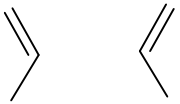
Kémiai összekapcsolási stratégiák

TRI

Homobifunkciós

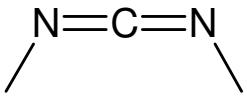
Azonos funkciós csoportok

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

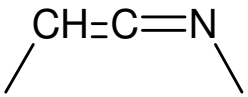
„Zero-length”

Nincs beépülés a két komponens közé

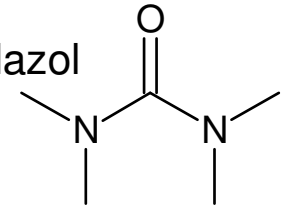
1. Karbodiimid



2. Woodward’ reagens



3. Diimidazol



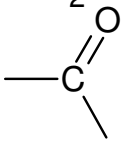
4. Oxidálószerek

-SH + HS-

5. Enzimek

Heterobifunkciós

Különböző funkciós csoportok

1. –NH_2 HS-
2. 

HS-
3. Fotoreaktív

-NH_2

-SH

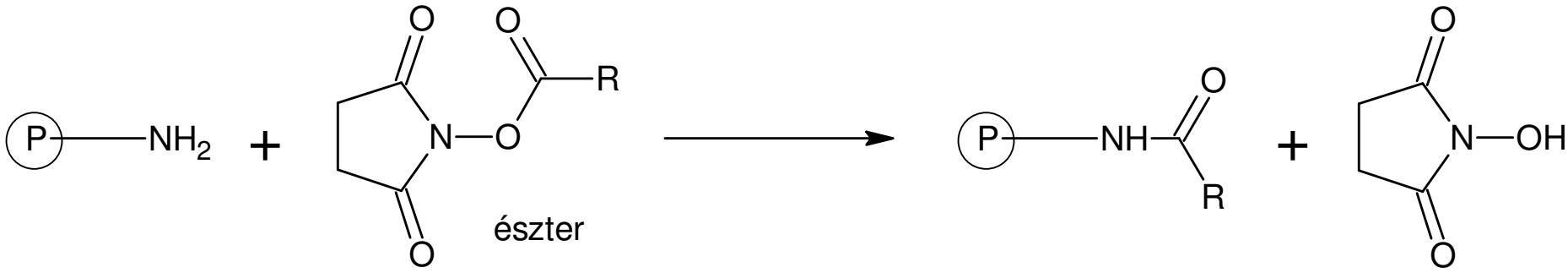
-COOH

-CHO

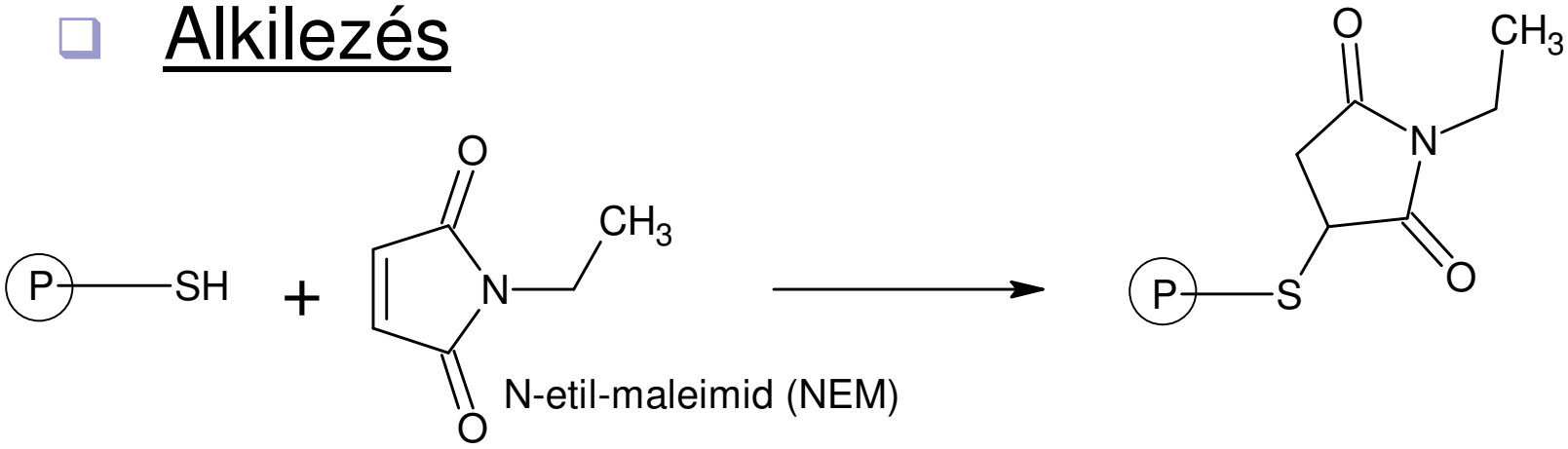
4. Fotoaffinitás

Kapcsoló reagens megválasztása

1. A reakció specificitása: acilezés, alkilezés
   - <u>Acilezés</u>



   - <u>Alkilezés</u>



- Nukleofilitás
  a) Egy perióduson belül

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

Tyr Lys, α Ser, Thr

  a) Egy oszlopon belül

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

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

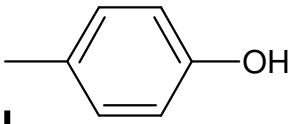
Edwards Jo, Pearson RG JACS 84 26 (1962)

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

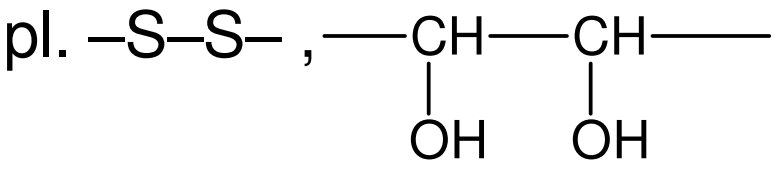
$> {}^{-}Cl >$ piridin $> {}^{-}OAc > H_2O$

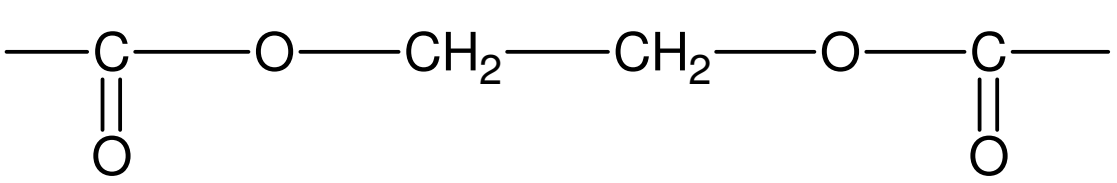
Σ $-SH > -NH_2 > -OH$

- <u>pH hatás</u> protonálás csökkenti a nukleofilitást

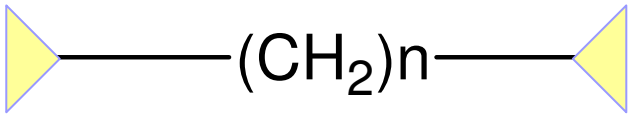
-SH	pK_a =	8.3
-$^{\varepsilon}NH_2$		10.8
		10.9
-OH		> 13

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

2. A reagens hidrofil/hidrofób jellege
   - pl. membrán
3. A reagens hasíthatósága
   - pl. –S–S– , 



4. A reagens mérete

pl. 

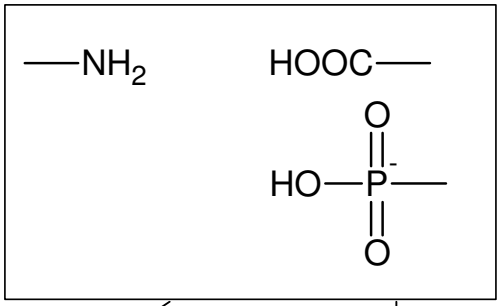
n = 0,1,2…

5. A reagens „riporter” sajátsága

pl. UV, F kromofór, spin-jelző

1. „Zero-length” kapcsoló reagensek

A) Savamid kötés



I. Kumulált kettős kötés
- a) Karbodiimid
- b) Woodward

II. Aktív karbonil
- a) CDI
- b) Szénsav származékok

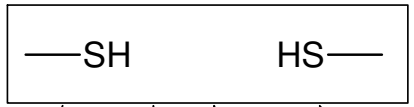
B) Shiff bázis (szekunder amin)

Spontán

Redukálószerek
(lásd ott)

