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

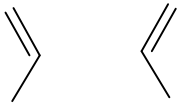
5. Conjugation
   - Chemical synthesis
   - Enzymatic synthesis (e.g. - NH_2 vs. - SH)
   - Gene technology

6. Analysis of conjugates
   - Purification
   - Structure determination

Strategies of bioconjugation

Homobifunctional

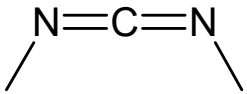
Identical functions

1. $-NH_2$ H_2N-
2. -SH HS-
3. -COOH HOOC-
4. -CHO OHC-
5. $-NH-NH_2$ H_2N-NH-
6. 

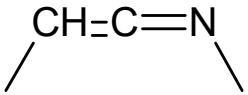
„Zero-length"

No insertion

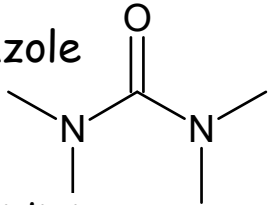
1. Carbodiimide



2. Woodward's reagent k



3. Diimidazole



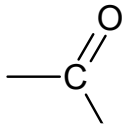
4. Oxidising agents

-SH + HS-

5. Enzymes

Heterobifunctional

Different functions

1. $-NH_2$ HS-
2.  HS-
3. Photoreactive

   $-NH_2$

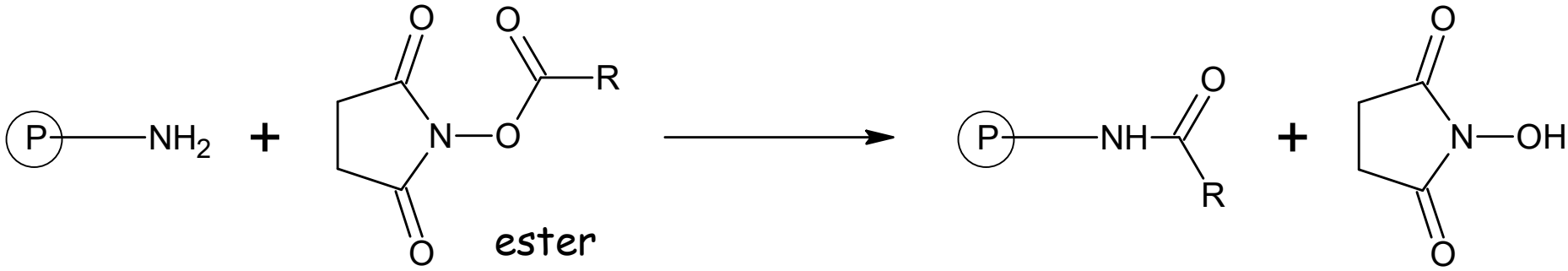
   -SH

   -COOH

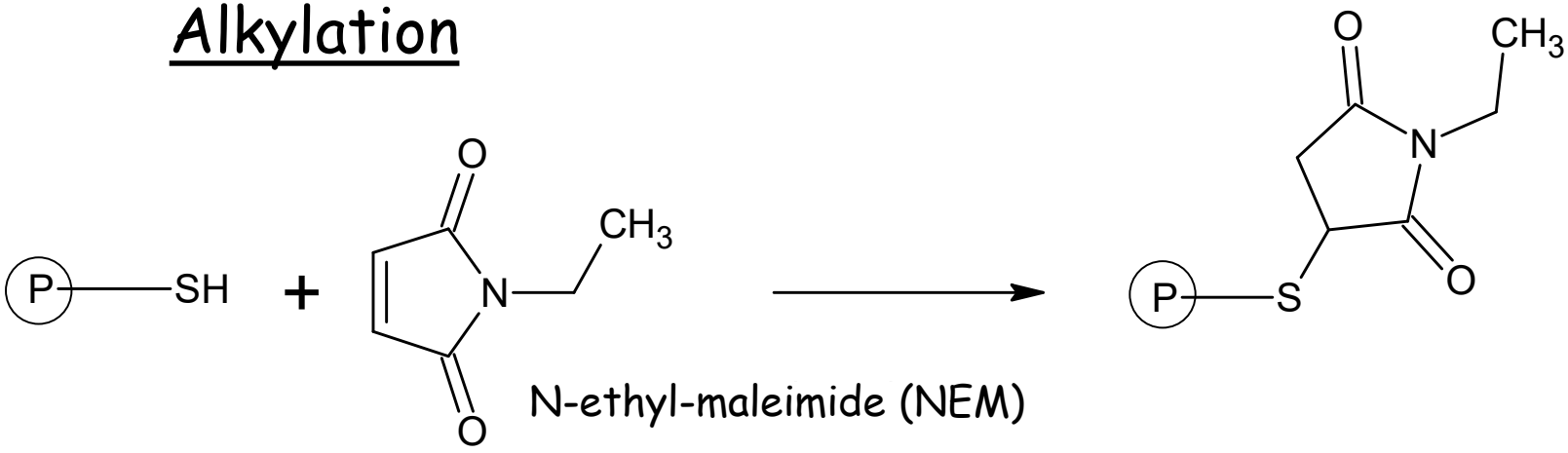
   -CHO
4. Photoaffinity labeling

Selection of coupling reagents

1. Specificity of the reaction
   - Acylation



Alkylation



Nucleophilicity order

a) Within a period

$^{-}NH_2 > {}^{-}OR > {}^{-}OH > {}^{-}OAr > R\text{-}NH_2 > NH_3 > H_2O$

Ser, Thr Tyr Lys, α

b) Within a column

$-SH > {}^{-}OH$; $F > Cl > Br > I$

Protonation effect

$^{-}NH_2 > NH_3$; $^{-}OAr > HOAr$; $^{-}OH > H_2O$

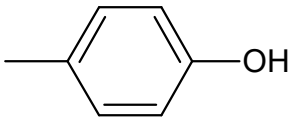
Edwards Jo, Pearson RG JACS 84 26 (1962)

$^{-}SR > {}^{-}SAr > {}^{-}I > {}^{-}CN > {}^{-}OH > {}^{-}N_3 > {}^{-}Br > {}^{-}OAr > {}^{-}Cl$

$> Pyridine > {}^{-}OAc > H_2O$

Σ $-SH > -NH_2 > -OH$

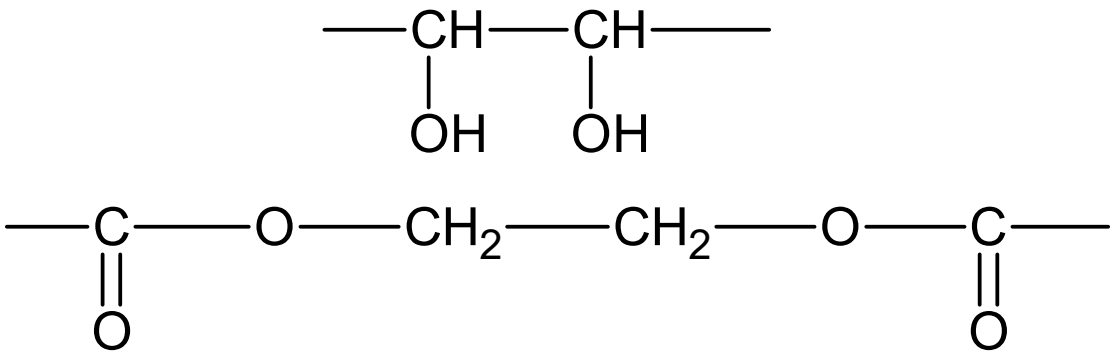
- **pH effect:** 1. protonation decreases nucleophilicity

-SH	pK_a =	8.3
-$^{\varepsilon}NH_2$		10.8
		10.9
-OH		> 13

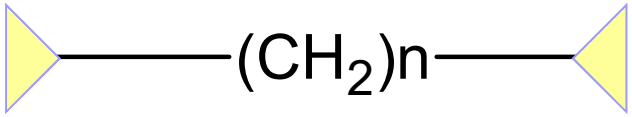
$$pH = pK_a + \log \frac{[A^-]}{[AH]}$$

2. Hydrophilic/hydrophobic character of the reagent
   e.g. membrane

3. Cleavability of the reagent e.g. -S-S-



4. Size of the reagent

e.g. 

n = 0,1,2...

5. „Reporter" feature of the reagent

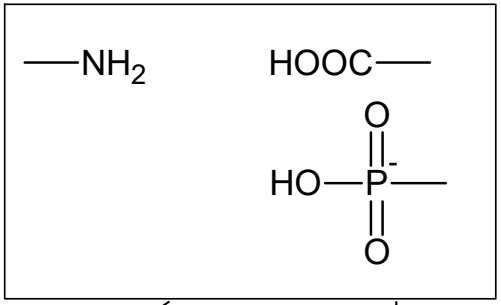
e.g. UV, F chromophore, spin-label

1. „Zero-length" coupling reagents

A) Amide linkage

B) Schiff base (secunder amine)

C) Disulphide linkage



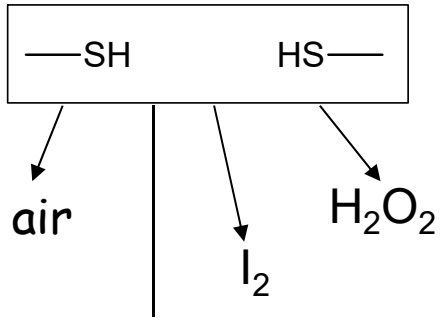
I. Cumulated unsaturated bond
   a) carbodiimide
   b) Woodward reagent

