Biokonjugátumok

Hudecz Ferenc

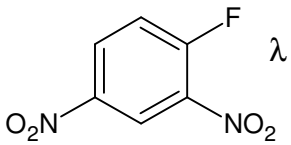
Előzmények

1) Fehérjék szerkezet – hatás összefüggéseinek kutatása (1925 –
   - Sumner, J.B., Graham V.A.: The nature of insoluble crease *Proc Soc Exp Biol Med* 22 504 (1925)
   - Melyik aminosav (oldallánc) „kell” és melyik „nem kell” a hatás megmaradásához
     Olcott, H.S., Fraenkel-Conrat, H.: Specific group reagents for proteins *Chem Rev* 41 151 (1947)
     Herriot, R.M.: Reactions of nativ proteins with chemical reagents *Adv Prot Chem* 3 161 (1947)

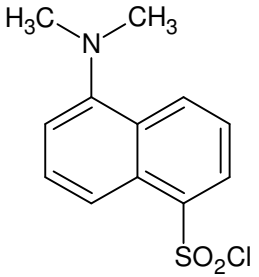
2) Szerkezet vizsgálat

Fehérjék

- N-terminális meghatározások



λ = 254 nm

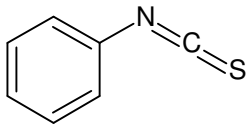


λ_s = 360 nm

λ_e = 480 nm

- Szekvencia meghatározás (1956)

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

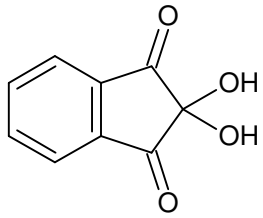


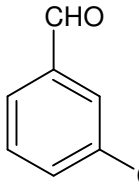
op.: -21 °C

- Aminosav meghatározás (1960)

  Moore, S., Stein, W.H.: Chromatographic determination of amino acids …

  Methods in Enzymol 6 819 (1963)