pl. HPO-IgG
Ferritin – avidin

C) Diszulfid kötés

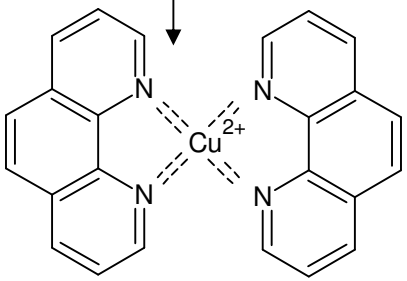


levegő

I_2

H_2O_2

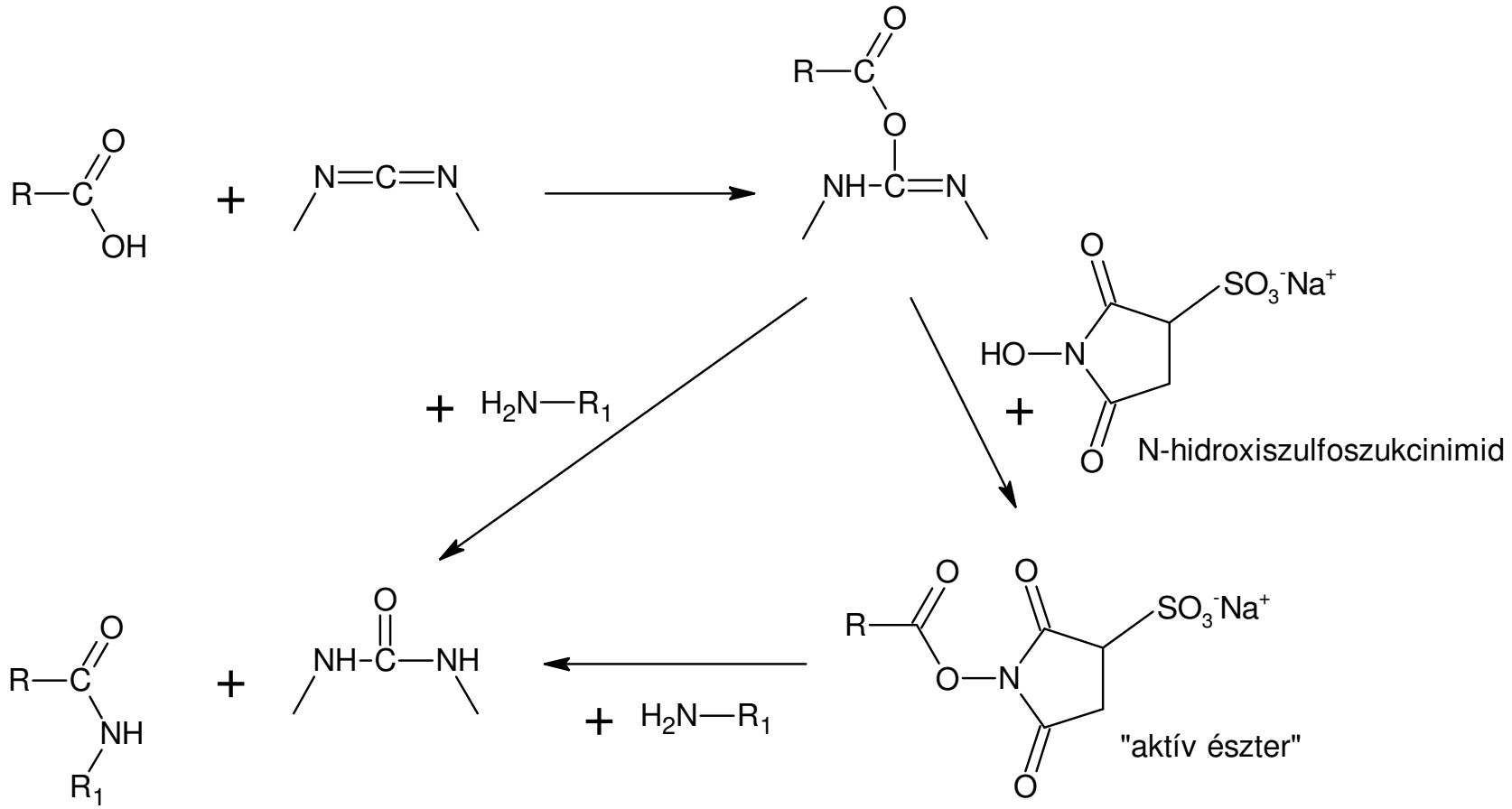
pl. membrán



CuP [di(1,10-fenantrolin)]

A) Savamid kötés <u>Kumulált kettős kötésű reagensek</u>

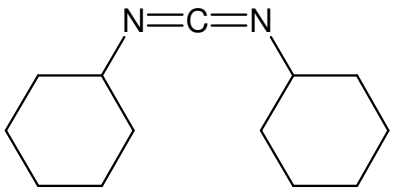
I. Karbodiimidek



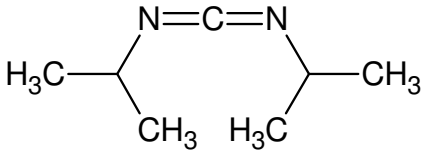
Staros JV Anal Biochem <u>156</u> 220 (1986)

A. Nem-vízoldékony karbodiimidek

Sheehan JC J Am Chem Soc 77 1067 (1955)



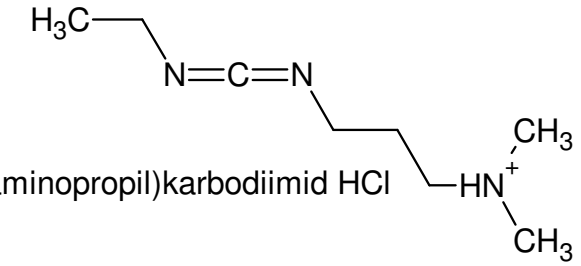
N,N' - diciklohexil-karbodiimid, DCC



N,N' - diizopropil-karbodiimid, DIC

B. Vízoldékony karbodiimidek

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



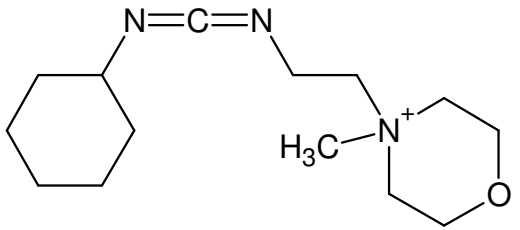
1-etil-3-(3-dimetilaminopropil)karbodiimid HCl

EDC [EDAC]

pH 4.7 - 6 (7.5) puffer

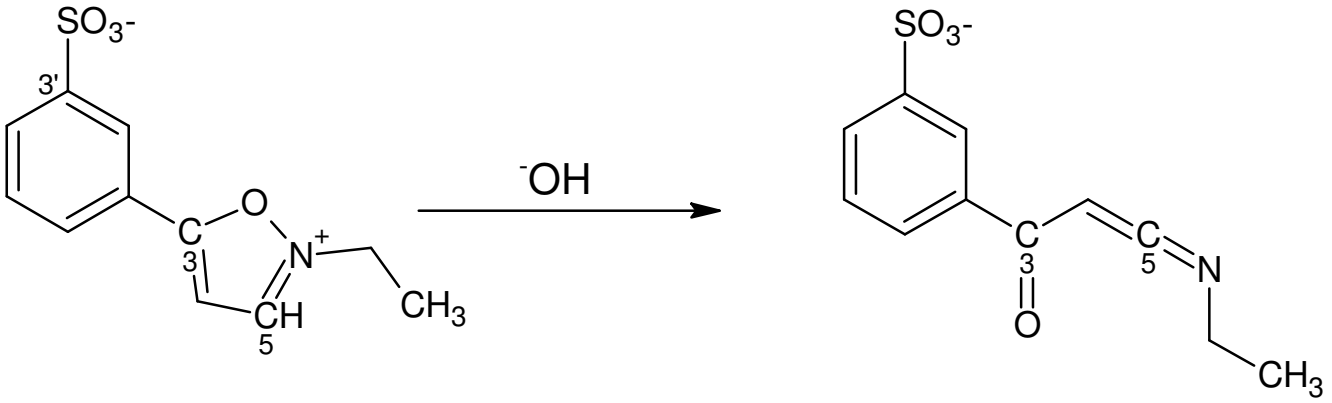
Mellékreakció: hidrolízis, tiolízis (tioészter)

Sheehan JC J Org Chem 21 439 (1956)



1-ciklohexil-3-(2-morfolinoetil)karbodiimid, CMC

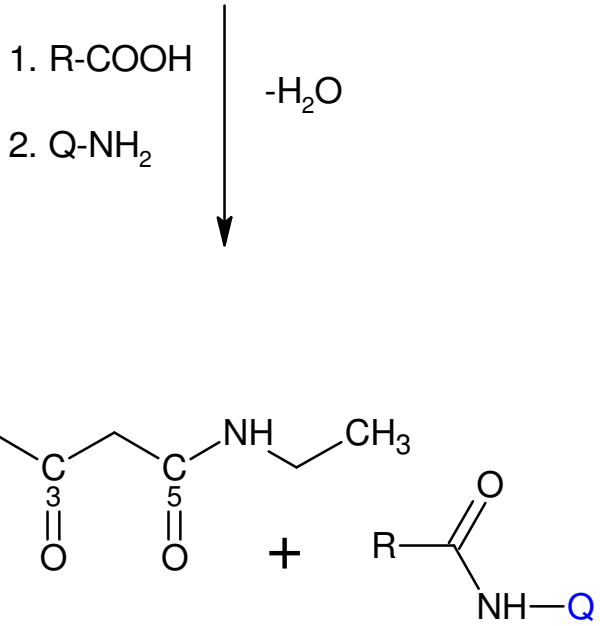
C. Woodward reagens



N-etil-3-fenil-izoxazolium-3'-szulfonát

ketoketénimin

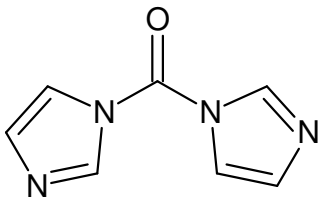
Woodward RB J Am Chem Soc 83 1010 (1961)



1) Polimer α-kimotripszin
2) Hemin – IgG konjugátum
3) Enzim – bilirubin konjugátum

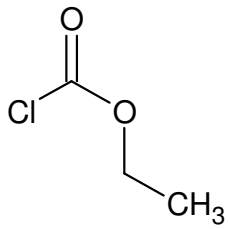
II. Aktív karbonil csoportot tartalmazó reagensek

