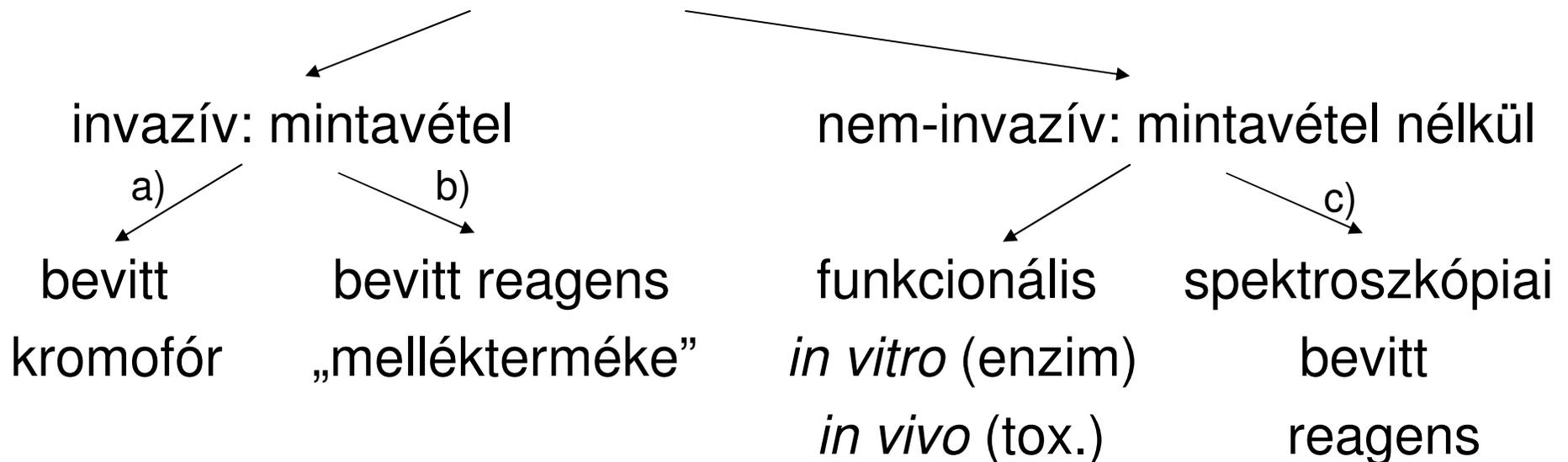


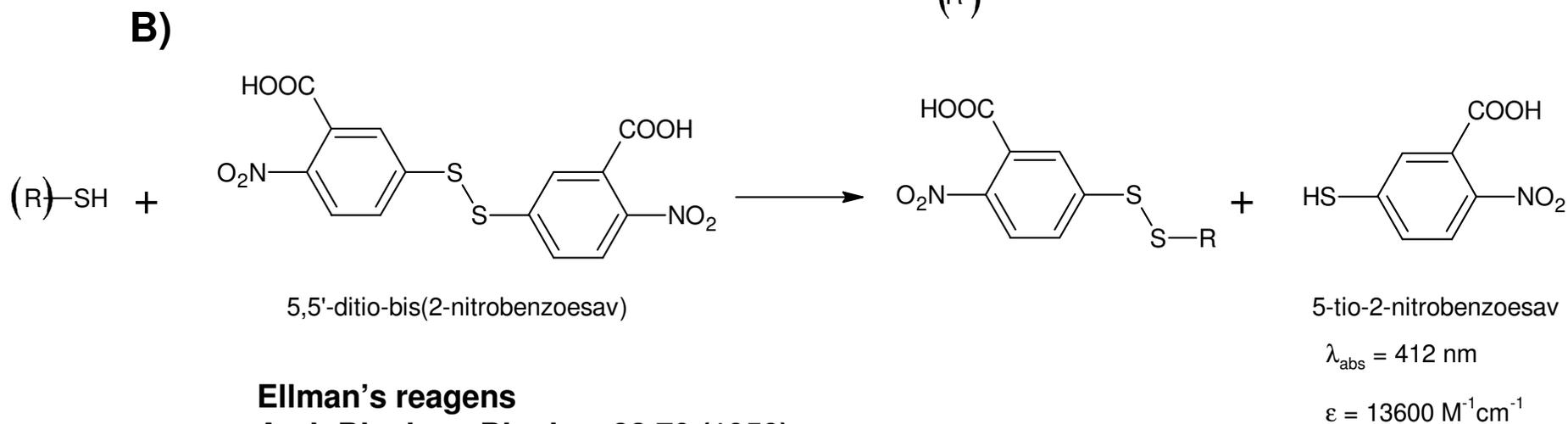
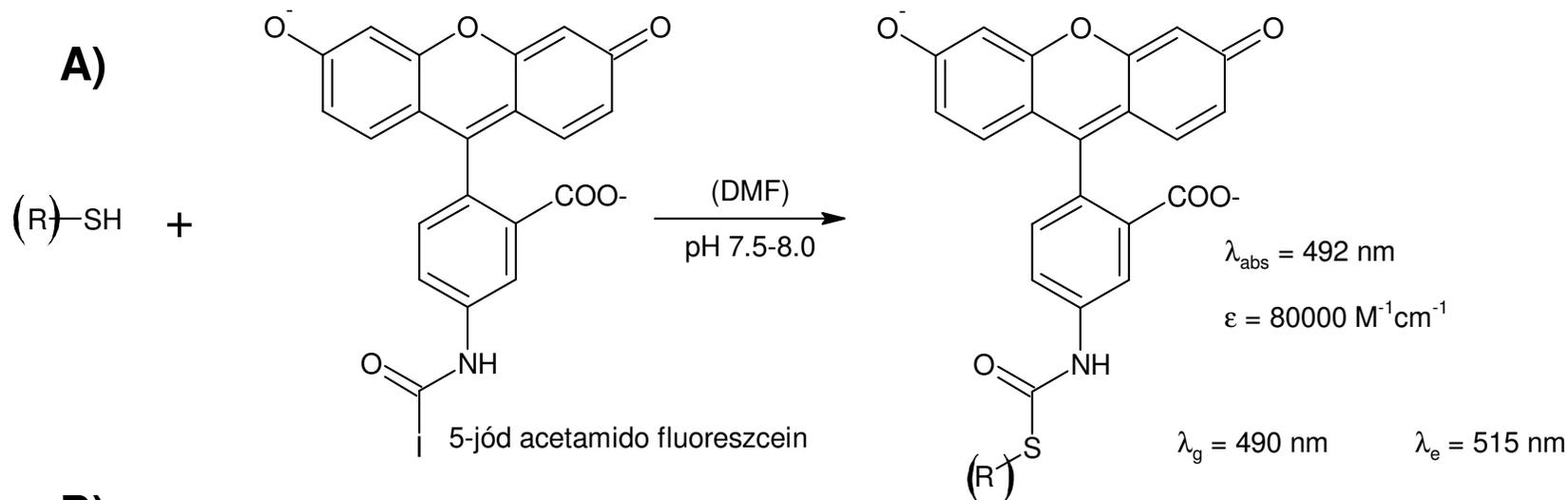
A kiépített/módosított funkciós csoport kimutatása

