# 7. Előadás

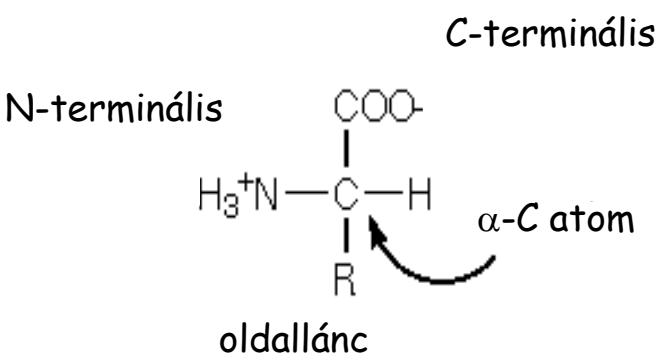
# Aminosavak

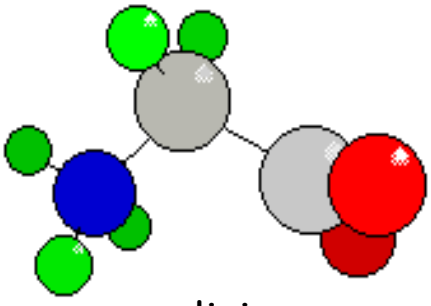
# Peptid hatóanyagok

| Peptide | Length | Method[a] |
|---|---|---|
| Oxytocin | 9 | C |
| Vasopressin analogues | | |
| Pitressin | 9 | C |
| Lypressin | 9 | C |
| Desmopressin | 9 | C, SP |
| Terlipressin | 12 | C, SP |
| Atosiban | 9 | C |
| Adrenocorticotropic hormone (ACTH) (1–24) | 24 | C |
| Insulin | 51 | E, S, R |
| Glucagon | 29 | E, SP, R |
| Secretin | 27 | E |
| Calcitonins | | |
| Human | 32 | C |
| Salmon | 32 | C, SP |
| Eel | 32 | C, SP |
| Dicarba-Eel (Elcatonin) | 31 | C, SP |
| Luteinizing hormone–releasing hormone (LH-RH) and analogues | 10 | C, SP |
| Leuprolide | 9 | C |
| Deslorelin | 9 | SP |
| Triptorelin | 10 | SP |
| Goserelin | 10 | SP |
| Buserelin | 9 | SP |
| Parathyroid hormone (PTH) (1–34) | 34 | SP |
| Corticotropin releasing factors | | |
| Human | 41 | SP |
| Ovine | 41 | SP |
| Growth hormone releasing factor (1–29) | 29 | SP |
| Somatostatin and analogues | 14 | C, SP |
| Lanreotide | 8 | SP |
| Octreotide | 8 | C |
| Thyrotropin releasing hormone (TRH) | 3 | C |
| Thymosin α-1 | 28 | SP |
| Thymopentin (TP-5) | 5 | C |
| Cyclosporin | 11 | E |
| Integrilin | 7 | C |

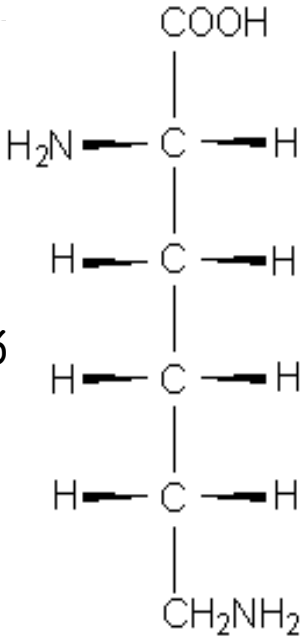
[a] Manufacturing methods: C = classical (solution-phase) chemical synthesis; E = extraction from natural sources; R = recombinant; S = semisynthesis; SP = solid-phase chemical synthesis.