A



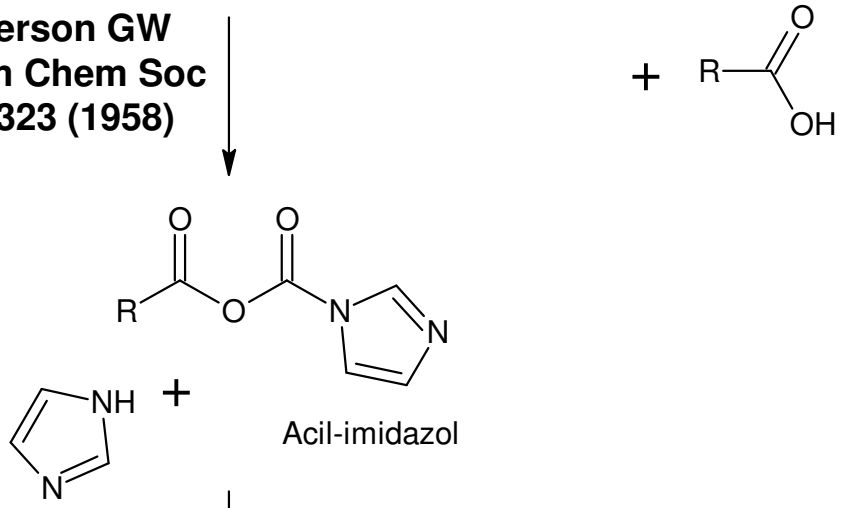
N,N' - karbidiimidazol, CDI

B

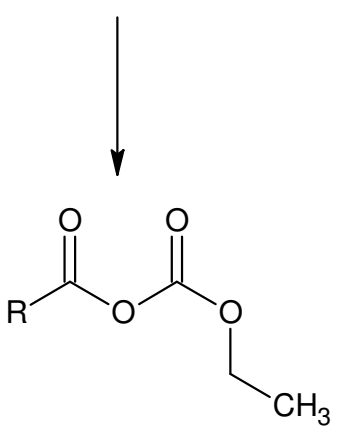


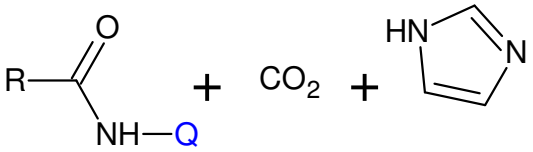
klórszénsavetilészter

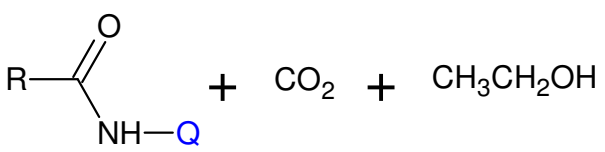
Anderson GW
J Am Chem Soc
<u>80</u> 4323 (1958)



Acil-imidazol





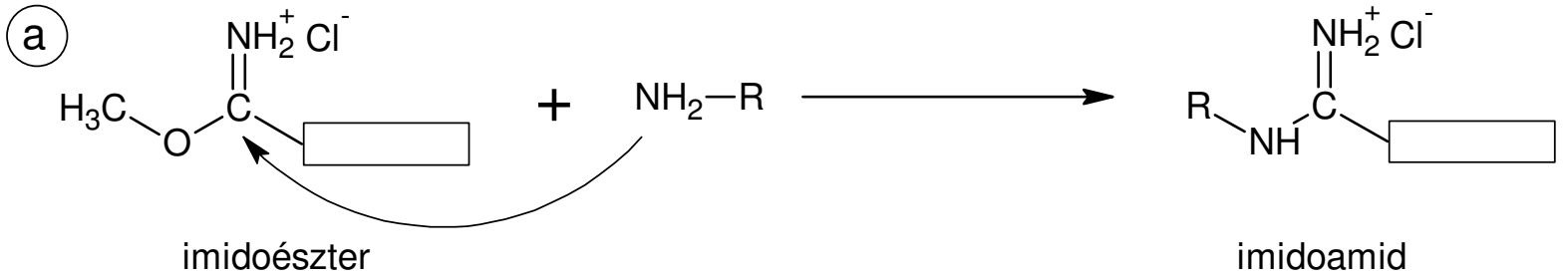


Enzim – ellenanyag → hisztokémia
Ganglionid – flourofor

2. Homobifunkciós kapcsoló reagensek

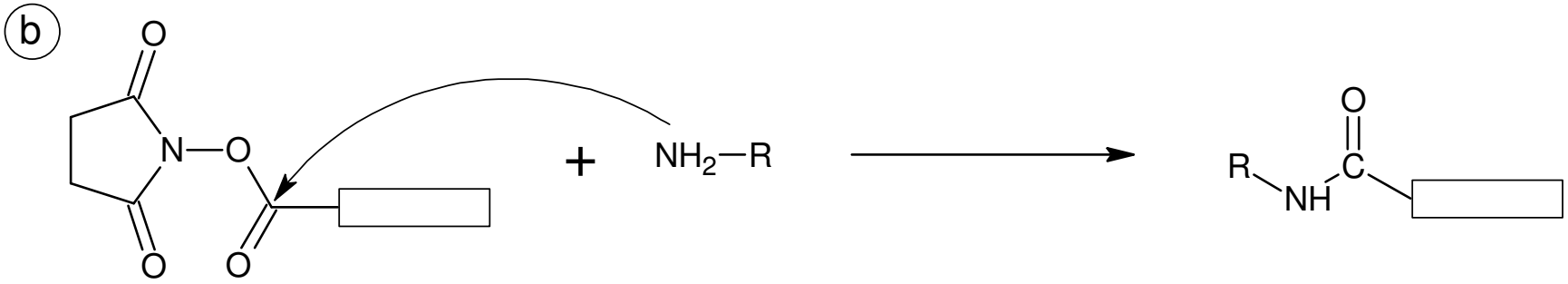
2.1. Funkciós csoportok

2.1.1. Amino csoport



imidoészter

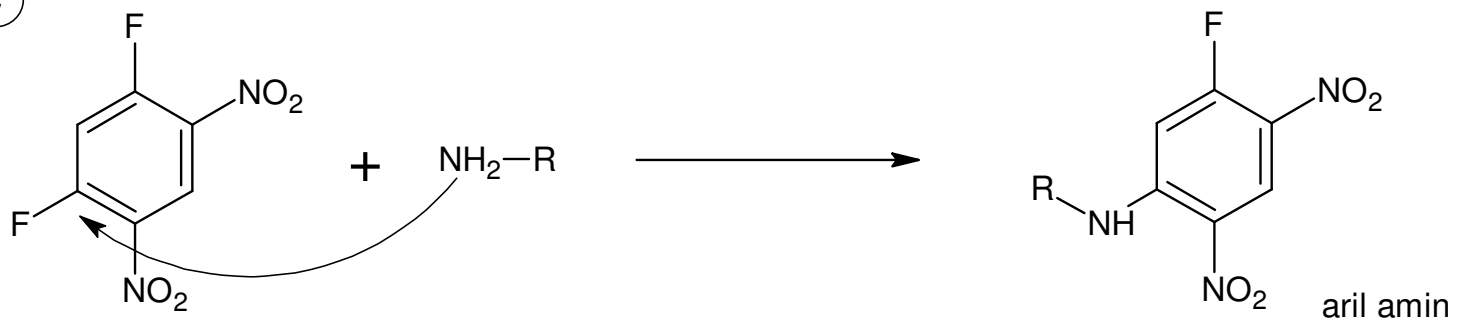
imidoamid



N-hidroxi szukcinimid észter

amid

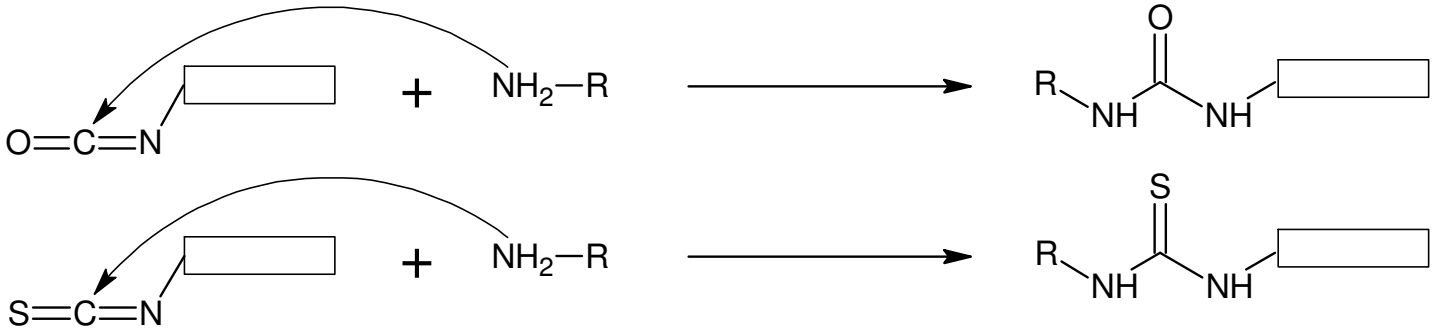
c



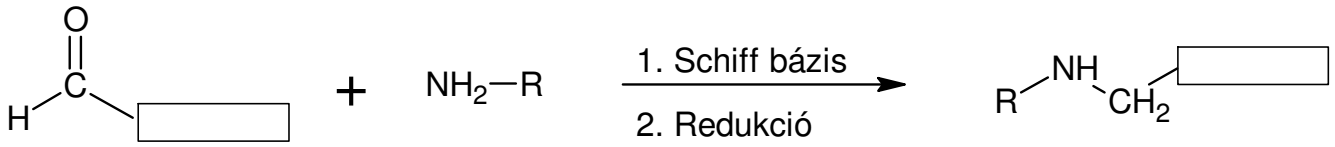
aril halogenid

aril amin

d



e

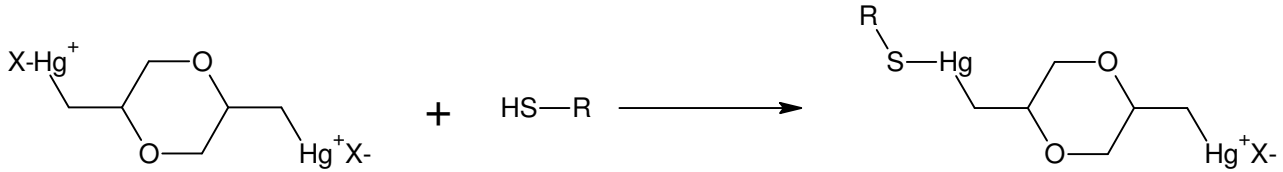


dialdehid

2.1.2. Tiol csoportok

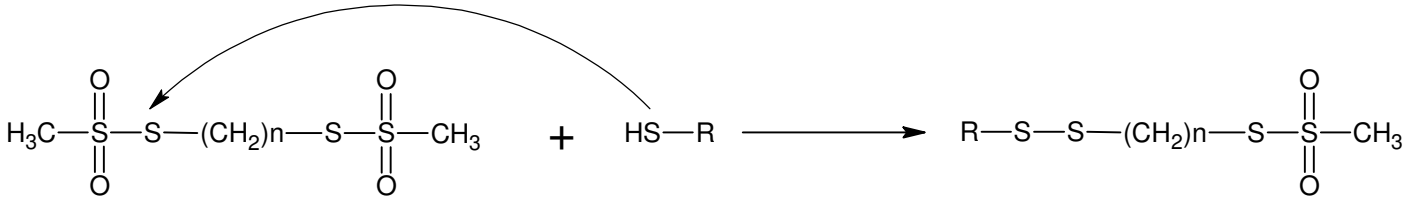
a) Hg^{2+} 1947-től

b)



