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
   - Limited reactivity (eg. – OH vs. - CHO)
   - Improved selectivity (e.g. - NH_2 vs. - SH)
   - Space considerations
   - Convenient chemistry (e.g. - COOH vs. - NH_2) → Transformation

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

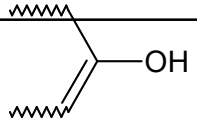
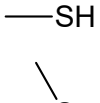
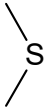
5. Conjugation
   - Chemical synthesis
   - Enzymatic synthesis (e.g. - NH_2 vs. - SH)
   - Gene technology

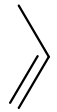
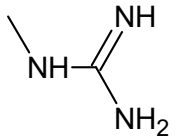
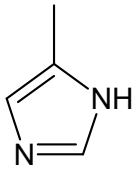
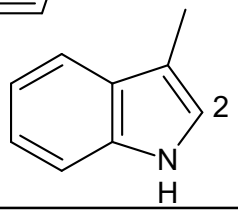
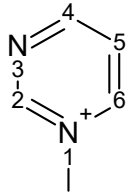
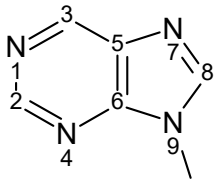
6. Analysis of conjugates
   - Purification
   - Structure determination

Design of bioconjugates

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

Target groups

Functional group	Proteins	Carbohydrates	Nucleic acids	Lipids
$-NH_2$	α(N), Lys	+ (N-glycoside)	-	+ Ser, ethanol amine
$-COOH/-PO(OH)_2$	α(C), Glu, Asp	-	+ 5′	+ Ser, fatty acids
-CHO	-	+	-	
-OH	Ser, Thr	+ primer, secunder (O-glycoside)	+ secunder 3′	+ glycerol, inositol, ganglioside
	Tyr	-	-	-
	Cys	-	-	-
	Met	-	-	-

Functional group	Proteins	Carbohydrates	Nucleic acids	Lipids
	Phe	-	-	-
$-CONH_2$	Gln, Asn	-	-	-
	Arg	-	-	-
	His	-	-	-
	Trp	-	-	-
	-	-	+4,6 S_N +2,3,4(5) S_E	-
	-	-	+2,6,8 S_N +1,3,4,7,8 S_E	-

Why do we need novel functional group?

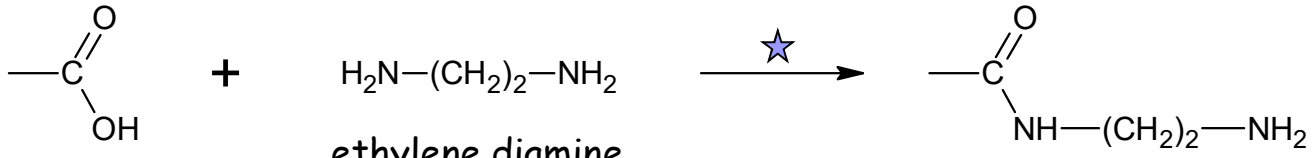
1. We need, but not present e.g. -COOH → $-NH_2$
2. We need higher reactivity e.g. -OH → -CHO
3. We need better selectivity e.g. $-NH_2$ → -SH
4. We need distance between the partners e.g. „spacer"

Tactics: „Trial and error"

1. - NH_2 function
2. - NH-NH_2 function
3. - COOH function
4. - CHO function
5. - OH function
6. - SH function

Establishment of amino function

A)



$H_2N—(CH_2)_3—NH_2$

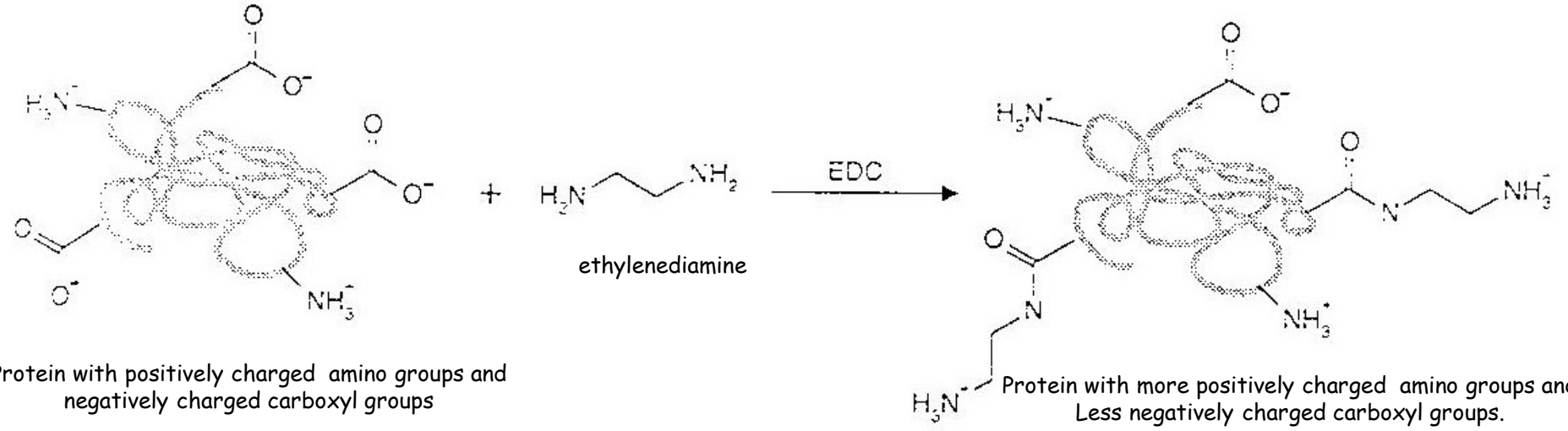
1,3 - diamino propane

$H_2N—(CH_2)_6—NH_2$

1,6 - diamino hexane

$H_2N—(CH_2)_2—NH—(CH_2)_2—NH_2$

3,3′ – imino-*bis*-propylamine

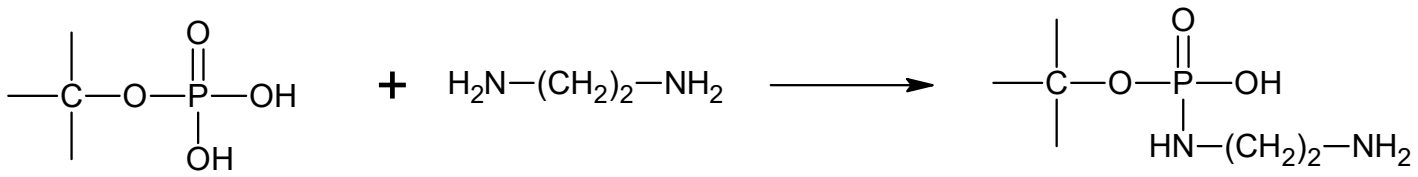


Protein with positively charged amino groups and negatively charged carboxyl groups

Protein with more positively charged amino groups and Less negatively charged carboxyl groups.

1. Activation ☆ needed: EDC; active ester; N,N' - carbonyldiimidazole
2. Decreasing the number of – and increasing the number of + charges and pI! (e.g. BSA pI 4.9 → pI 9.5-11)
3. Indirect (spacer) → hydrophobic interaction
4. Applications: proteins e.g. HRP, only 2 NH_2 groups
   glycoproteins → e.g. sialic acid

B)

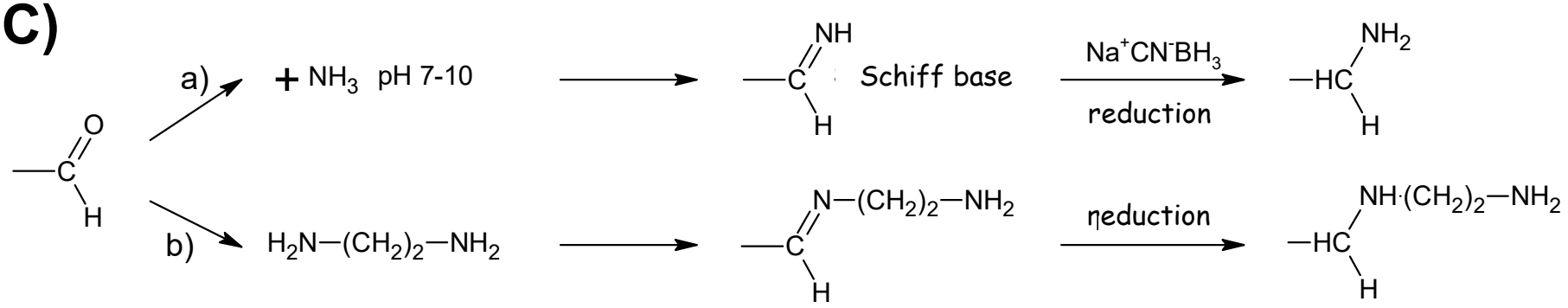


phosphoramidate

Applications: RNA, DNA (5'-OH)

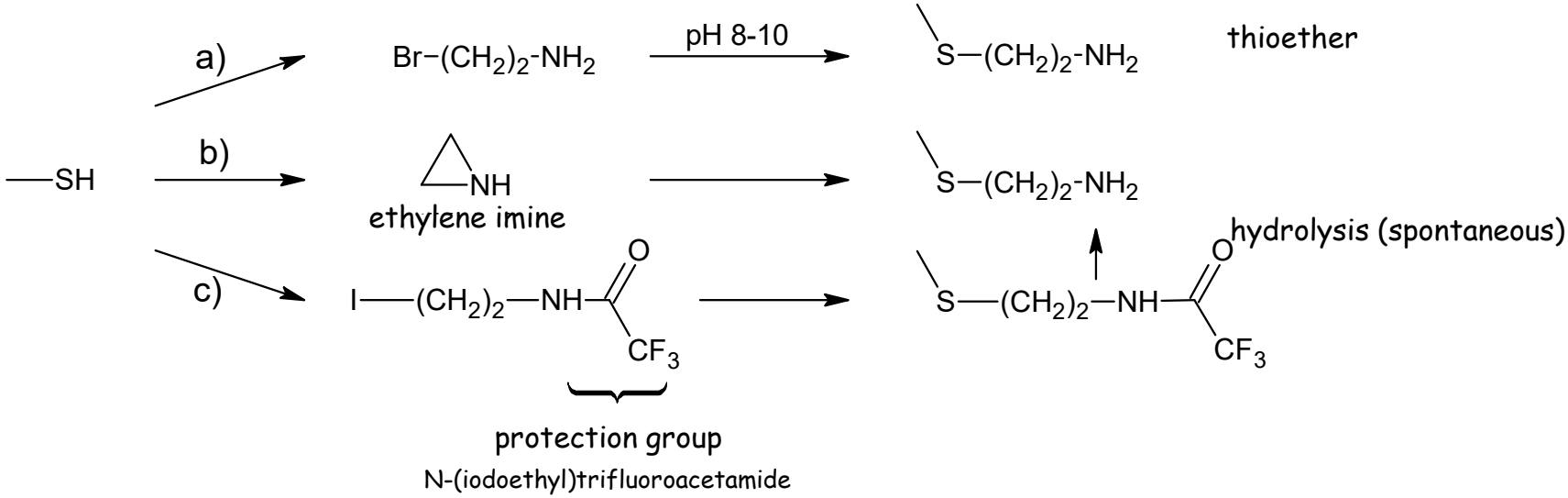
Chu, BCF et al. *Nucleic Acid Res* 14 5591 (1986)