- Követelmények: 1) érzékeny
2) kvantitatív
3) gyors
4) kis anyagmennyiség

- Megközelítések

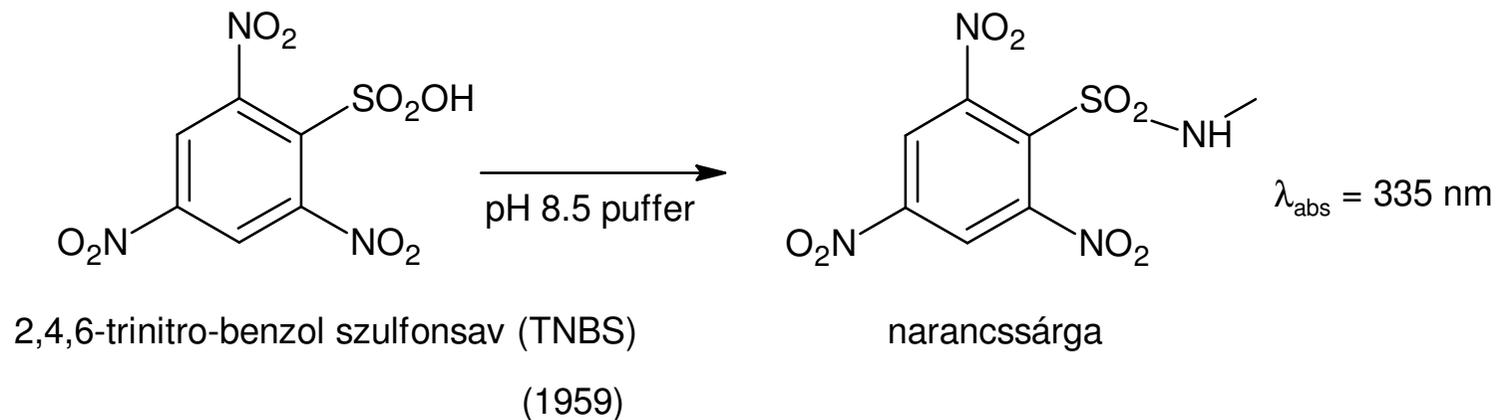
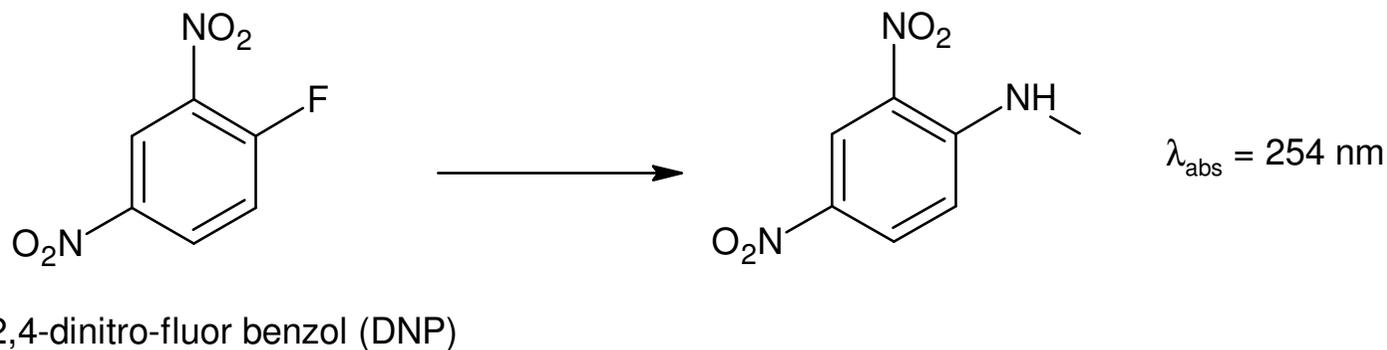


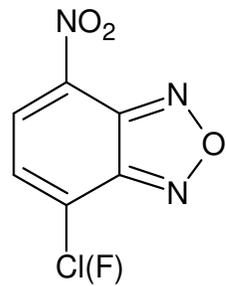
1) –SH csoport kimutatása



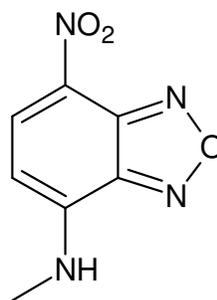
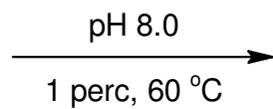
Ellman's reagents
Arch Biochem Biophys 82 70 (1959)

2) $-\text{NH}_2$ kimutatása – I. A) típus



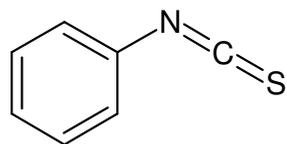


4-klór-7-nitro-benzo-2-oxa-1,3-diazol
(NBD-Cl)

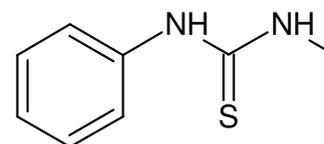
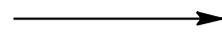


$\lambda_g = 470 \text{ nm}$

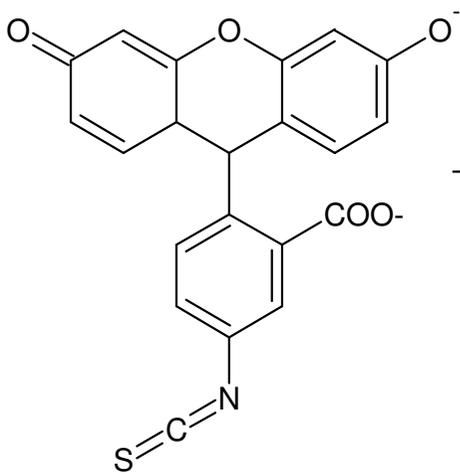
$\lambda_{em} = 530 \text{ nm}$



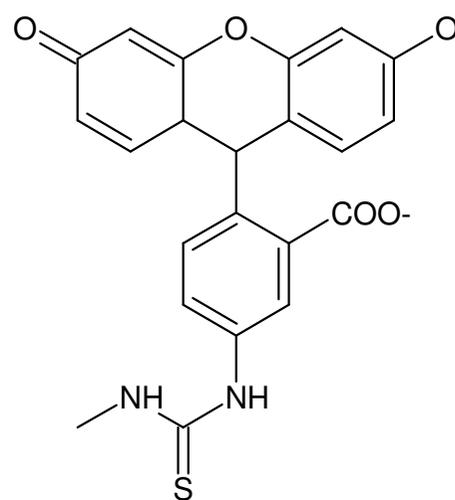
fenil-izotiocianát (PITC)