II. Active carbonyl
   a) carbonyldiimidazole (CDI)
   b) derivatives of carbonic acid

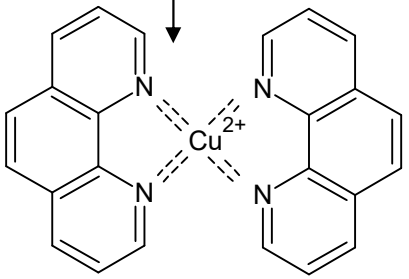
Spontaneous

Reducing agents

e.g. HPO-IgG
Ferritin – avidin



e.g. membrane

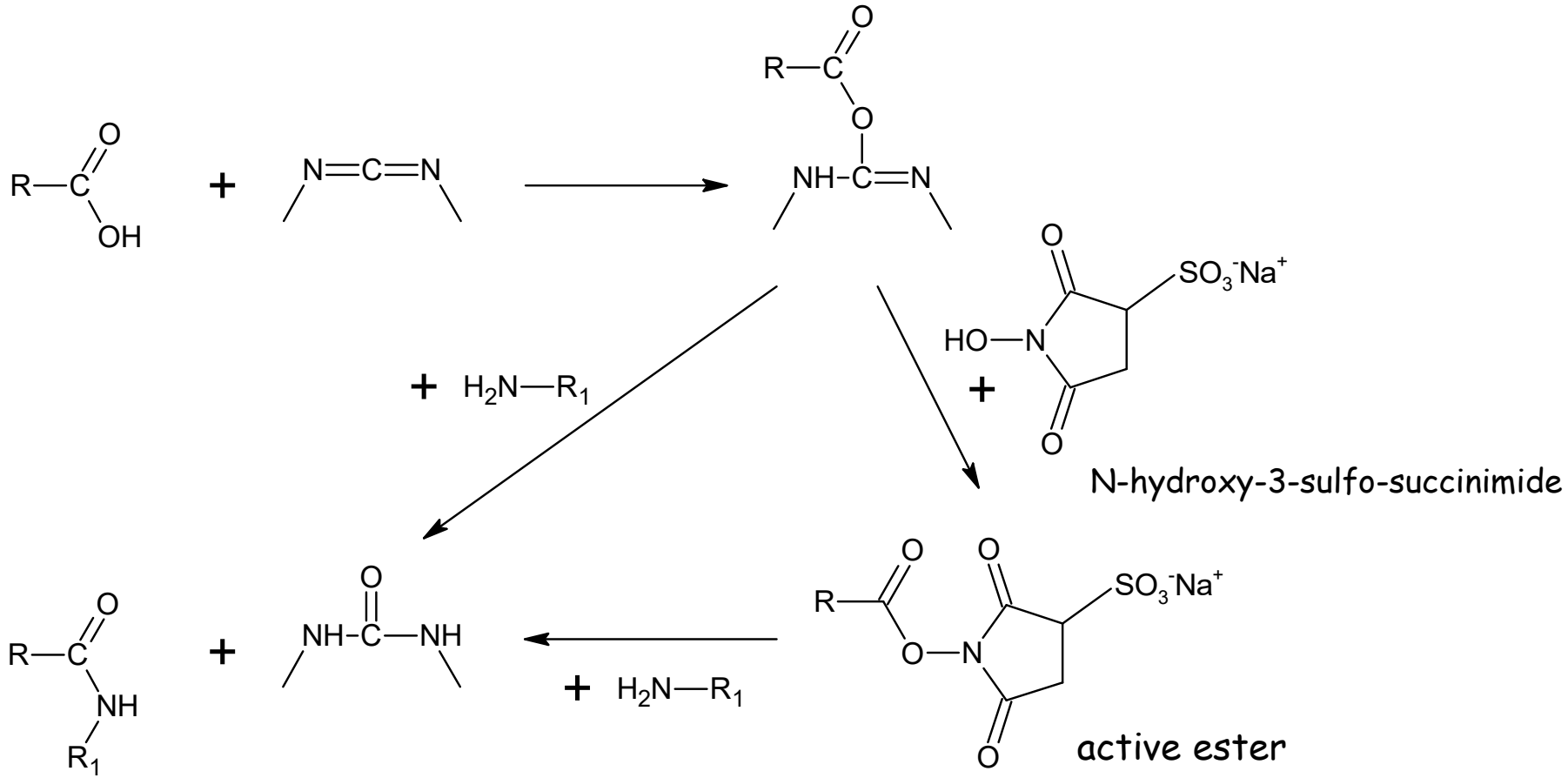


CuP [di(1,10-fenantrolin)]

A) Amide linkage

(reagents with cumulated unsaturated bond)

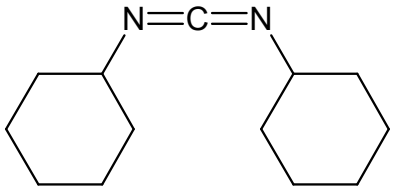
I. Carbodiimides



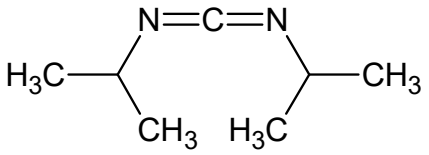
Staros JV Anal Biochem 156 220 (1986)

A. Non-water soluble carbodiimides

Sheehan JC J Am Chem Soc 77 1067 (1955)



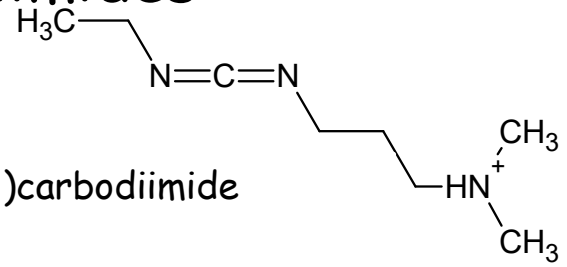
N, N' – dicyclohexylcarbodiimide (DCC)



N, N' – diisopropylcarbodiimide (DIC)

B. Water soluble carbodiimides

Sheehan JC et al. J Org Chem 26 2525 (1961)

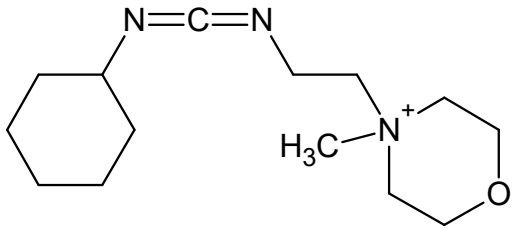


1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
(EDC, EDAC)

pH 4.7 - 6 (7.5)

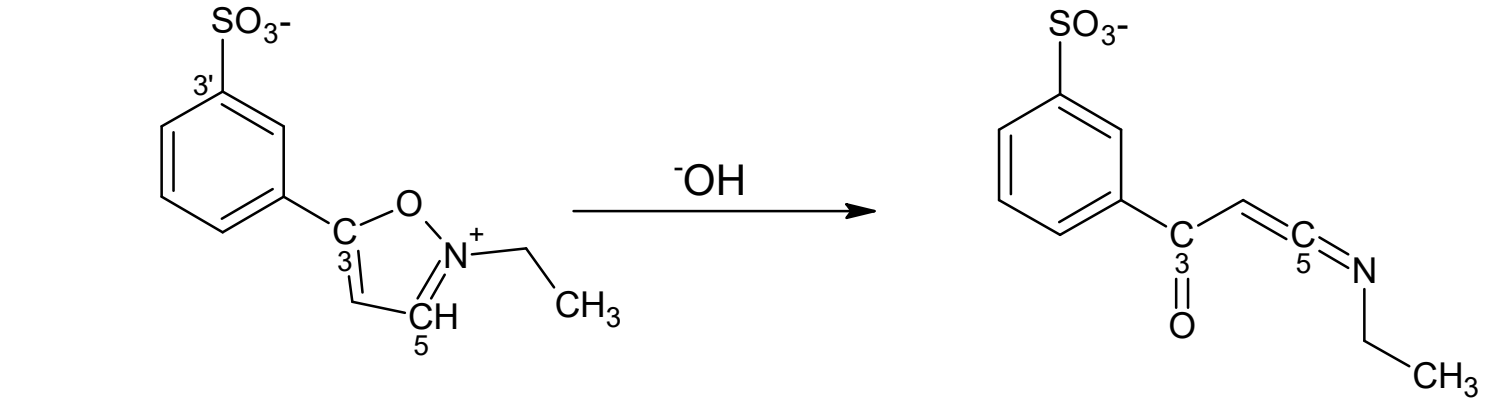
Side reaction: hydrolysis, thiolysis

Sheehan JC J Org Chem 21 439 (1956)



1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide (CMC)

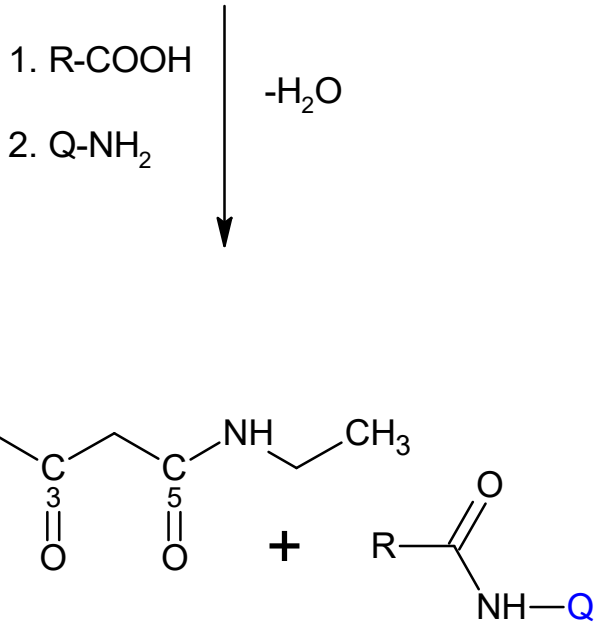
C. Woodward reagent



N-ethyl-5-phenylisoxazolium-3'-sulfonate

ketoketene-imine

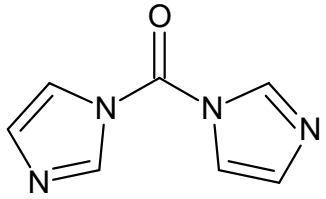
Woodward RB J Am Chem Soc 83 1010 (1961)