C)



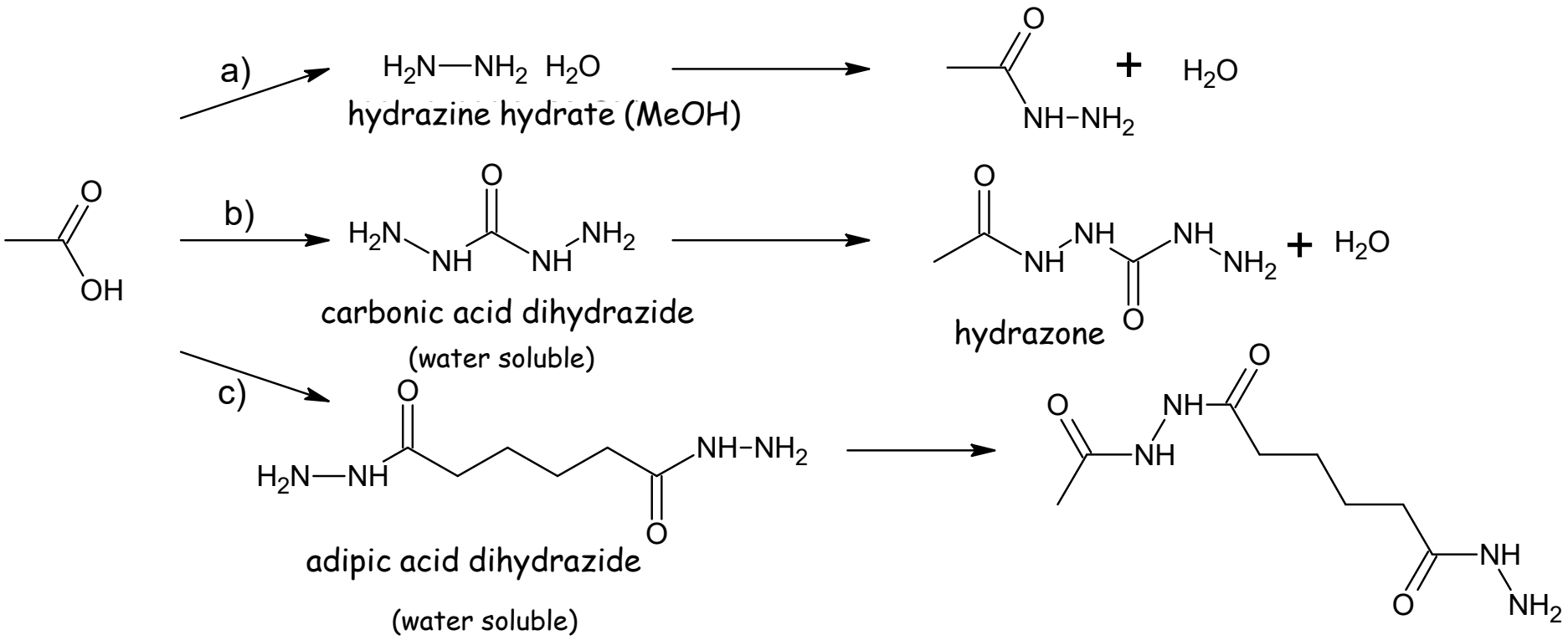
1. Spontaneous, pH 7-10, 10x molar access
2. Increasing the number of + charges, pI value
3. Direct: a); indirect: b)
4. <u>Applications</u>: glycoproteins, carbohydrates

D) Lindley, H. *Nature* 178 647 (1956)

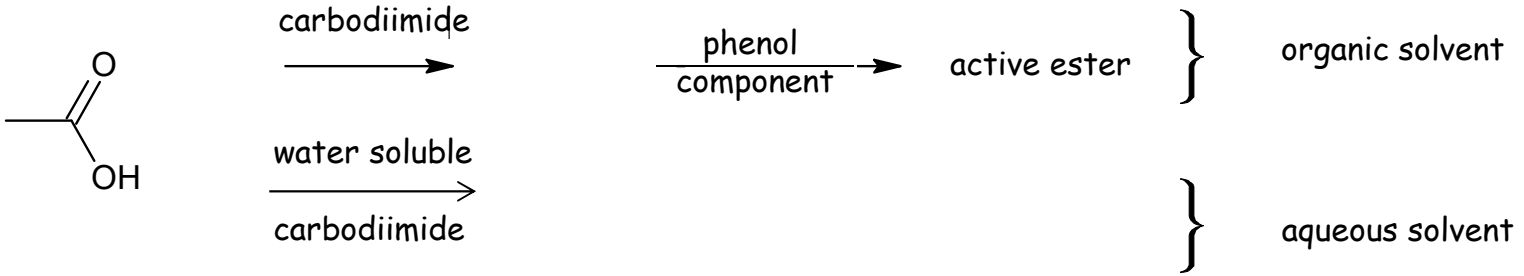


1. Spontaneous, pH 8-10, 10x molar access (DTT), in MeOH, 6 M guanidium·HCl
2. Increasing the number of + charges, pI value
3. Indirect
4. Applications: proteins

Establishment of hydrazino function

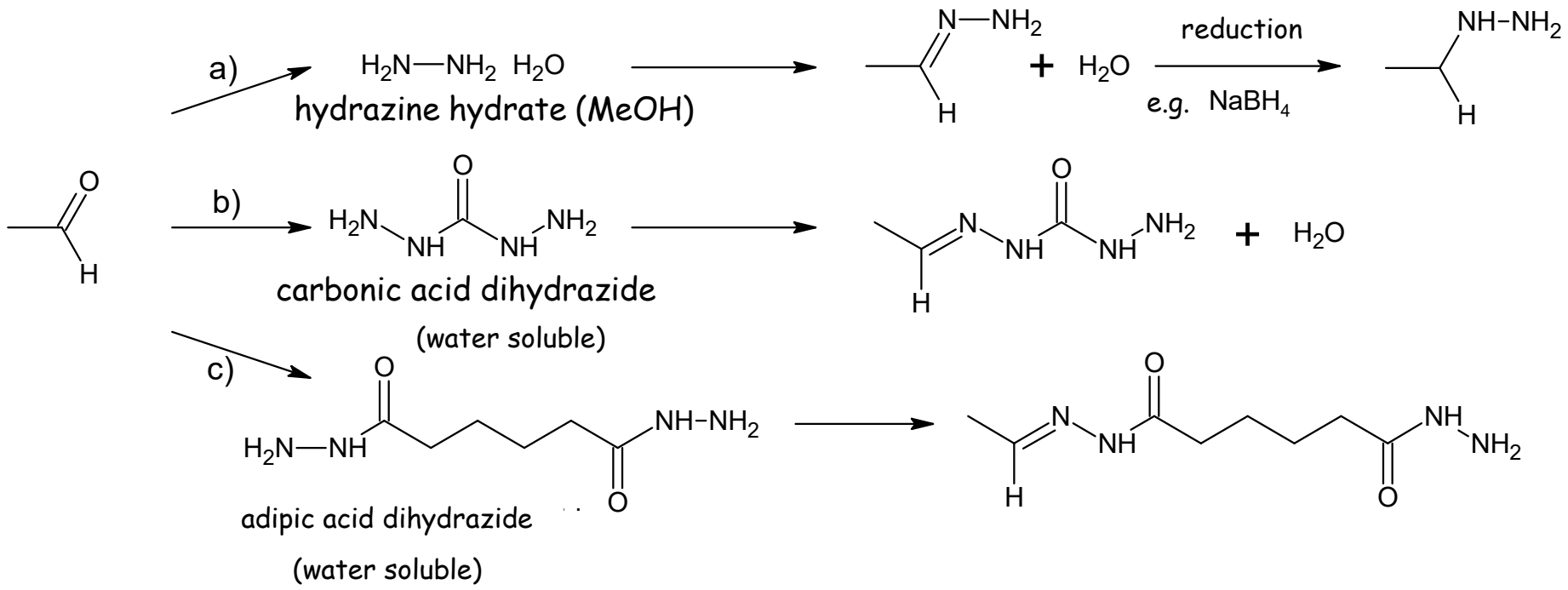


1. „Pre-activation" is needed (transformation to more active substance)

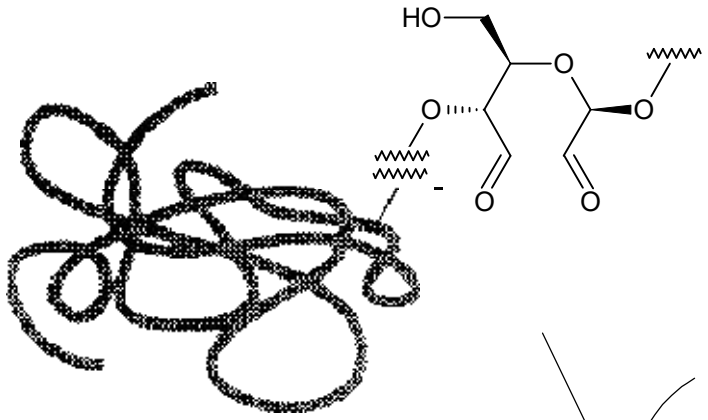


2. Decreasing number of negative charges, increased pI value
a) direct; b) indirect (spacer)

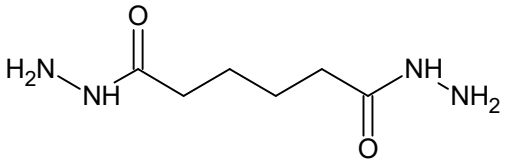
3. Applications: proteins, microtiter plates (e.g. elimination of charges)