# Aminosavak: alaptulajdonságok

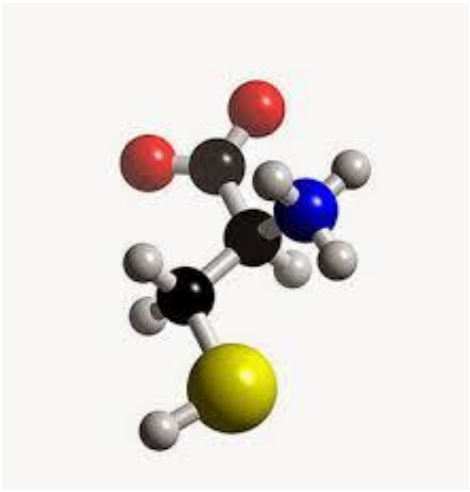


glicin

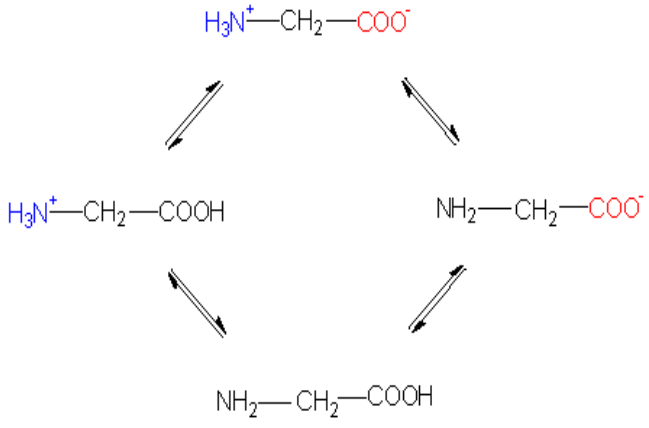


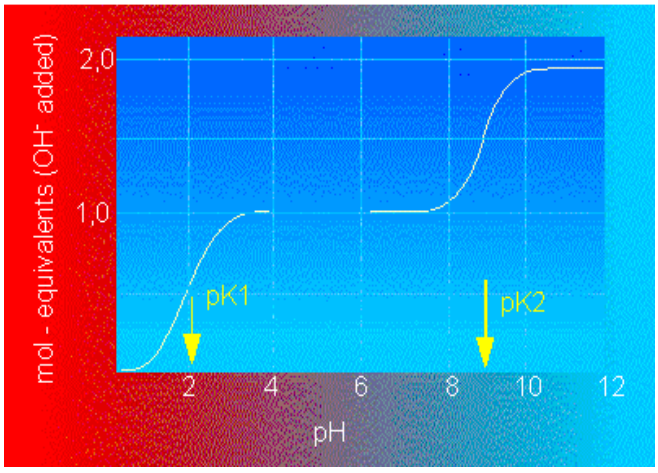

lizin
(Fischer projekció



cisztein

α-zwitter ion és ω oldallánc



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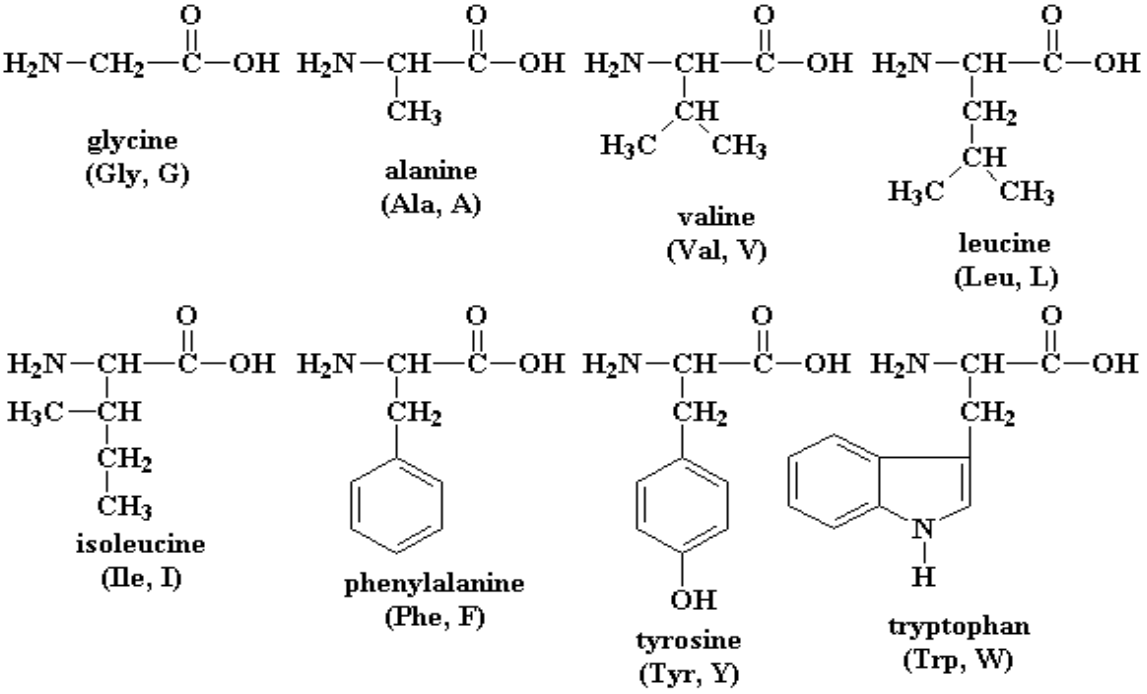