3,6-bis(merkurimetil)dioxán X = Cl^-, NO_2^-

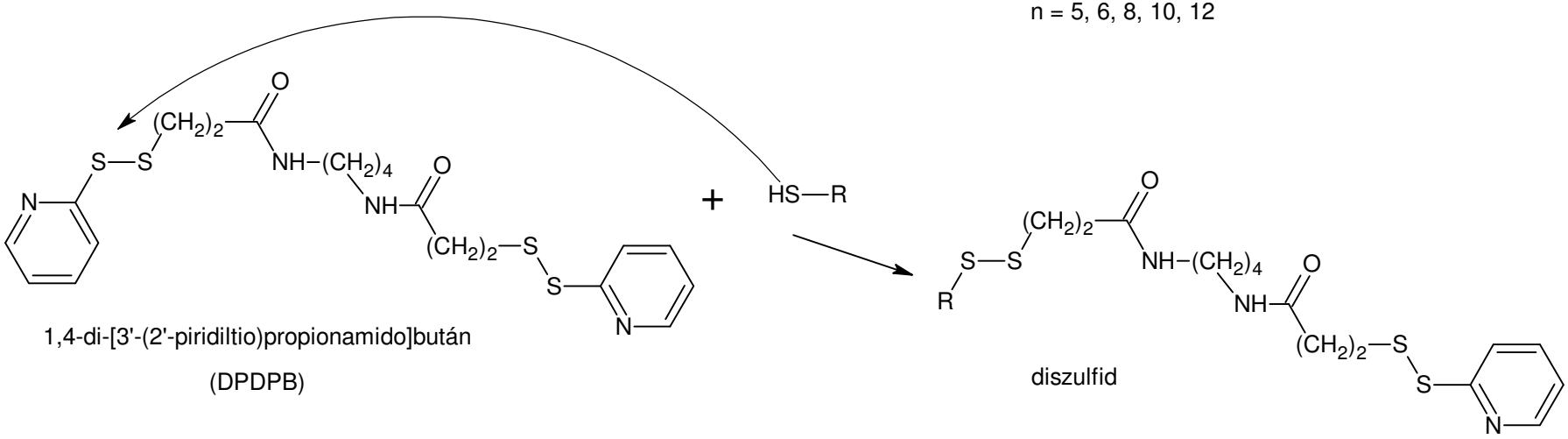
c)



polimetilén bis(metántioszulfonát) diszulfid + metánszulfonsav

n = 5, 6, 8, 10, 12

d)

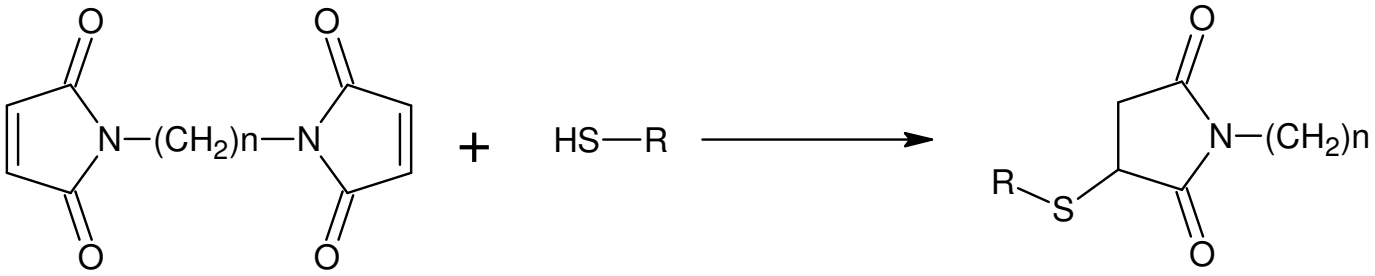


1,4-di-[3'-(2'-piridiltio)propionamido]bután

(DPDPB)