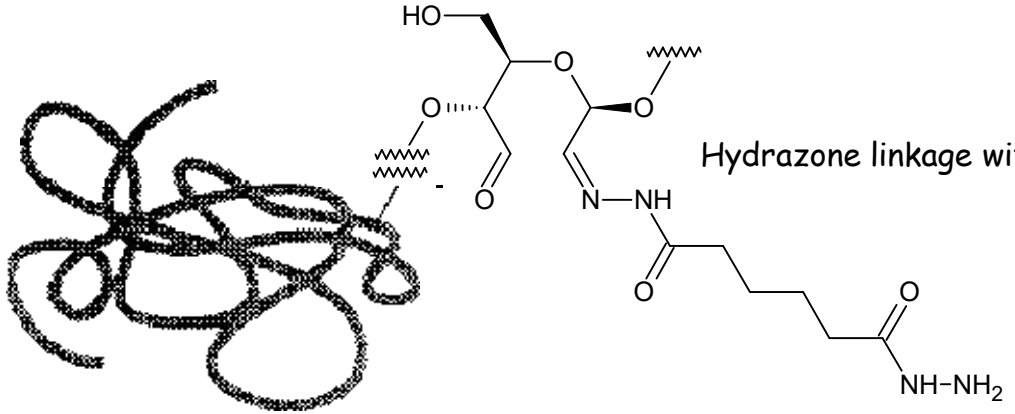
1. Spontaneous
2. No change in pI value
3. Applications: glycoproteins (antibodies, enzymes), carbohydrates (dextran, affinity chromatography)



Glycoprotein with periodate oxidized oligosaccharide (aldehyde)

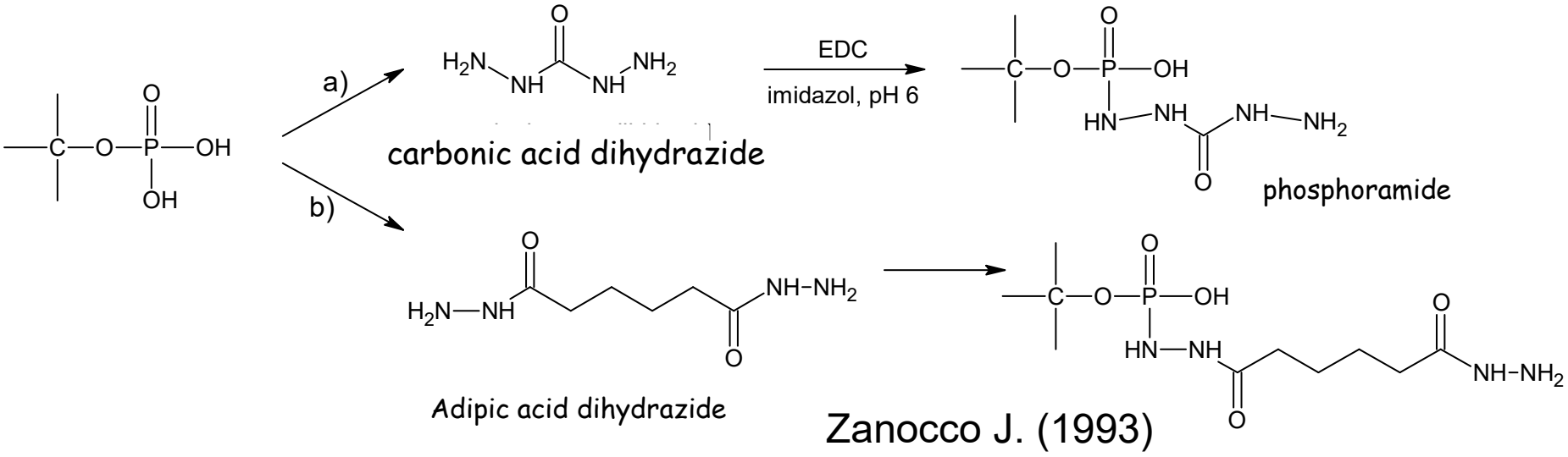


Adipic acid dihydrazide



Hydrazone linkage with terminal hydrazide

Ghosh, SS et al. *Anal Biochem* 178 43-51 (1998)



1. Preactivation
2. Decreasing the of - charges, pI value
3. Applications: RNS, DNS (5'-OH)

Why do we need to establish function?

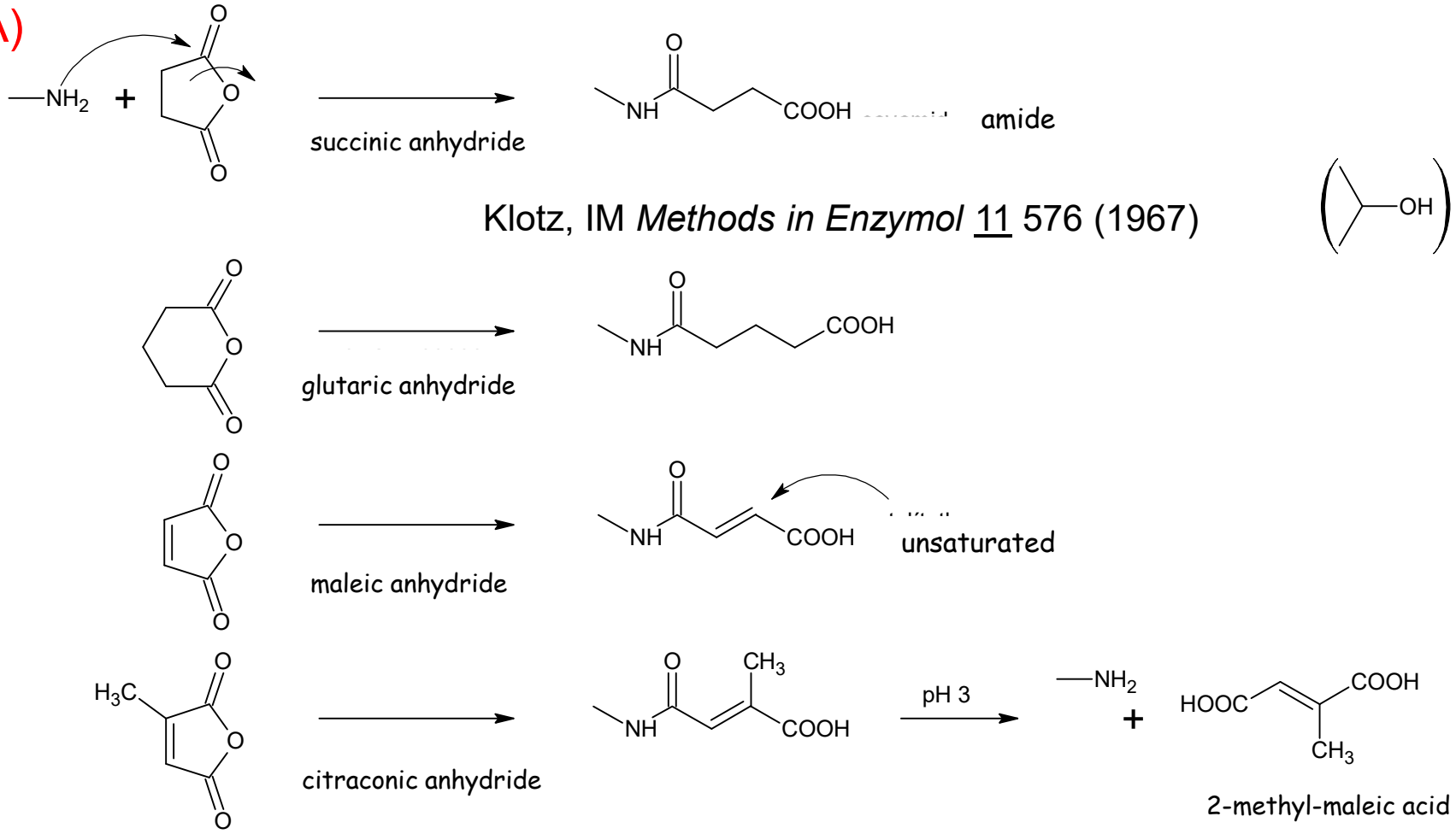
1. We do not have the desired one, e.g. -COOH → $-NH_2$
2. Increased reactivity, e.g. -OH → -CHO
3. Selectivity, e.g. $-NH_2$ → -SH
4. Distance e.g. „spacer"

Tactics: „Trial and error"

1. - NH_2 fuction
2. - NH-NH_2 function
3. - COOH function
4. - CHO function
5. - OH function
6. - SH function

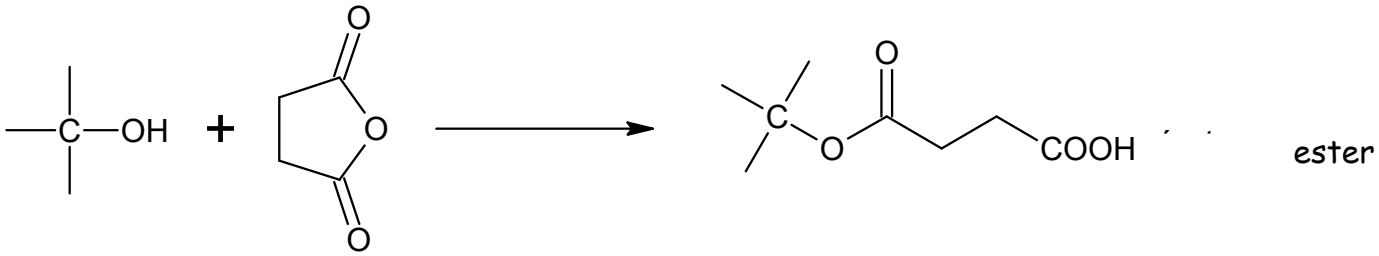
Establishment of carboxyl function

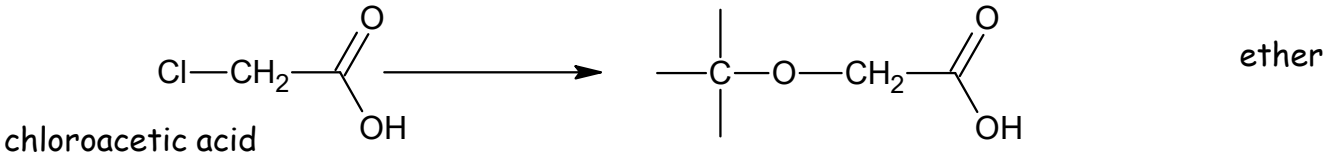
A)



1. Spontaneous reaction
2. Decreasing the number of + , increasing the number of - , decreasing the pI value
3. Indirect
4. Applications: proteins, carbohydrates (amino)

B)