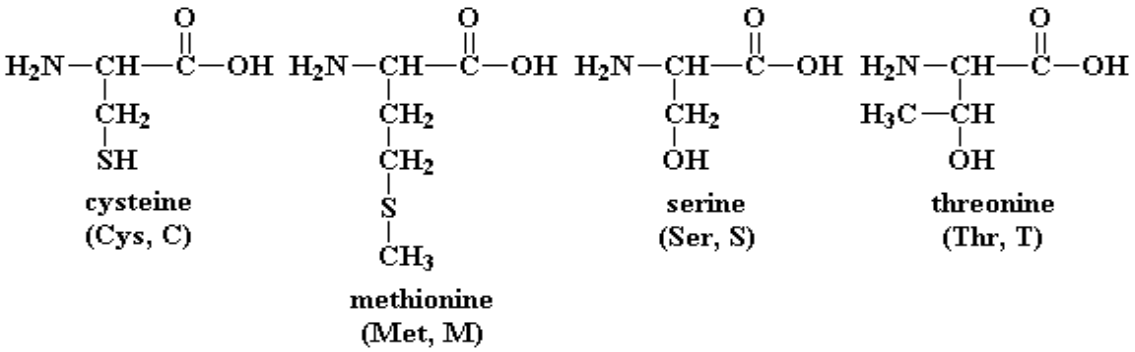


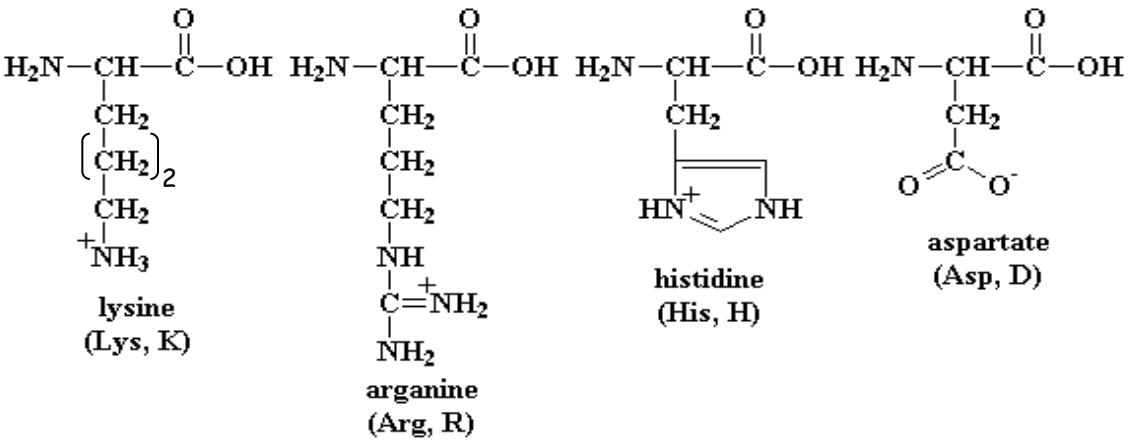


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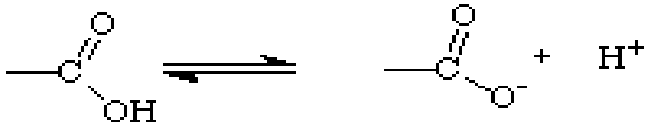
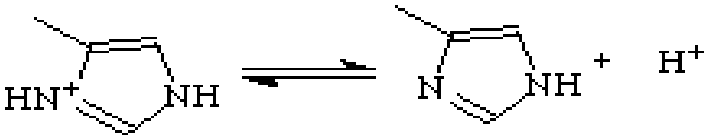
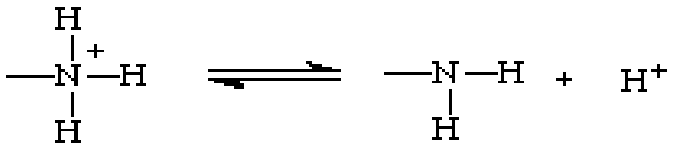
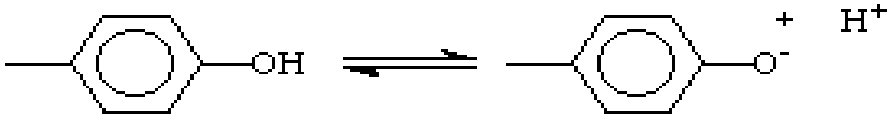
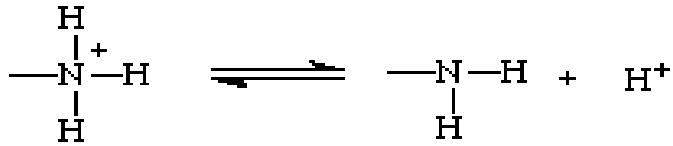
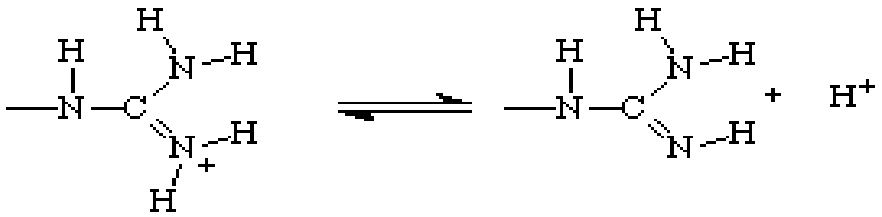
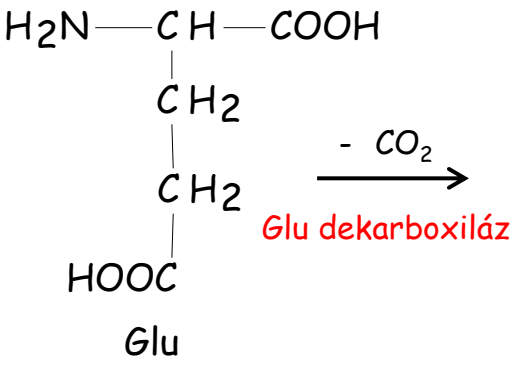
# Funkciós csoportok

| Group | Acid ⇌ Base + $H^+$ | $pK_a$ |
|---|---|---|
| Terminal carboxyl |  | 3.1 |
| aspartic acid or glutamic acid | | 4.4 |
| Histidine |  | 6.5 |
| Terminal Amino |  | 8.0 |
| Cysteine | | 8.5 |
| Tyrosine |  | 10.0 |
| Lysine |  | 10.0 |
| Arginine |  | 12.0 |