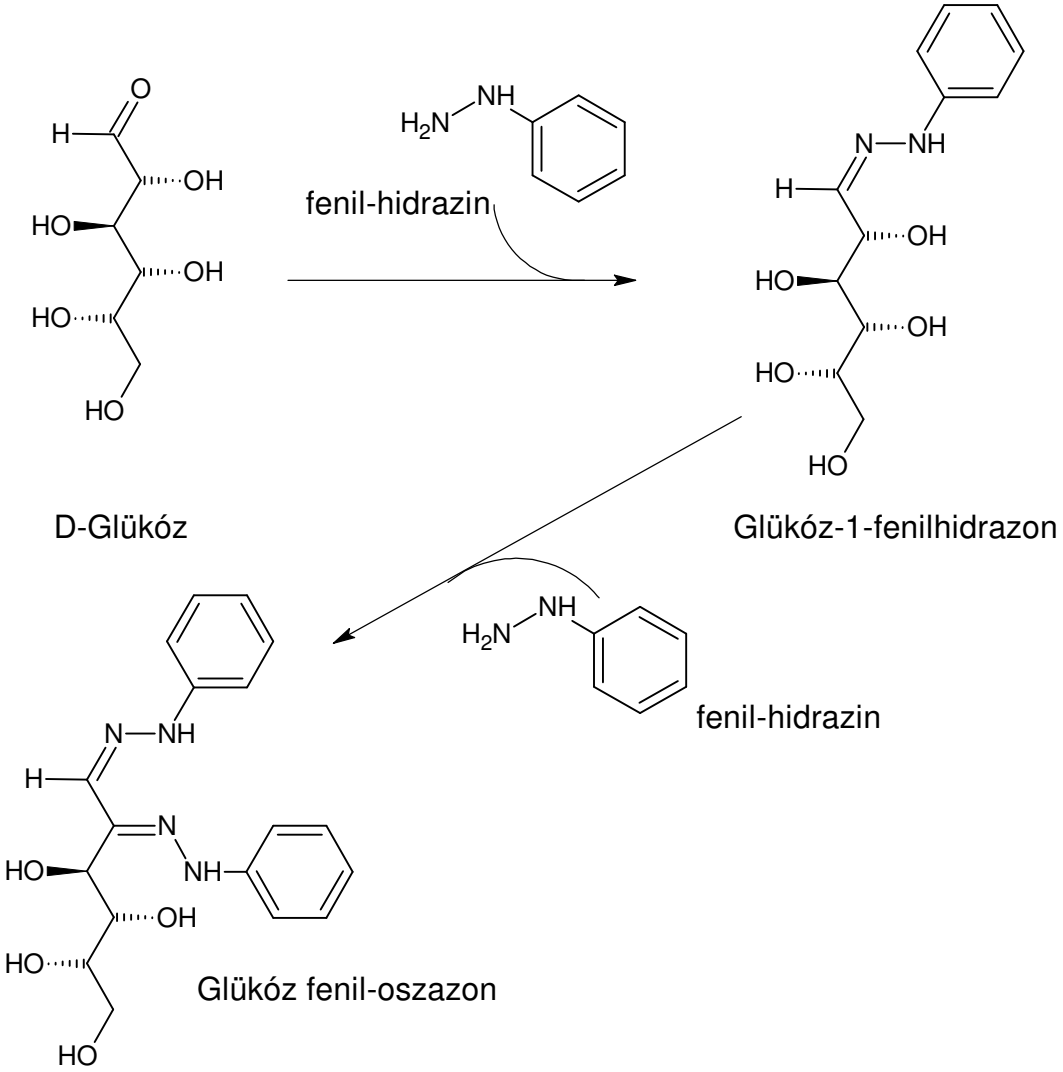


λ_s = 340 nm

λ_e = 455 nm

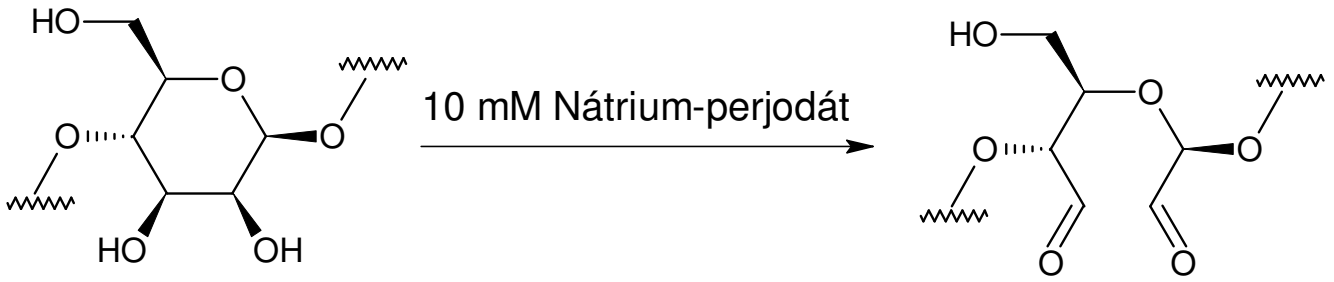
Szénhidrátok

- E. Fischer 1884 Redukáló monoszacharidok



Szénhidrátok

- Reakció oxo-csoporttal

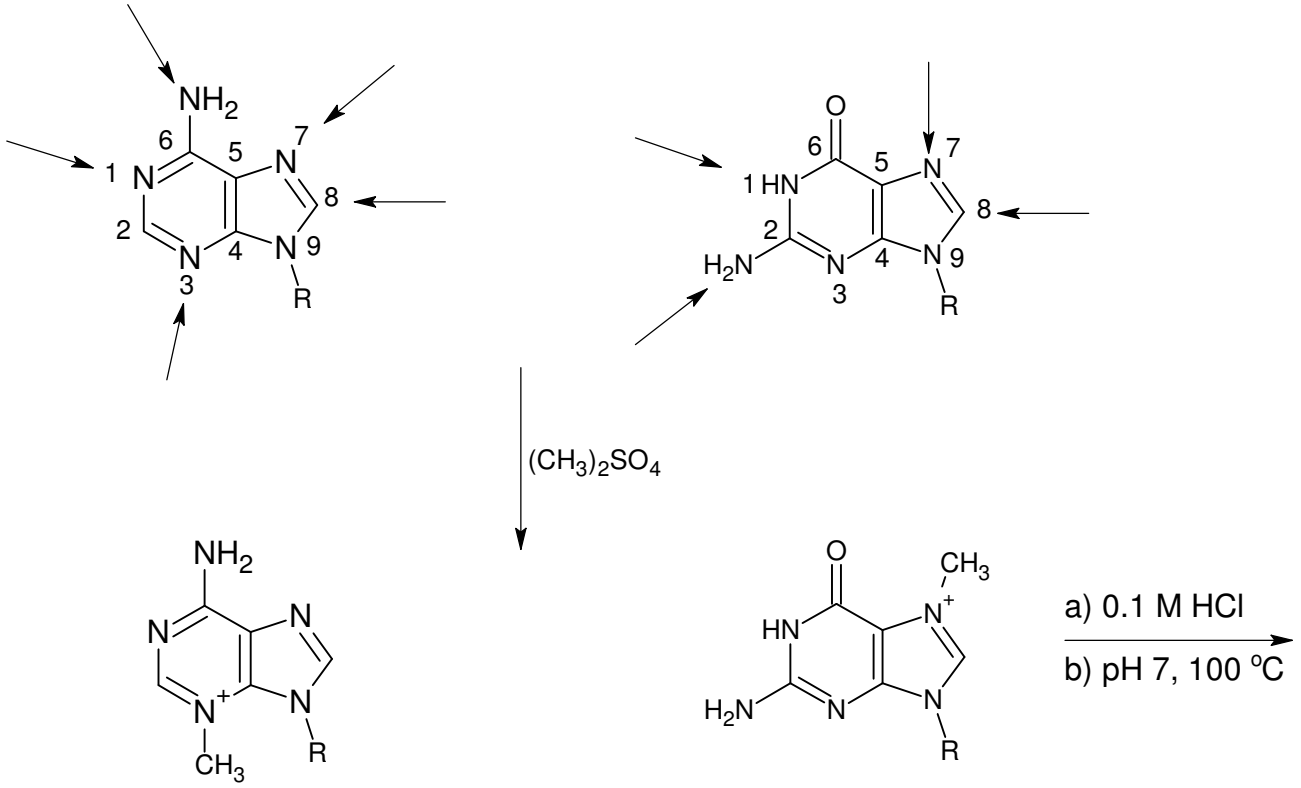


β-D-Mannóz részlet poliszacharid láncban

C - C kötés hasadása aldehid részlet oxidálásával

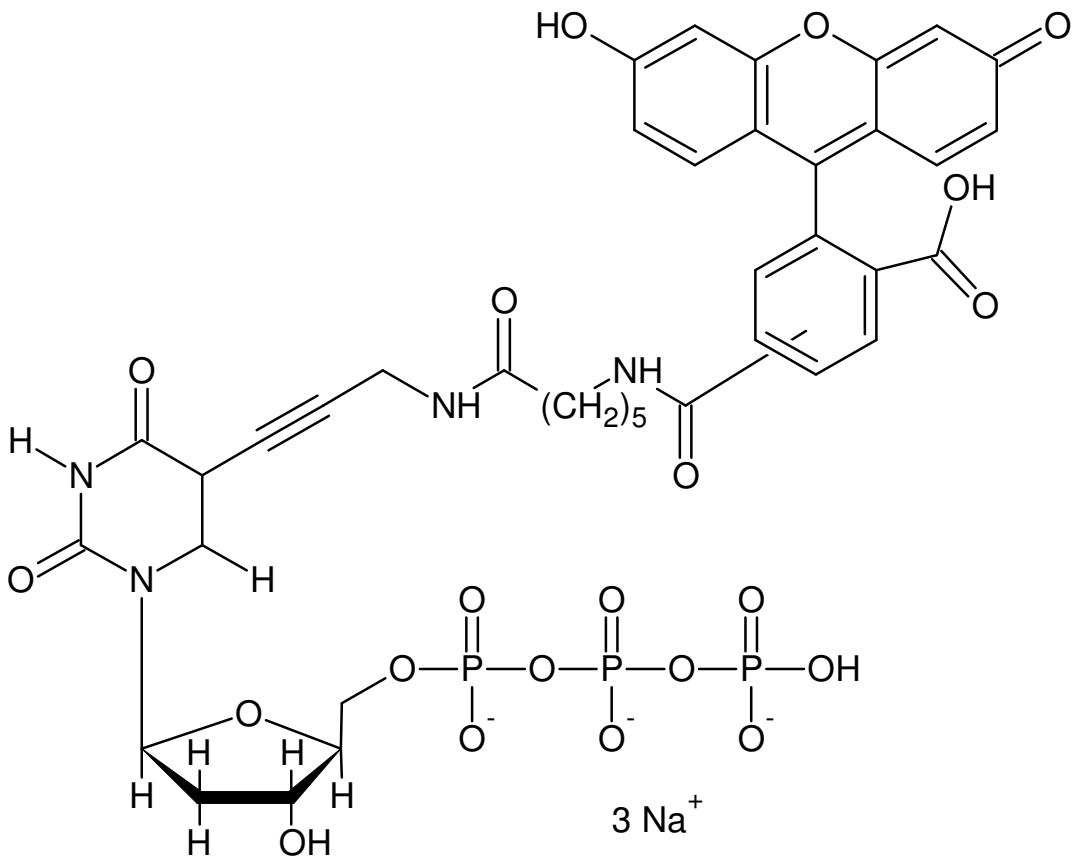
oxo-csoport „előállítása” hidroxil-csoportból

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



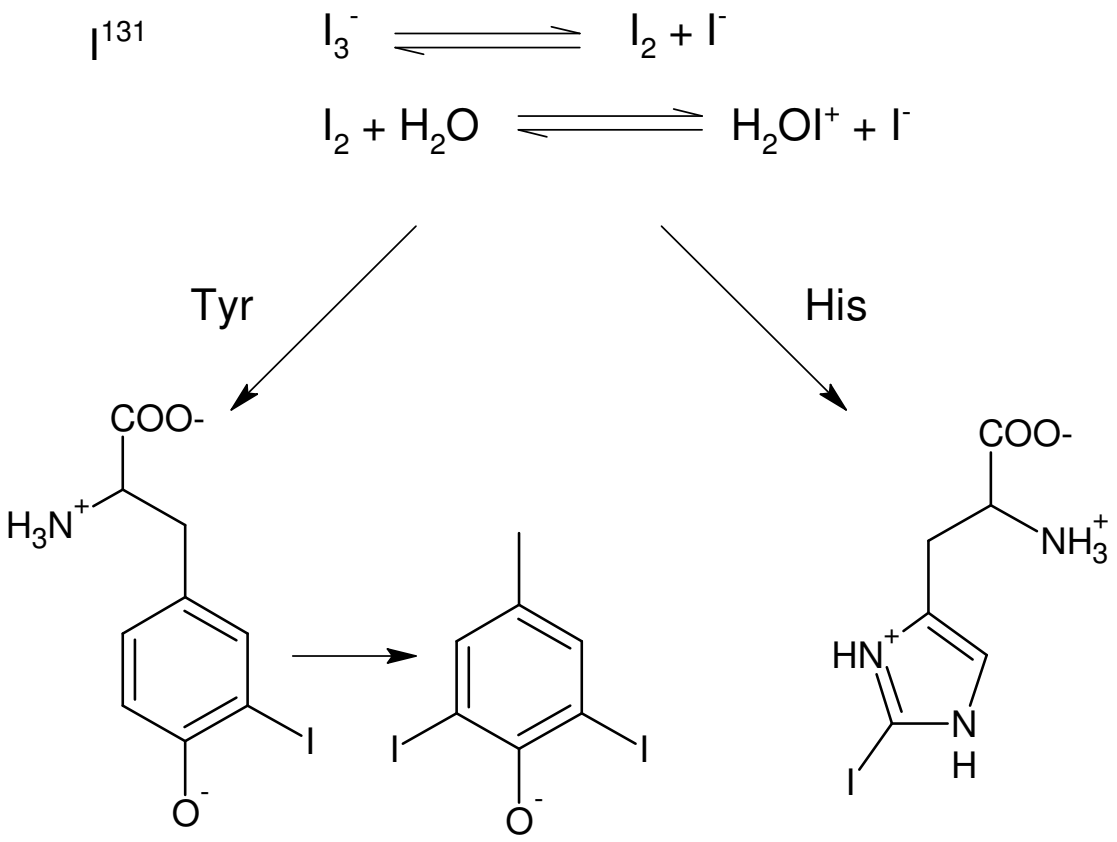
  - Sanger, F. et al. *PNAS* 74 5463 (1977)
  - Smith, L.M. et al. *Nature* 321 674 (1986)