$\lambda_{abs} = 254 \text{ nm}$



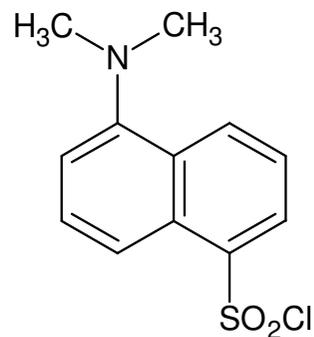
FITC



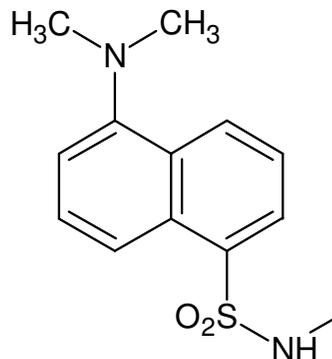
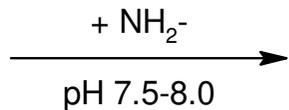
$\lambda_g = 494 \text{ nm}$

$\lambda_e = 520 \text{ nm}$

-NH₂ kimutatása – II. A) típus



Dansyl-klorid



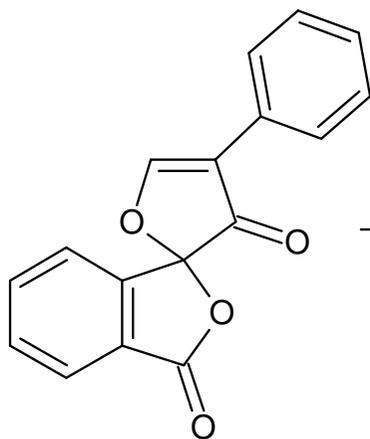
[Melléktermék: Dns-OH, fluoreszkál]

$\lambda_{\text{abs}} = 254 \text{ nm}$

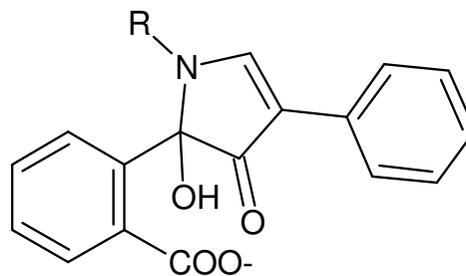
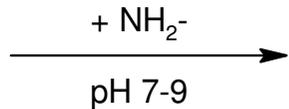
$\lambda_{\text{g}} = 360 \text{ nm}$

$\lambda_{\text{e}} = 480 \text{ nm}$

F

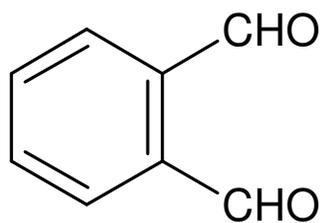


Fluoresztamin

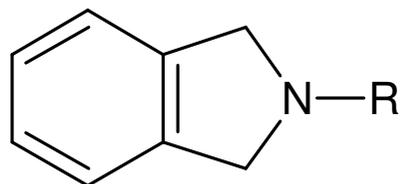
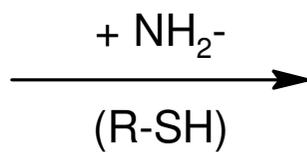


Fluoreszcens

[Melléktermék: R-N= helyett O, nem F]

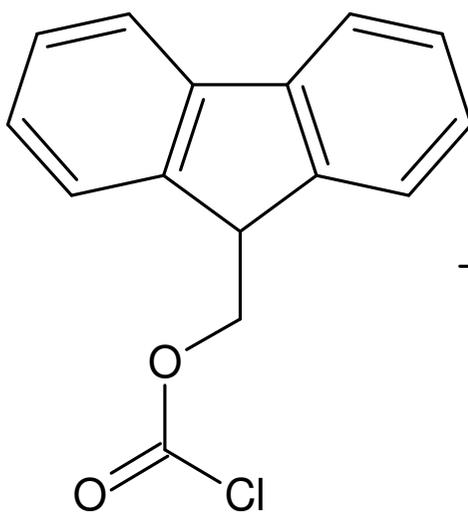


O-Ftálaldehyd

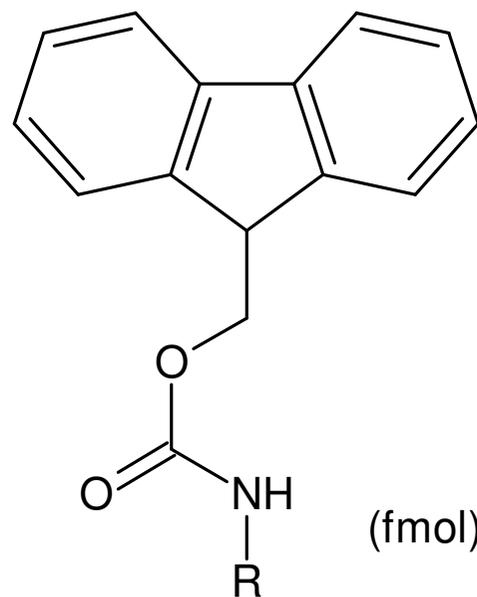
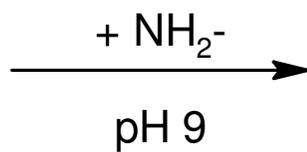