# Néhány nem fehérjealkotó természetes aminosav

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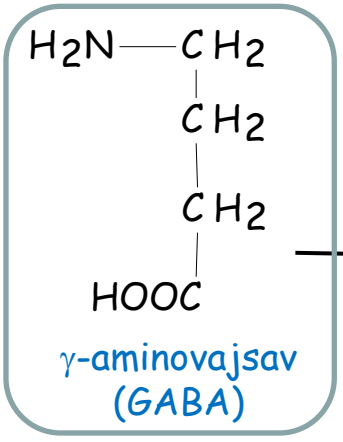
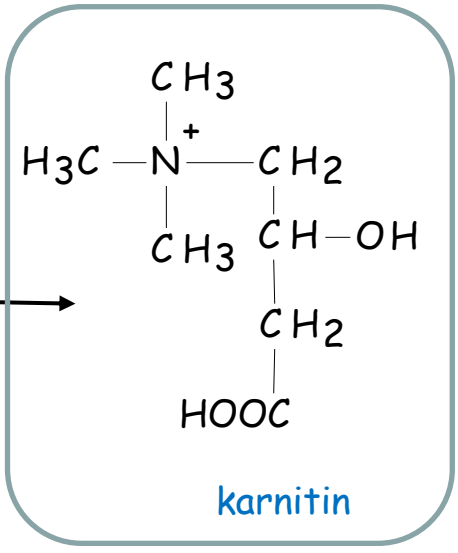
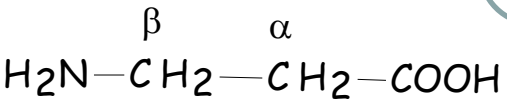


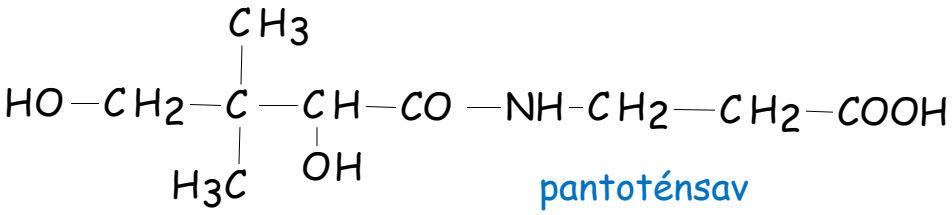

γ-aminovajsav (GABA)



