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
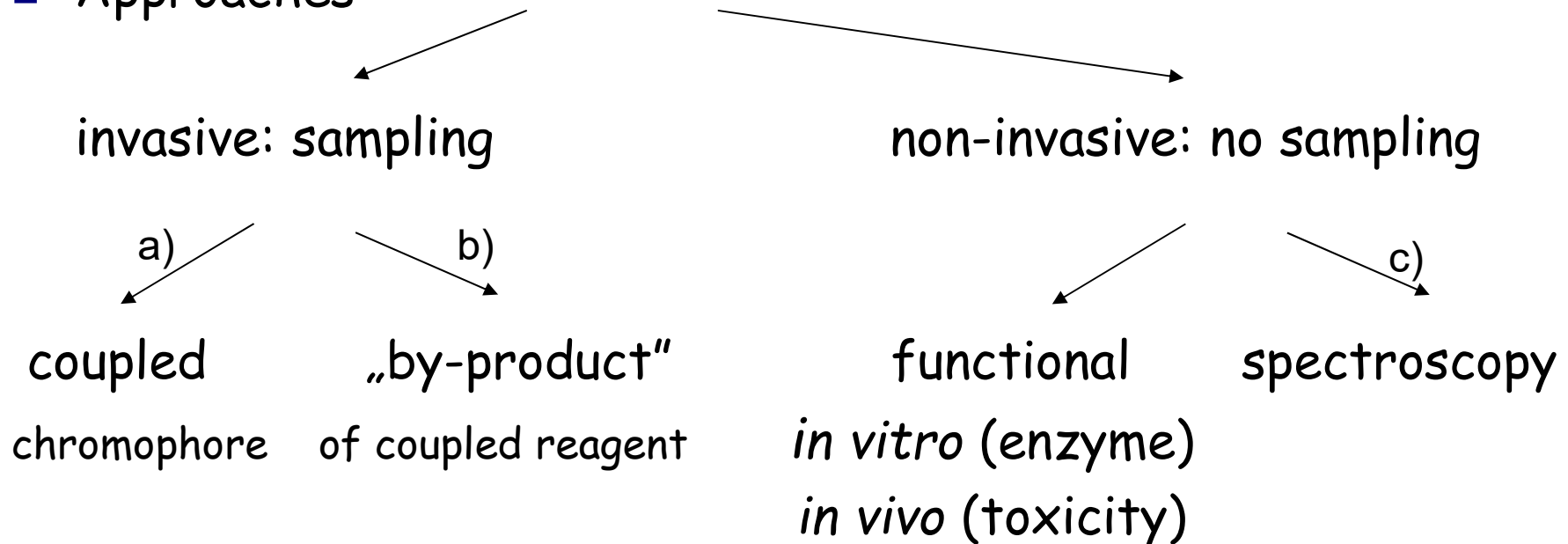   Purification
    Structure determination

Detection of the functional group

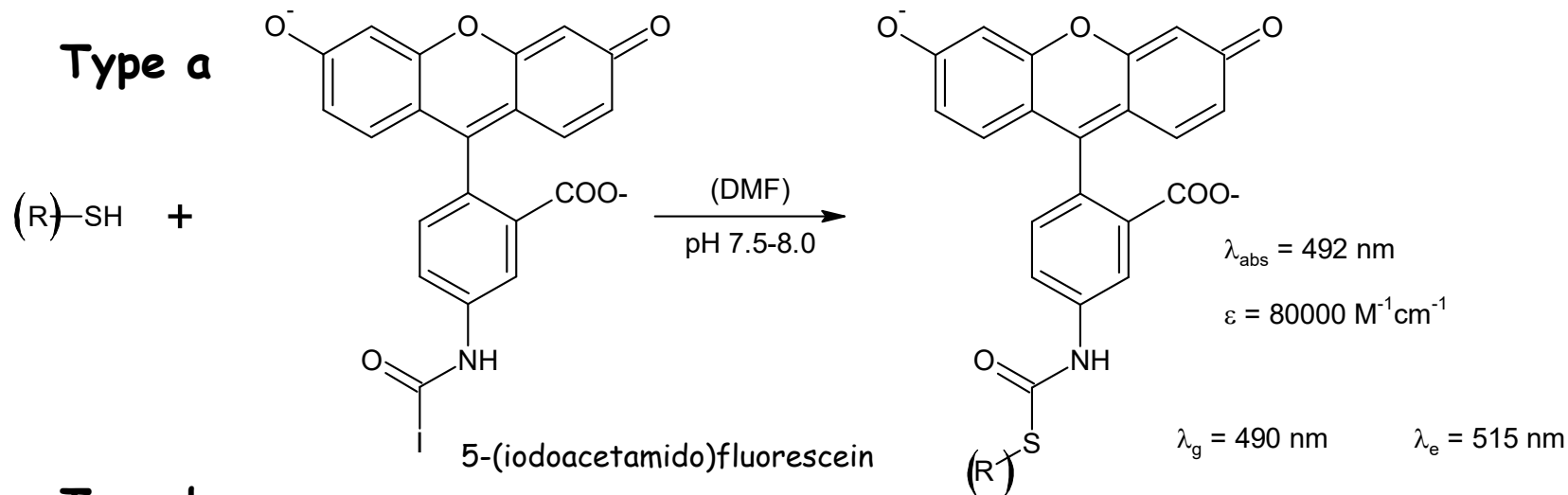- Requirements: 1) sensitive
                   2) quantitative
                   3) quick
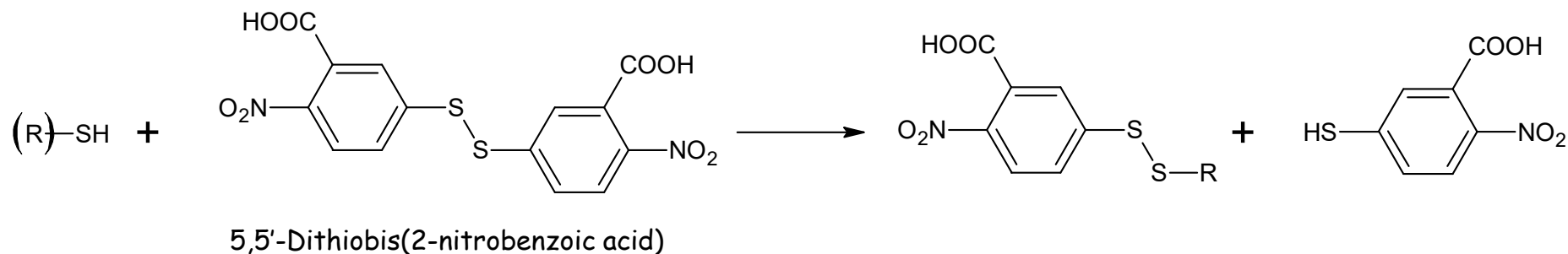   4) small sample size