$$\lambda_g = 360 \text{ nm}$$

$$\lambda_e = 455 \text{ nm}$$



9-fluorenil-metil-kloroformát



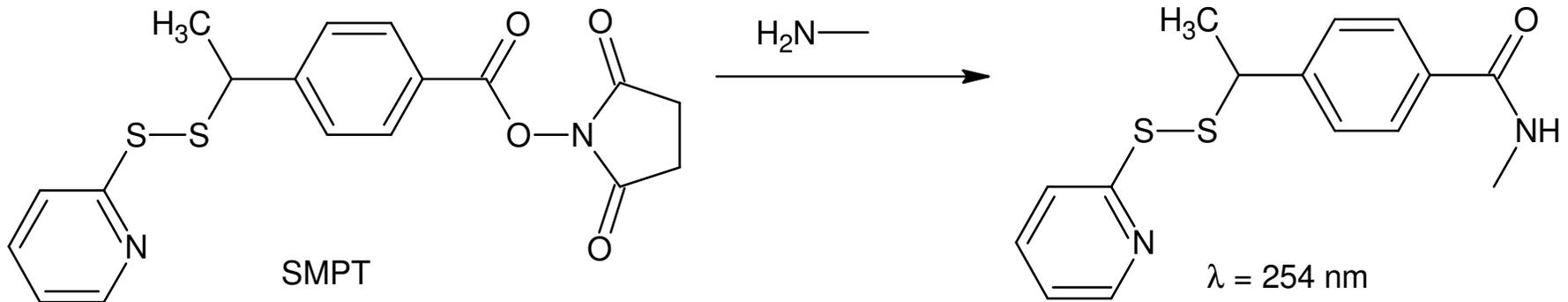
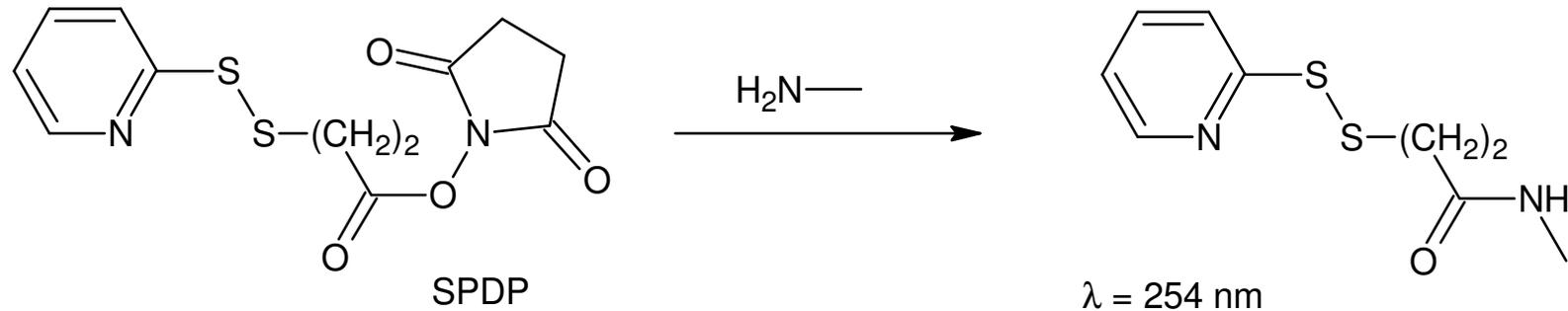
(fmol)

$$\lambda_{\text{abs}} = 260 \text{ nm}$$

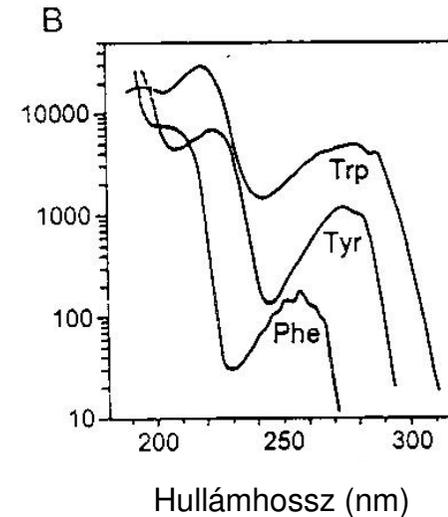
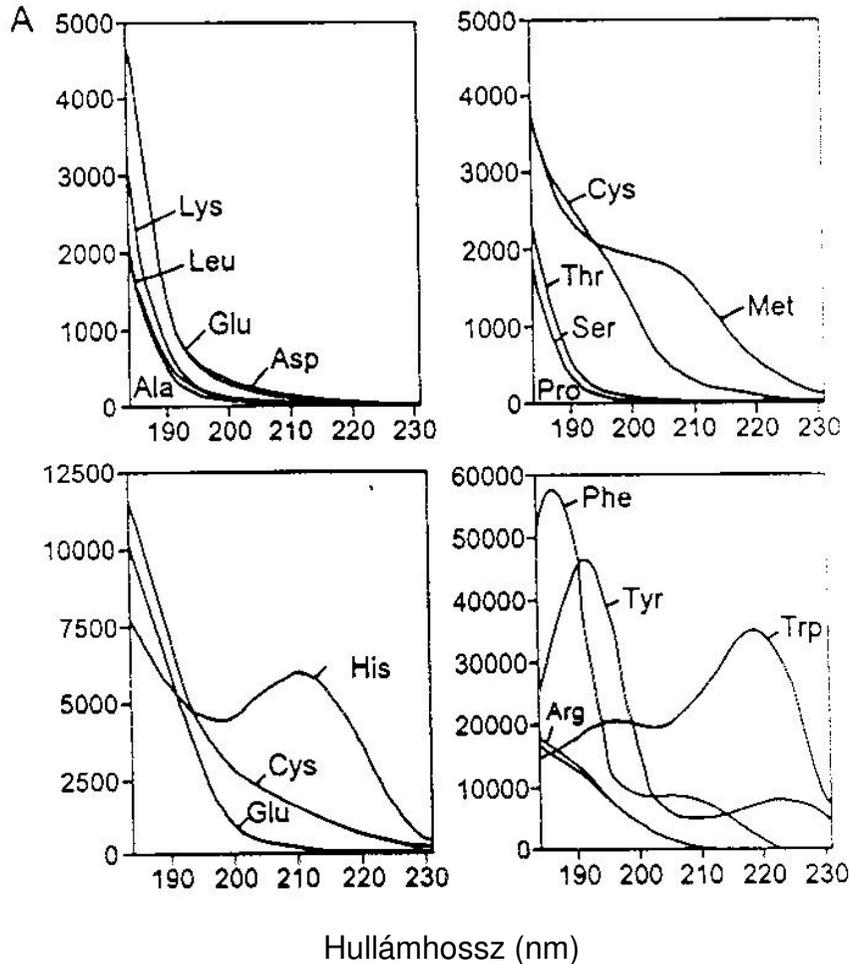
$$\lambda_g = 270 \text{ nm}$$