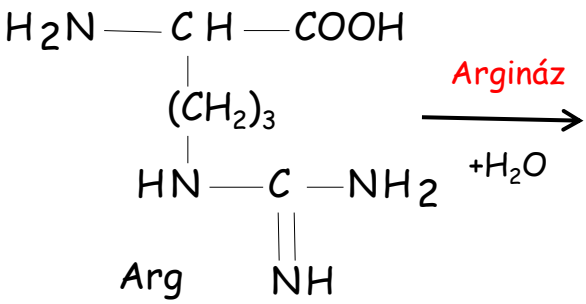

karnitin

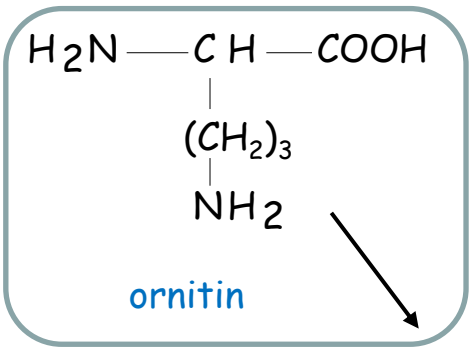



β-Alanin

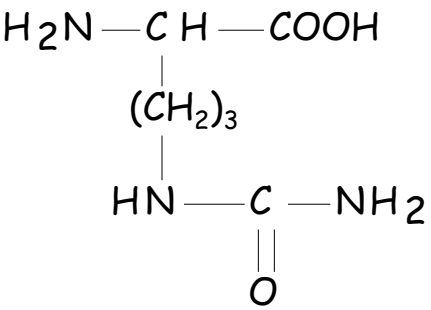



pantoténsav

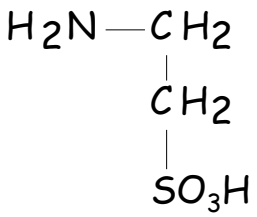



ornitin

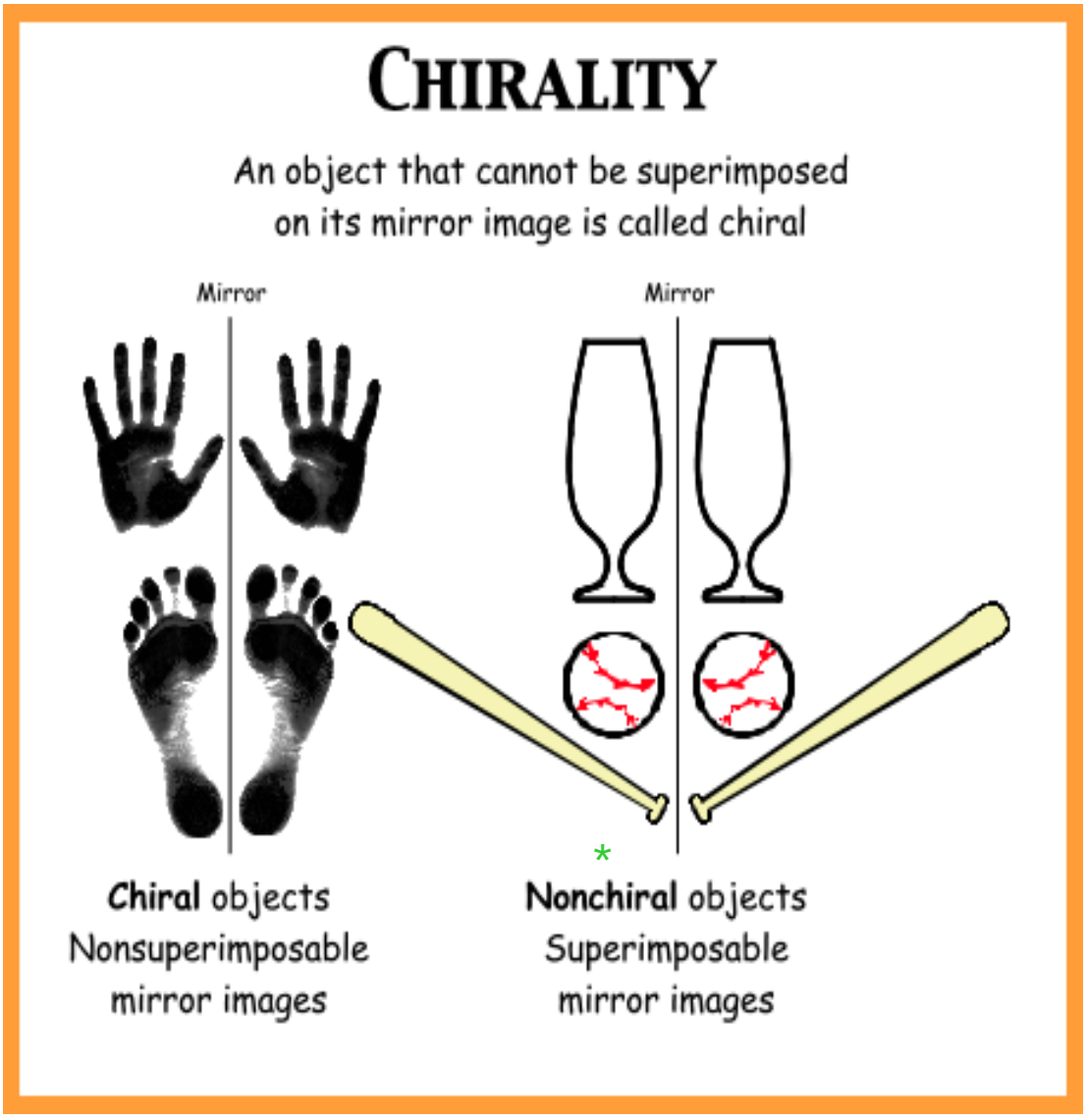


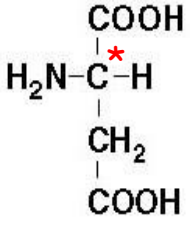

citrulin
(citrullus lat. görögdinye)



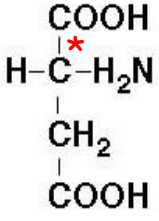

taurin (epesav alkotórész)

# Aminosavak: kiralitás

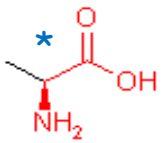



L– Aspartic Acid




D–Aspartic Acid



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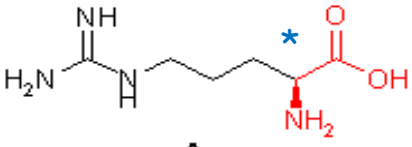

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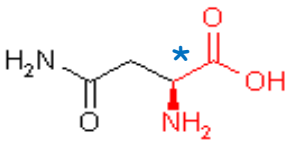

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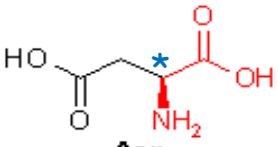

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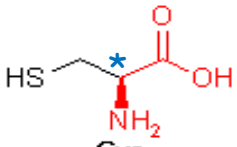

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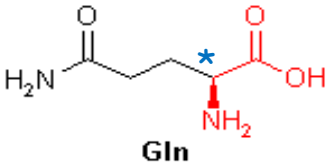
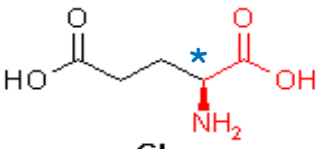


Gln



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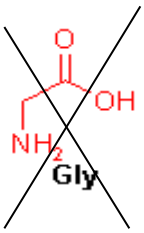

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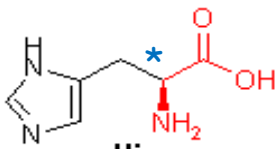