- Approaches

invasive: sampling   non-invasive: no sampling

a)   b)   c)

coupled   „by-product"   functional   spectroscopy

chromophore   of coupled reagent   *in vitro* (enzyme)
                                            in vivo (toxicity)

1) Detection of –SH group

Type a

$(R)\!-\!SH$ + 5-(iodoacetamido)fluorescein $\xrightarrow[\text{pH 7.5-8.0}]{\text{(DMF)}}$

λ_{abs} = 492 nm

ε = 80000 M^{-1}cm^{-1}

λ_g = 490 nm λ_e = 515 nm

Type b

$(R)\!-\!SH$ + 5,5'-Dithiobis(2-nitrobenzoic acid)

Ellman's reagent
Arch Biochem Biophys 82 70 (1959)

λ_{abs} = 412 nm

ε = 13600 M^{-1}cm^{-1}

2) Detection of –NH$_2$ group

Type a
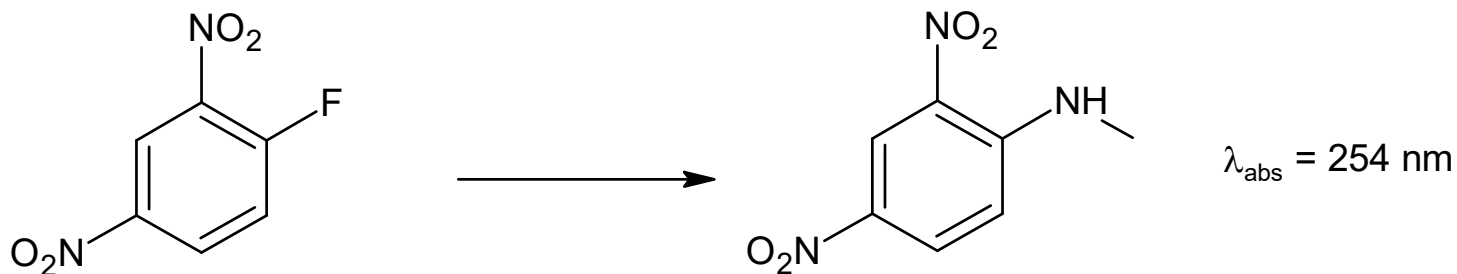
1-Fluoro-2,4-dinitrobenzene (DNP)
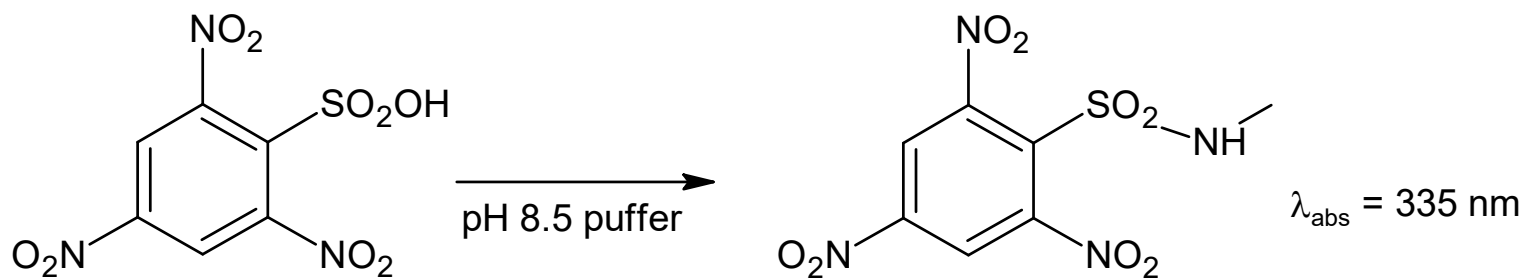
λ_{abs} = 254 nm

2,4,6-Trinitrobenzenesulfonic acid (TNBS)

(1959)

pH 8.5 puffer

orange colour

λ_{abs} = 335 nm

NO₂
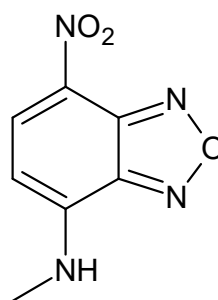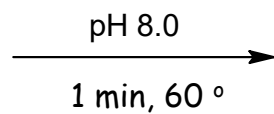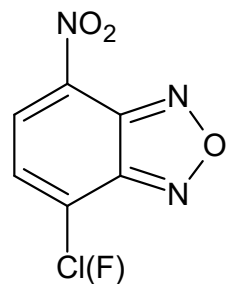
λ_g = 470 nm

λ_{em} = 530 nm

**4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole
(NBD-Cl)**

pH 8.0

1 min, 60 °
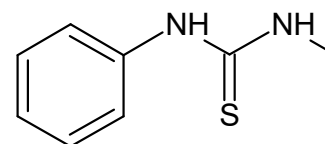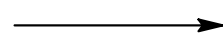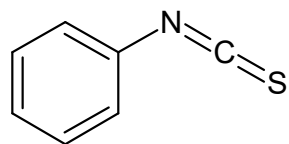
λ_{abs} = 254 nm

phenyl isothiocyanate (PITC)
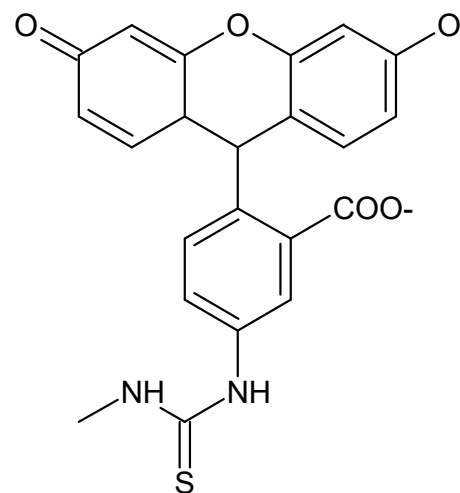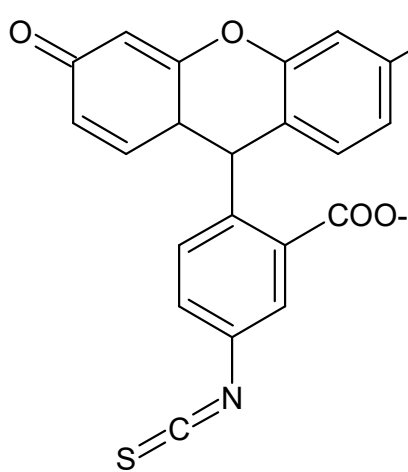
λ_g = 494 nm