$$\lambda_e = 315 \text{ nm}$$

-NH₂ kimutatása – C) típus

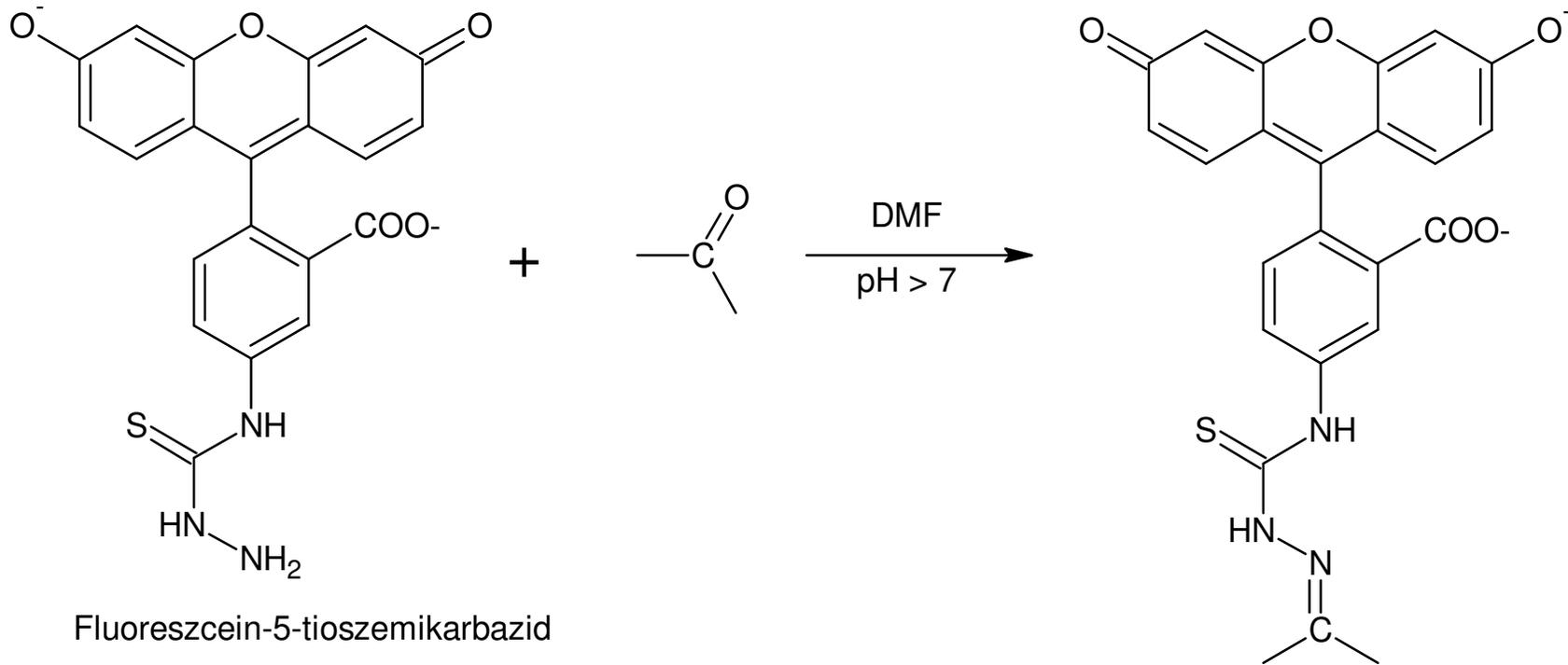


Aminosavak és Gly-Gly dipeptid UV spektrumai



A felvételek az aminosavak vizes oldataiban (pH 5-6 ill. pH 3 – Cys) készültek

Aldehid/keton csoport kimutatása

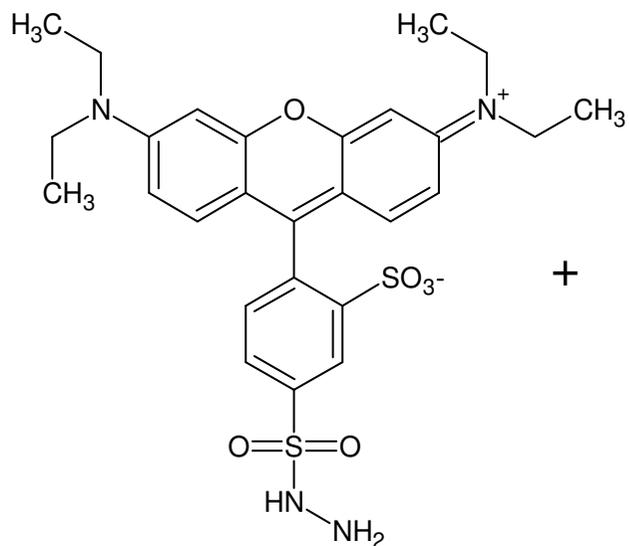


Fluoreszcein-5-tioszemikarbazid

$\lambda_g = 492 \text{ nm}$ $\lambda_e = 516 \text{ nm}$

$\epsilon_{492} = 85000 \text{ M}^{-1}\text{cm}^{-1}$

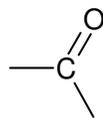
Alkalmazás: oxidált RNS, glikoproteinek, sejtek



"Lissamin" Rhodamine B szulfonil hidrazin

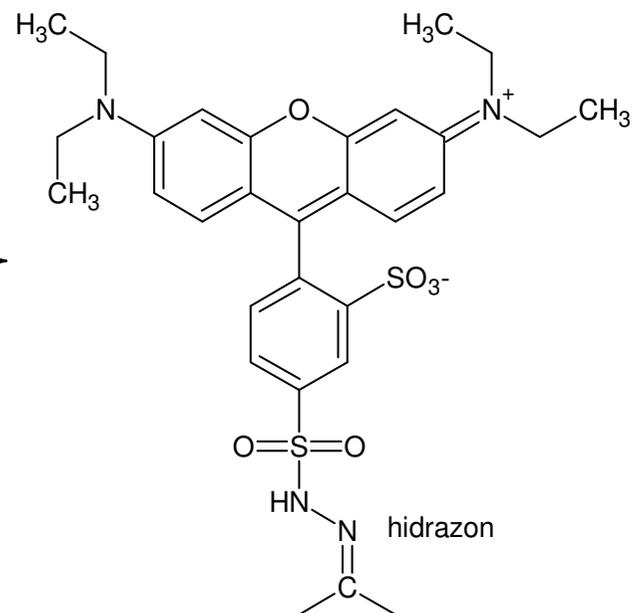
$\lambda_g = 560 \text{ nm}$ $\lambda_e = 585 \text{ nm}$

$\epsilon_{560} = 95000 \text{ M}^{-1} \text{ cm}^{-1}$

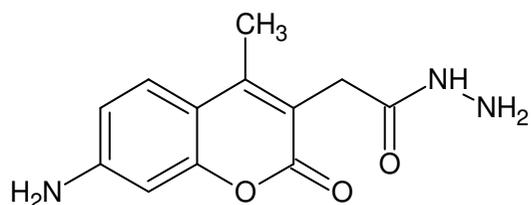


+

DMF



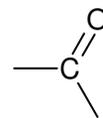
hidrazon



7-amino-4-metil kumarin 3-acetil-hidrazid
(AMCA-hidrazid)

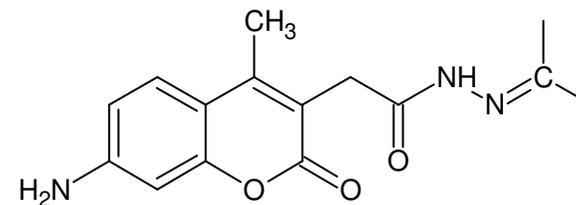
$\lambda_g = 345 \text{ nm}$ $\lambda_e = 440-460 \text{ nm}$