diszulfid

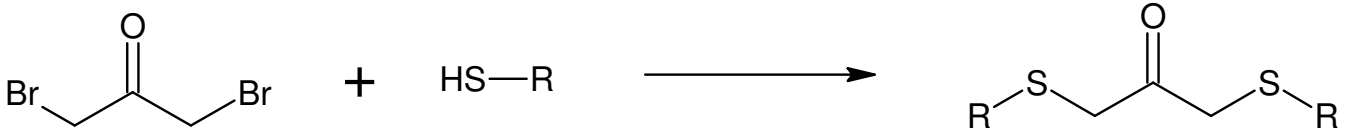
e



N,N'-metilén-bis-maleimid

tioéter n = 1, 3, 6, 8, 12

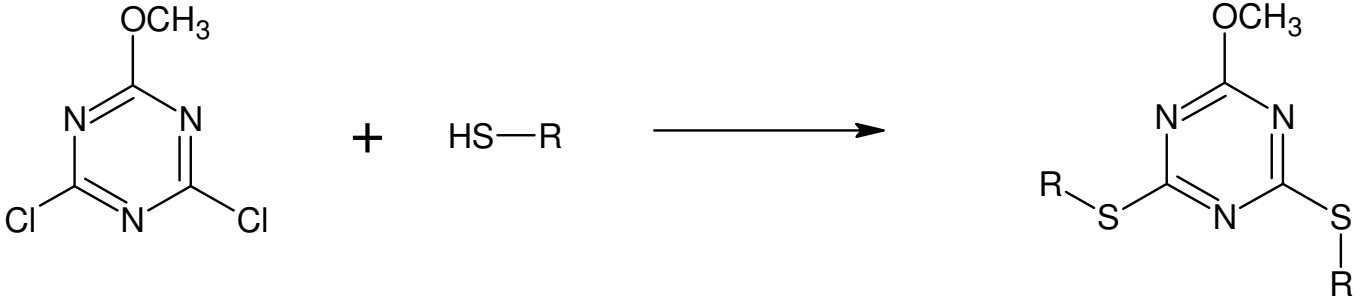
f



1,3-dibrómaceton

tioéter

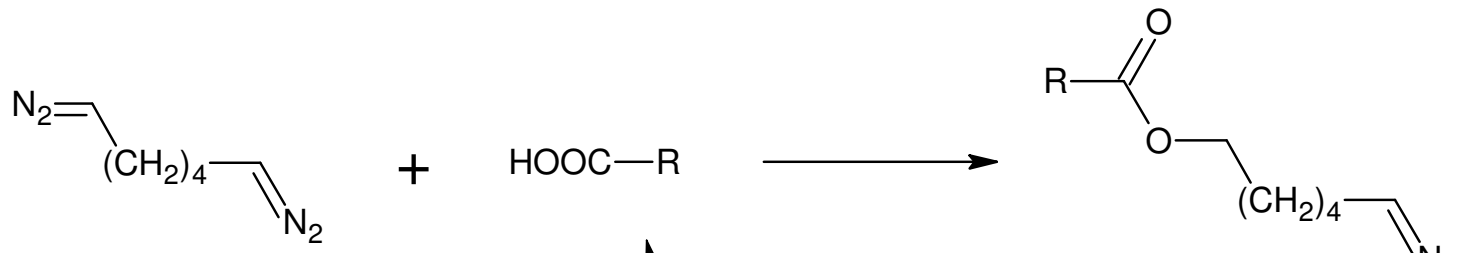
g



2,4-diklór-6-metoxi-S-triazin

tioéter

2.1.3. Karboxil csoportok



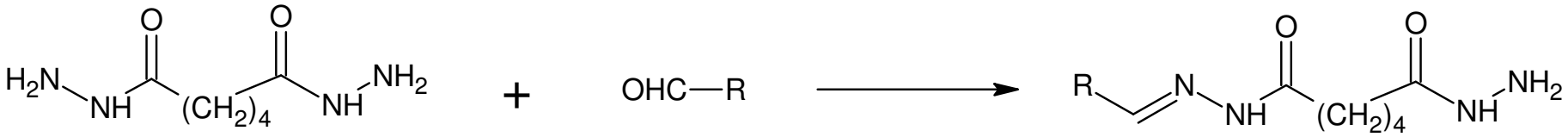
bis-diazo-hexán

észter

EDC

imid

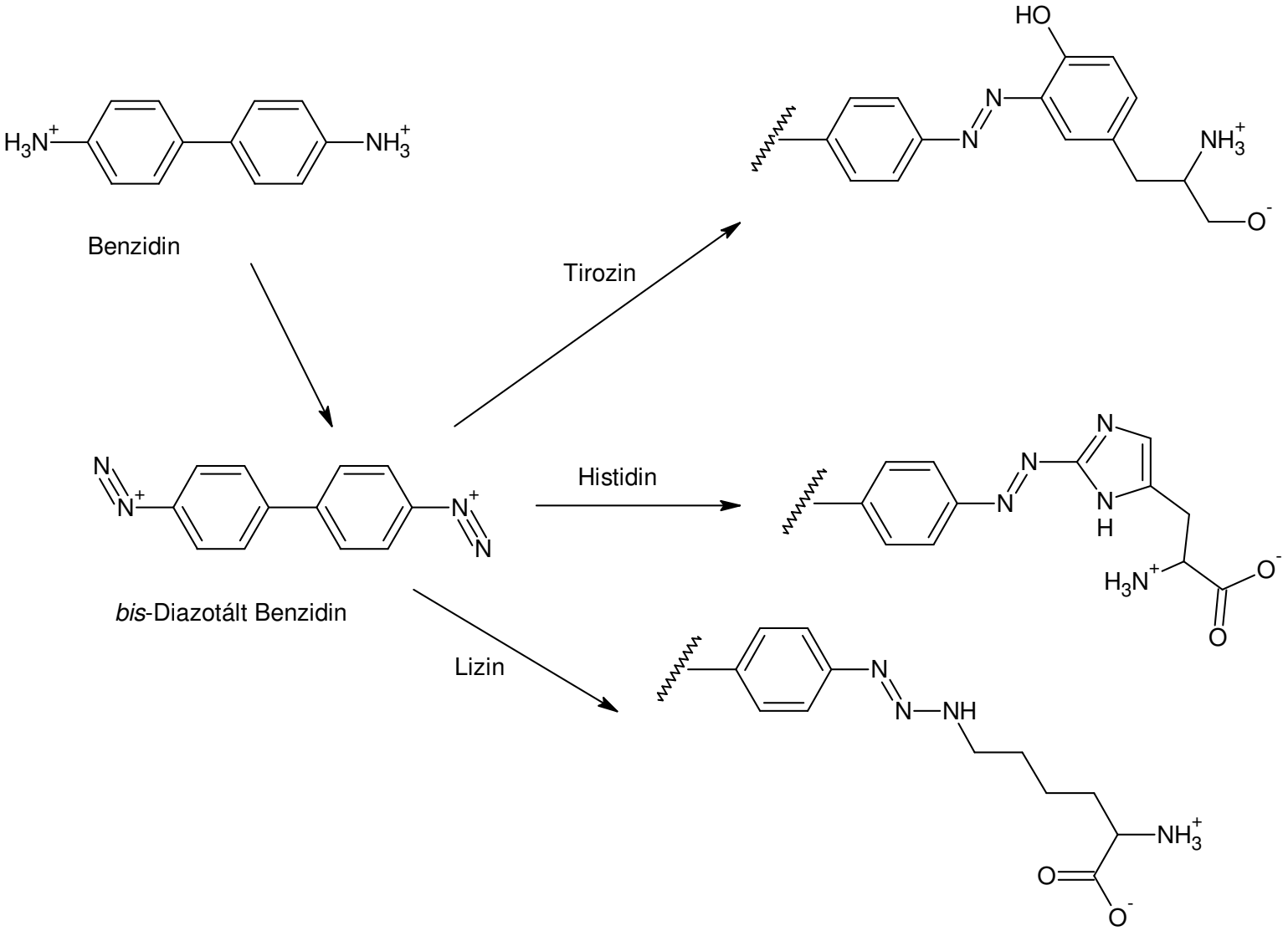
2.1.4. Aldehid csoportok



Adipinsav hididrazid

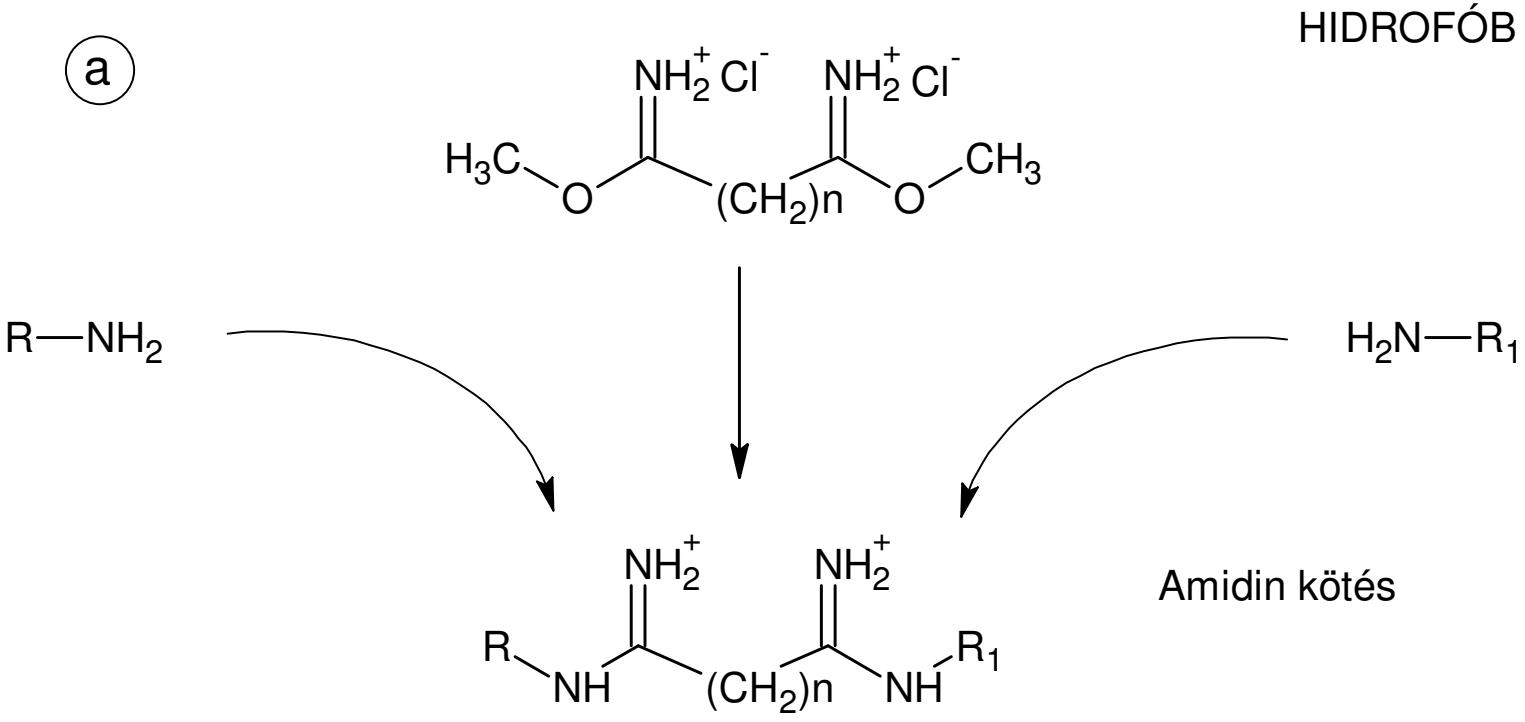
hidrazon

2.1.5. Bis-benzidin származékok – vegyes célcsoport (karcinogén 1985)



2.2. Összekötők (spacer)

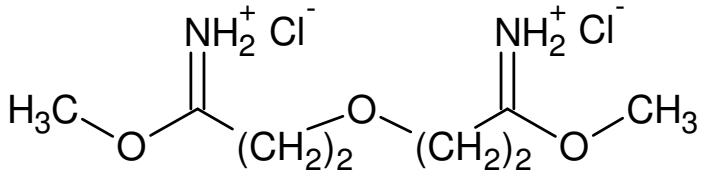
2.2.1. Nem hasítható



n = 1 dimetil malonimidát (DMM)

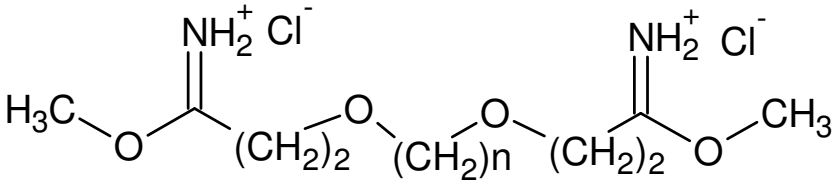
n = 2 - 10 (n = 4, DMA n = 5, DMP n = 6, DMS

b



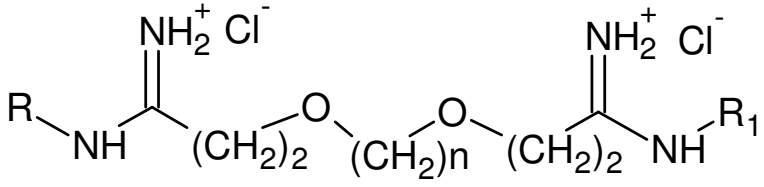
HIDROFIL

DODP



n = 1, 2, 4

n = 1 DMDP



<u>Alkalmazás</u>:

pH 8 – 9, TÖLTÉS, vízoldékony

fixálás elektronmikroszkópia

multienzim komplexek

immunkomplexek szerkezete

protein – A – immobilizáció

2.2.2. Hasítható

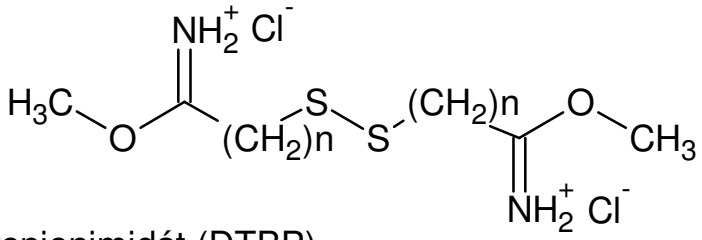
Alkalmazás: elektroforézis → hasítás

molekuláris mozgás a membránban

IA ag asszociáció

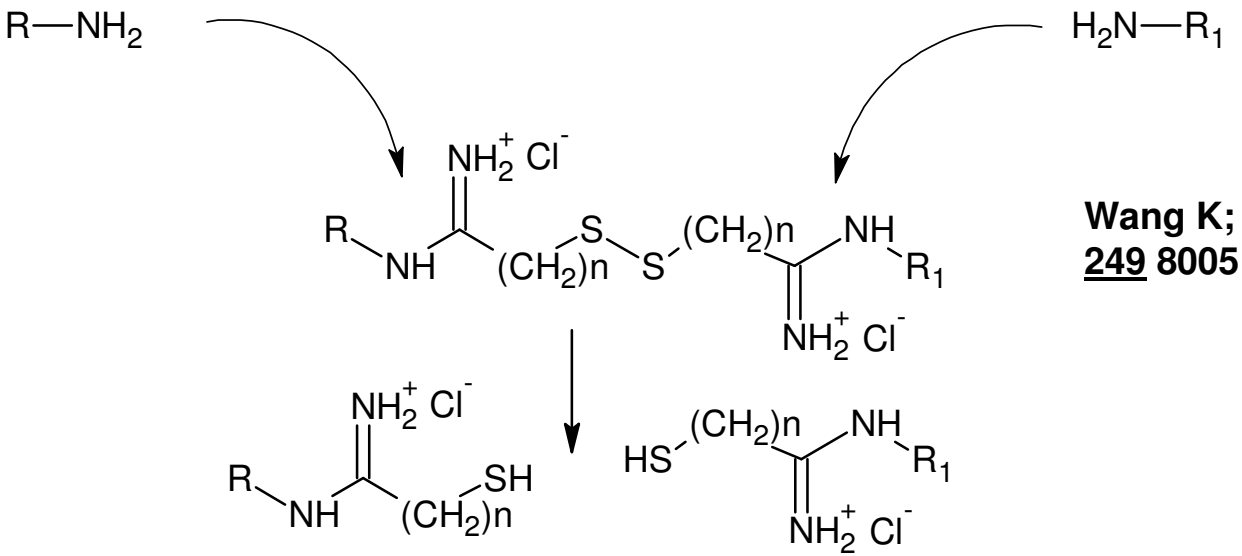
Shivdagani RA J Immunol 141 1252 (1988)

a DISZULFID / HIDROFÓB



n = 2 Dimetil 3,3'-ditiobis propionimidát (DTBP)

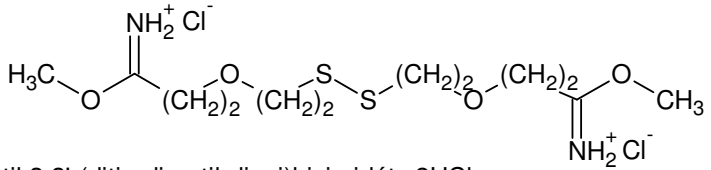
n = 3 DTBB, 4 DTBV, 6 DTBE



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

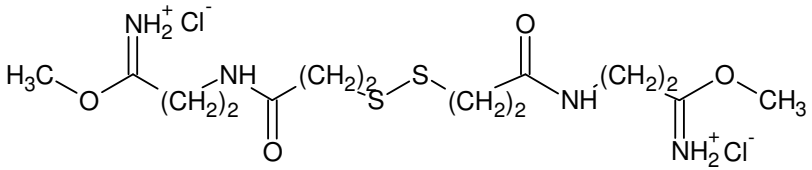
DISZULFID / HIDROFIL

1



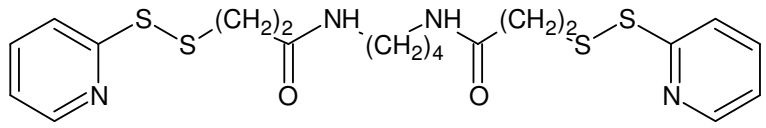
Dimetil 3,3'-(ditio-dimetil-dioxi)bisimidát · 2HCl

2

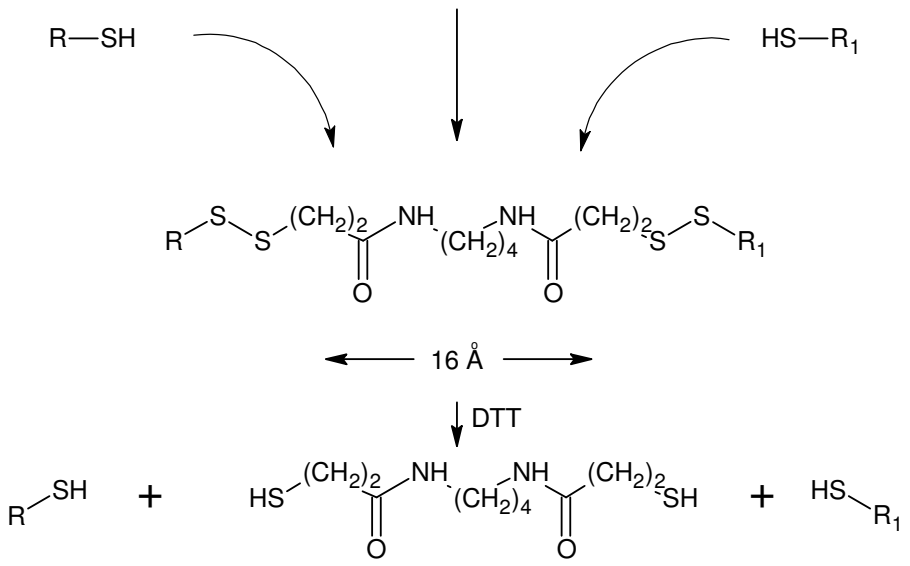


Dimetil 3,3'-(ditio-dimetilén-diamido)bispropionimidát · 2HCl

3

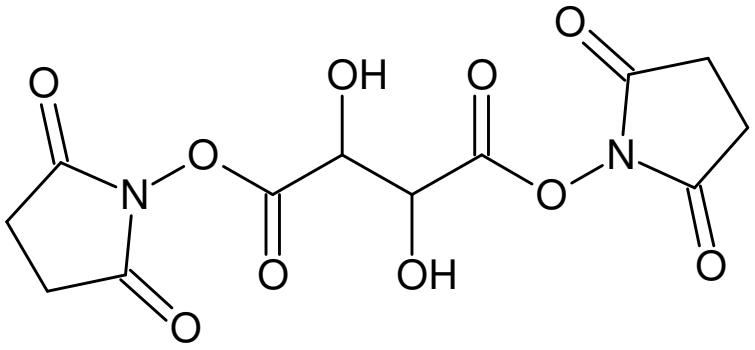


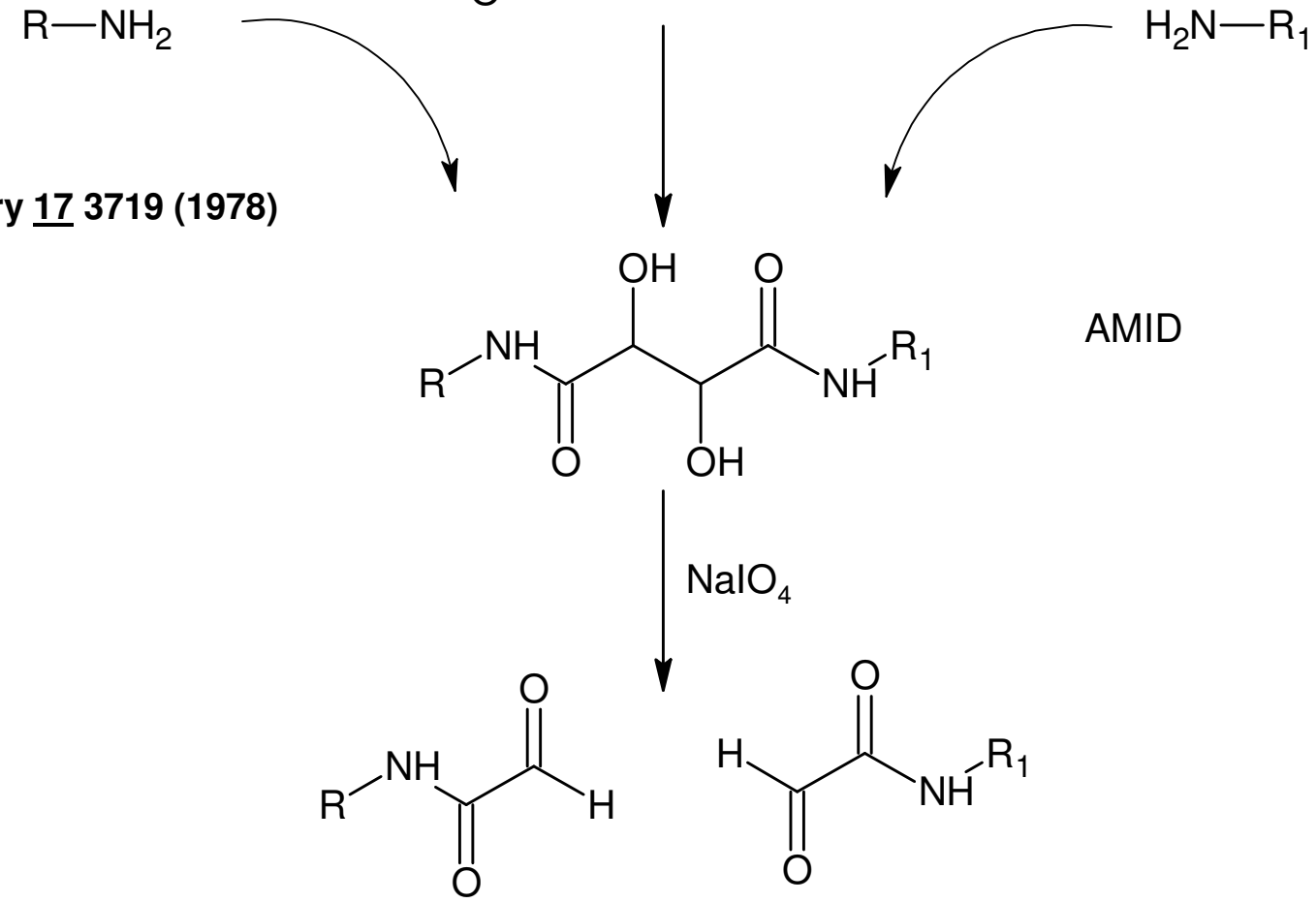
1,4-di-[3'-(2'-piridilditio)propionamido]bután DPDPB



b Vicinális diol

Diszukcinimit tartarát (DST)