λ_e = 520 nm

FITC
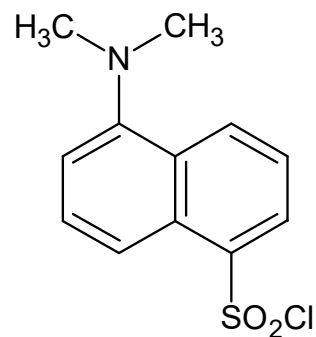
Dansyl chloride
(1-(**D**imethyl**a**mino)**n**aphthalene-
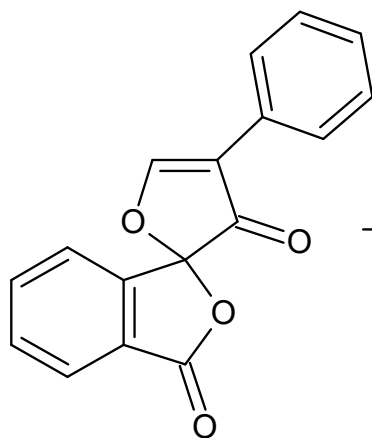5-**sulfonyl** chloride)

+ NH$_2$-
pH 7.5-8.0
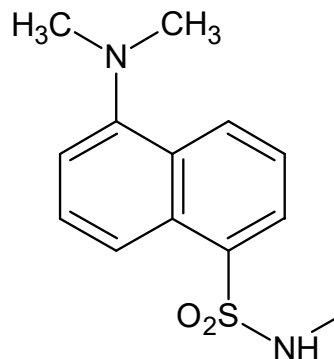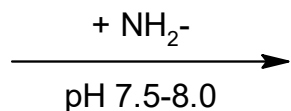
By-product: Dansyl-OH
(fluorescent)

λ_{abs} = 254 nm

λ_g = 360 nm
λ_e = 480 nm ⎤ F

Fluorescamine

+ NH$_2$-
pH 7-9
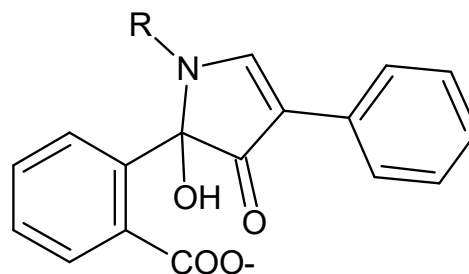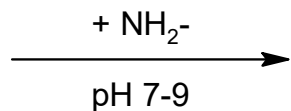
Fluorescent
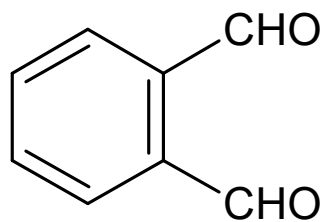(But!
By-product: N replaced by O
non-fluorescent)
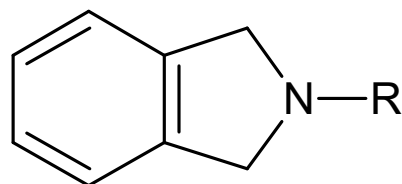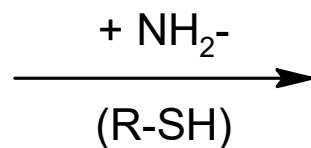
ortho-phthalaldehyde
(OPA)

$\lambda_g = 360$ nm

$\lambda_e = 455$ nm

9-fluorenyl-metil-chlorophormate
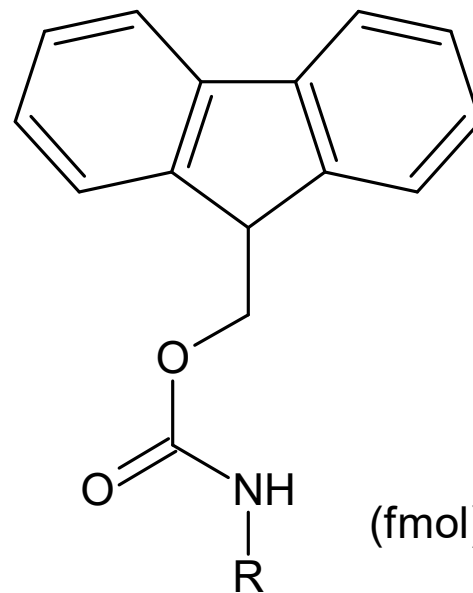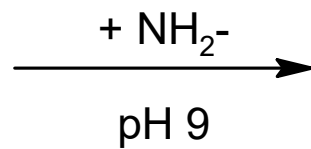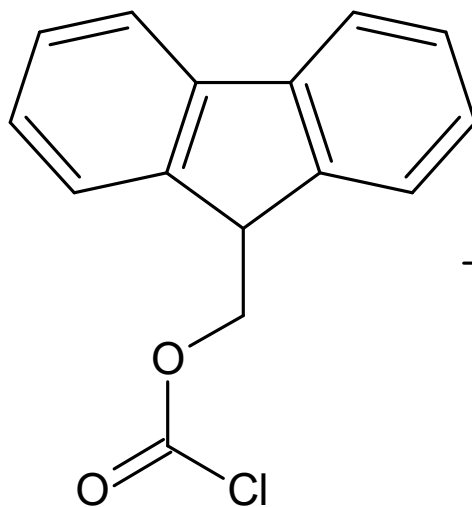
(fmol)