$\epsilon_{345} = 13900 \text{ M}^{-1} \text{ cm}^{-1}$



+

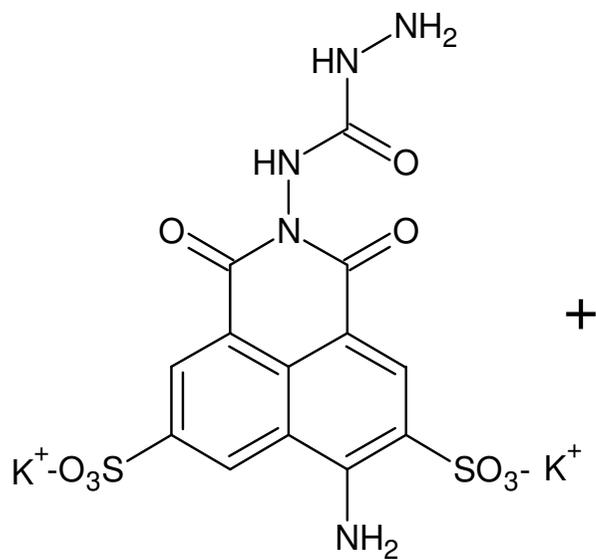
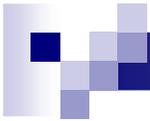
DMSO



hidrazon

Kettős jelölés

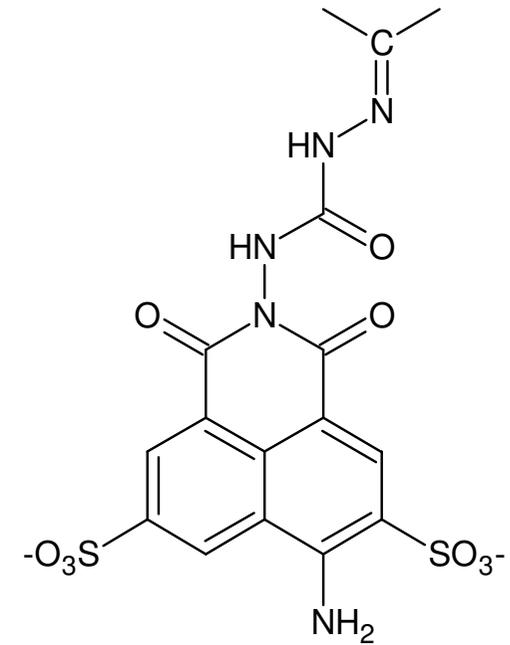
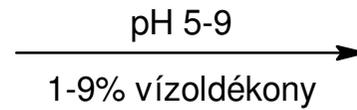
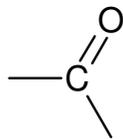
stabil



Lucifer sárga CH

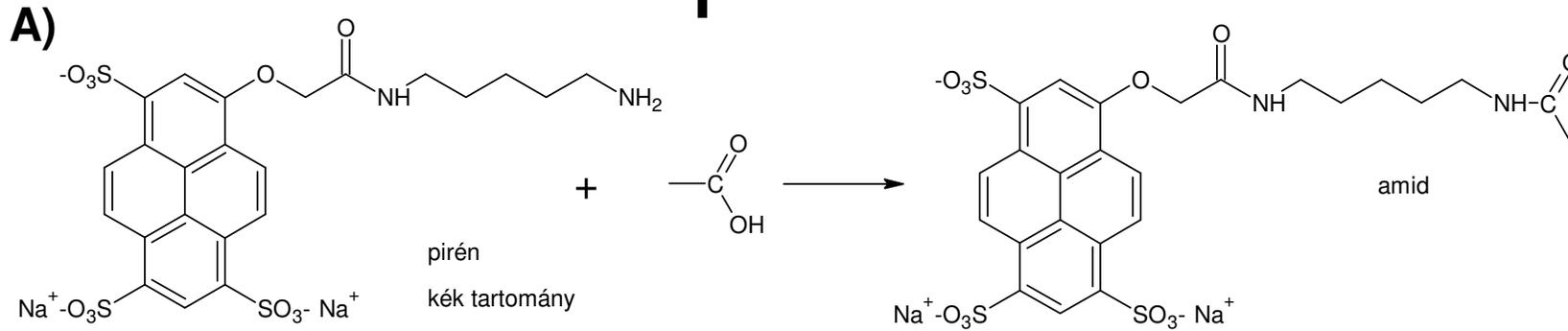
$\lambda_g = 428 \text{ nm}$ $\lambda_e = 534 \text{ nm}$

$\epsilon_{428} = 12000 \text{ M}^{-1}\text{cm}^{-1}$



Alkalmazás: glikolipidek, neuronok

Karboxil csoport kimutatása

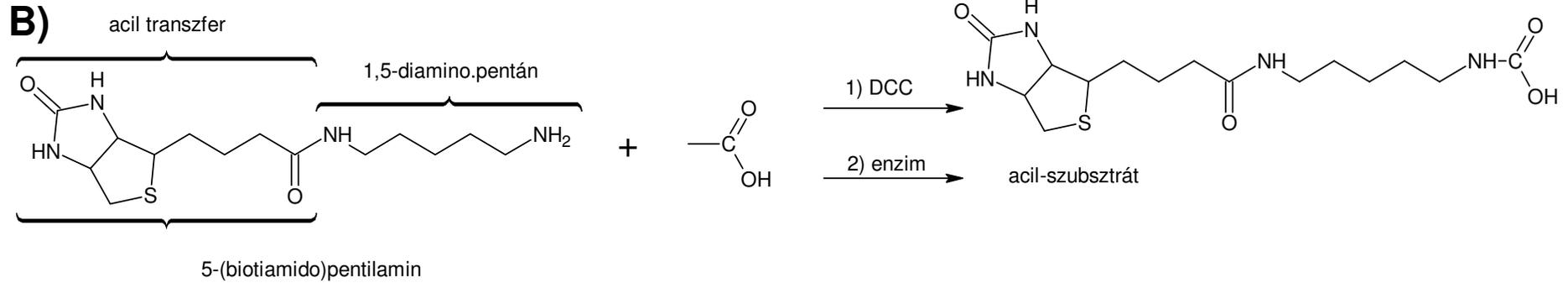


Cascade Blue Cadaverin

$\lambda_g = 377,398 \text{ nm}$ $\lambda_e = 422 \text{ nm}$
 $\epsilon = 27000 \text{ M}^{-1}\text{cm}^{-1}$