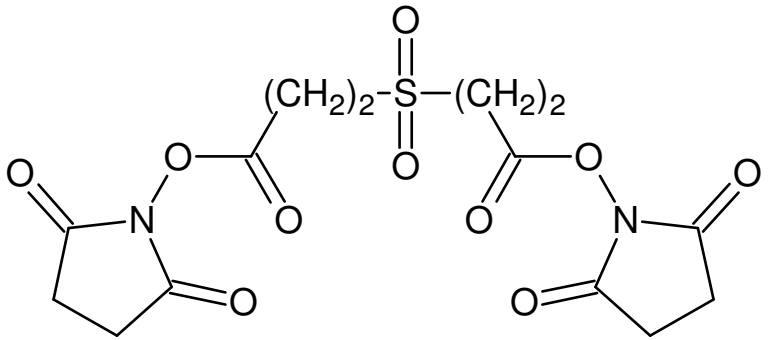




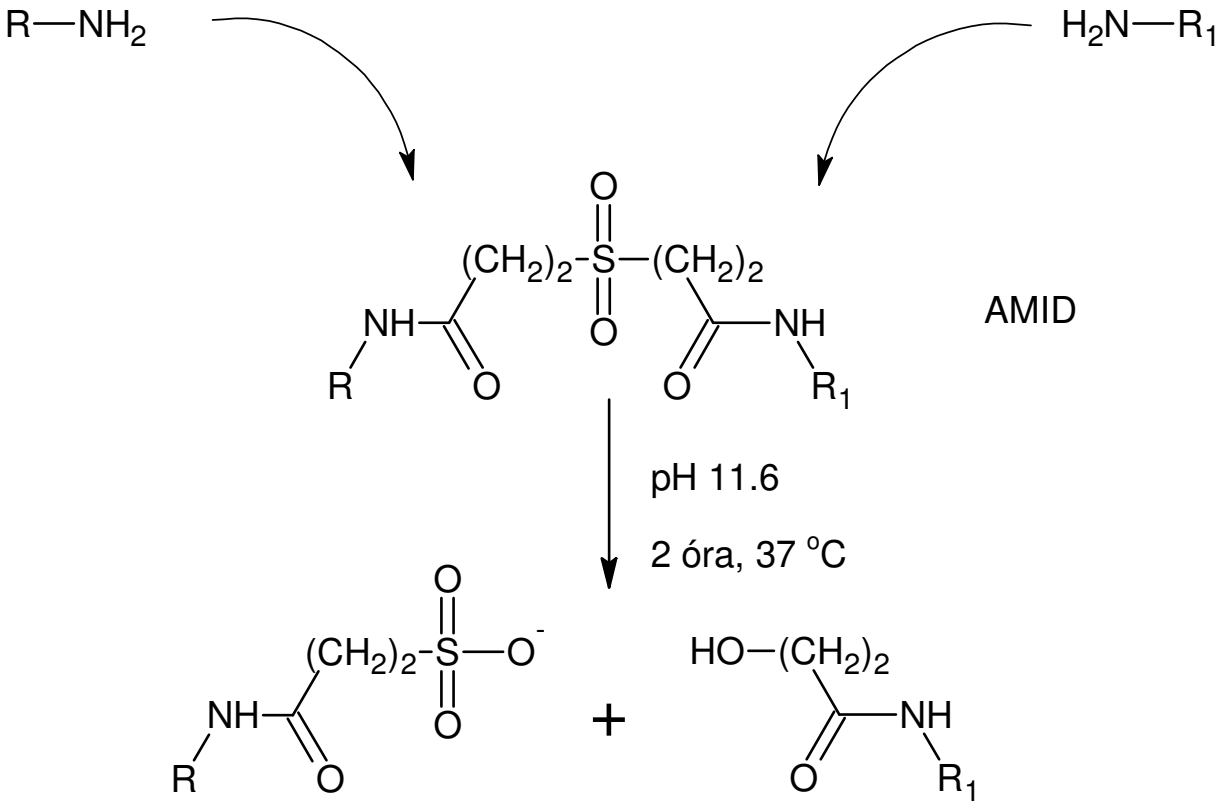
Smith RJ, Biochemistry 17 3719 (1978)

Ⓒ Bázisérzékeny kötés

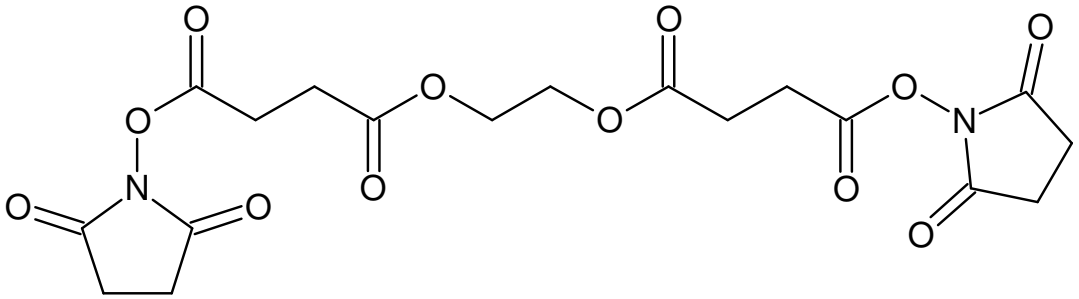
C1



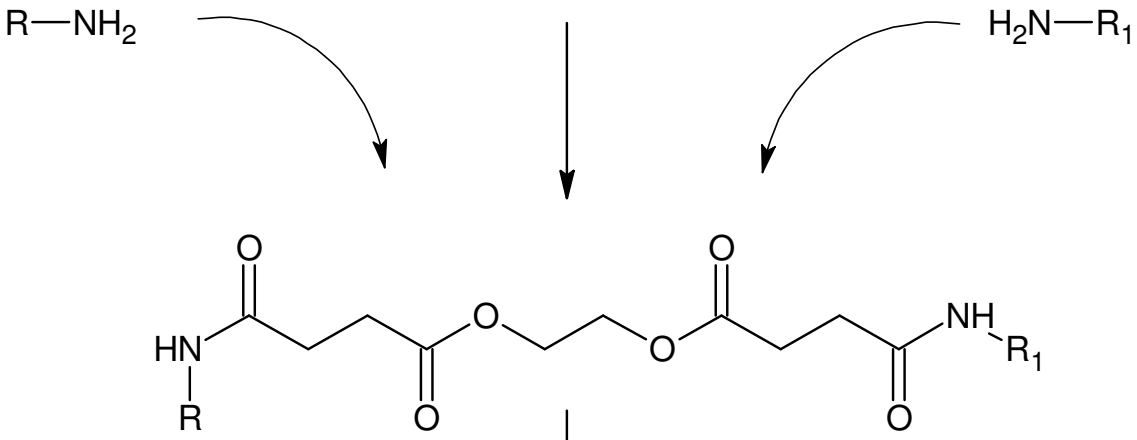
Bis[2-(szukcinimido-oxikarbonil)etil]szulfon (BSES) nem vízoldékony

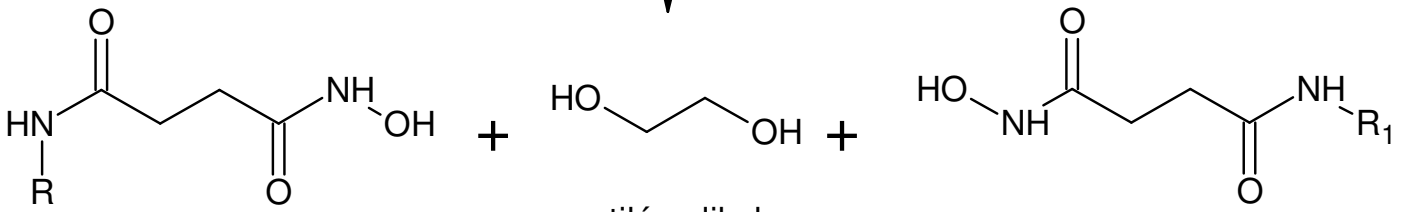


C2



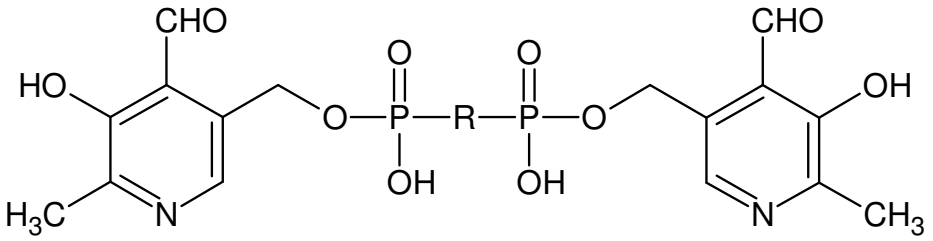
Etilén-glikol-bis(szukcinimidil szukcinát) (EGS) nem vízoldélony