$\lambda_{abs} = 260$ nm

$\lambda_g = 270$ nm

$\lambda_e = 315$ nm

Type b/c

SPDP

H₂N—
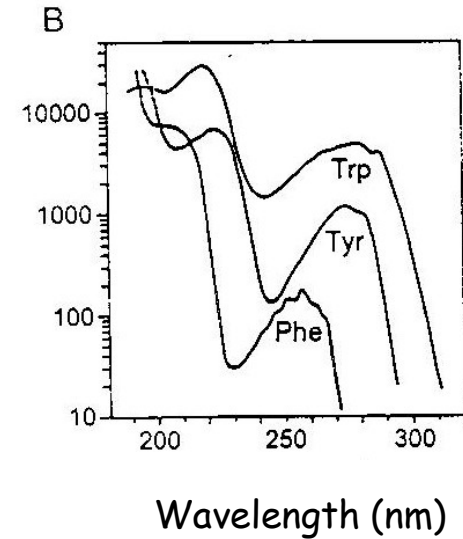
$\lambda = 254$ nm
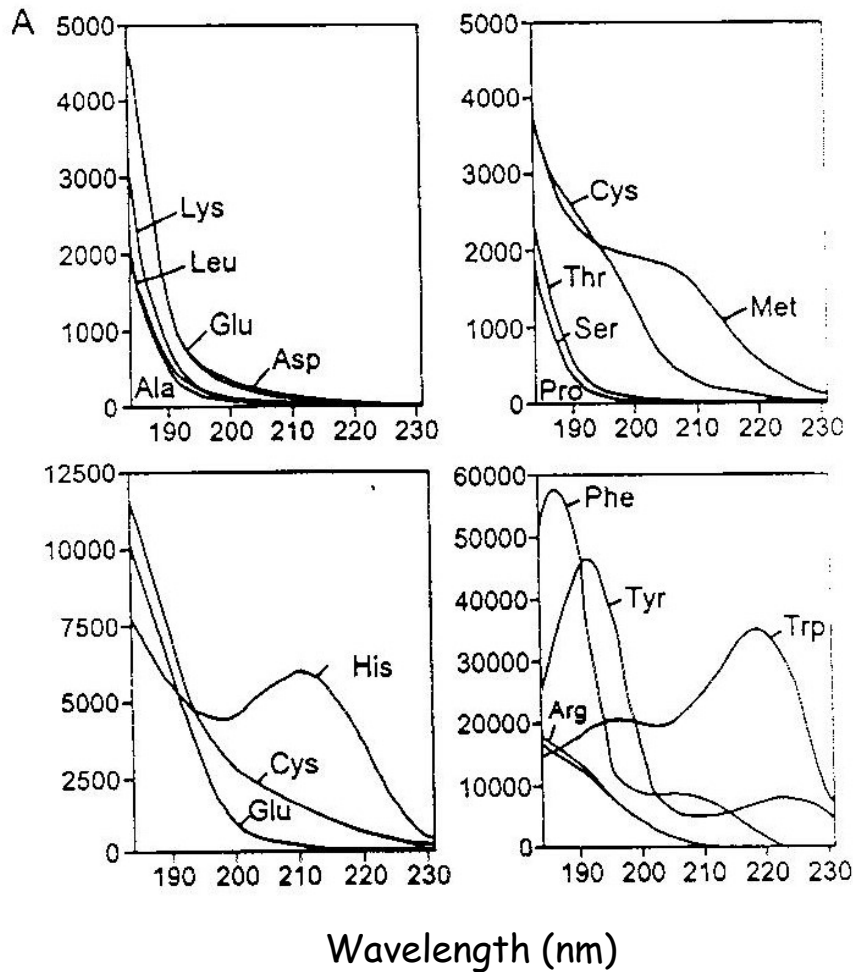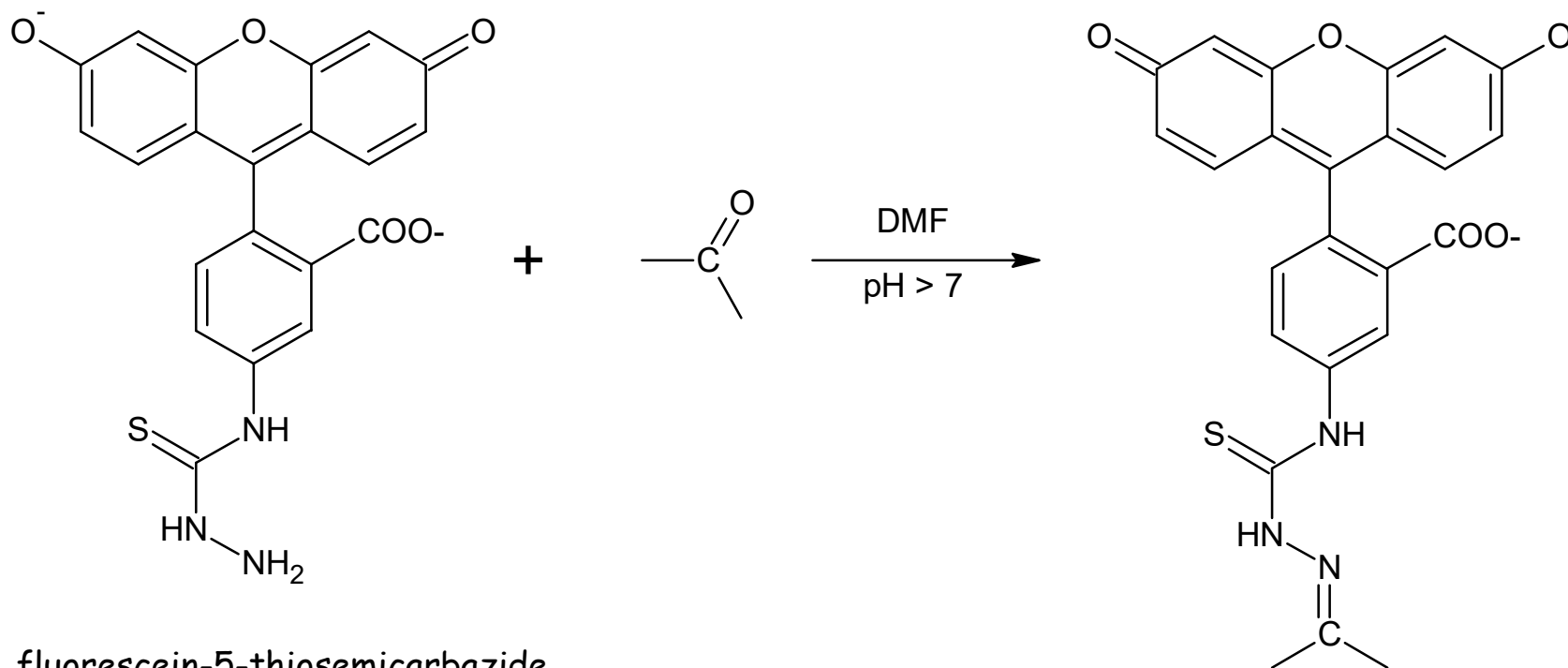
SMPT