1) Polymer a-chymotripsin
2) Hemin – IgG conjugate
3) Enzyme – bilirubin conjugate

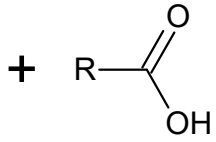
II. Active carbonyl reagents

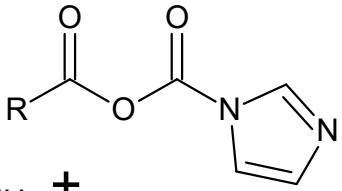
A

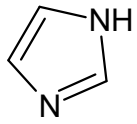


N,N'-carbonyldiimidazole (CDI)

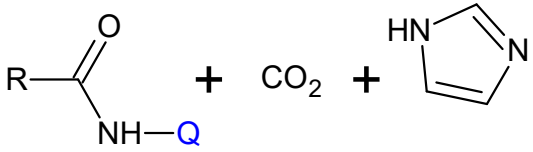
Anderson GW
J. Am. Chem. Soc. 80 4323 (1958)



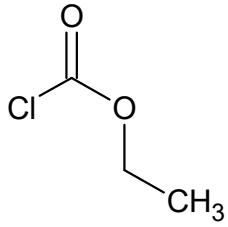




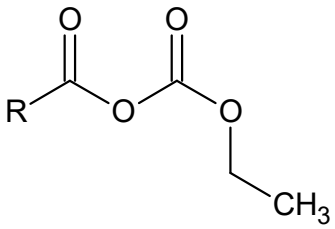
Acyl imidazole



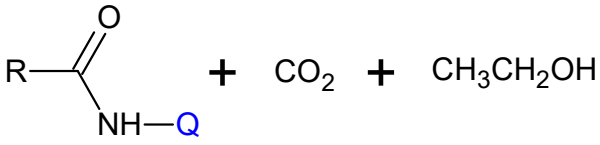
B



Carbonochloridic acid ethyl ester



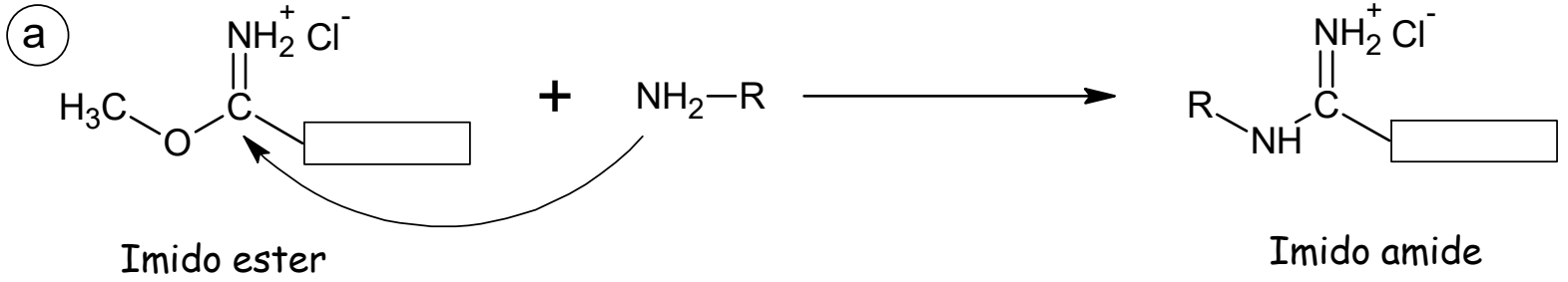
Enzyme – antibody → histochemistry
Ganglionid – fluorophore



2. Homobifunctional coupling reagents

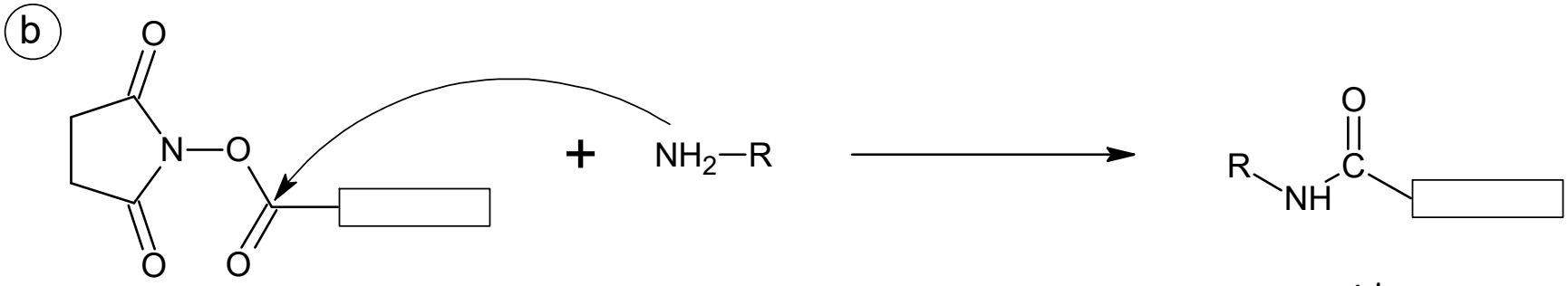
2.1. Functional groups

2.1.1. Amino group



Imido ester

Imido amide



N-hydroxy-succinimide ester

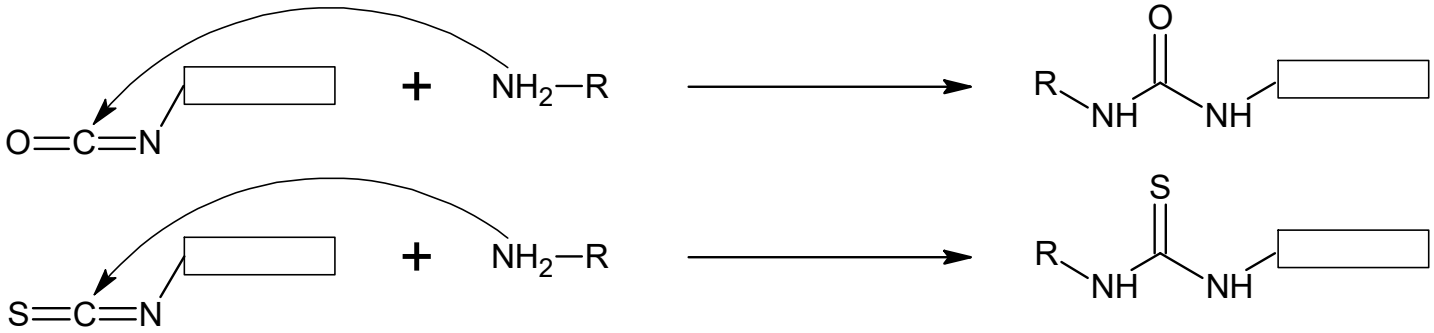
amide

c

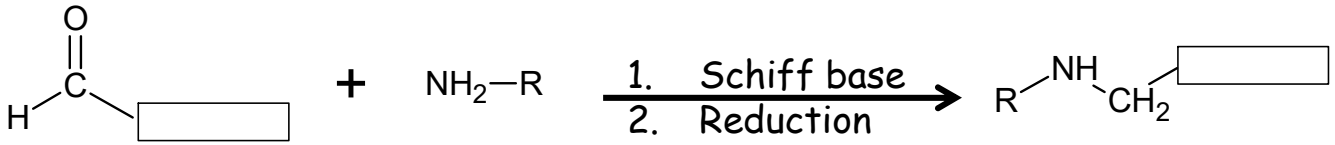
aryl halogenide

aryl amine

d



e

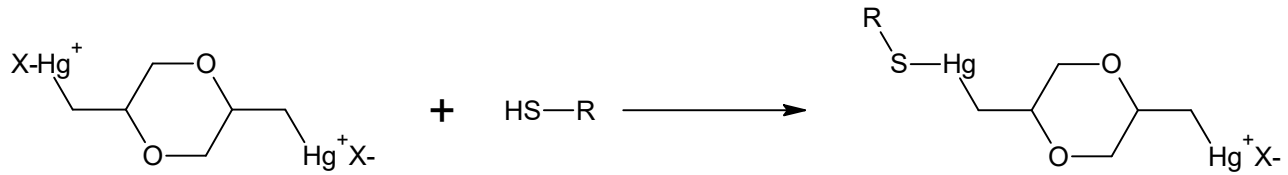


dialdehyde

2.1.2. Thiol function

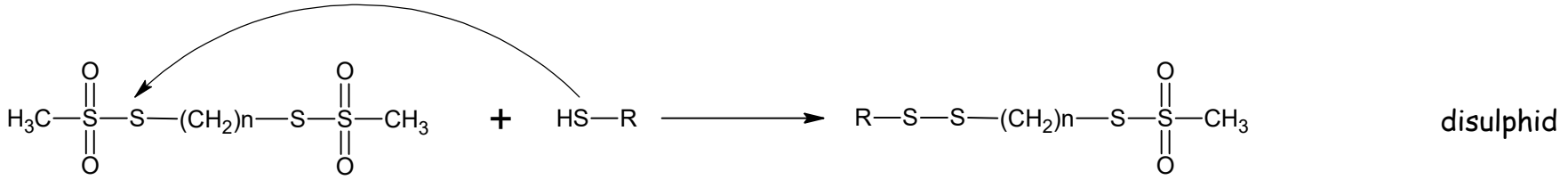
a) Hg^{2+} Since 1947

b)



3,6-bis(acetatomercurimethyl)dioxane $X = Cl^-, NO_2^-$

c)



