green light emission (λ_{max} = 560 nm) occurs at pH 8.

http://www.photobiology.info/Branchini2.html

2. Example: small – big, indirect, amide bond

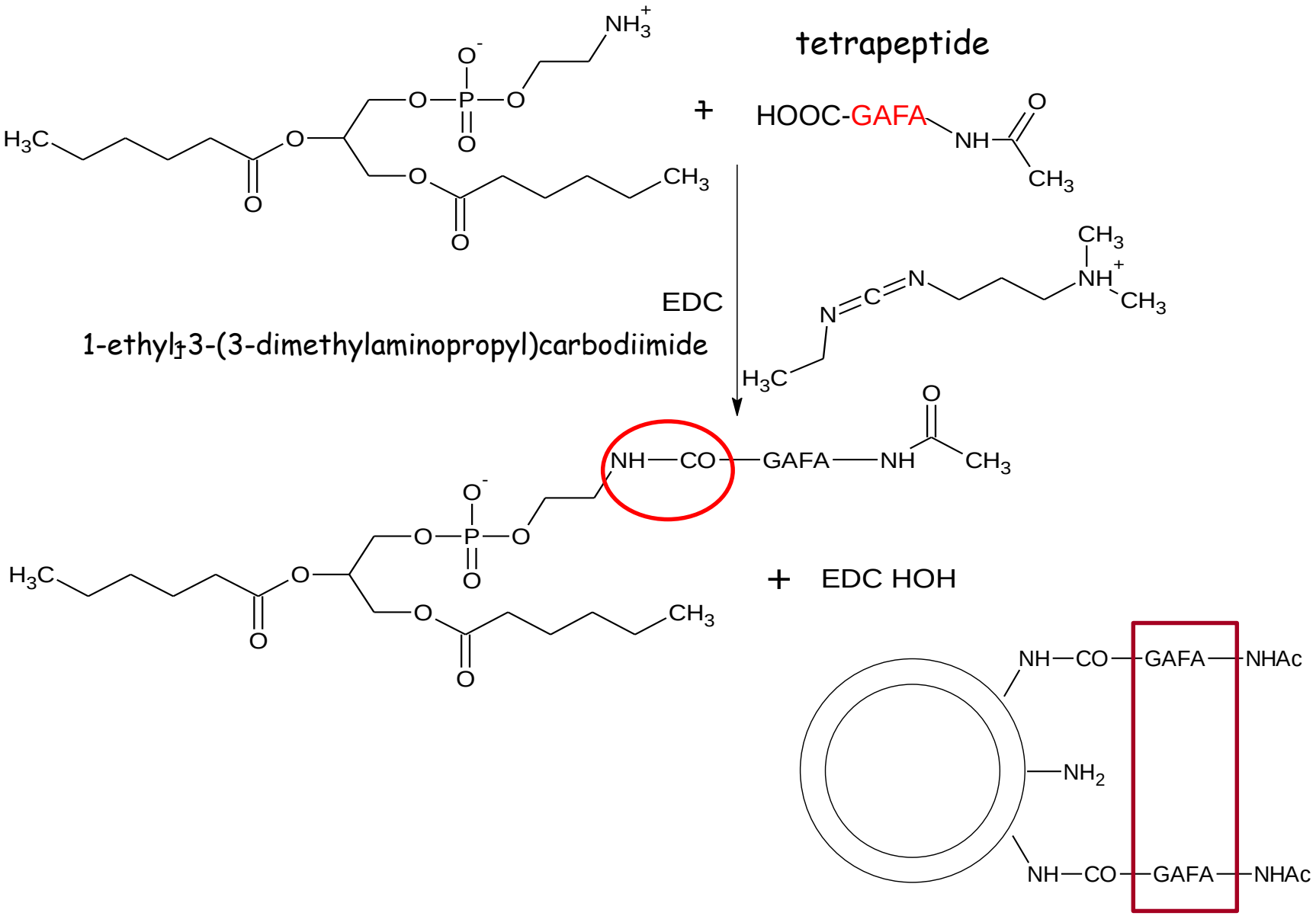
Liposome – hapten conjugate



PE liposome

phosphatidylethanolamine

2. Example


tetrapeptide
HOOC-GAFA
EDC
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDC HOH
NH—CO—GAFA—NHAc
NH2
NH—CO—GAFA—NHAc