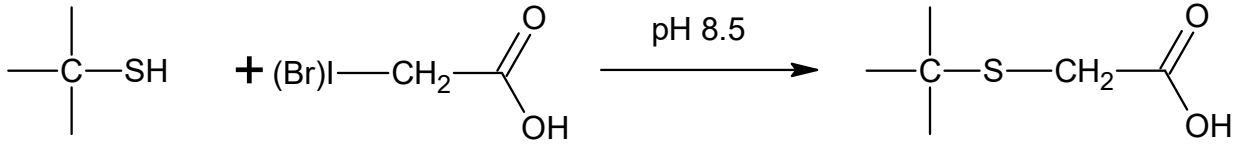


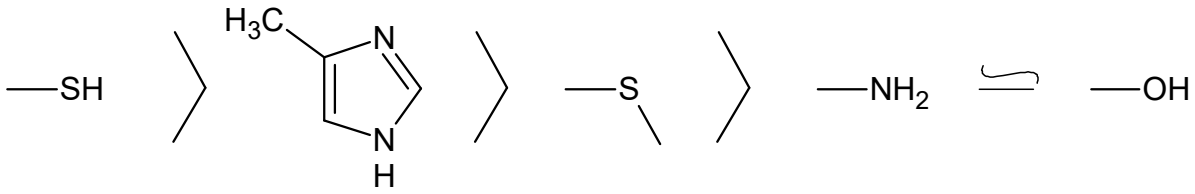


Plotz, PH *Biochemistry* 21 301 (1982)

1. Spontaneous reaction
2. Indirect
3. Applications: carbohydrates, proteins

C)



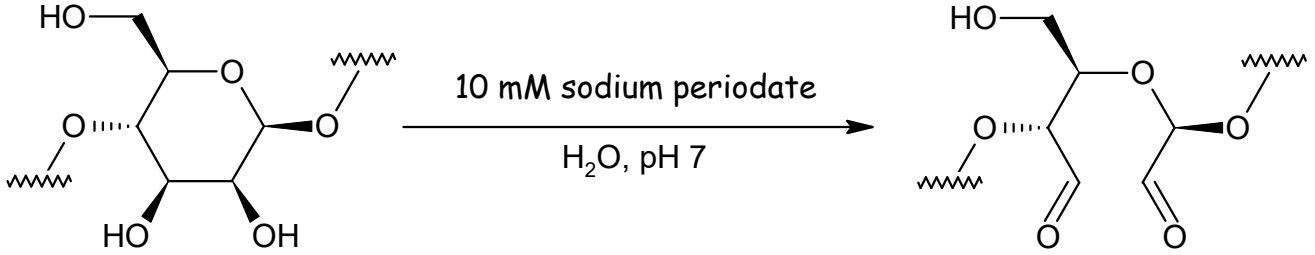


-I > -Br > -Cl > -F

Establishment of aldehyde function

A) Oxidation of vicinal diol

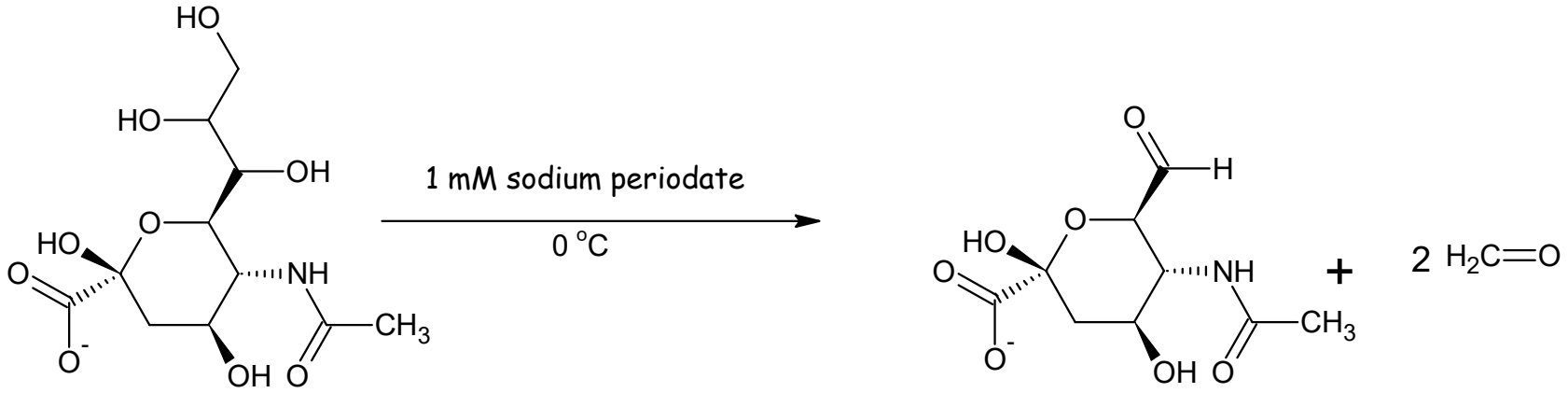
Bobbit, JM *Adv Carbohyd Chem* 11 1-41 (1956)



β-D-mannose in the chain

Split of the C-C bond
(formation of aldehyde function)

Van Lenten, L *J Biol Chem* 246 1889 (1971)
(terminal, vicinal, cis)

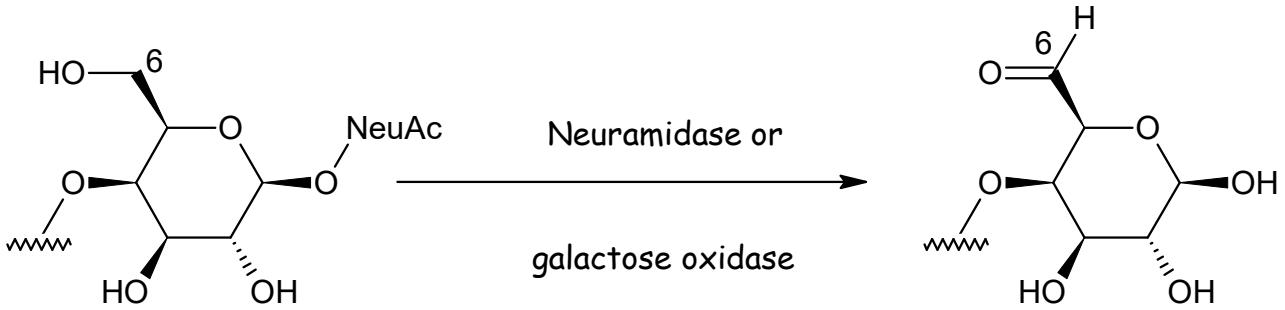


N-acety-D-neuraminic acid moiety

oxidation of the sialic moiety

B) Enzymatic oxidation

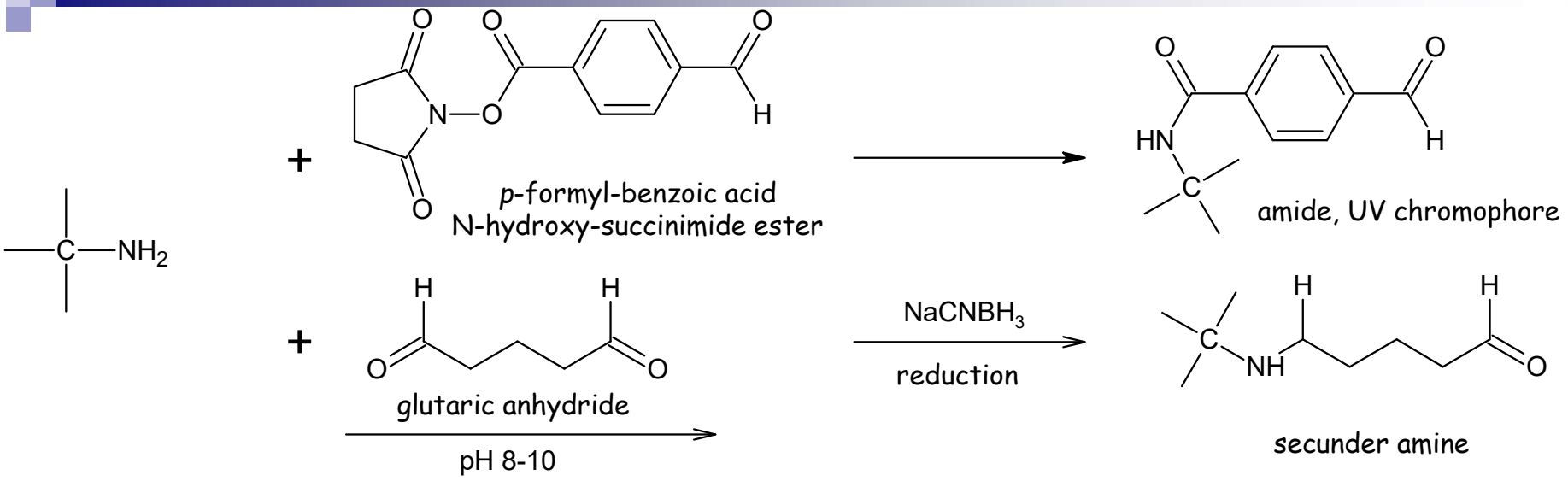
Avigad, E et al. *J Biol Chem* 237 2736 (1962)



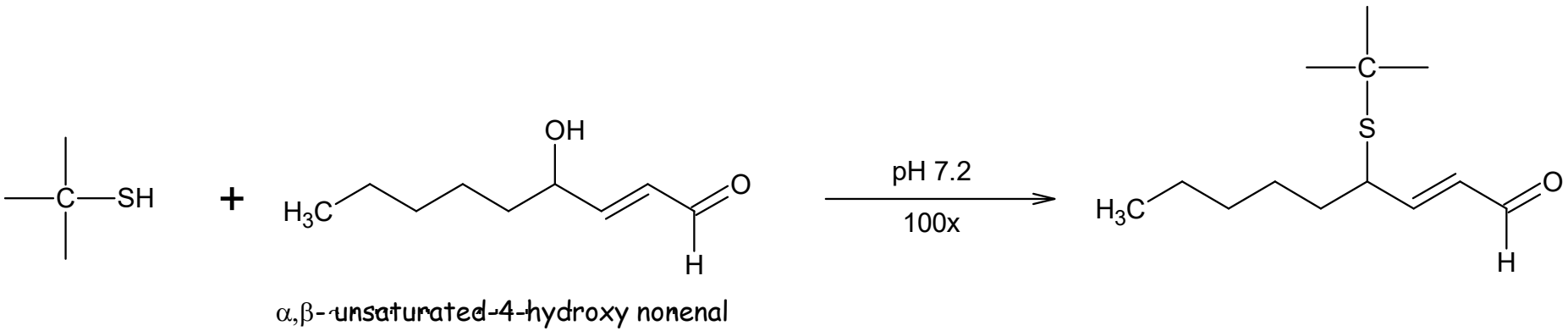
β-D-galactose

[N-Ac-D-galactose]