Kombináció: Lucifer sárga

$\lambda_g \sim 400$ λ_e $\begin{cases} 422 \\ 534 \end{cases}$

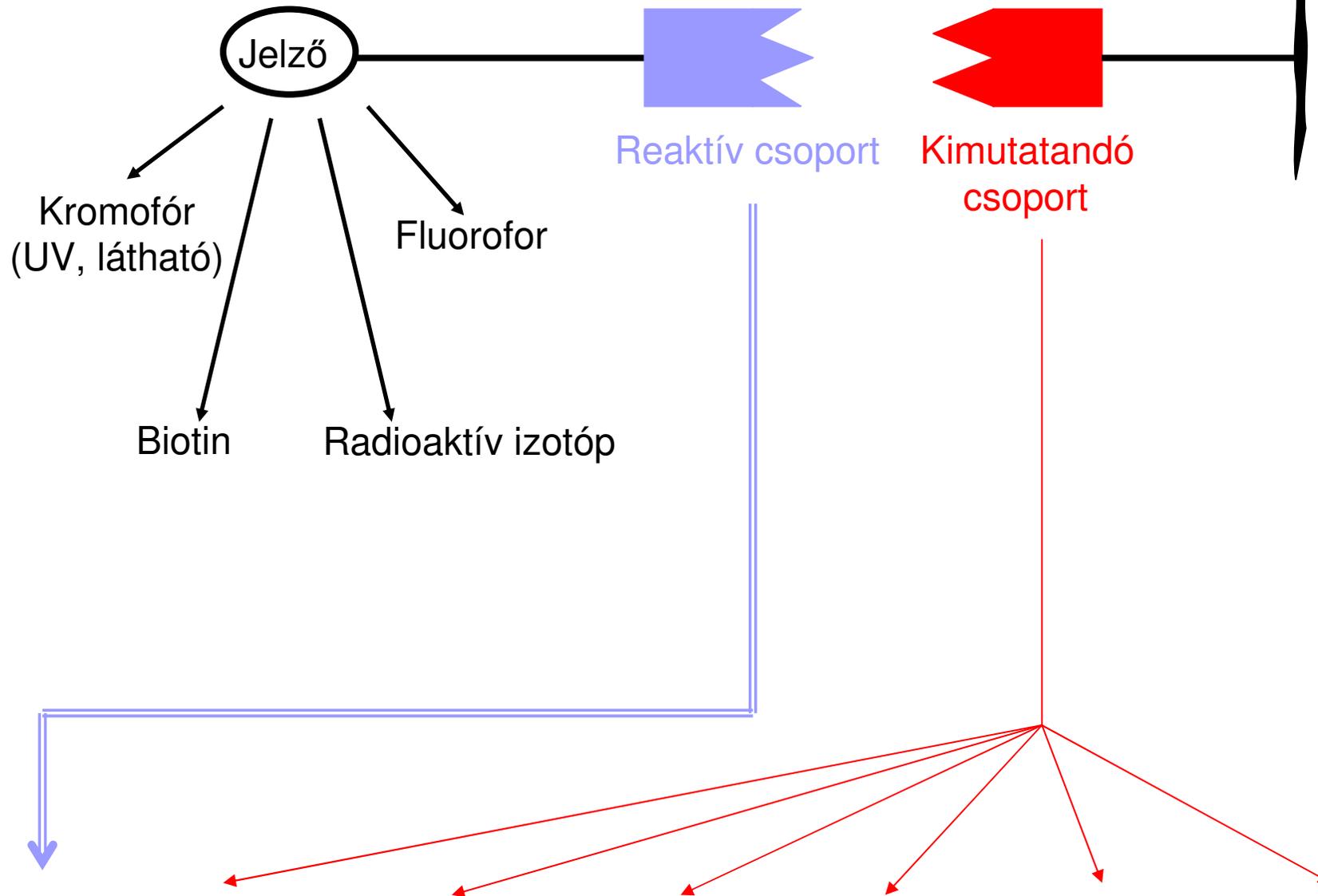


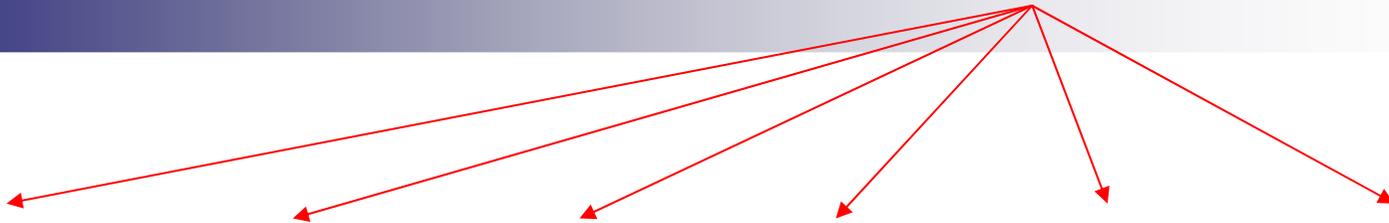
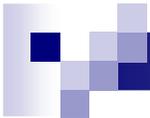
XIIIa faktor
(Transzglutamináz) } meghatározása plazmában

Magas szint \longrightarrow RÁK máj/vese

Lee et al. Clin Chem 34 906 (1998)

Összegzés





-NH₂

-OH

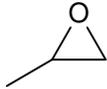
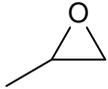
-SH

-COOH

-CHO

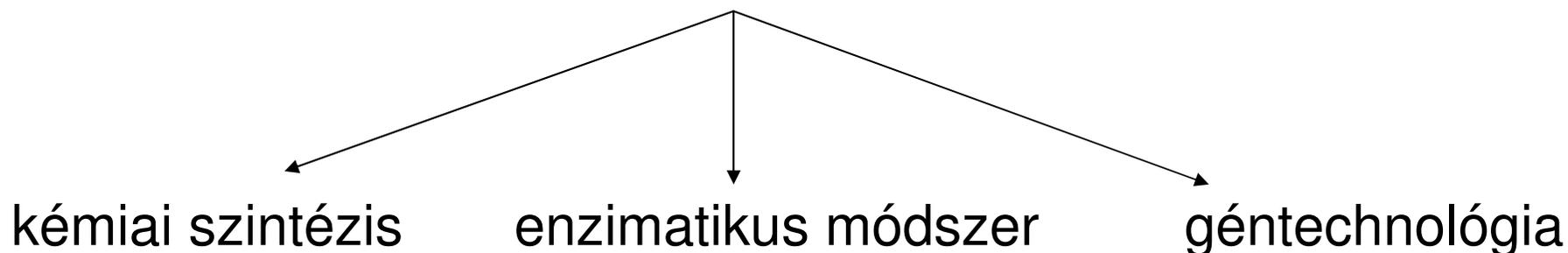
Aktív H

REAKTÍV
C
S
P
O
R
T

<p>—COOH —COOR —CO—O OC— —N=C=O —N=C=S —CHO  —F(aril) —SO₂Cl</p>	<p>—CO—O OC— —N=C=O  —Cl(alkil)</p>	<p>—F(aril/alkil) —S—S—R —CH=CH₂</p>	<p>—NH₂ —CH₂N₂</p>	<p>—NH—NH₂ —NH₂</p>	<p>—N⁺≡N</p>
---	---	---	---	---	-------------------------



- 
1. Reaktív csoportok
 2. Reaktív csoportok létrehozása
 3. Reaktív csoportok kimutatása
 4. Reaktív csoportok összekapcsolása