- ChromaTide fluoreszcein-12-dUTP szerkezete (C-7604)

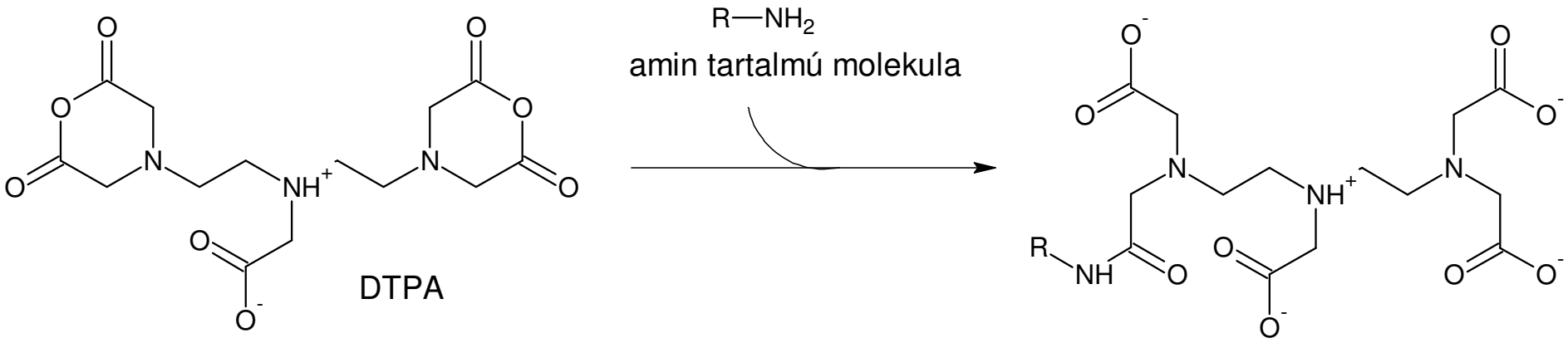


2) Biopolimerek kimutatása sejtben, szövetben

- Fehérjék jelölése radioaktív izotóppal
  - 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)

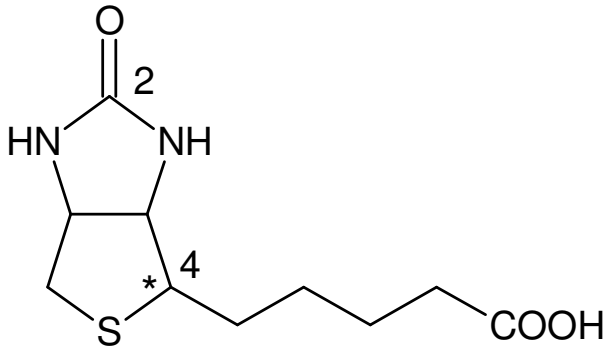


Fehérjék jelölése biotinnal

- 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)



D-Biotin

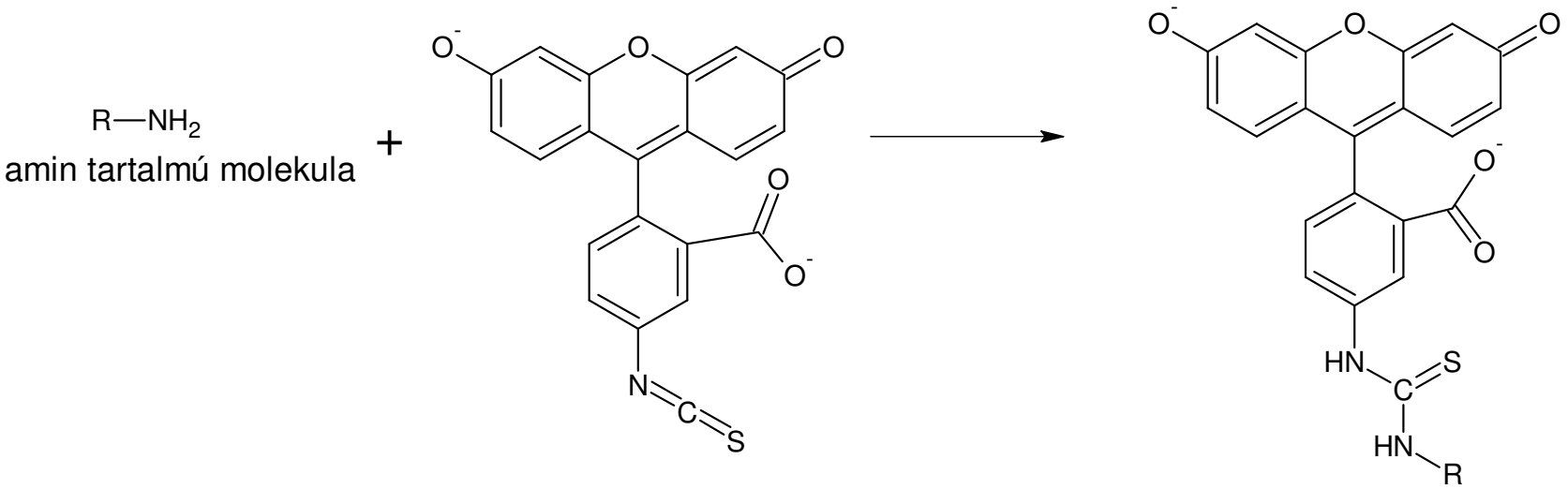
$K_a = 10^{15}\ M^{-1}$

op.: 232-233 °C

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

Biopolimerek jelölése fluorofórokkal

- McKinney, R. et al. Factors affecting the rate of reaction of fluorescein isothiocyanat with serum proteins *J Immunol* 93 232 (1964)