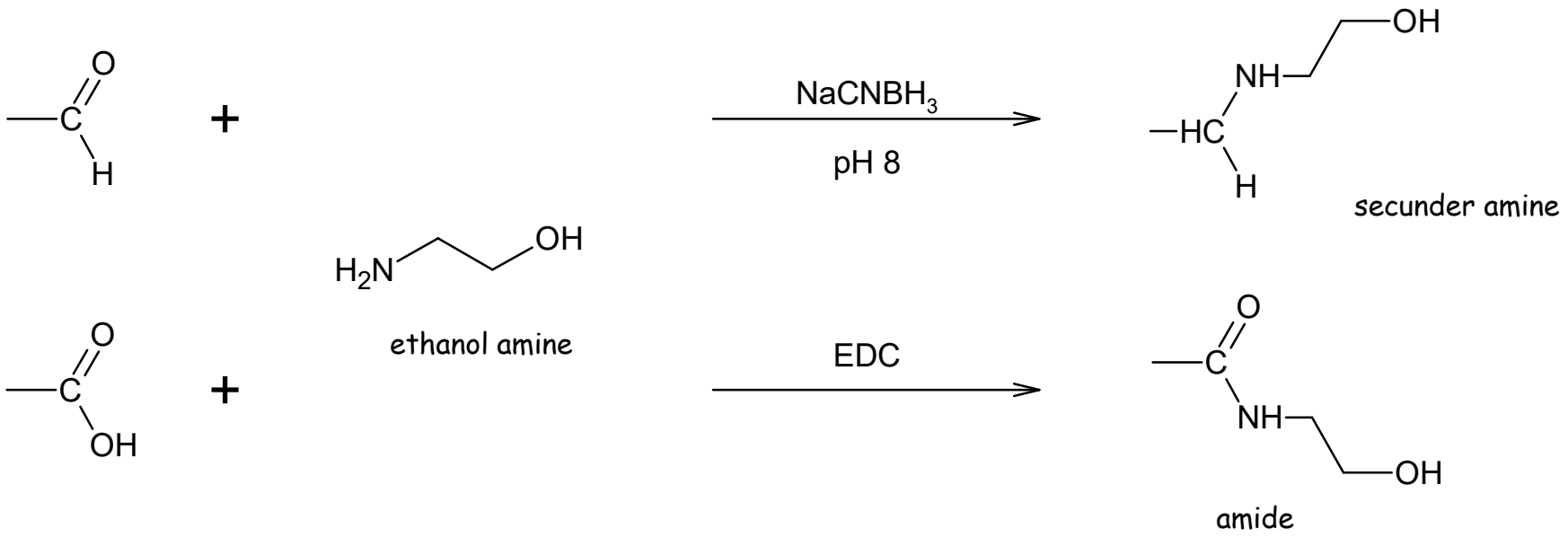
1. Spontaneous reaction
2. Direct
3. Applications: carbohydrates, glycoproteins



1. Spontaneous reaction
2. Indirect
3. <u>Applications</u>: protein, nucleic acid, carbohydrates ($-NH_2$)



Establishment of hydroxy function



1. Activation/reduction
2. Indirect
3. Applications: proteins, carbohydrates

Why do we need to establish function?

1. We do not have the desired one, e.g. -COOH → $-NH_2$
2. Increased reactivity, e.g. -OH → -CHO
3. Selectivity, e.g. $-NH_2$ → -SH
4. Distance e.g. „spacer"

Tactics: „Trial and error"

1. - NH_2 fuction
2. - NH-NH_2 function
3. - COOH function
4. - CHO function
5. - OH function
6. - SH function

Establishment of thiol function

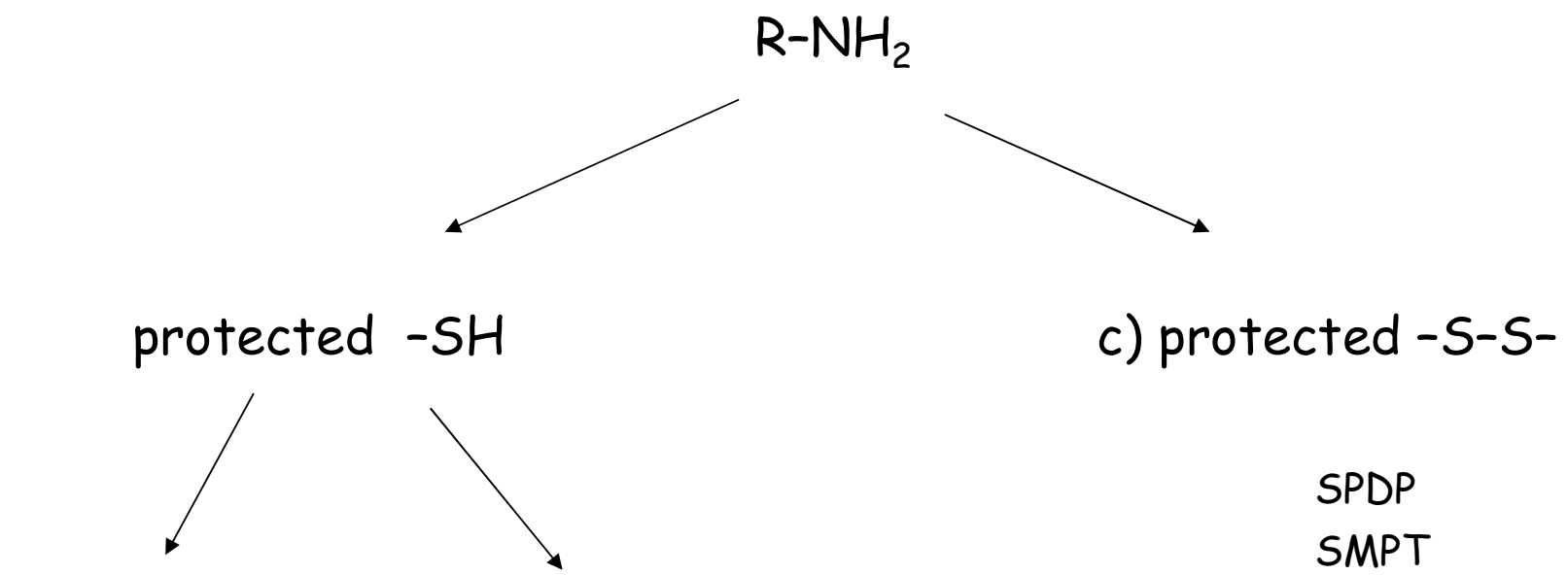
Pro: relatively low abundance in proteins

Con: easy to oxidize (-SH → -S-S-)

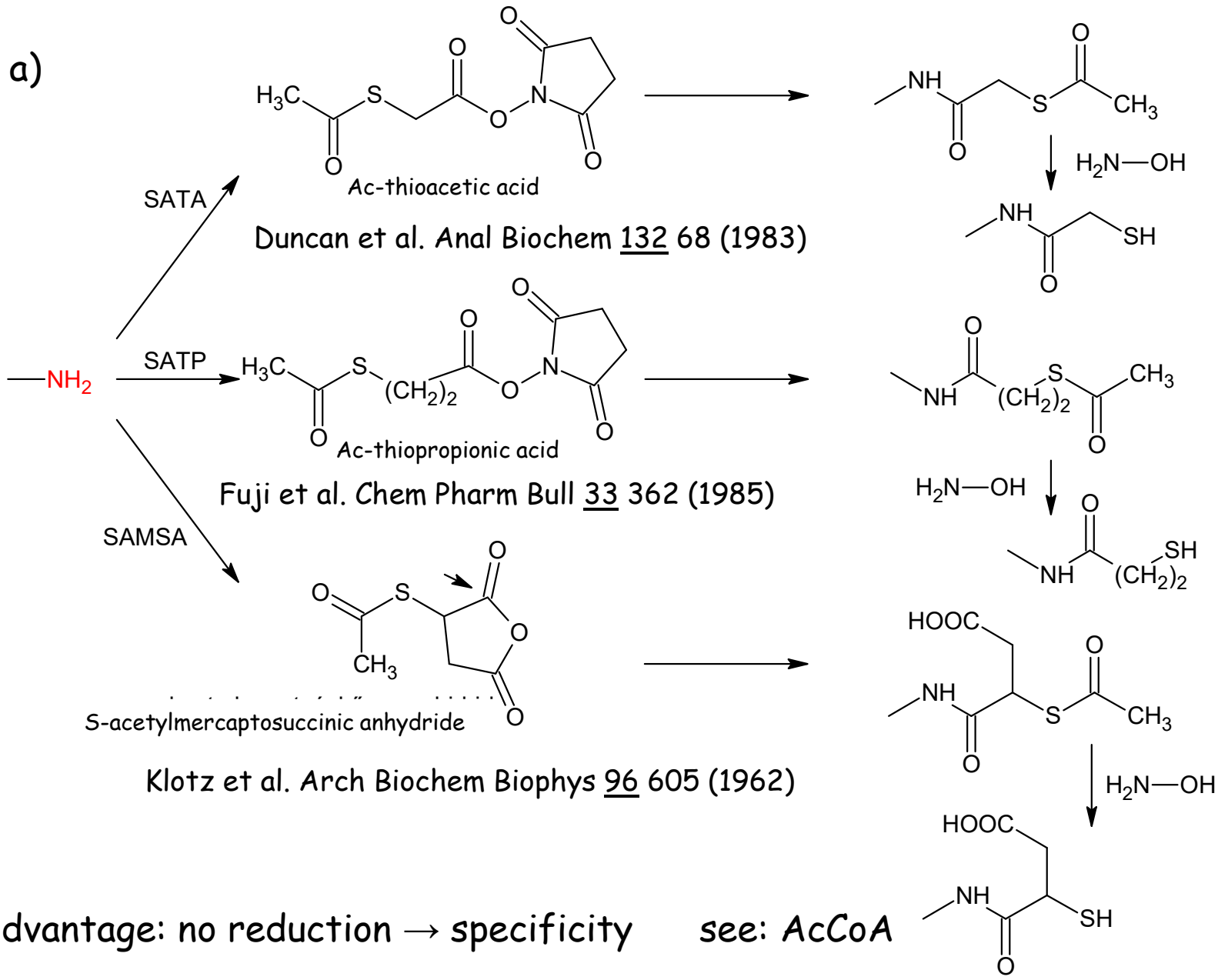
1. oxigene/nitrogen atmosphere
2. EDTA use (0.01 – 0.1M) → to avoid metal catalysis
   (pl. for BSA reaction c = 0.1M)