H₂N—

$\lambda = 254$ nm
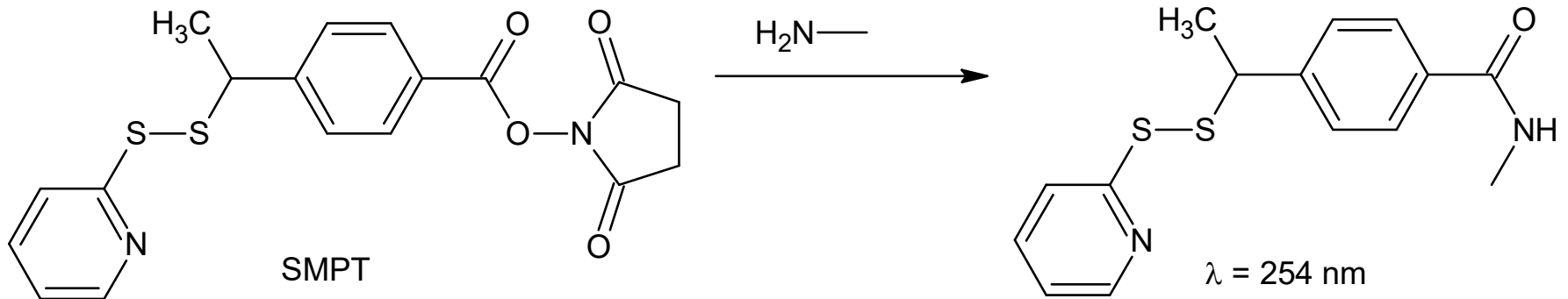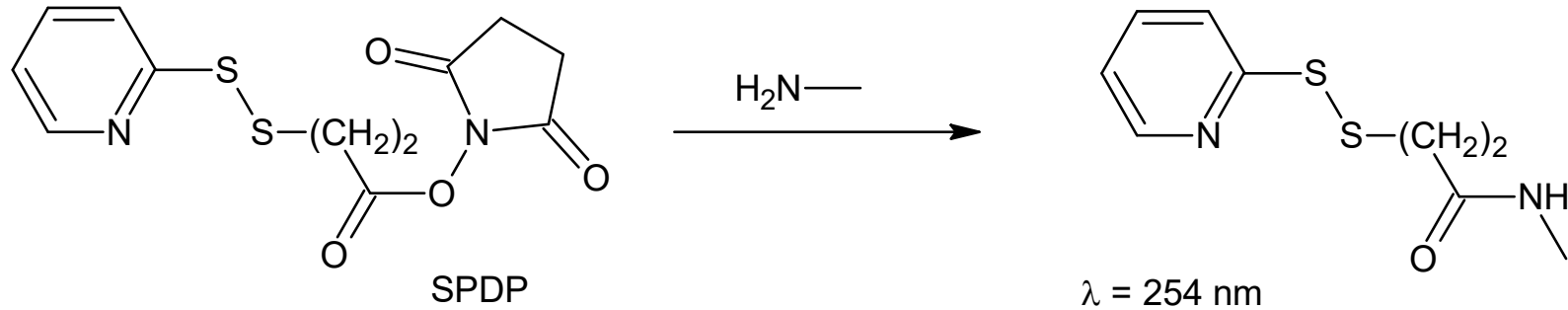
UV spectra of amino acids and Gly-Gly dipeptide

Wavelength (nm)

Recorded in aqueous solutions
[pH 5-6 or pH 3 (Cys)]
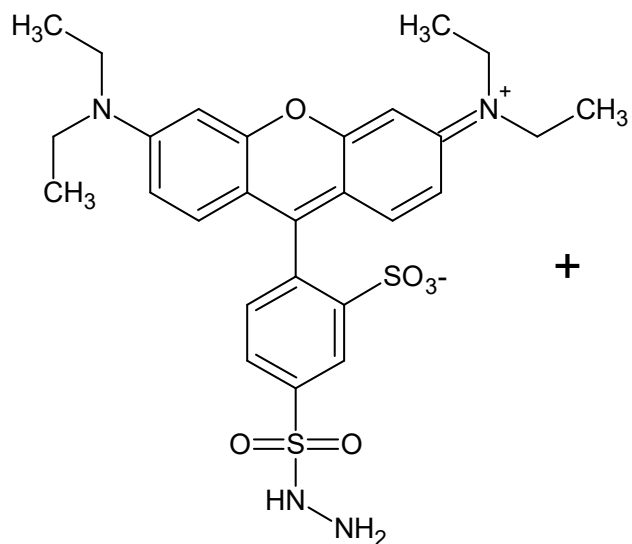
3) Detection of aldehyde/keto function

fluorescein-5-thiosemicarbazide

λ_g = 492 nm λ_e = 516 nm

ε_{492} = 85000 $M^{-1}cm^{-1}$
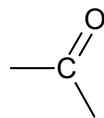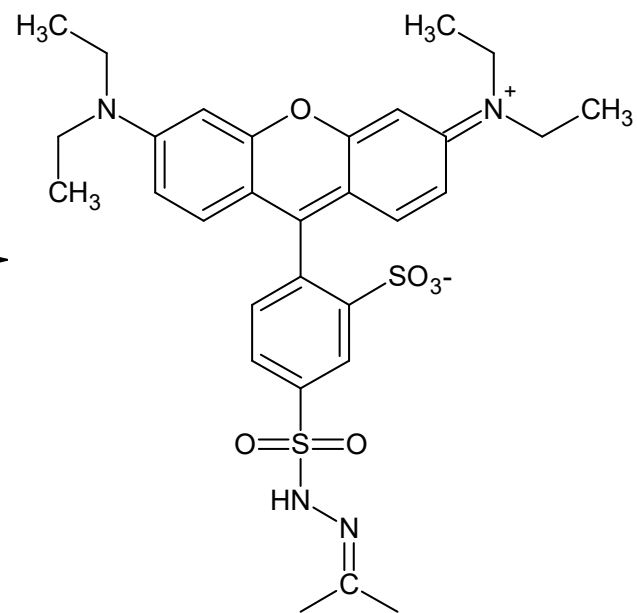
<u>Application</u>: oxidized RNS, glycoproteins, cells

Lissamine™ Rhodamine B Sulfonyl hydrazine

λ_g = 560 nm λ_e = 585 nm
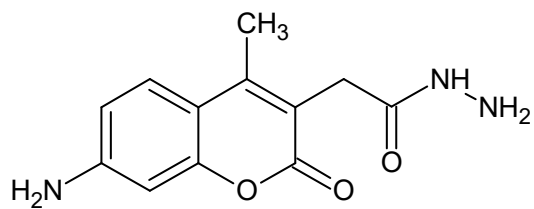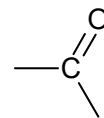
ε_{560} = 95000 M^{-1}cm^{-1}

Lissamine™ Rhodamine B Sulfonyl hydrazone
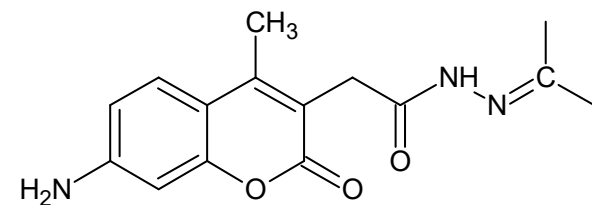
7-amino-4-methylcoumarin-3-acetyl hydrazide
(AMCA-hydrazide)