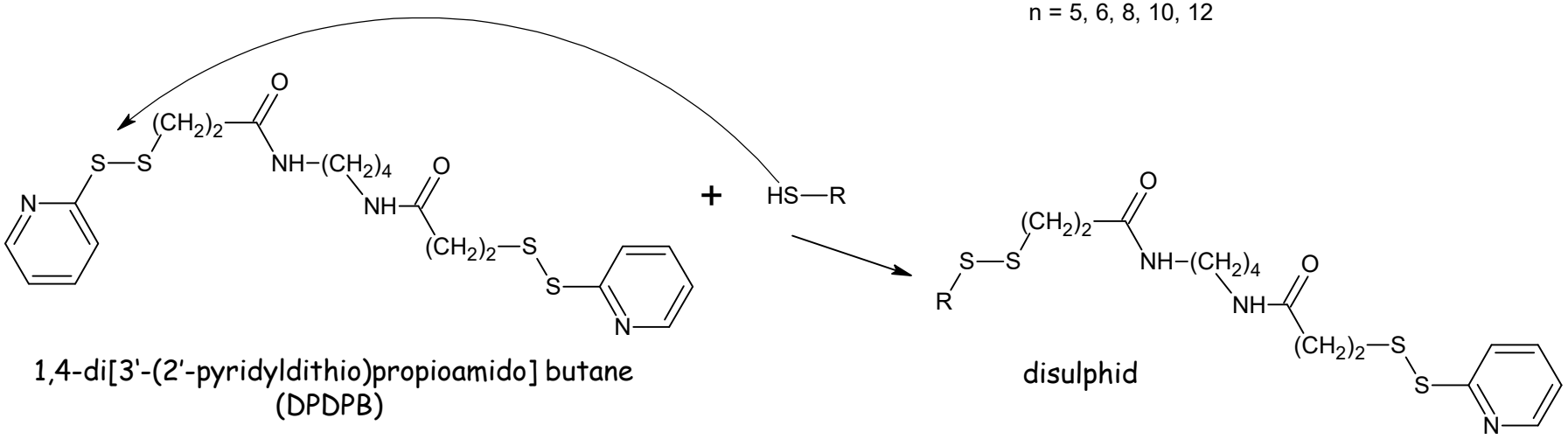
polymethylene bis-(methanesulfonate ester)

disulphid methanesulfonic acid

disulphid

n = 5, 6, 8, 10, 12

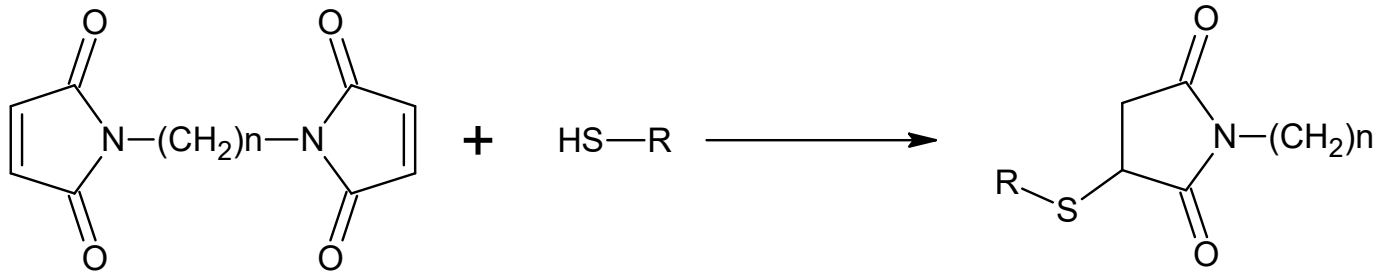
d)



1,4-di[3'-(2'-pyridyldithio)propioamido] butane
(DPDPB)