3. Example: big–big, indirect, disulphide bond

Immunotoxin conjugate

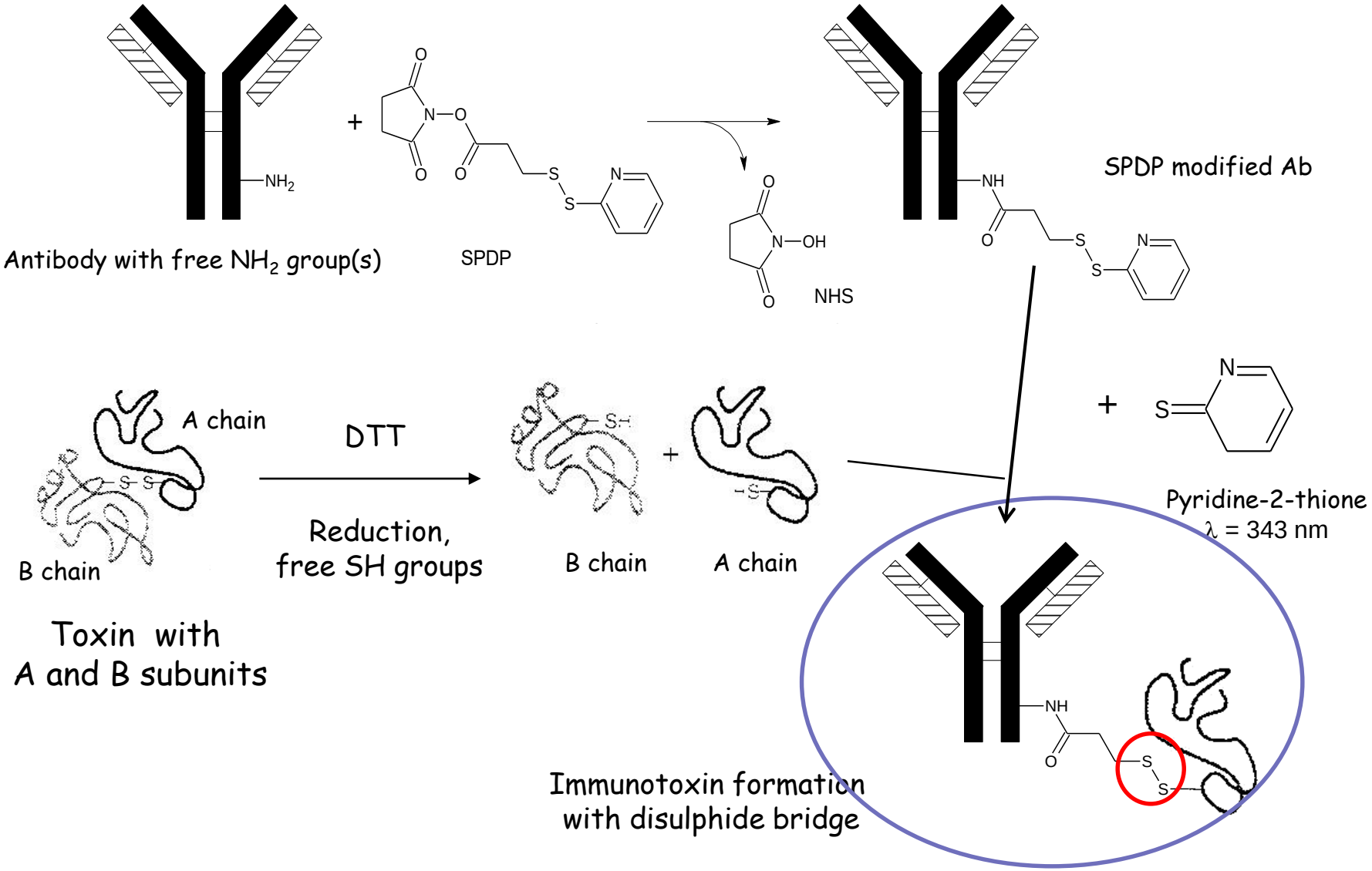
1. step: introduction of protected -SH group(s) of antibody (Ab + SPDP → amide bond