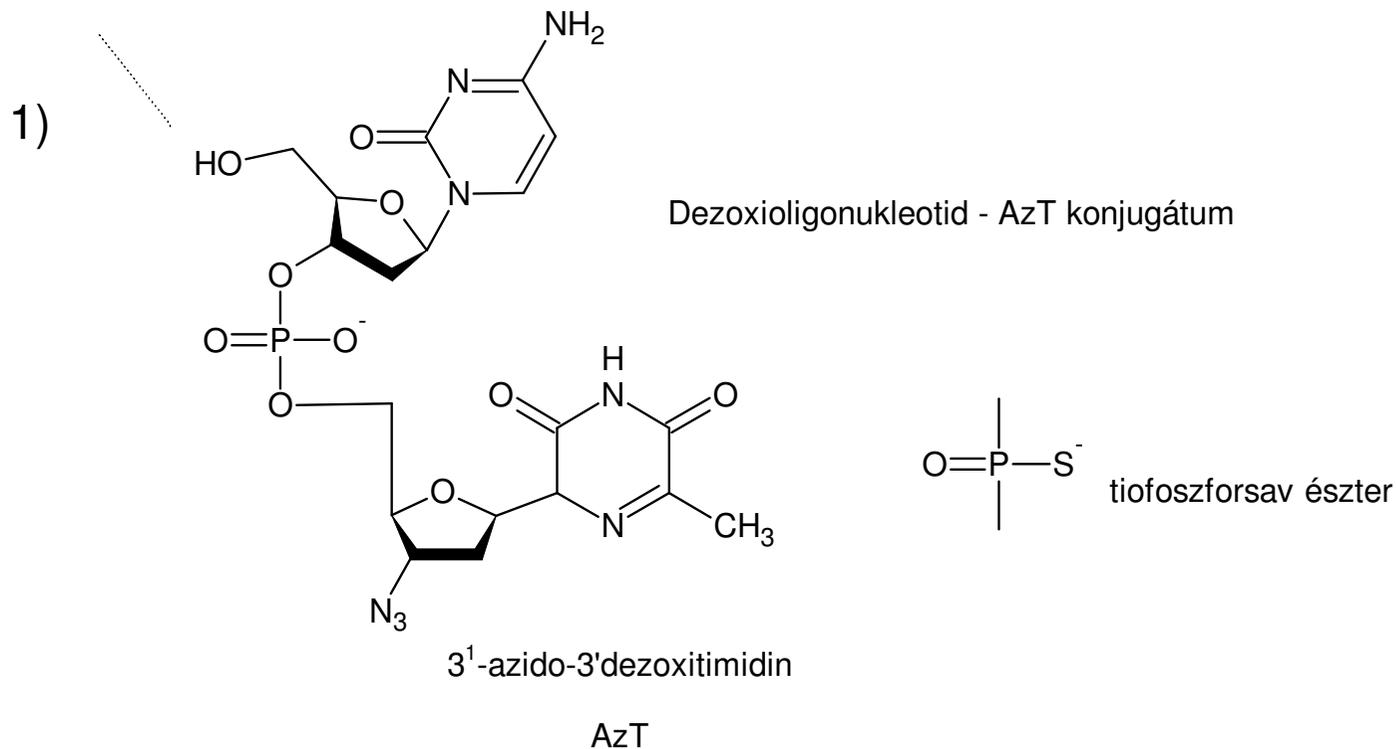


Szemponatok:

- reaktivitás
- specifitás
- izolálás, tisztítás
- alkalmazás, felhasználás

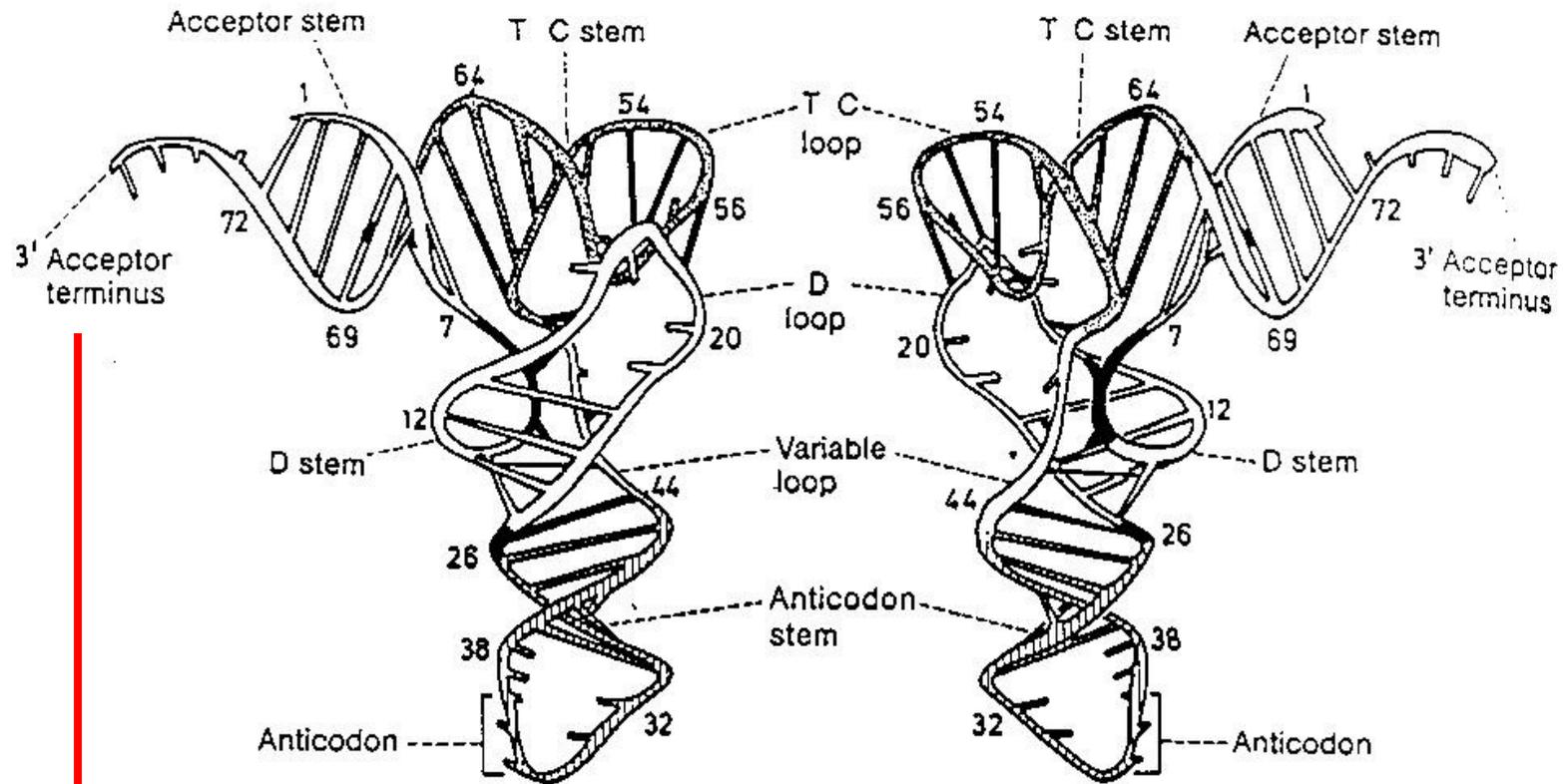
■ Példák

1. AzT-oligonukleotid
2. B- és T-sejt epitóp – peptidszintézis
3. Aminosav-transzport – enzimatis
4. Epitóp azonosítás – géntechnológiai



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

tRNS



P. Zamencik
M. Hoogland (1957)