disulphid

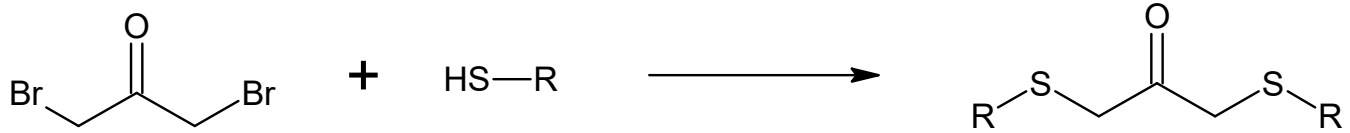
e



N, N'-methylene-bis-maleimide

thioether n = 1, 3, 6, 8, 12

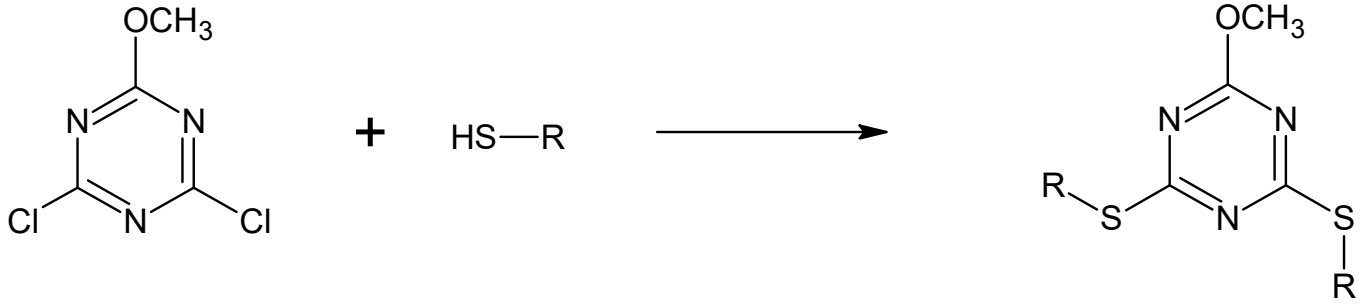
f



1,3-dibromoacetone

thioether

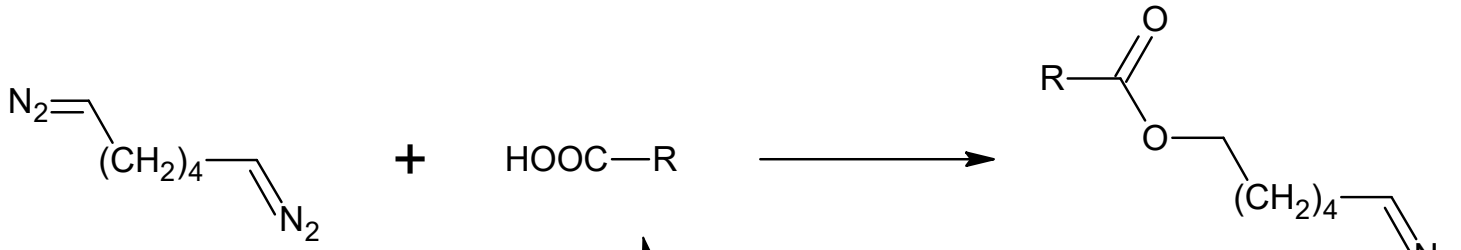
g



2,4-dichloro-6-methoxy-1,3,5-triazine

thioether

2.1.3. Carboxyl function



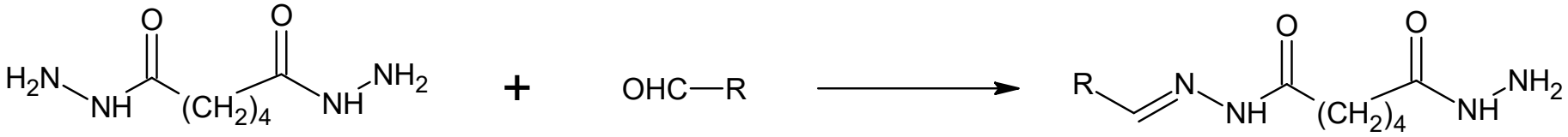
1,6-bis(diazo) hexane

ester

EDC

imid

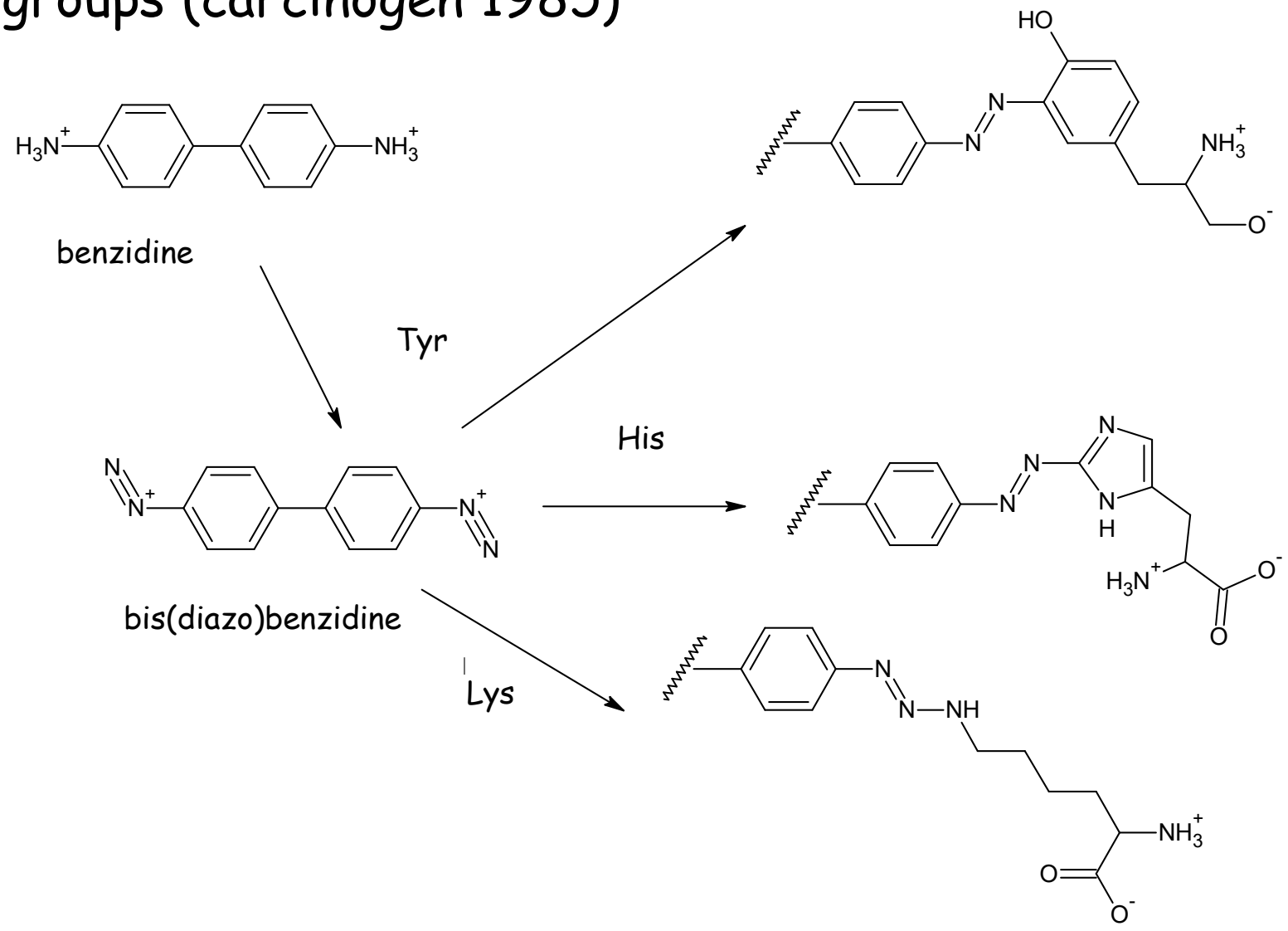
2.1.4. Aldehyde function



adipic acid hydrazide

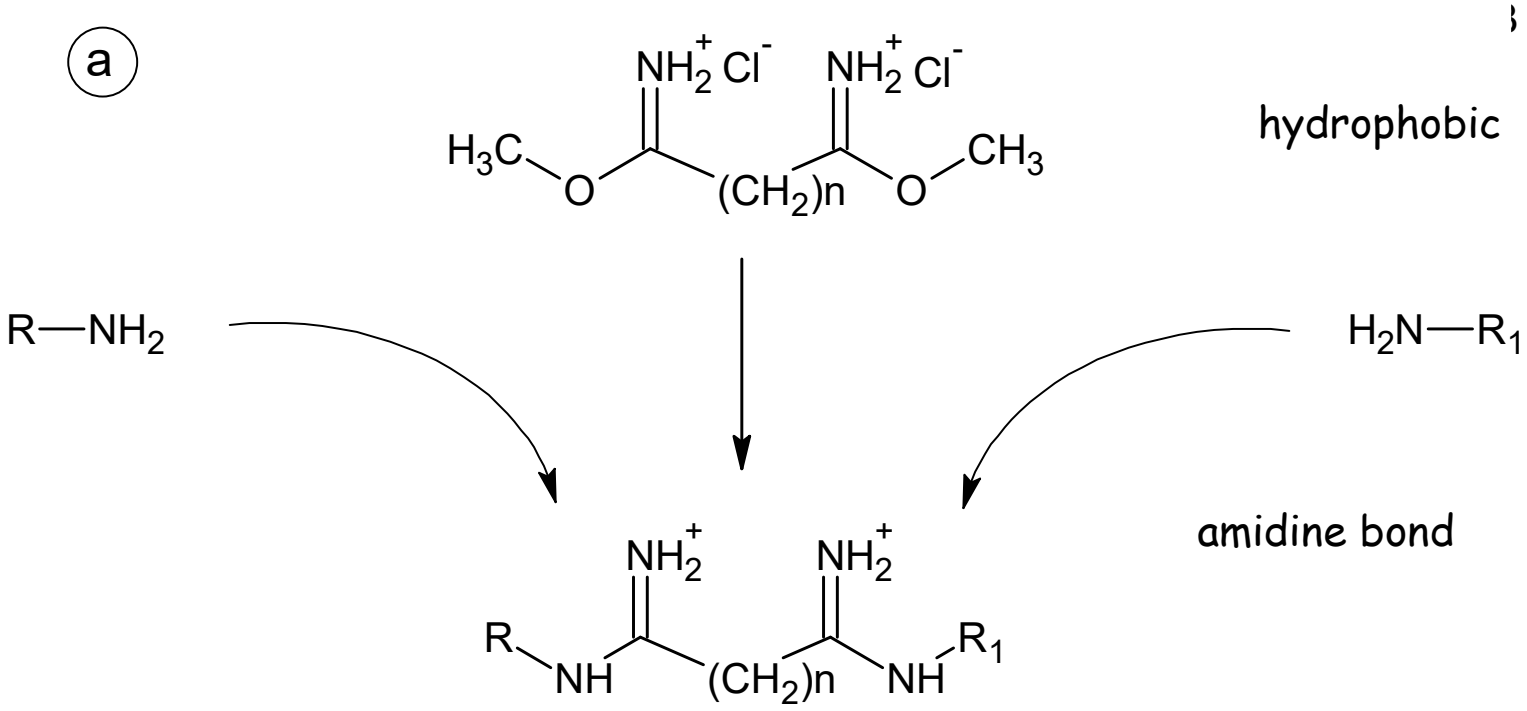
hydrazone

2.1.5. Bis-benzydine derivatives – mixed functional groups (carcinogen 1985)



2.2. Spacers

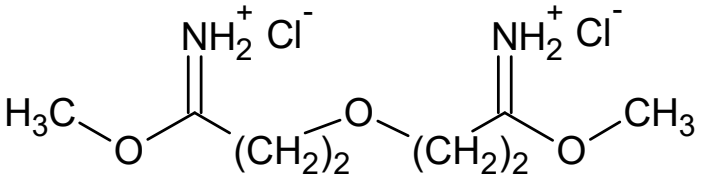
2.2.1. Non-cleavable



n = 1 dimethyl malonimidate (DMM)

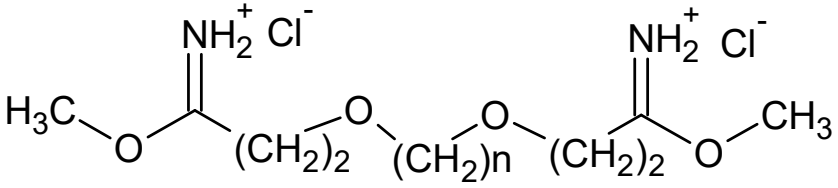
n = 2 - 10 (n = 4, DMA n = 5, DMP n = 6, DMS

b



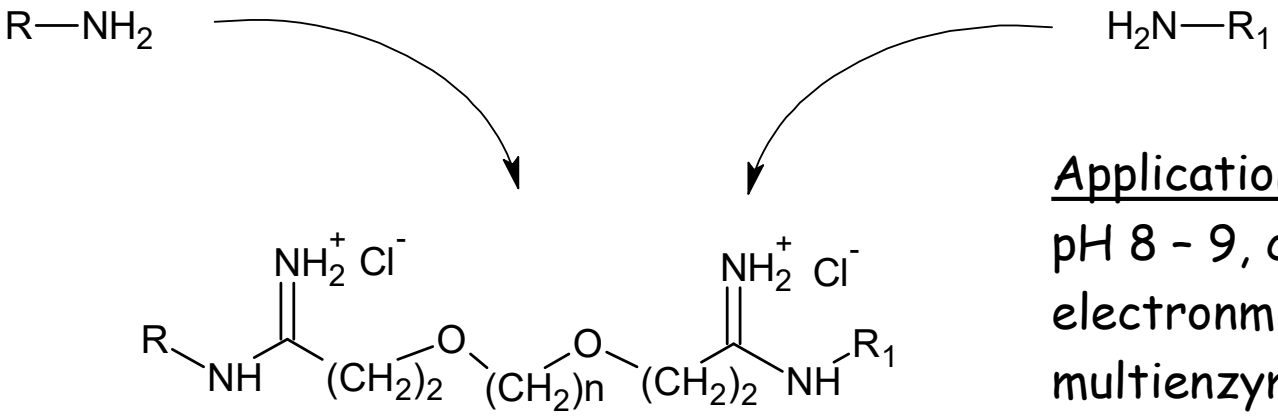
hydrophil

DODP



n = 1, 2, 4

n = 1 DMDP



<u>Applications</u>:

pH 8 – 9, charge, water soluble

electronmicroscopy

multienzyme complexes

immuncomplexes structure

protein – A – immobilization

2.2.2. Cleavable

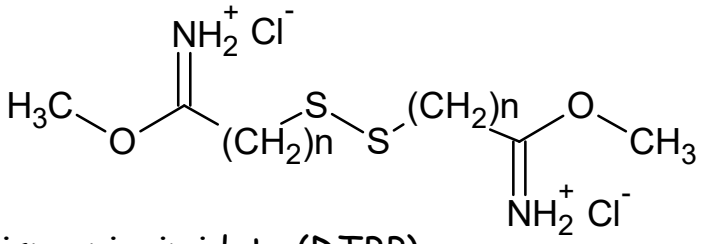
Application: electrophoresis → cleavage

molecular movement in cellular membrane

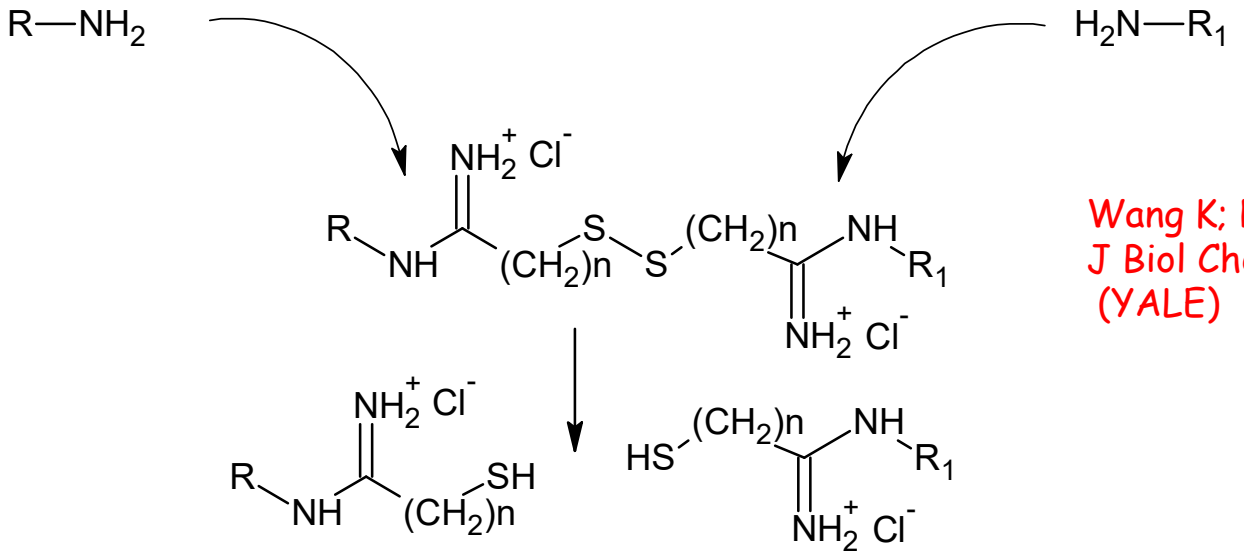
IA ag association

Shivdagani RA J Immunol 141 1252 (1988)

(a) Disulphide bond

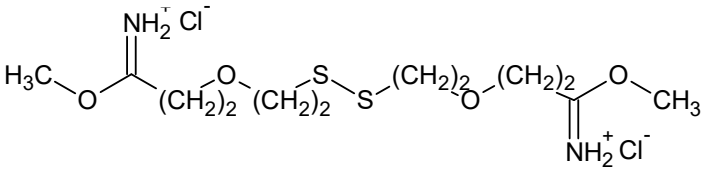


n = 2, Dimethyl 3,3'-dithiobispropionimidate (DTBP),
n = 3, DTBB, n = 4, DTBV, n = 6, DTBE



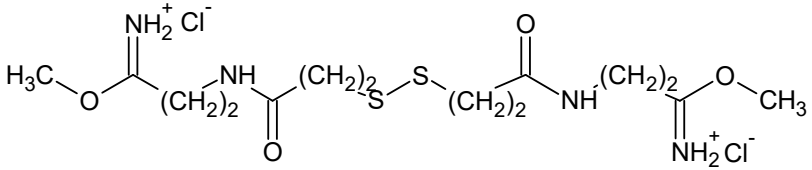
Wang K; Richards F:
J Biol Chem 249 8005 (1974)
(YALE)

1



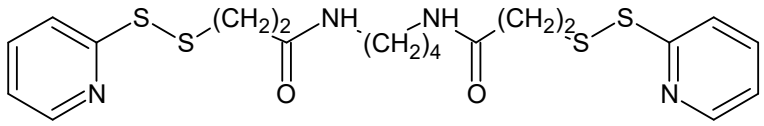
Dimethyl 3,3'(dithio-dimethyl-dioxi) bisimidate . 2HCl

2

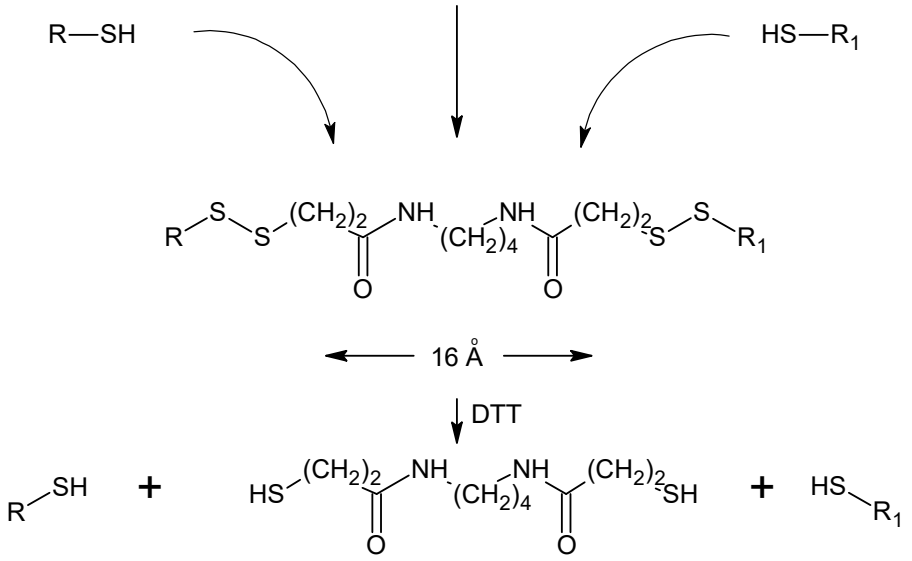


Dimethyl 3,3'(dithio-dimethylene-diamido) bispropioimidate . 2HCl

3

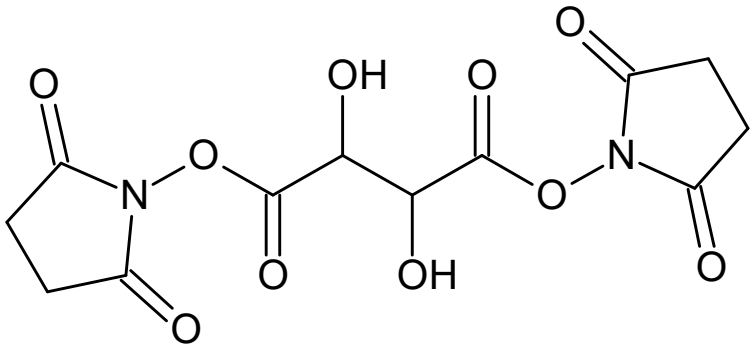


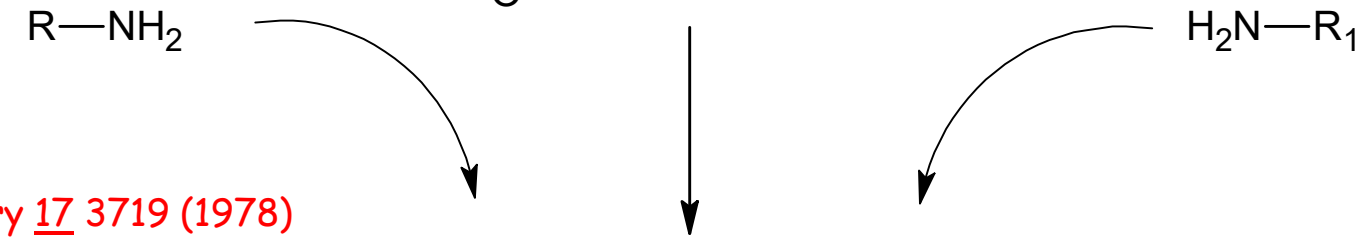
1,4-di[3'-(2'-pyridyldithio)propioamido] butane



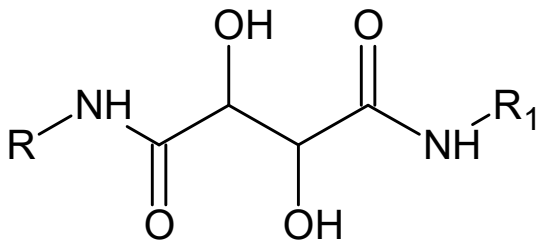
b Vicinal diol

Disuccinimidyl tartarate (DST)

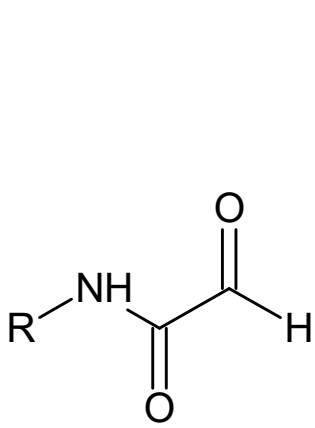


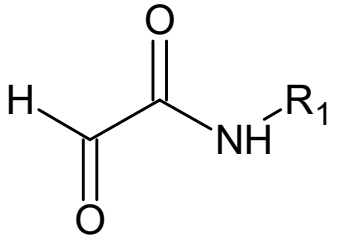


Smith RJ, Biochemistry 17 3719 (1978)