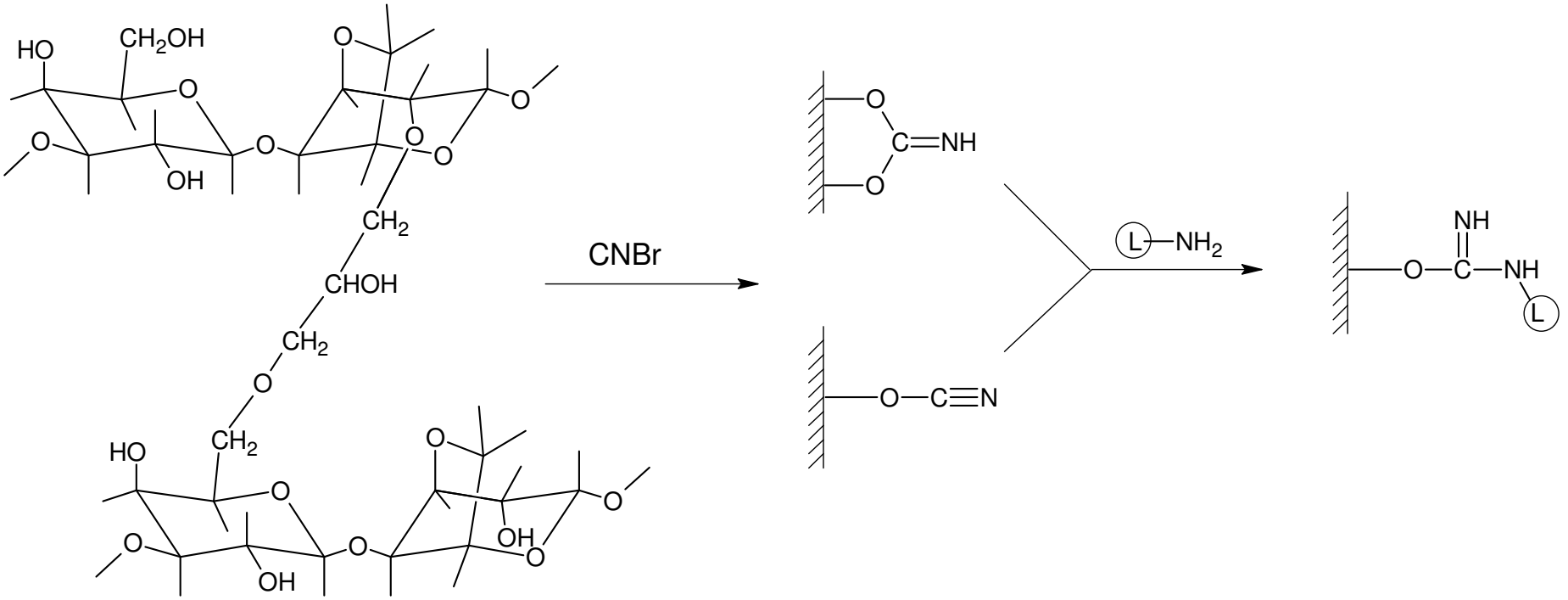


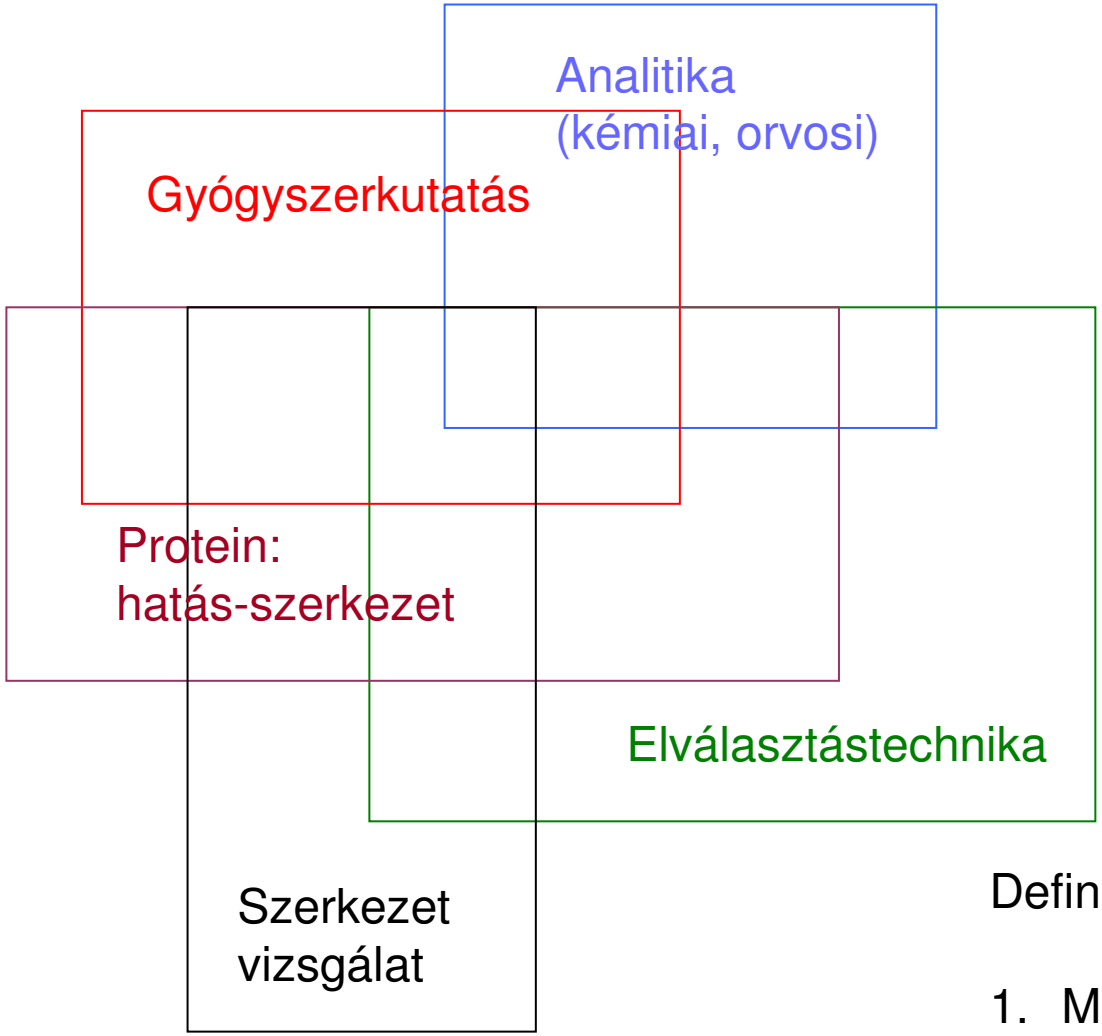
Fluoreszcein izotiocianát

Tiourea kötés létrehozása

2) Affinitás kromatográfia

- 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)



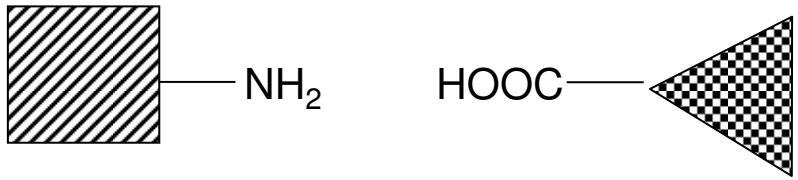


Definíció

1. Mindkét komponens aktív
2. Kovalens kötés

3. Lehet több komponens is

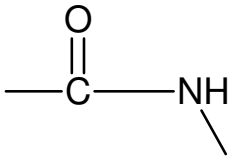
- Megközelítési lehetőségek
  1. A partnerek mérete
     - Kicsi – kicsi
     - Kicsi – nagy
     - Nagy – nagy
  2. A kötés jellege
     - Közvetlen