A) From amino function
B) From hydroxy function
C) From oxo function
D) From carboxyl function
E) From disulphid linkage

A) From amino function



a)



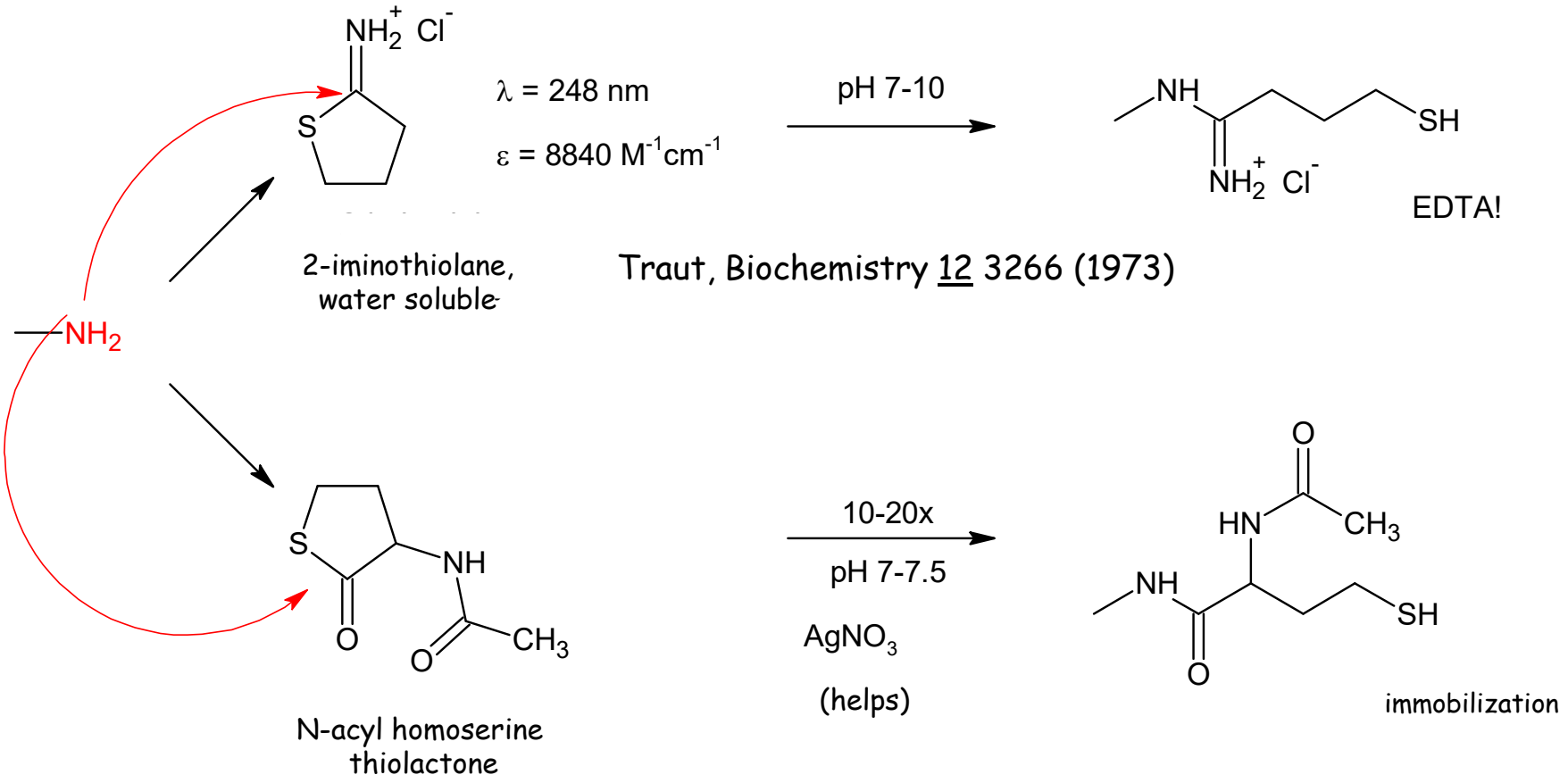
Duncan et al. Anal Biochem 132 68 (1983)

Fuji et al. Chem Pharm Bull 33 362 (1985)

Klotz et al. Arch Biochem Biophys 96 605 (1962)

Advantage: no reduction → specificity see: AcCoA

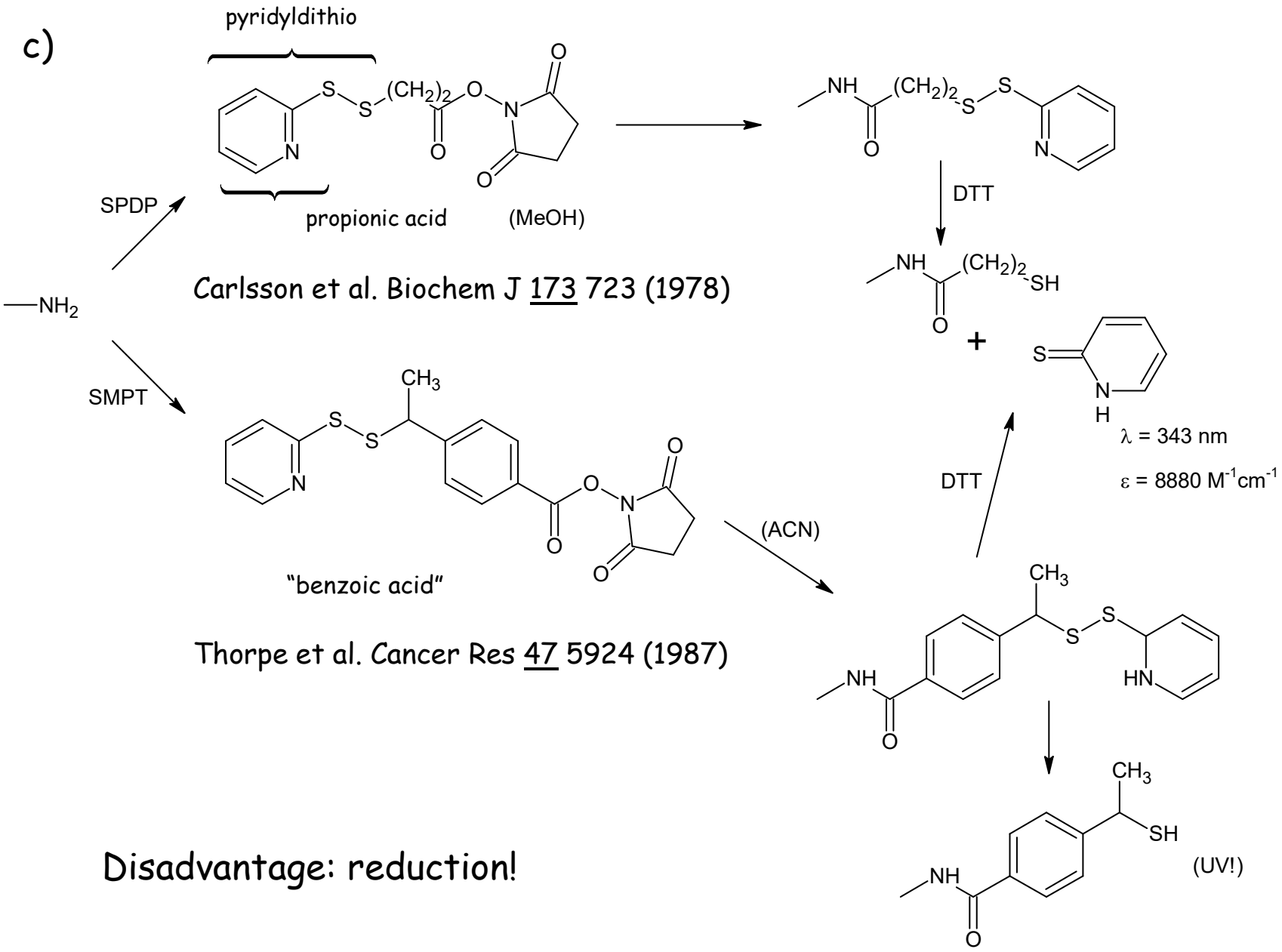
b)



Traut, Biochemistry 12 3266 (1973)

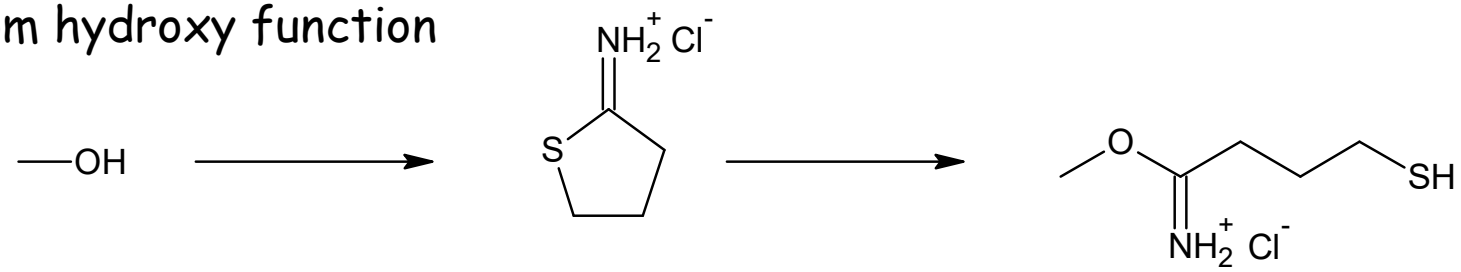
Eldjarn et al. Acta Chem Scand 17 2610 (1963)

c)



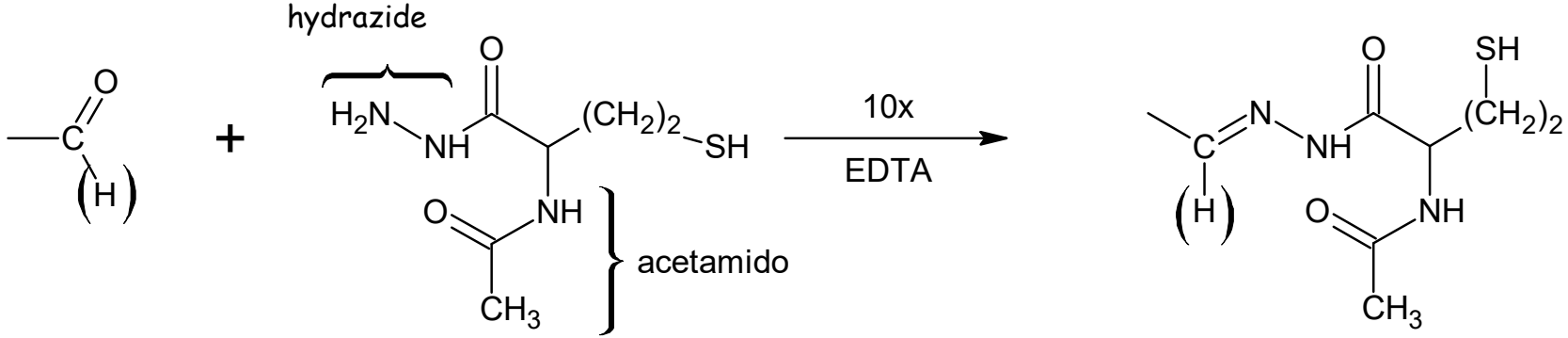
Disadvantage: reduction!