λ_g = 345 nm λ_e = 440-460 nm

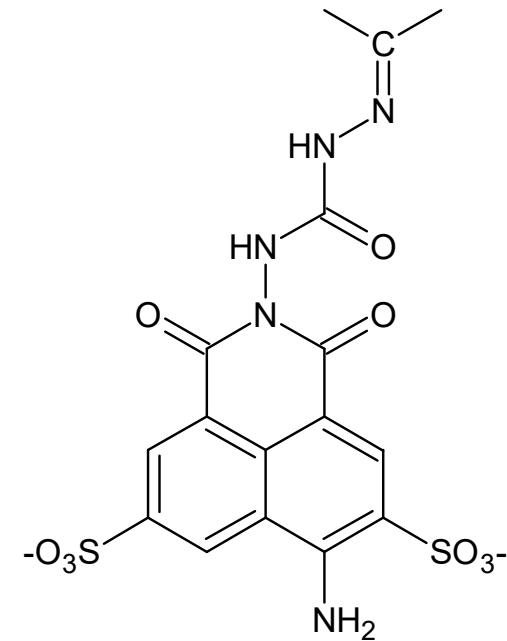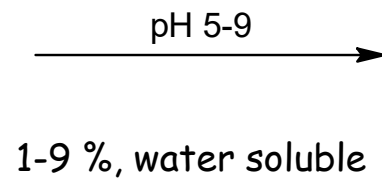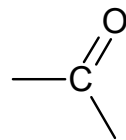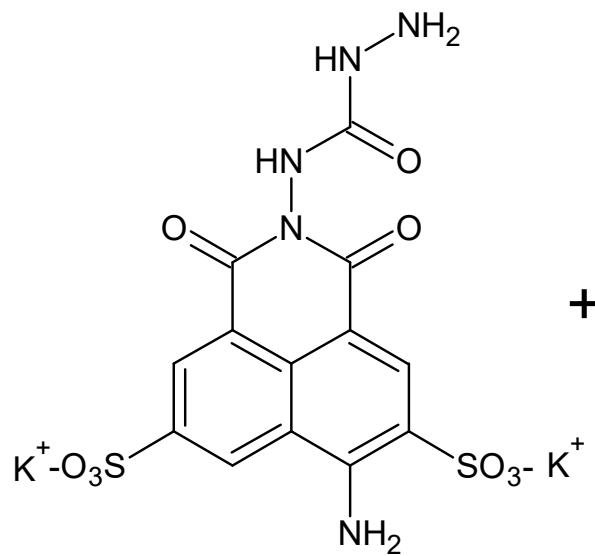ε_{345} = 13900 M^{-1}cm^{-1}

7-amino-4-methylcoumarin-3-acetyl hydrazone
(AMCA-hydrazide)

λ_g = 428 nm λ_e = 534 nm

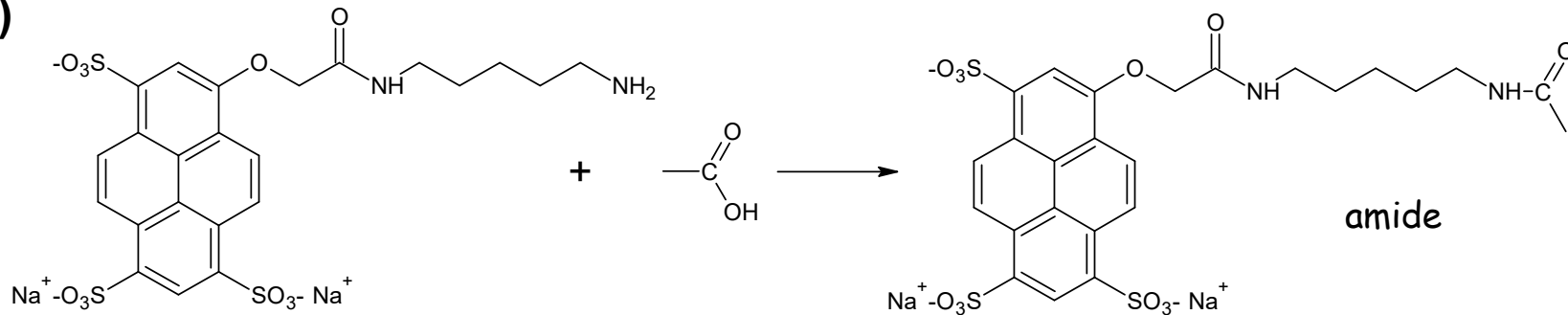ε_{428} = 12000 M^{-1}cm^{-1}

Lucifer yellow CH

Application: glycolipids, neurons

4) Detection of – COOH group

A)

Cascade Bule cadaverin

Combination with Lucifer yellow

λ_g = 377,398 nm λ_e = 422 nm

ε = 27000 $M^{-1}cm^{-1}$

λ_g ~ 400 nm λ_e

422 nm

534 nm

B)

acyl - transfer

1,5 –diamino-pentane

1) DCC

2) enzyme

acyl - substrate

5 (biotiamide)pentilamin
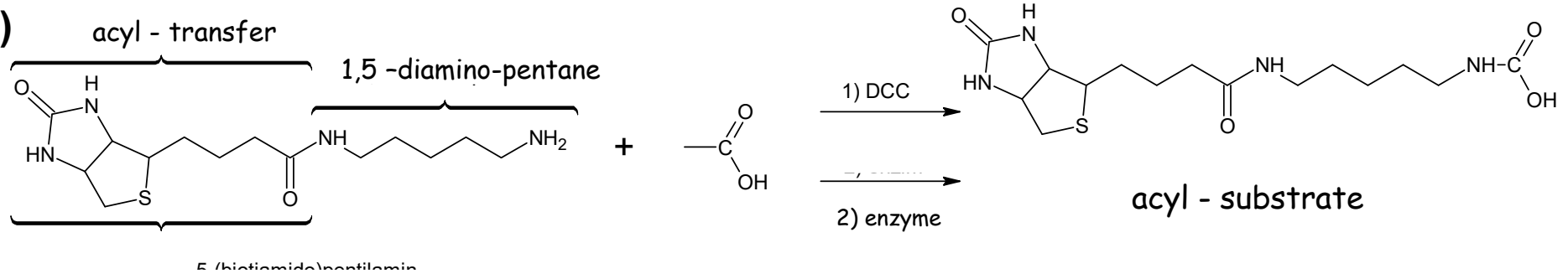
Lee et al. Clin Chem <u>34</u> 906 (1998)

Summary

Reporter

Reactive group

"To be detected" group

Chromofore
(UV, visible)