2. step: creation of free -SH groups of the toxin subunits (cleavage of the -S-S- bond, DTT)

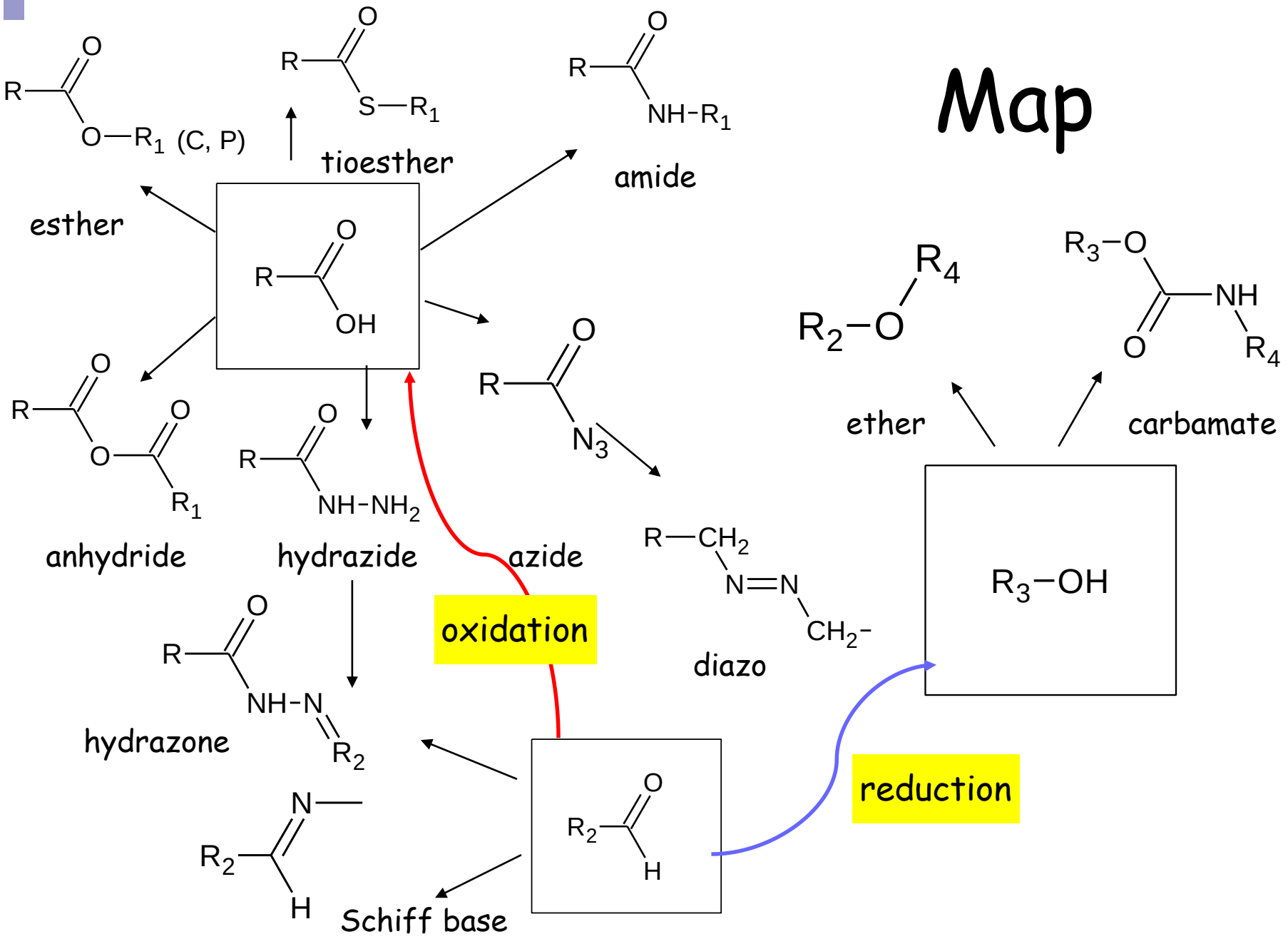
3. step: free –SH partner reacts with the component having „protected" SH function (AB-SSP + Toxin-SH → disulphid linkage)