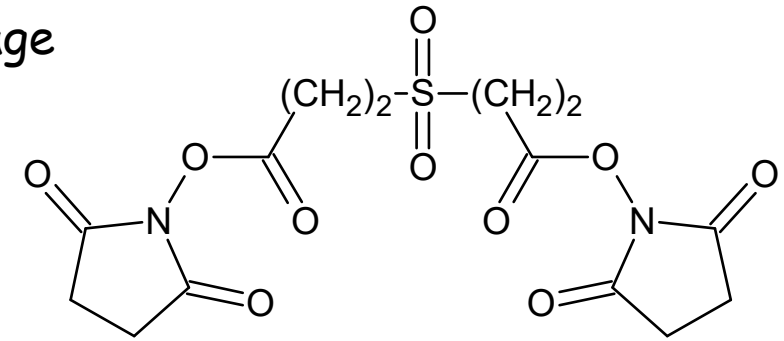
Amide



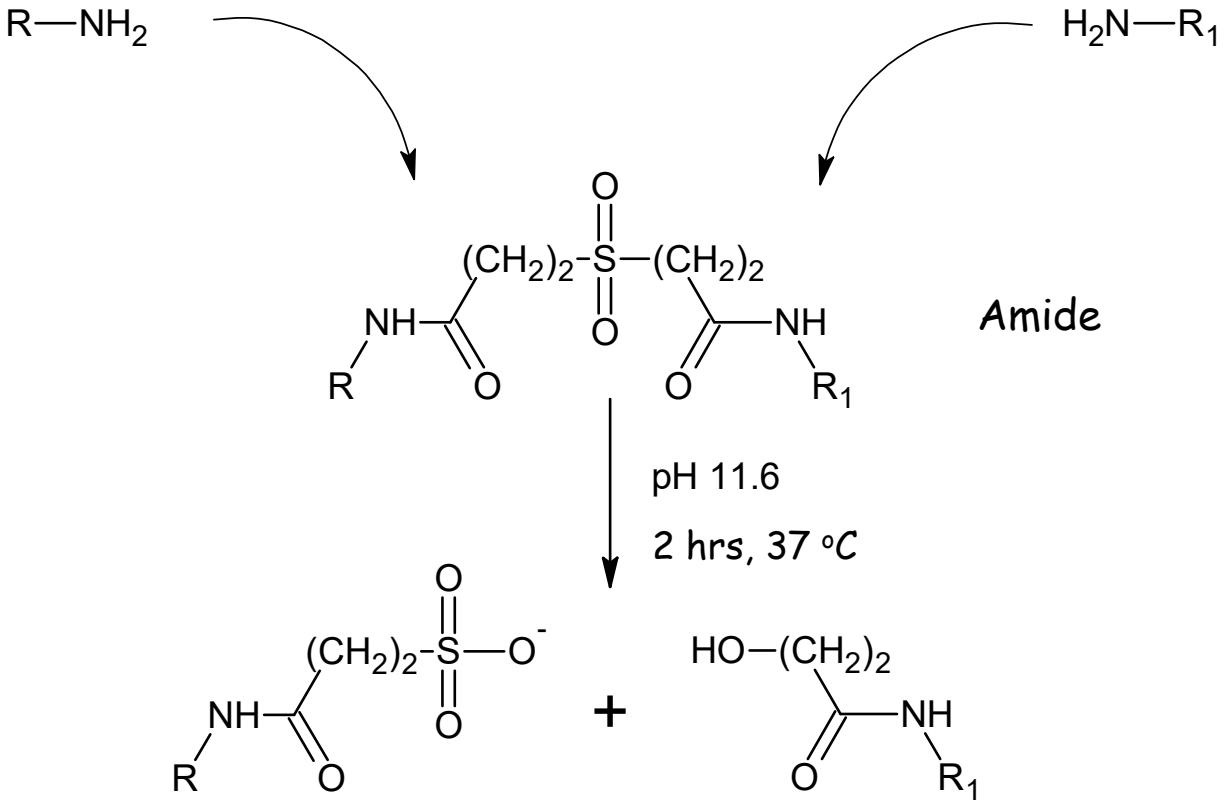


(c) Base sensitive linkage

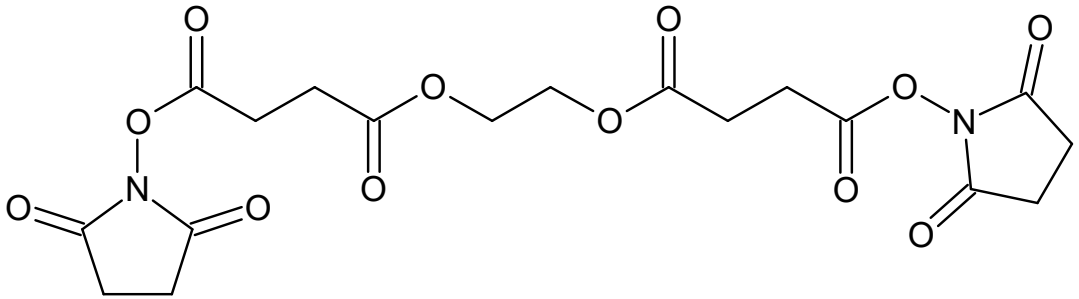
C1



Bis[2-(N-succinimidyl-oxycarbonyloxy)ethyl] sulfone (BSES) Non water soluble

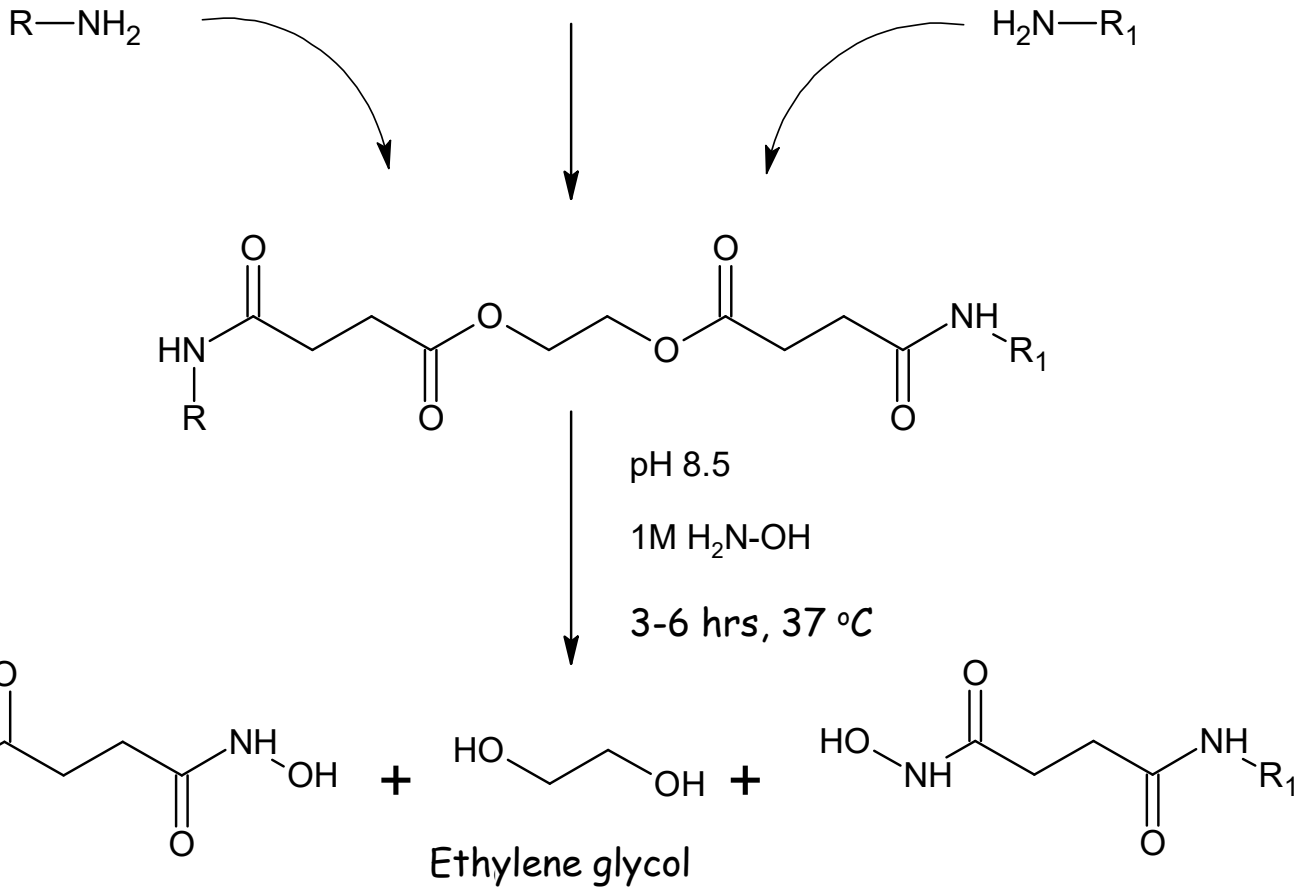


C2

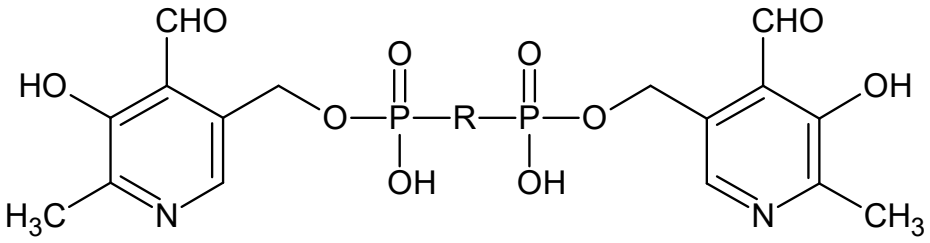


Ethylene glycol-bis(succinic acid N-hydroxysuccinimide ester) (EGS)