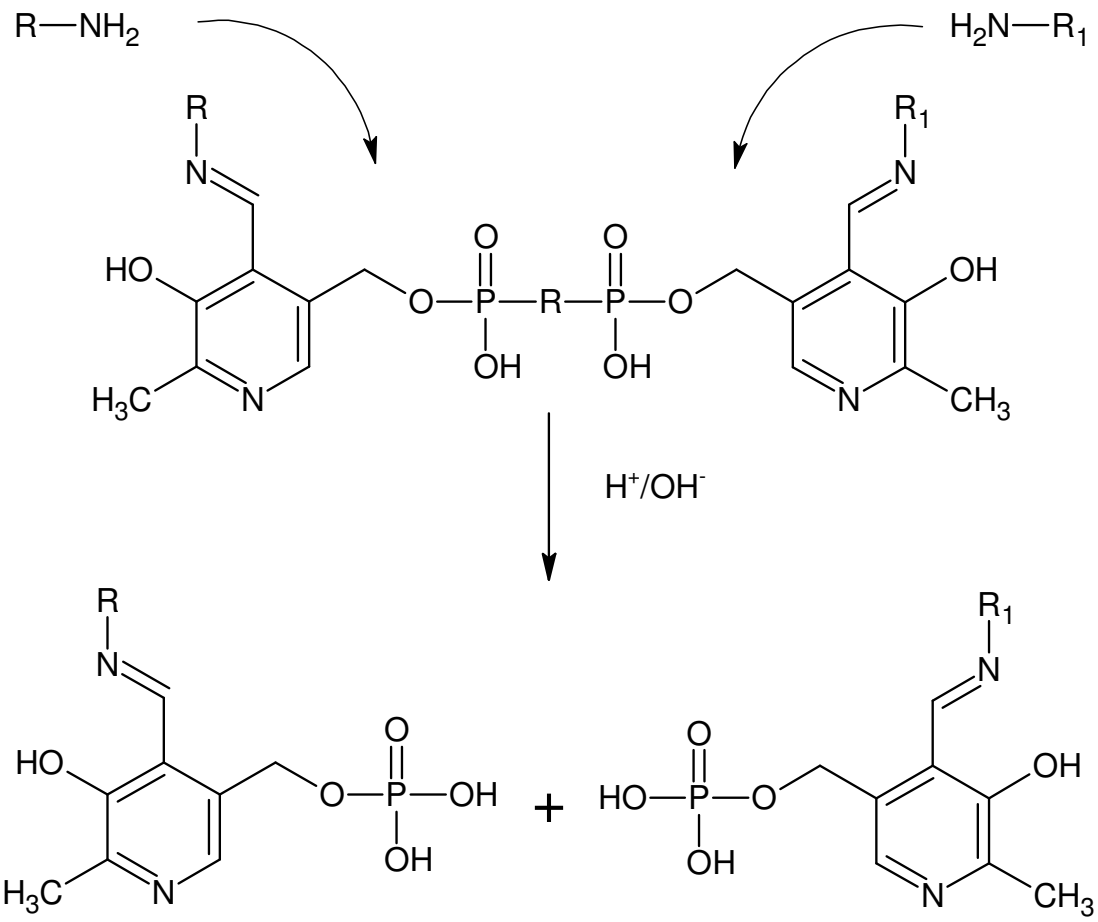


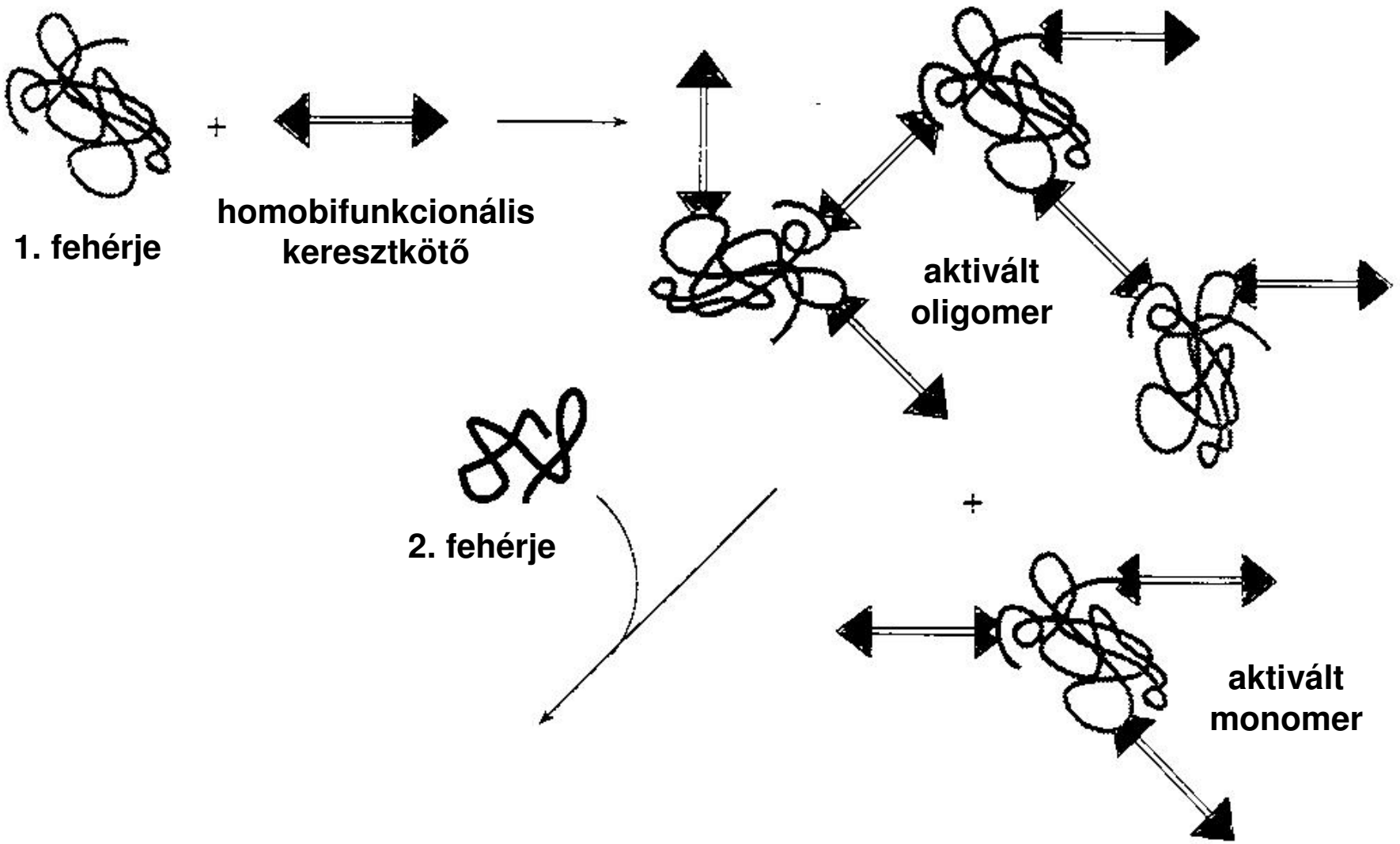
etilén glikol

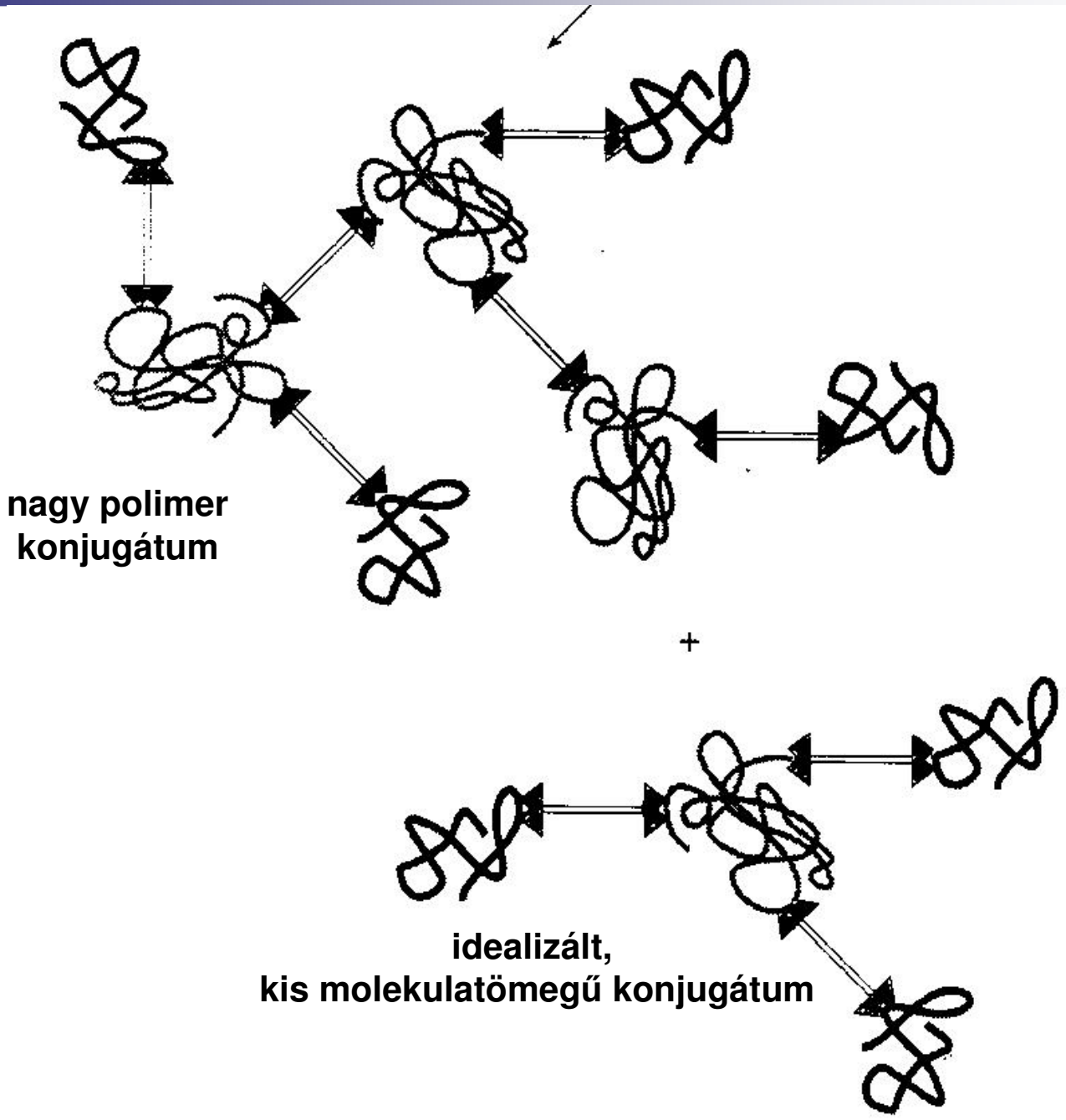
C3



P^1,P^2-bis(5'-piridoxál)difoszfát (Bis-PLP)




1. fehérje
homobifunkcionális
keresztkötő
aktivált
oligomer
2. fehérje
aktivált
monomer


nagy polimer
konjugátum
+
idealizált,
kis molekulatömegű konjugátum