3. Example

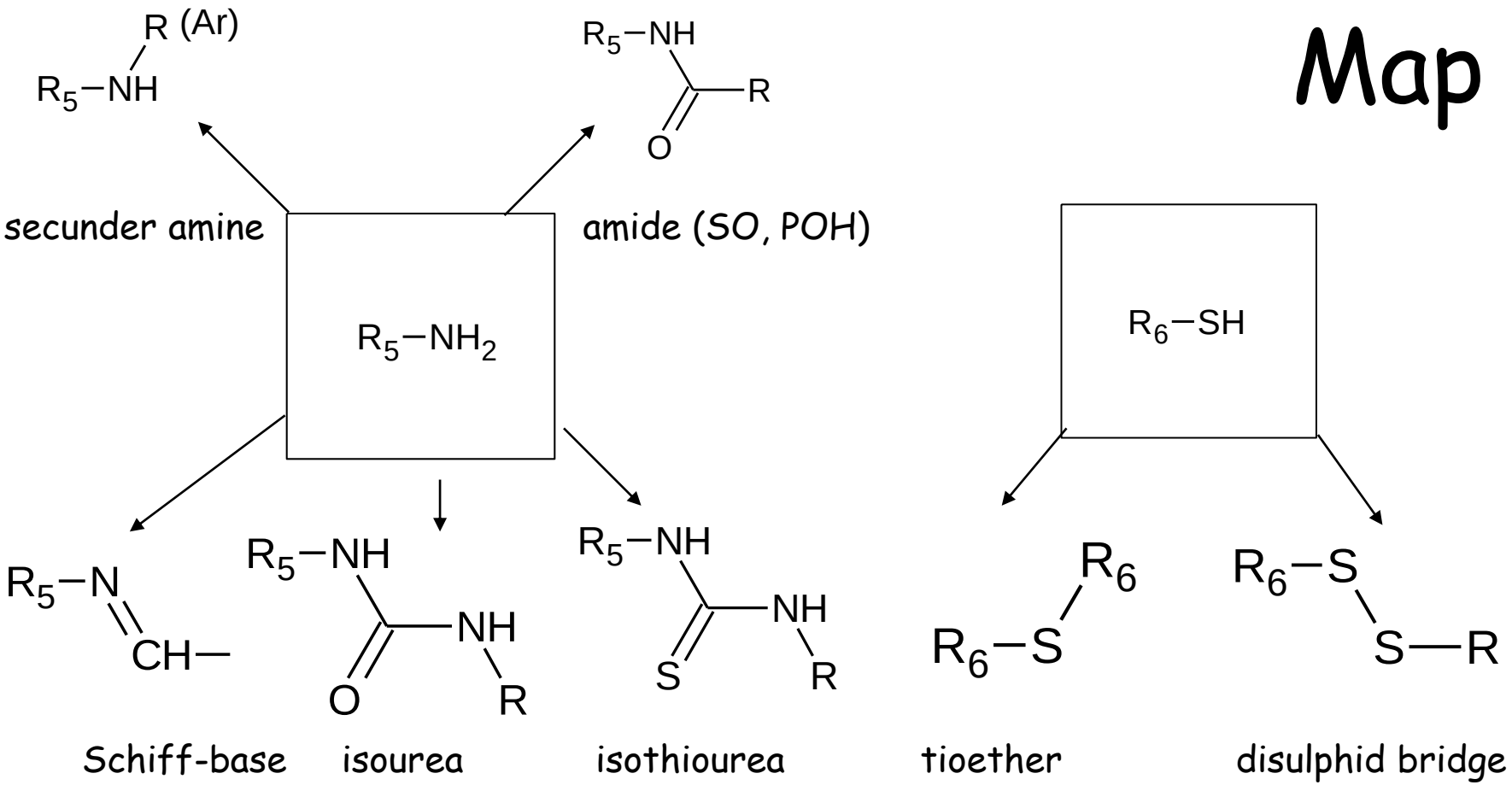
- Carlsson, J. et al. Biochem J 173 723 (1978)



Map


esther
tioesther
amide
anhydride
hydrazide
azide
ether
carbamate
oxidation
diazo
hydrazone
reduction
Schiff base

Map



1) Introduction of functional groups ? (-*COOH*, -*CHO*, -*OH*, -*SH*, -NH_2)
2) Elimination of functional groups?