B) From hydroxy function



Tarentino et al. Glycobiology 3 279-285 (1993)

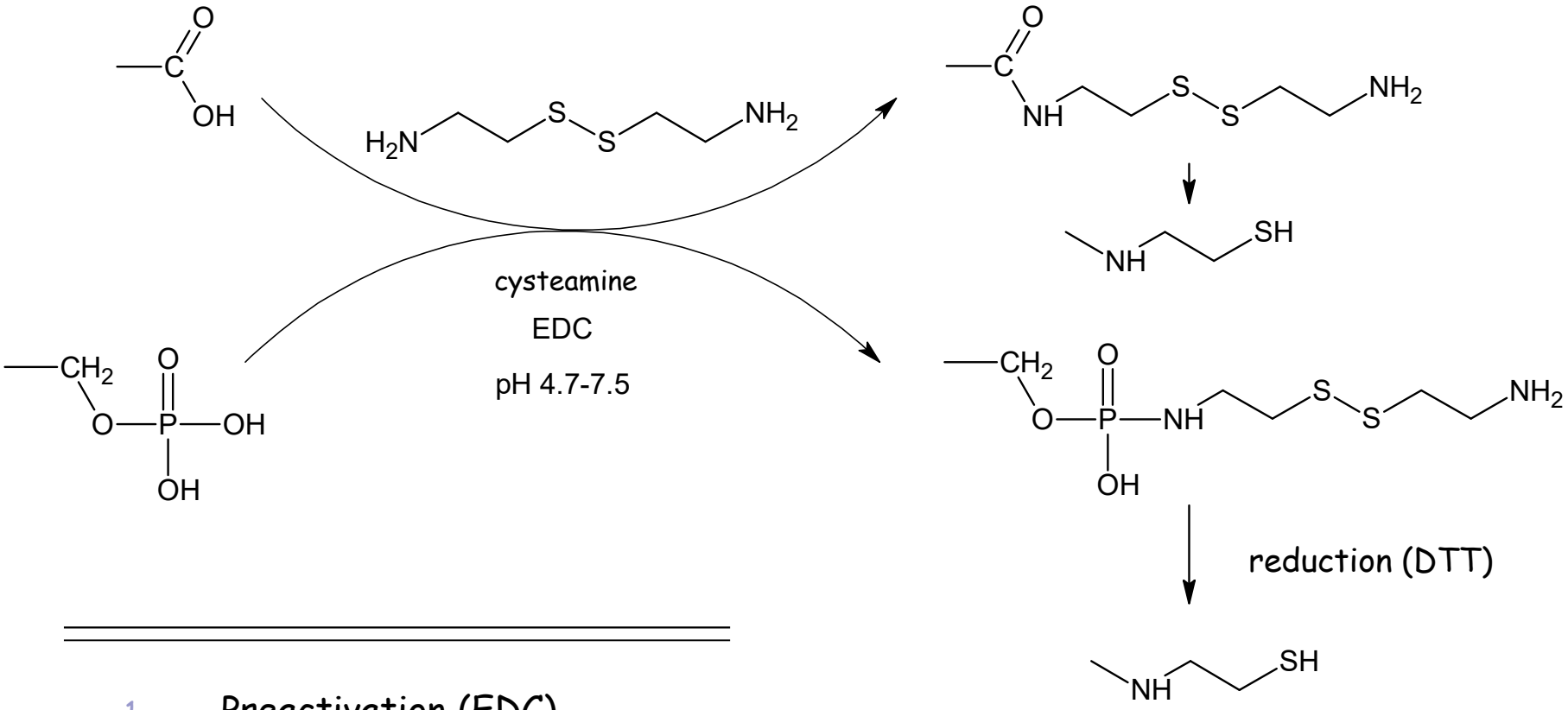
C) From oxo function



AMBH "butyric acid" (EtOH) hydrazone linkage

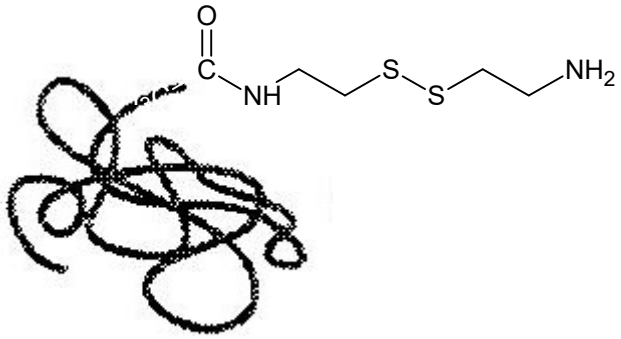
Taylor et al. Biochem Int 1 353 (1980)

D) From carboxyl function



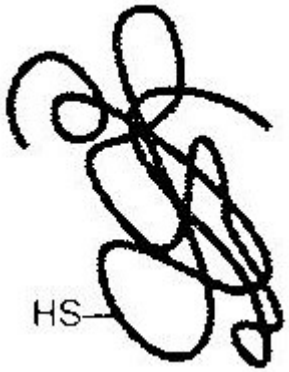
1. Preactivation (EDC)
2. From negative to positive charge
3. <u>Application</u>: RNS, DNS (5'-OH), protein

But:

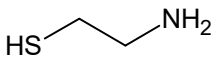


Protein modified with cysteamine

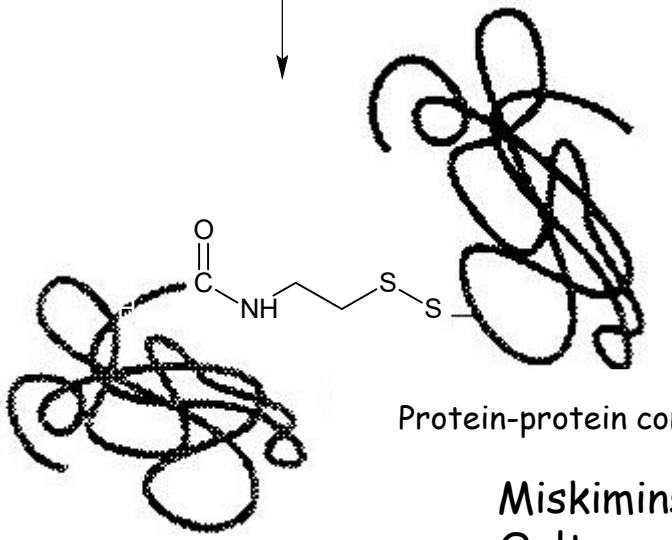
+



Protein with thiol function incorporated



2-mercaptoethylamine

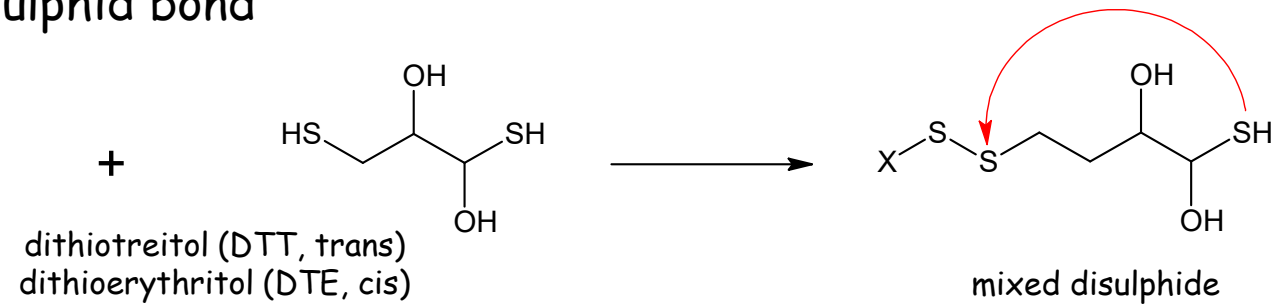


Protein-protein conjugate with disulphide bond

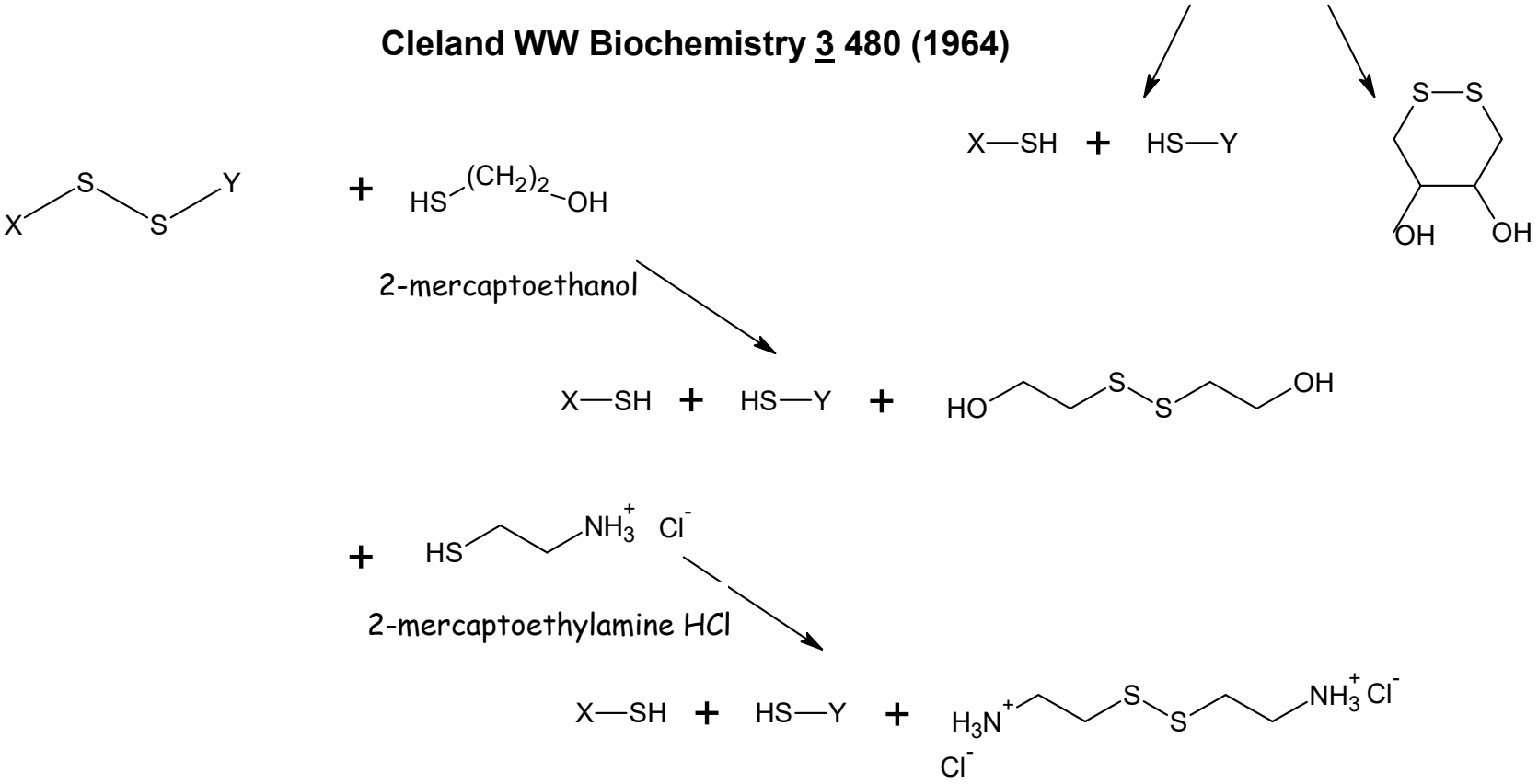
Insulin – diphteria toxin chain A
Antibody – toxin

Miskimins et al. 1979
Oeltmann et al. 1981

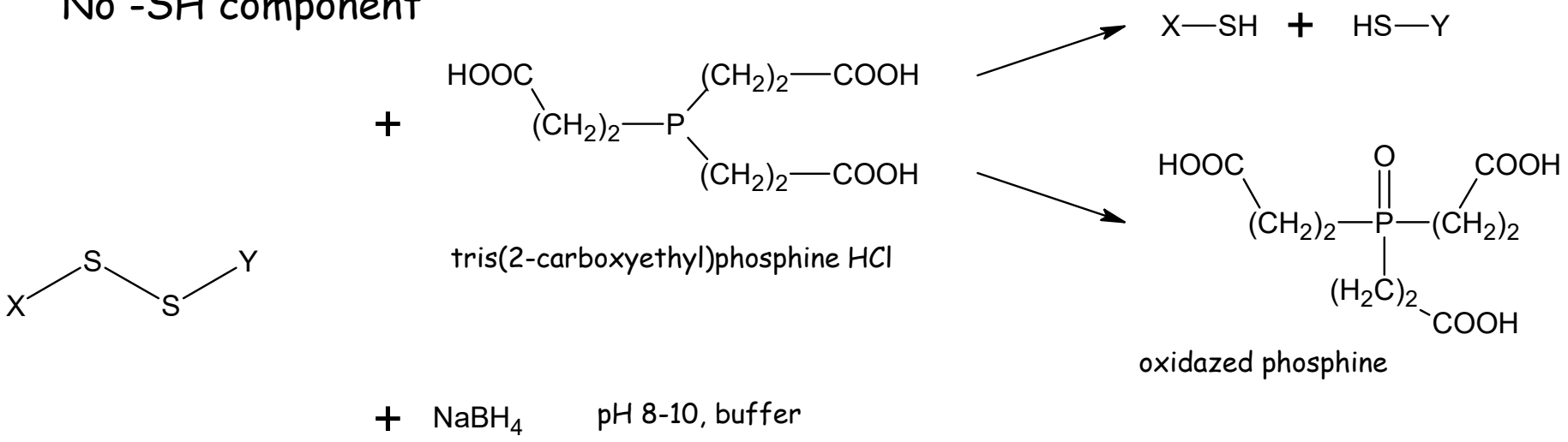
E) From disulphid bond



Cleland WW Biochemistry 3 480 (1964)



No -SH component



Gailit J Anal Biochem 214 334 (1993)

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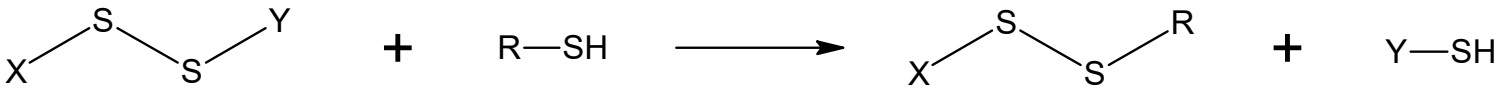
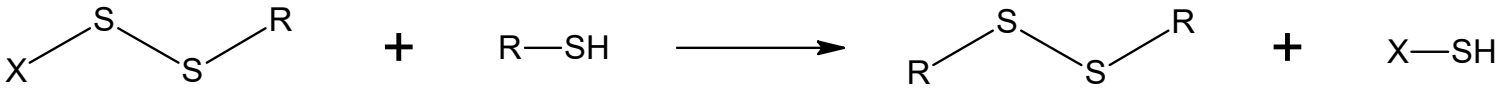
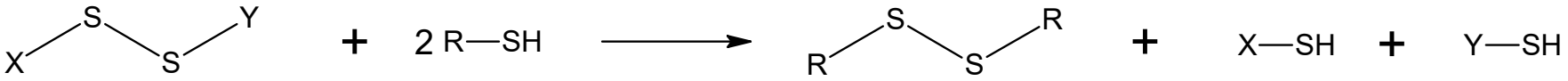
1. Spontaneous
2. Molar excess is highly important ! (min. 20x)
3. Applications: proteins (structural studies)
   establishment –S–S–X linkage
4. Denaturating agents: urea, guanidin, SDS
5. Other: Cys, HS-CH_2-COOH (tioglikolsav)

Reducing reagents with -SH group



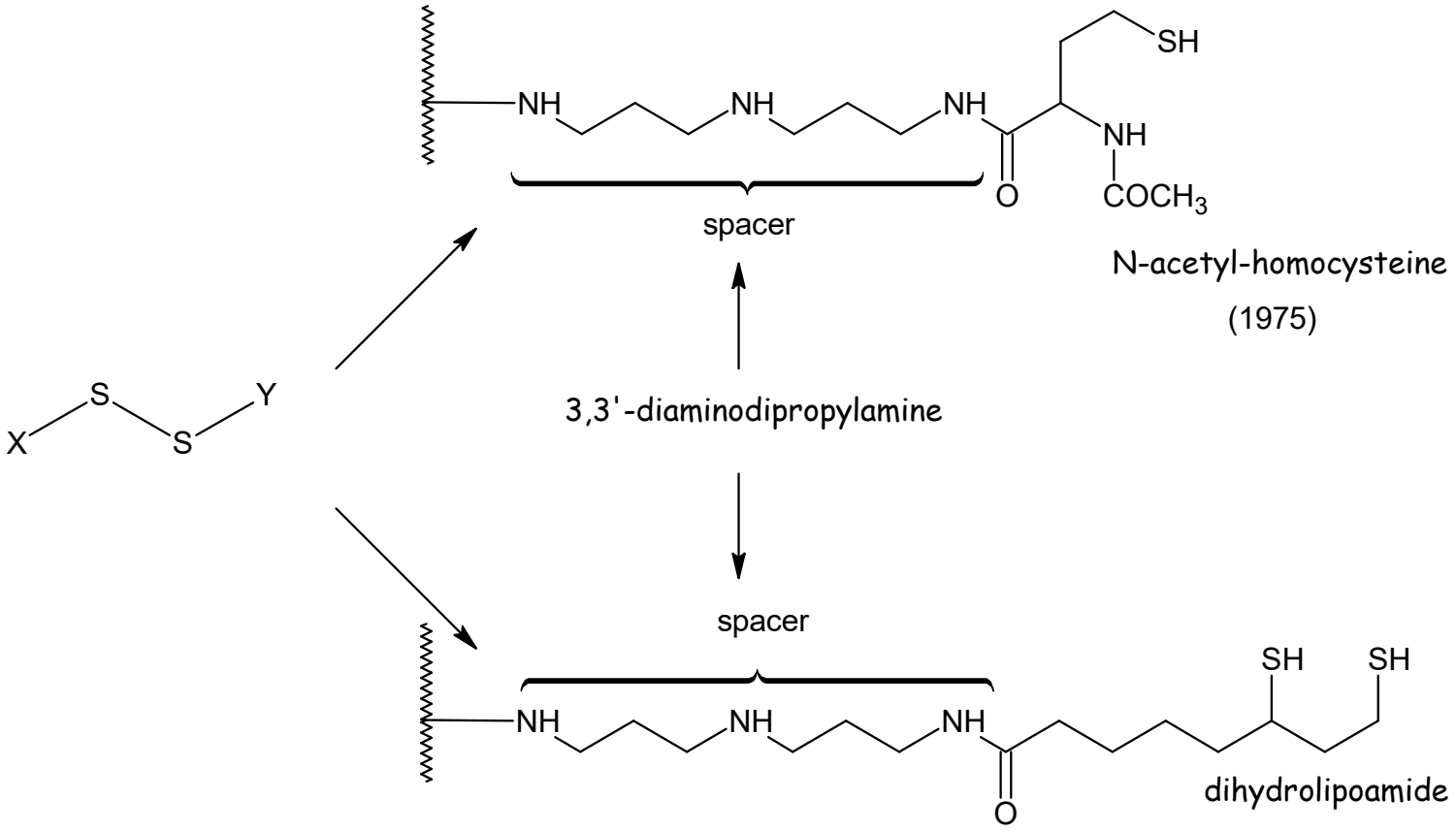
'mixed disulphid





symmetrical disulphid

Establishment of thiol function by immobilized reducing agents



Advantages: „no" by-products (oxidált redukálószer)
immobilized reducing agent
regeneration

Summary

From?

To what?	NH_2	$NH\text{-}NH_2$	COOH	CHO	OH	SH
NH_2			+	+		+
$NH\text{-}NH_2$			+	+		
COOH	+			(+)	+	+
CHO	+		(+)		+	+
OH			+	+		
SH	+		+	+	+	