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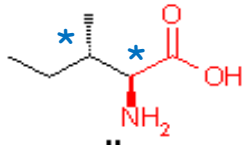

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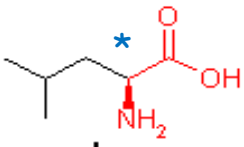

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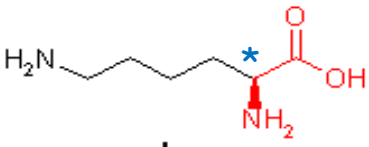

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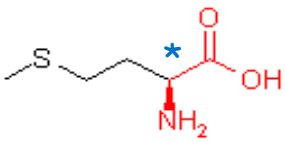

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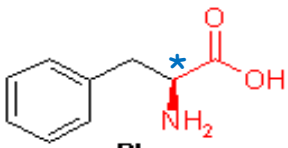

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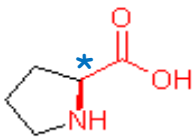

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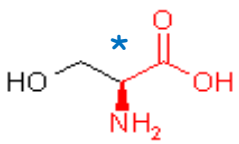

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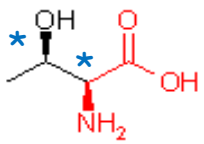

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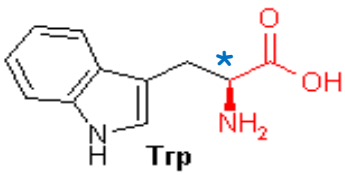

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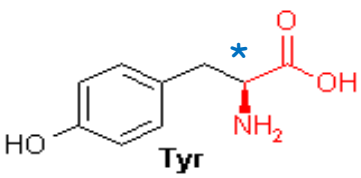
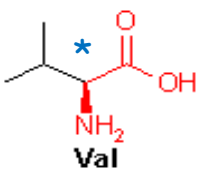
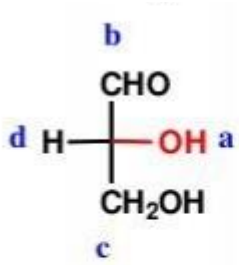


Tyr



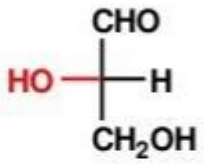
Val

# D/L nomenklatura és kiralitás



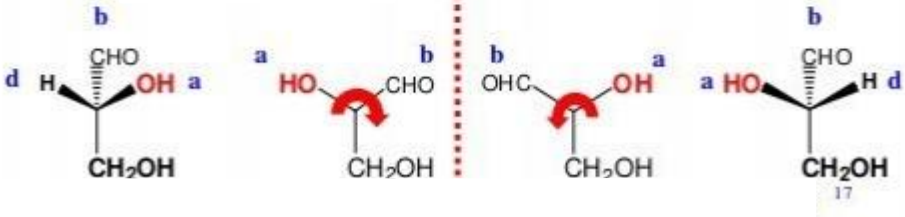

D(+)-glicerinaldehid

R(+)-glicerinaldehid

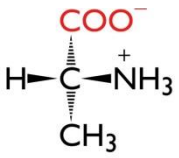



L(-)-glicerinaldehid

S(-)-glicerinaldehid

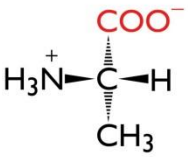



## Egy királis centrum (Ala)




D-alanin

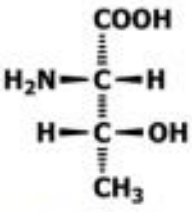
R-konfiguráció



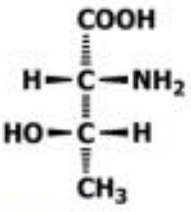

L-alanin

S-konfiguráció

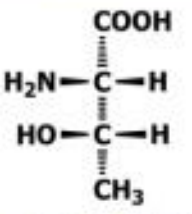
## Két királis centrum (Thr)




L-Thr = (2S,3R)-Thr

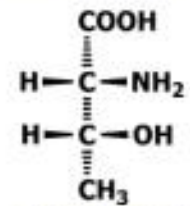



D-Thr = (2R,3S)-Thr




L- allo-Thr

= (2S,3S)-Thr




D-allo-Thr

= (2R,3R)-Thr

# Aminosavak előállítása

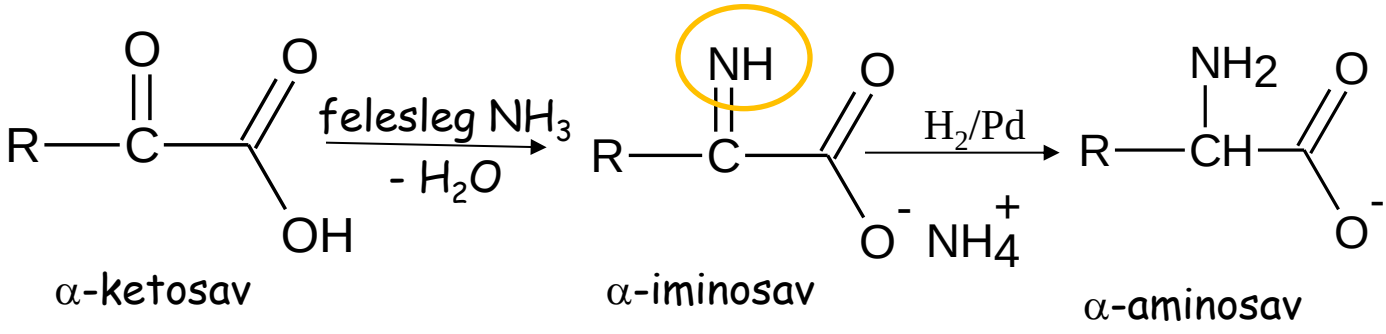
1. Fehérjék hidrolízise
2. Kémiai szintézis
3. Rezolválás