Fluorofore

Biotin

Radioactive isotope
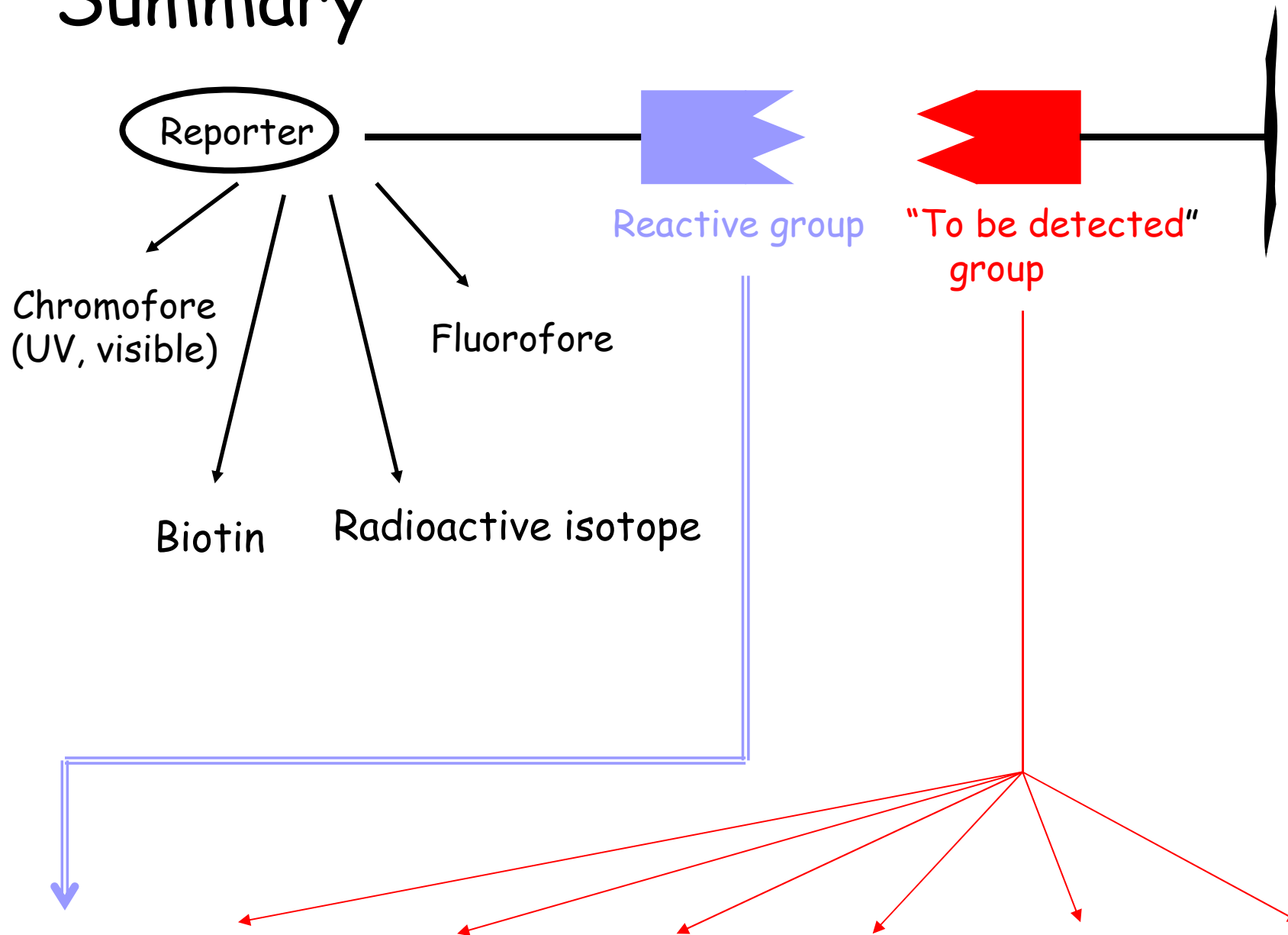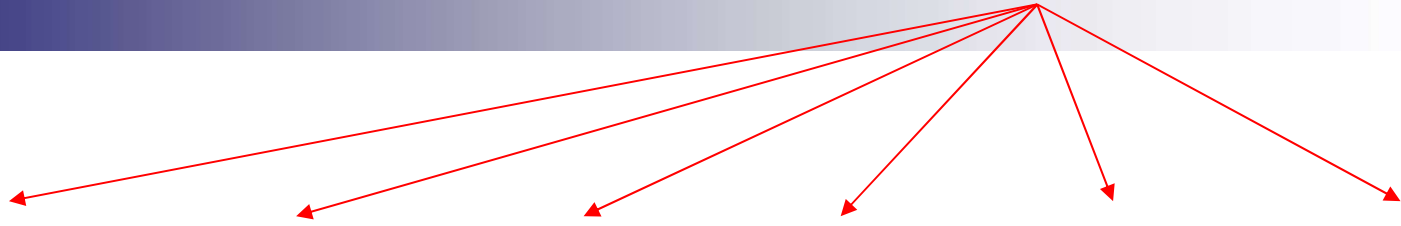
REACTÍVE GROUP
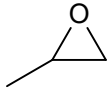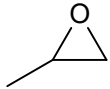
-NH$_2$	-OH	-SH	-COOH	-CHO	Active H
—COOH					
—COOR					
—CO—O / OC—	—CO—O / OC—				
—N=C=O	—N=C=O				
—N=C=S					
—CHO					
epoxide (O)	epoxide (O)				
—F(aril)	—Cl(alkil)	—F(aril/alkil)			
—SO$_2$Cl		—S—S—R		—NH—NH$_2$	
			—NH$_2$	—NH$_2$	
			—CH$_2$N$_2$		
		—CH=CH$_2$			—N$^+$≡N