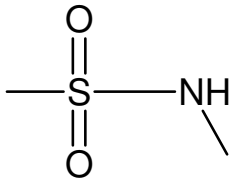
     - Közvetett

3. A kötés típusa

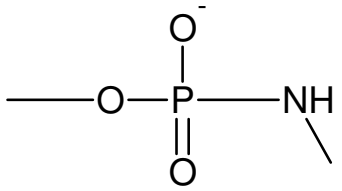
- Savamid



karbonsav

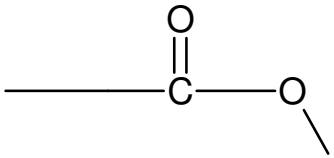


szulfonsav

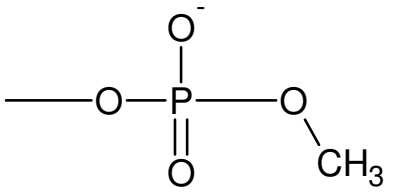


foszforamidát

- Savészter

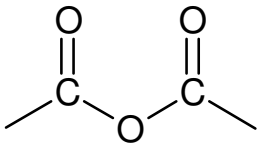


karbonsav

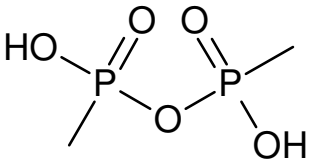


foszforsav

- Savanhidrid



karbonsav



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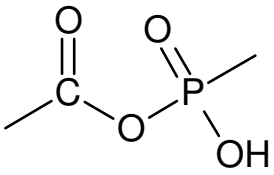
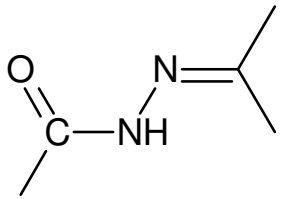
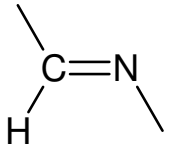
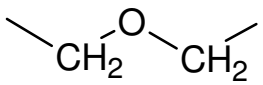


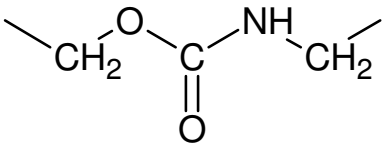
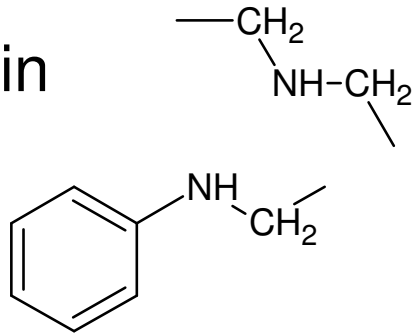
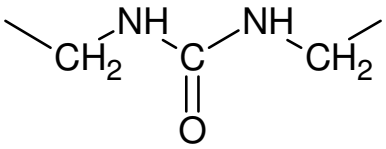
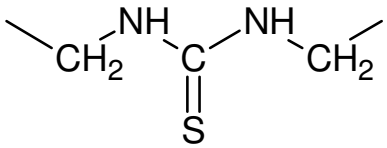
„vegyes”

- Hidrazon 

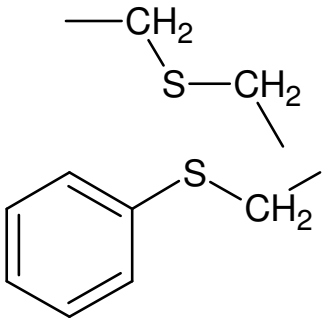
Aldehid + hidrazon
- Schiff bázis 

Aldehid + amin
- Éter 

Alkohol

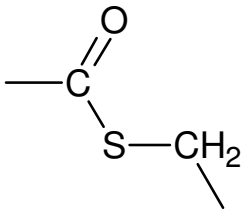
- Karbamát  Alkohol
- Szekunder amin (N-glikozid)  Amin
  + aldehid
  + aril-halogenid
- Izourea  Amin
- Izotiourea  Amin