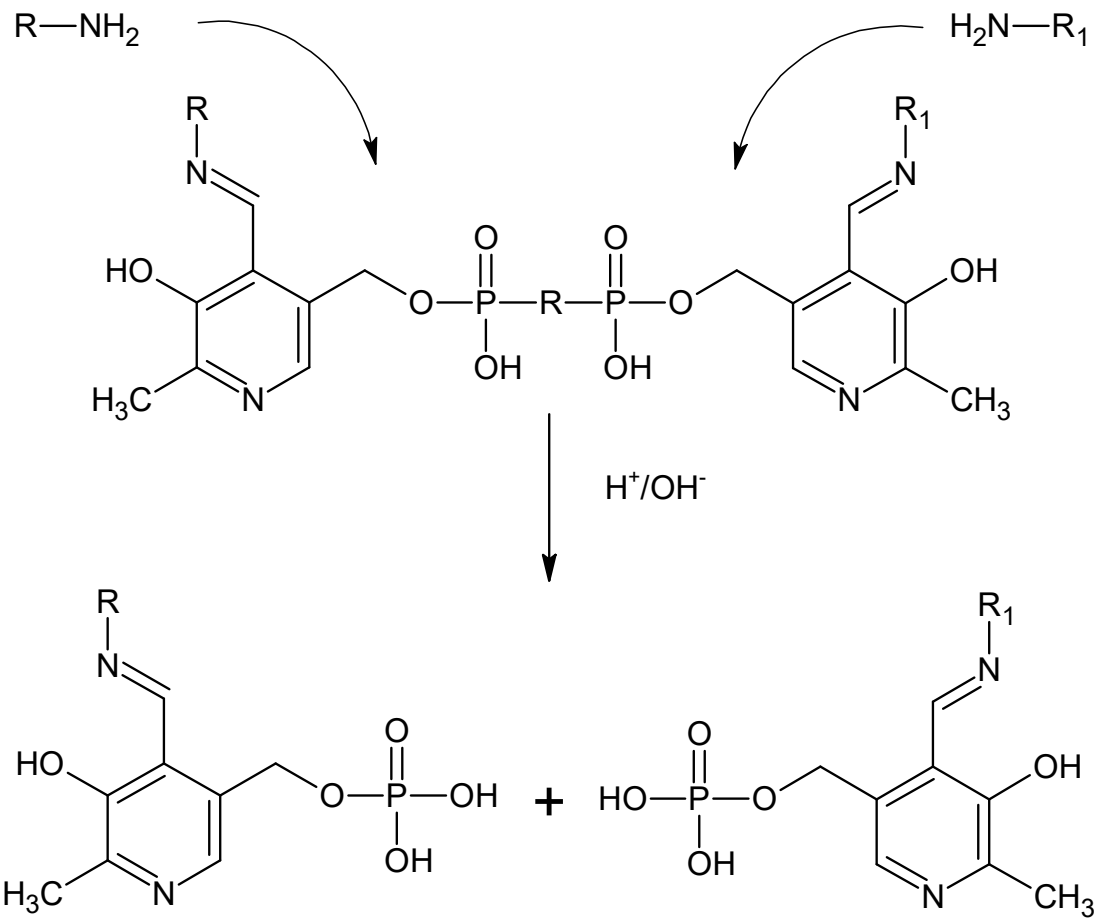
Non water soluble

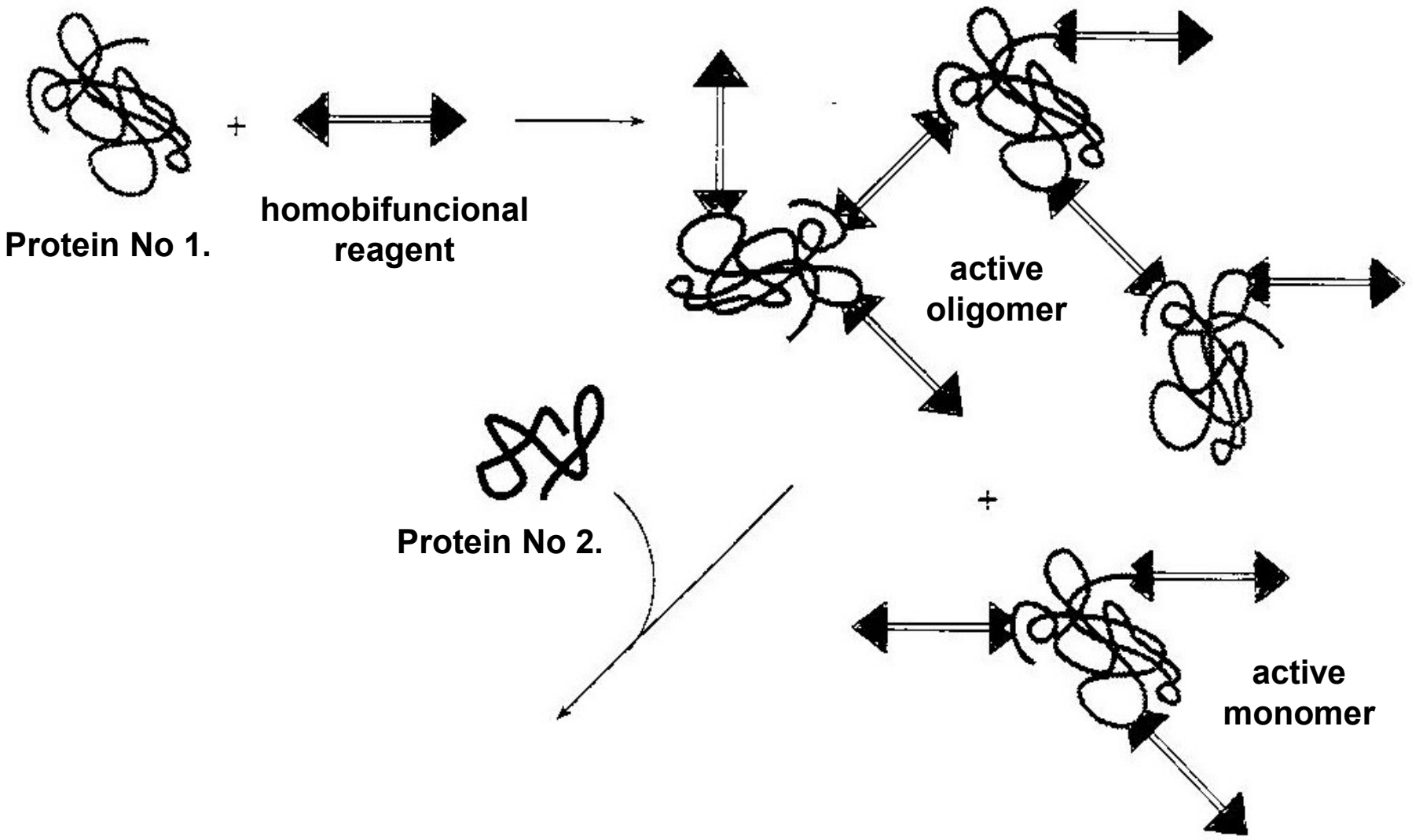


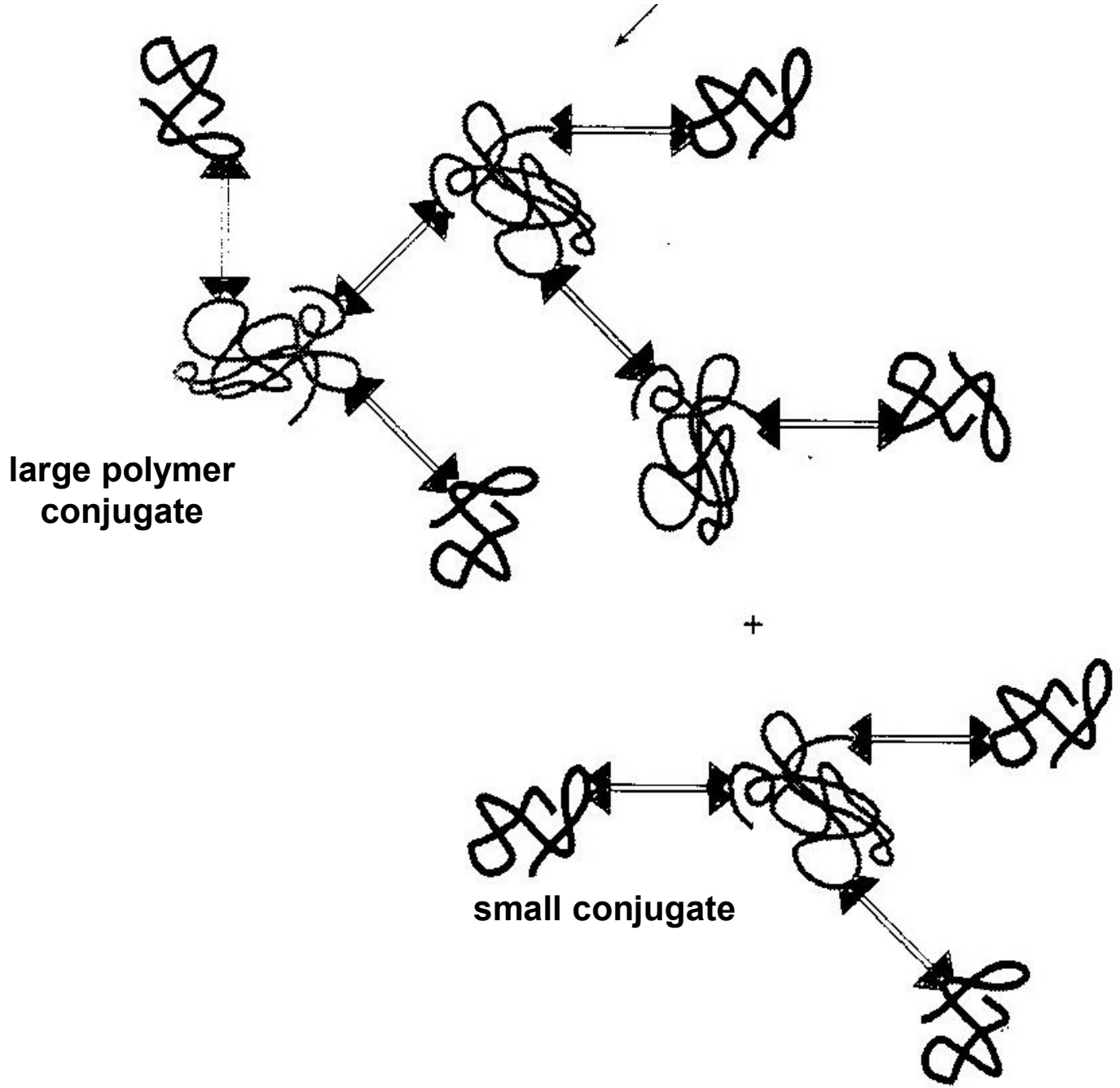
C3



P'P2-bis(5'-pyridoxal) diphosphate




Protein No 1.
homobifuncional
reagent
active
oligomer
Protein No 2.
active
monomer


large polymer
conjugate
+
small conjugate