1. Reactive groups
2. Establishment of reactive groups
3. Detection of reactive groups
4. Coupling of reactive groups

Chemical synthesis   Enzymatic method   Gentechnology
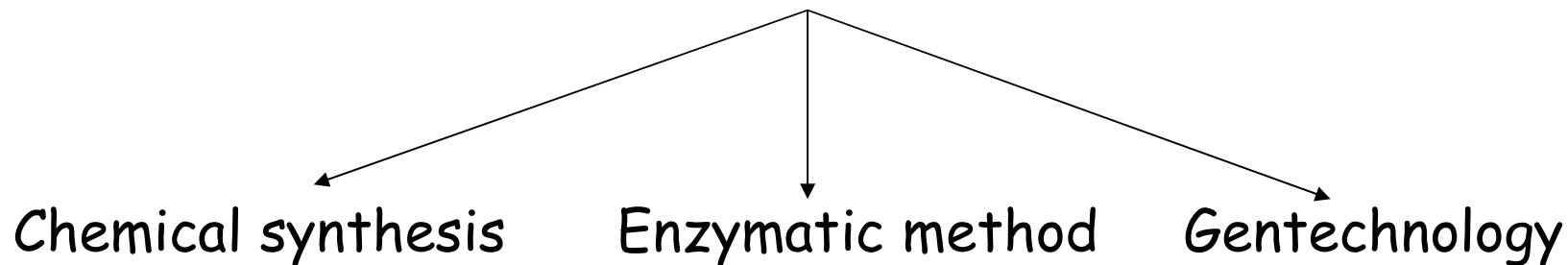
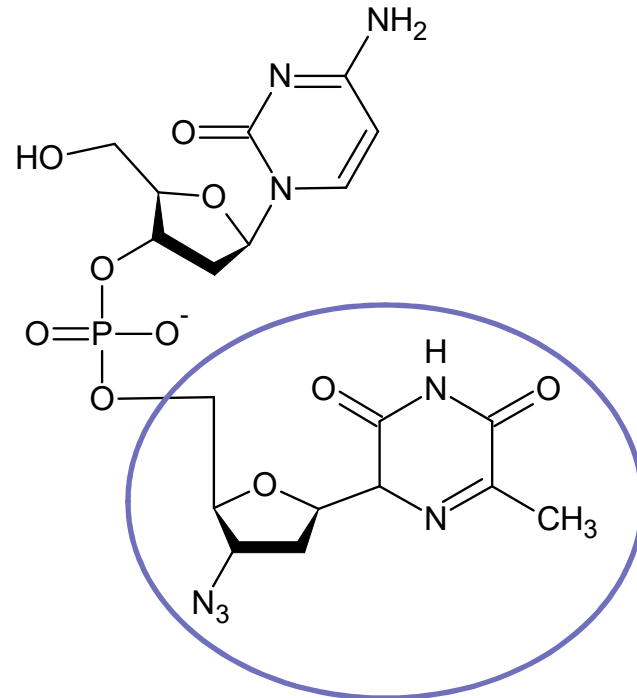Considerations: reactivity
                     specificity
                     isolation, purification
                     applications

Examples
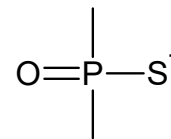
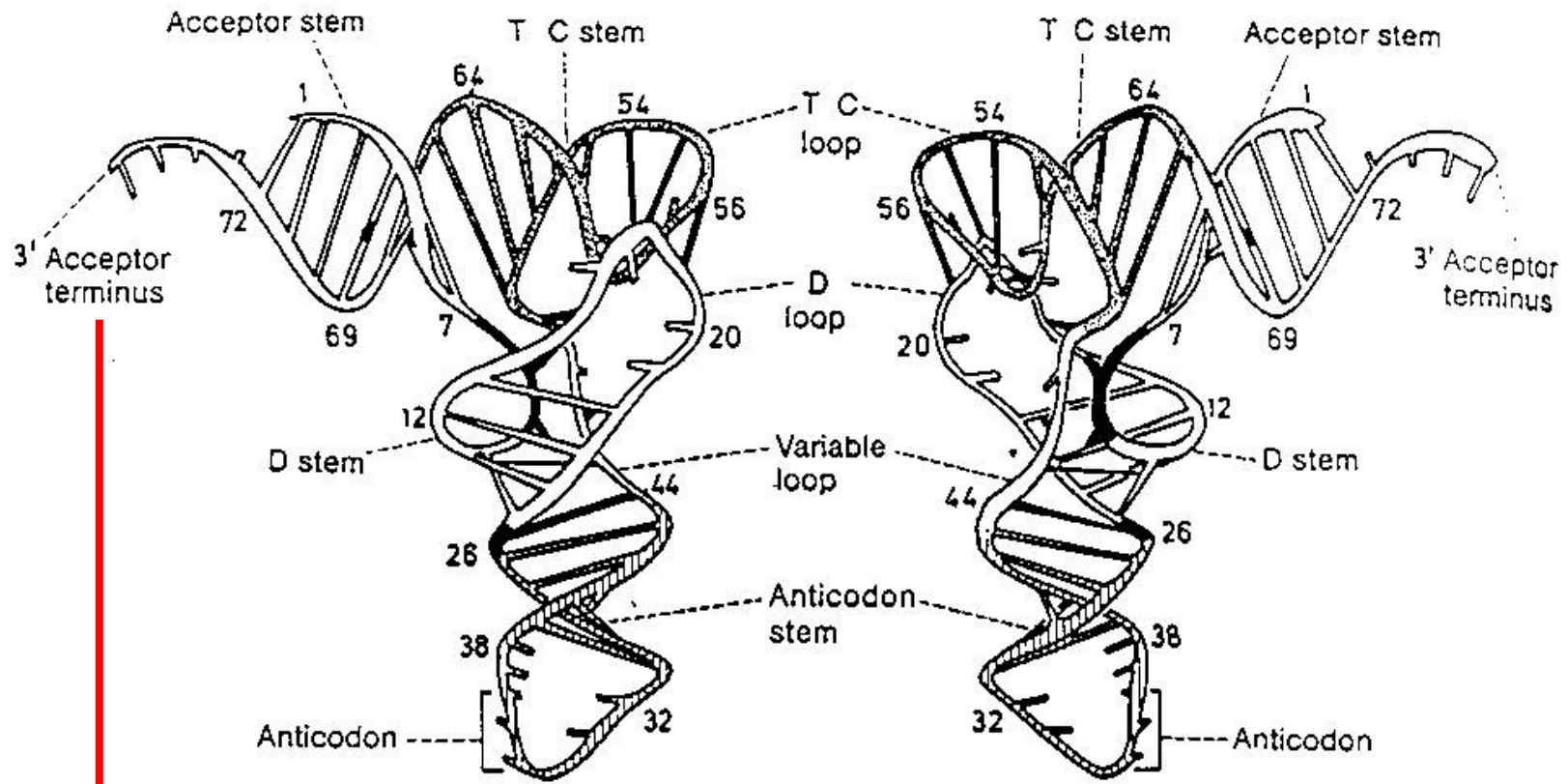1. AzT-oligonucleotide
2. Amino acid-transport – enzymatic

1)

Deoxyoligonucleotide – AzT conjugate

thiophosphoric acid ester

AzT

3'-Azido-3'-deoxythymidine

A. Rich, A. Klug (1974) X-Ray   tRNS

P. Zamencik
M. Hoogland (1957)

Adenine

+ AMP

+ tRNS

2P$_i$

aminoacyl-tRNA
synthetase

Anhydride

H$_3$N$^+$—CH + ATP ⇌ H$_3$N$^+$—CH + PP$_i$

aminoacyl-adenylate
(aminoacyl-AMP)

Ribose-adenine