- Tioéter



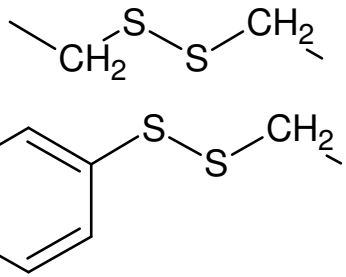
Tiol

- Tioészter



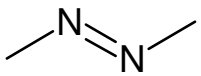
Tiol

- Diszulfid



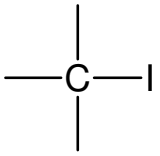
Tiol

- Diazo

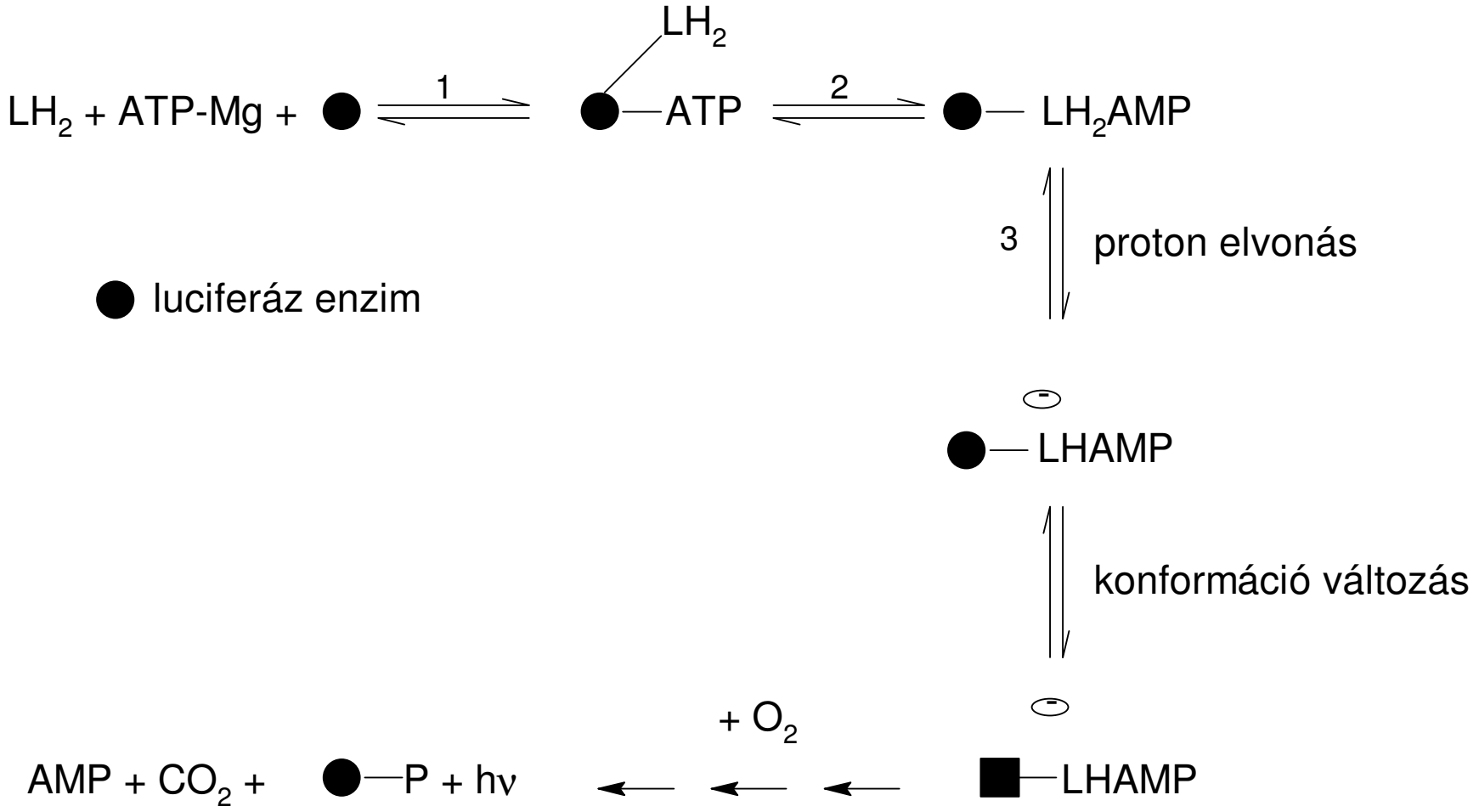


Azid

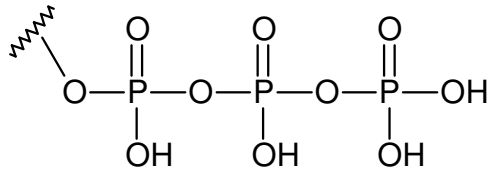
- C – X



1. Példa: kicsi – kicsi, közvetlen, anhidrid kötés „Firefly” lumineszcencia



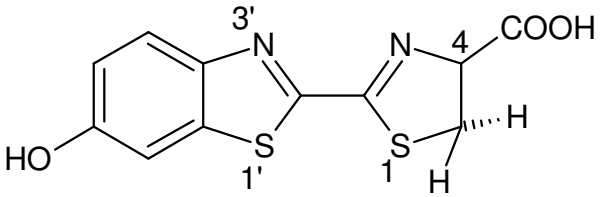
1. Példa



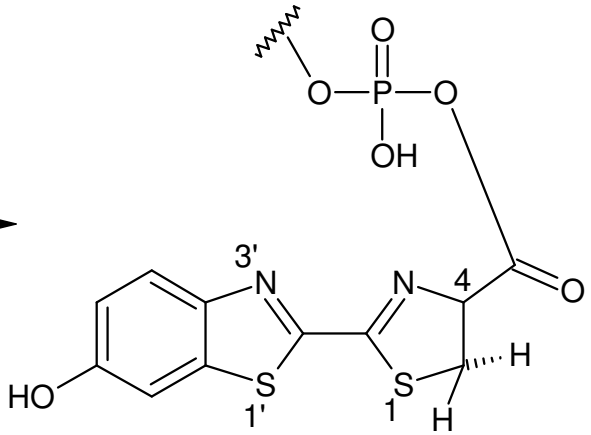
ATP

Luciferáz

Mg^{2+}



D (-) luciferin (LH_2)

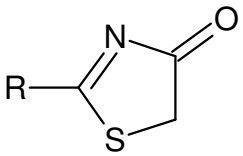


Luciferil-adenilát

+ ADP

Adenilsav } andidrid

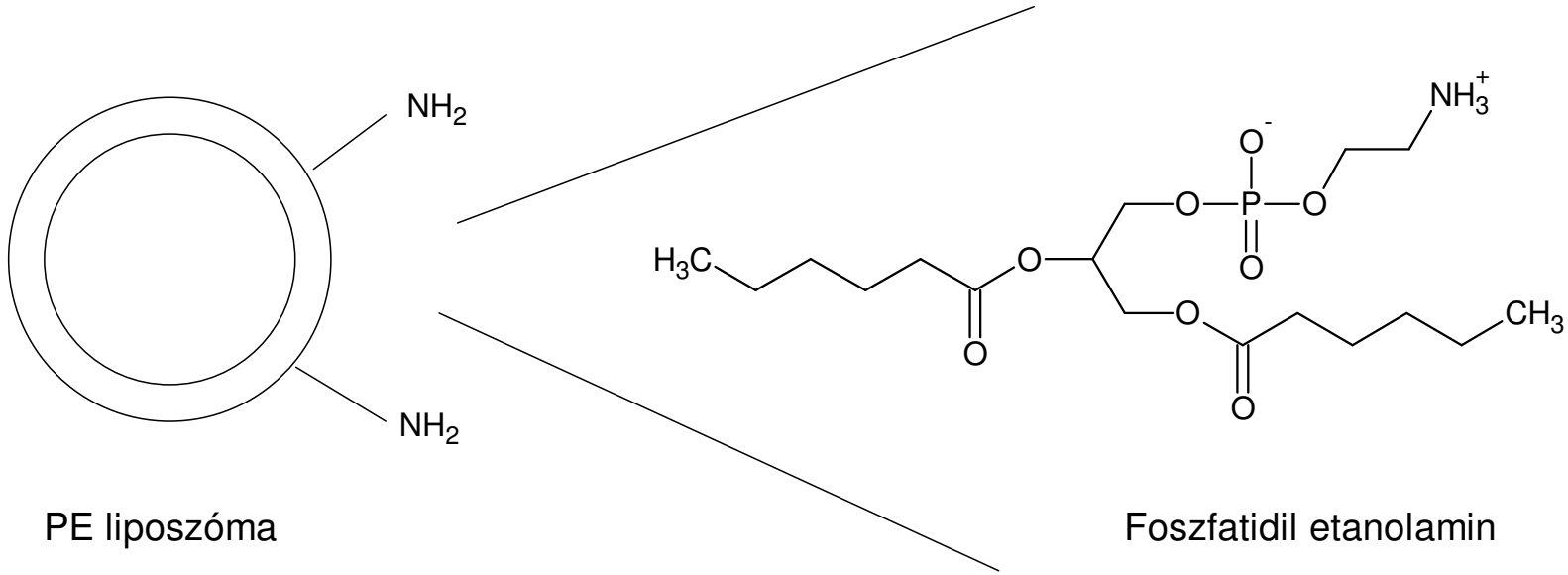
"Karbonsav" }



oxiluciferin

2. Példa: kicsi – nagy, közvetlen, amid kötés

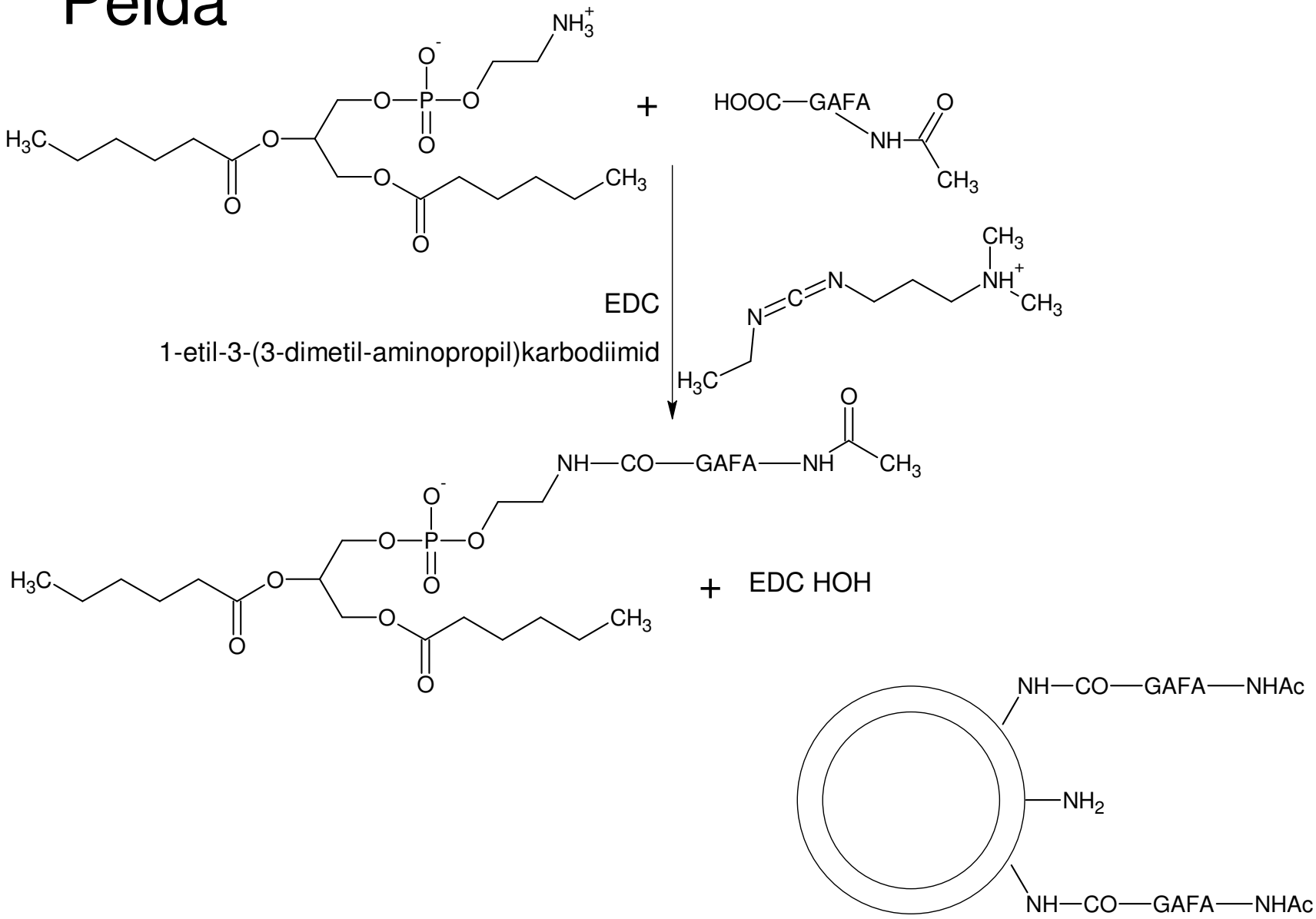
Liposzoma – haptén konjugátum



PE liposzóma

Foszfatidil etanolamin

2. Példa


NH3+
O-
O
P
O
O
H3C
O
O
O
CH3
+
HOOC—GAFA—NH
O
CH3
EDC
1-etil-3-(3-dimetil-aminopropil)karbodiimid
CH3
N
C
N
NH+
CH3
H3C
NH—CO—GAFA—NH
O
CH3
+ EDC HOH
NH—CO—GAFA—NHAc
NH2
NH—CO—GAFA—NHAc

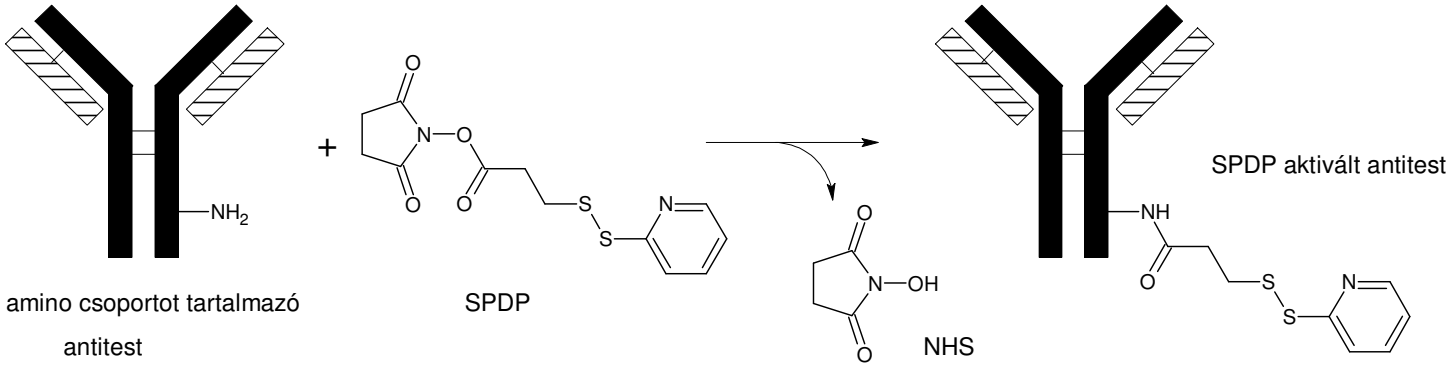
3. Példa: nagy – nagy, közvetett,diszulfid kötés

Immunotoxin konjugátum

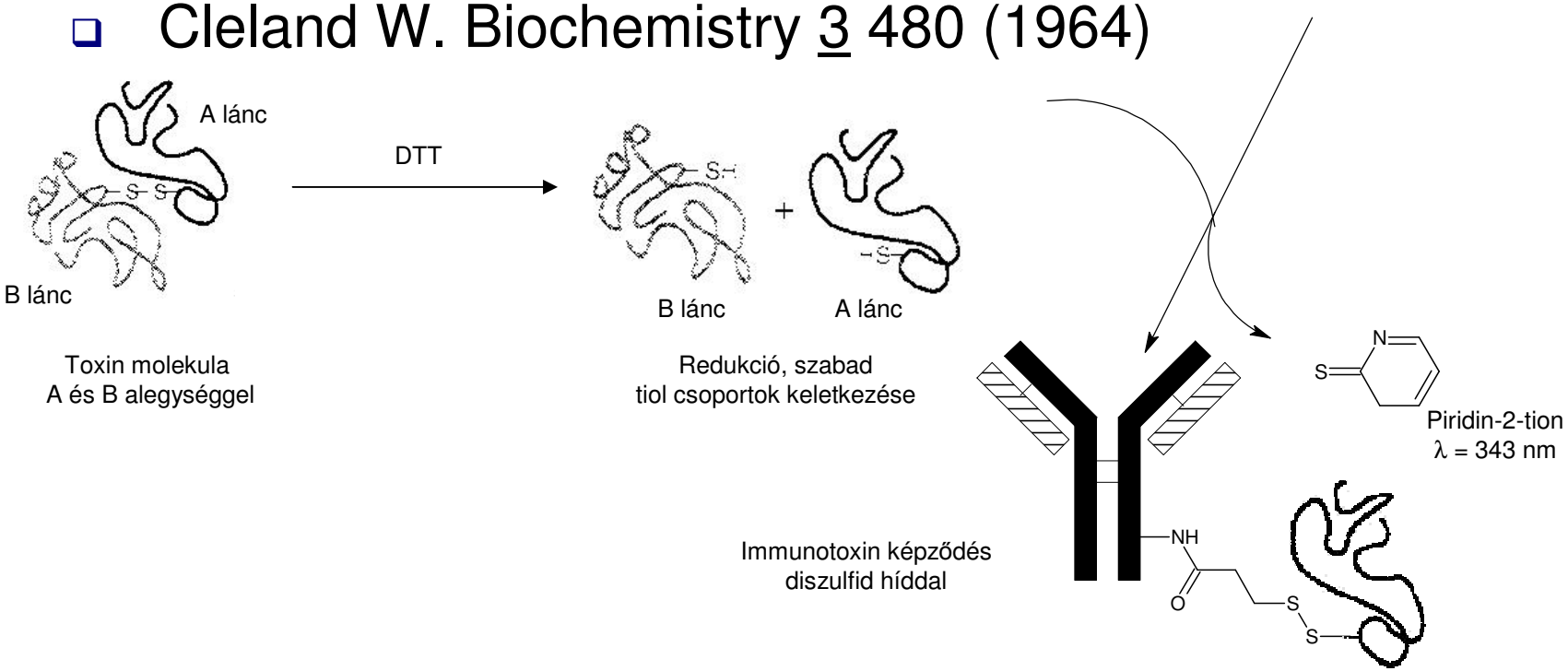
1. lépés: védett -SH csoport kiépítése az ellenanyag molekulán (Ab + SPDP → amidkötés)
2. lépés: szabad -SH csoport létrehozása a toxin alegységben (-S-S- kötés hasítása, DTT)
3. lépés: az –SH partner reakciója a „védett” SH tartalmú komponenssel (AB-SSP + Toxin-SH → diszulfid kötés)

3. Példa

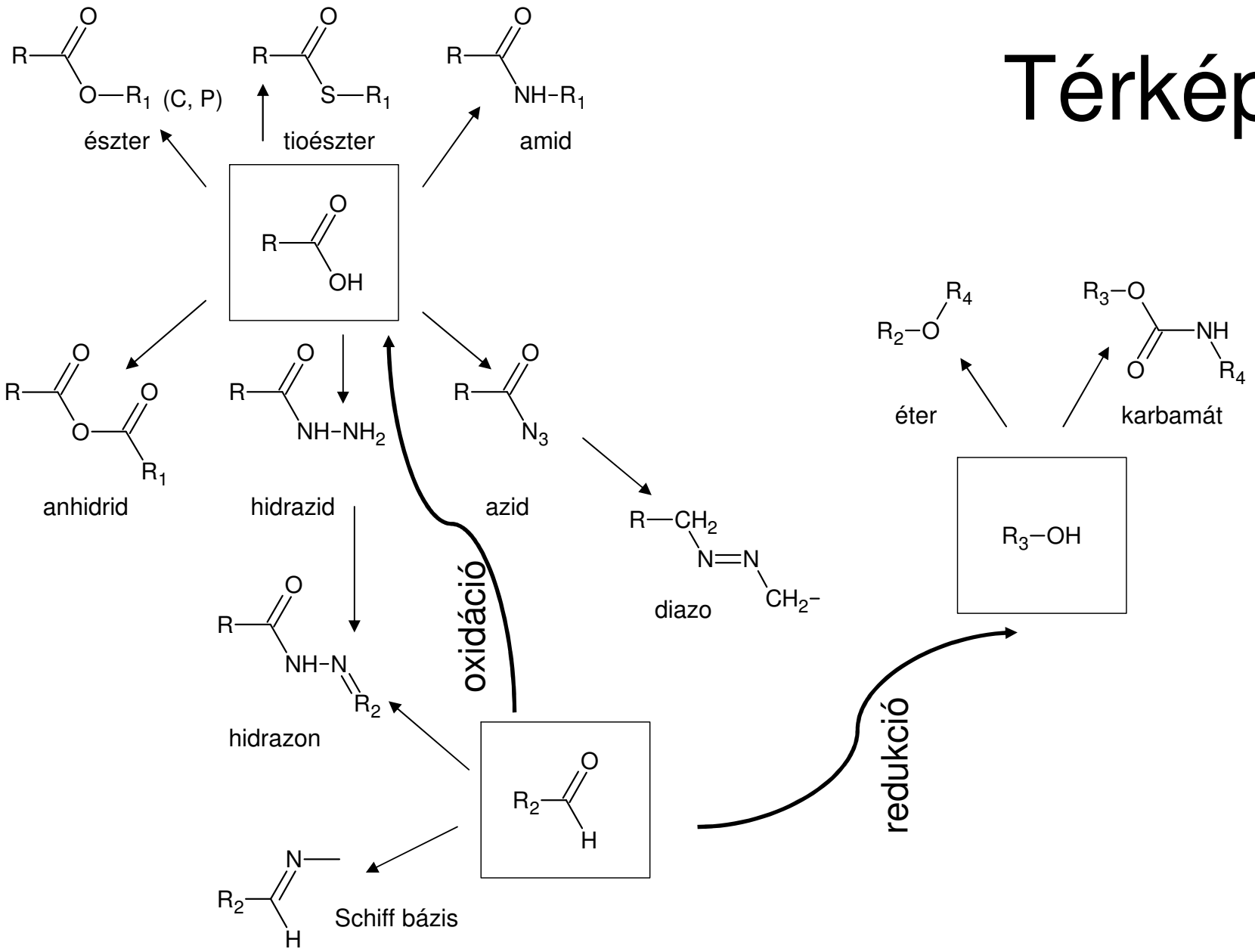
- Carlsson, J. et al. Biochem J 173 723 (1978)



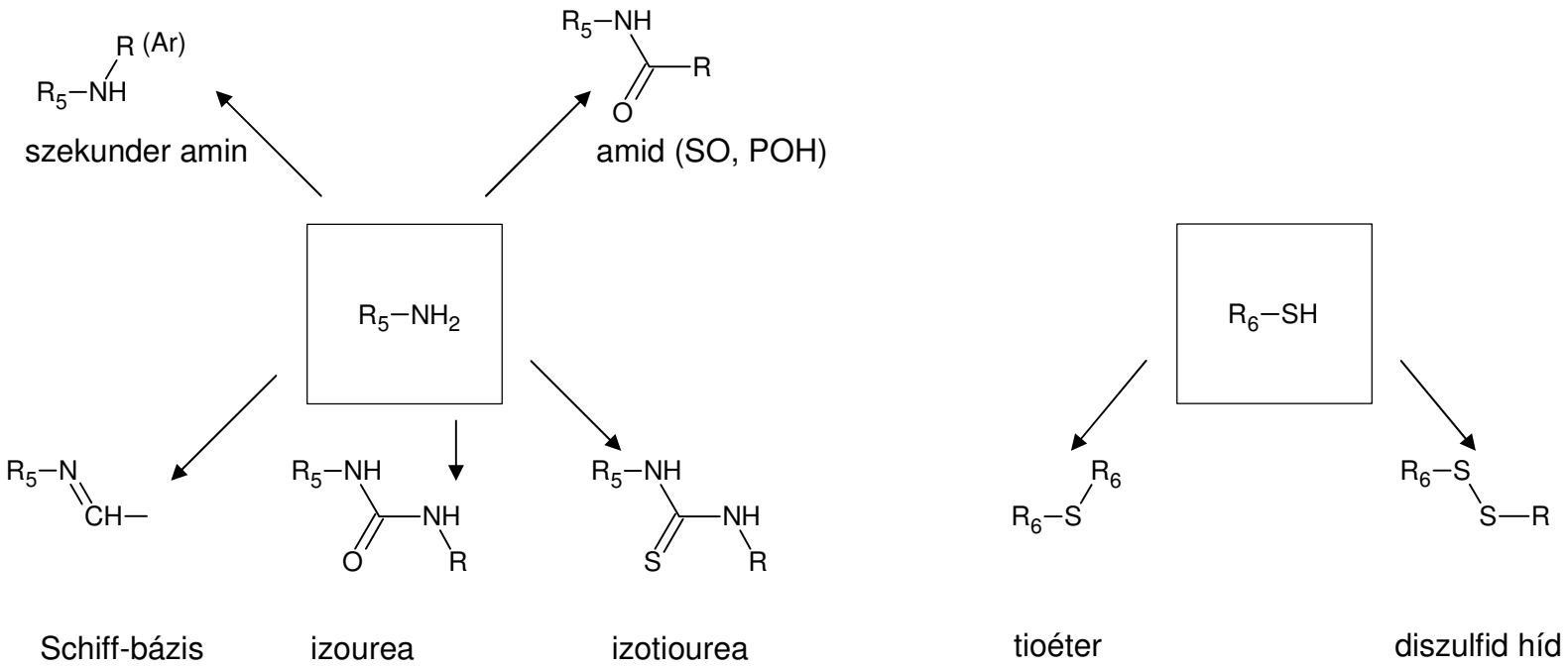
- Cleland W. Biochemistry 3 480 (1964)



Térkép


(C, P)
észter
tioészter
amid
anhidrid
hidrazid
azid
diazo
éter
karbamát
oxidáció
redukció
hidrazon
Schiff bázis

Térkép



1) Hogyan lehet funkciós csoportot kiépíteni? (-COOH, -CHO, -OH, -SH, -NH_2)
2) Hogyan lehet funkciós csoportot eltüntetni?