*Kémiai szintézis*

1. Miller-kisérlet

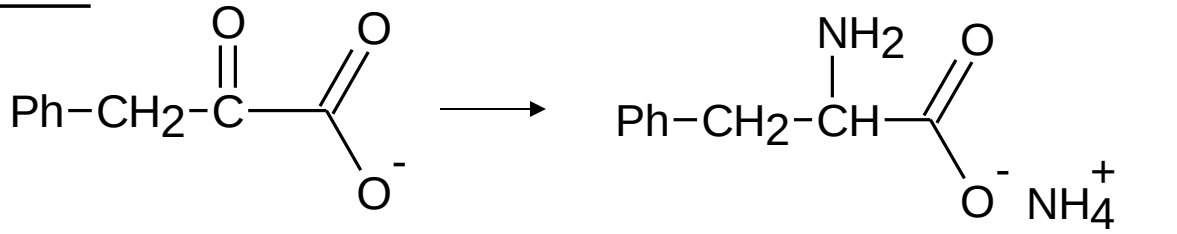
$H_2, H_2O, NH_3, CH_4 \xrightarrow[\text{kisülés}]{\text{elektromos}}$ aminosavak

2. Reduktív aminálás



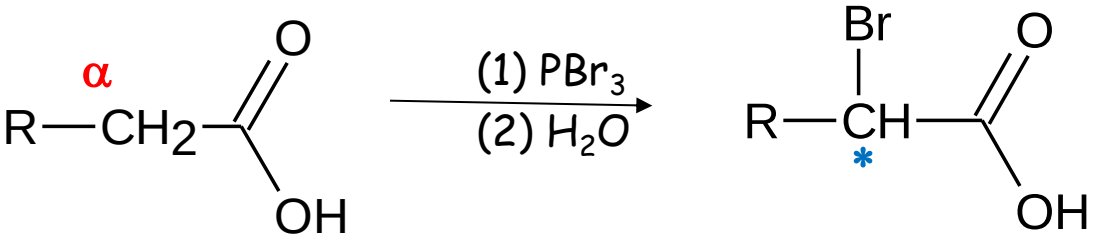

α-ketosav α-iminosav α-aminosav

Példa:

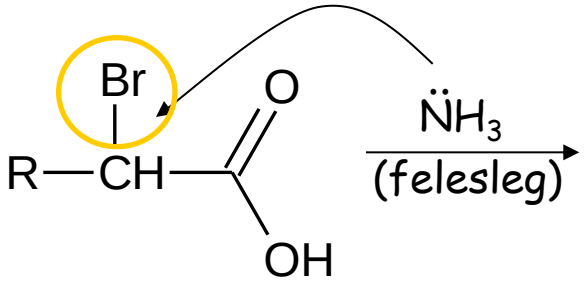



3-fenil-2-oxopropánsav (D,L)-Phe $NH_4^+$ só (30 %)

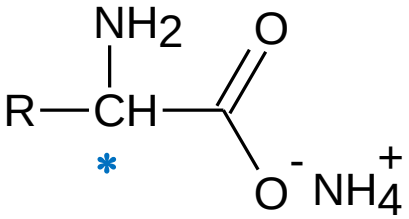
3. Aminálás ($S_N$)




α-bróm-karbonsav

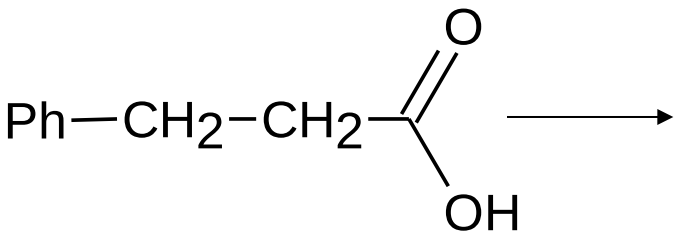



α-bróm-karbonsav

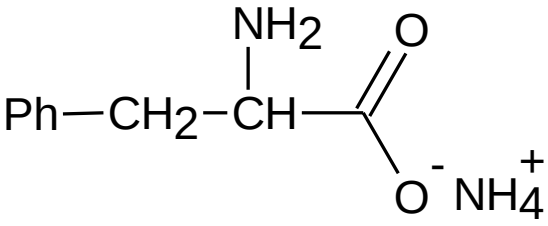



α-aminosav (racém)

Példa:

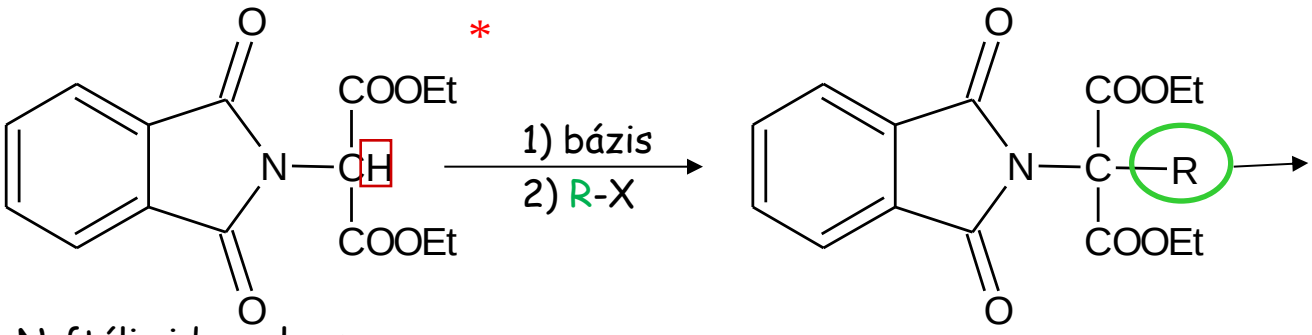



3-fenilpropánsav

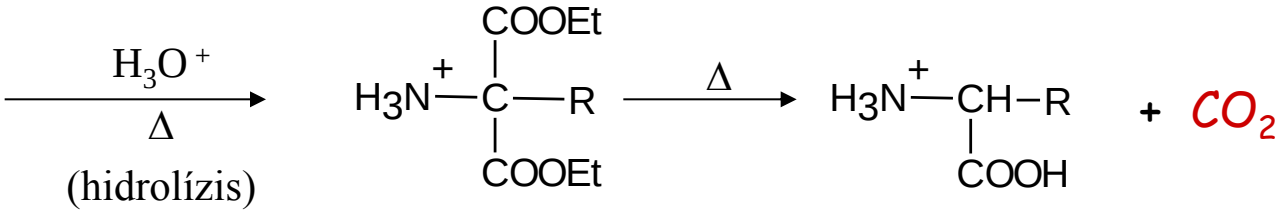



(D,L)-Phe $NH_4^+$ só
(30 - 50 %)

## 4. Gabriel-féle malonészter szintézis (Met, Asp)

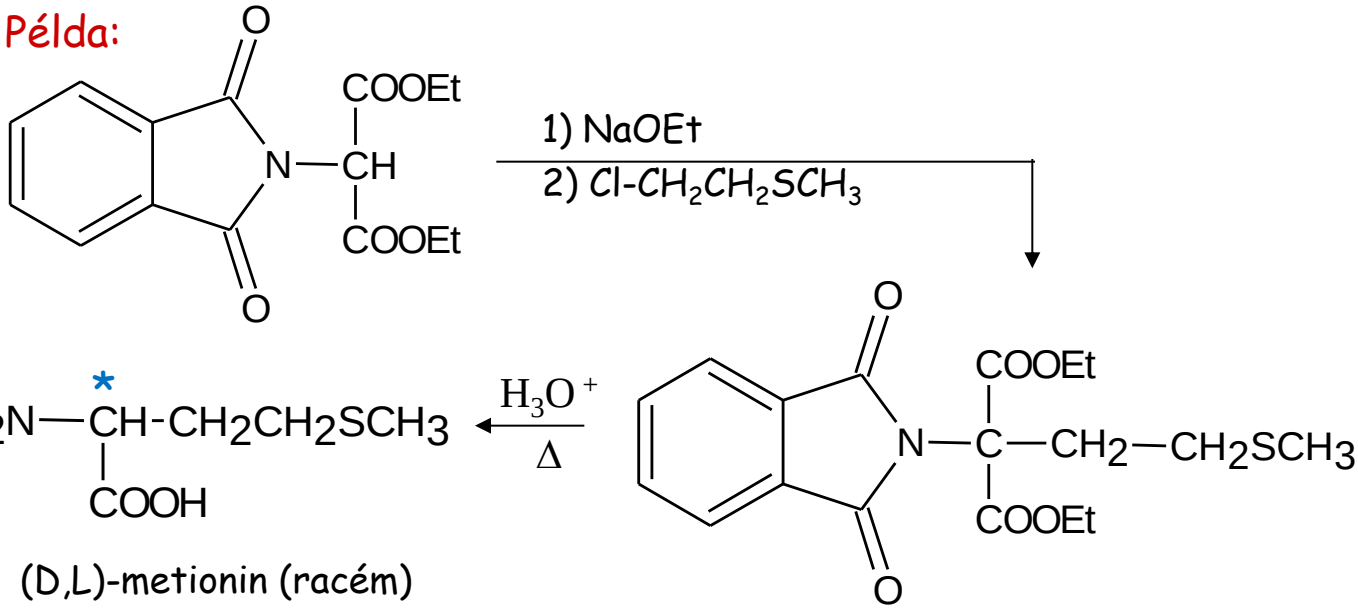



N-ftálimido malonsav dietilészter




α-aminosav

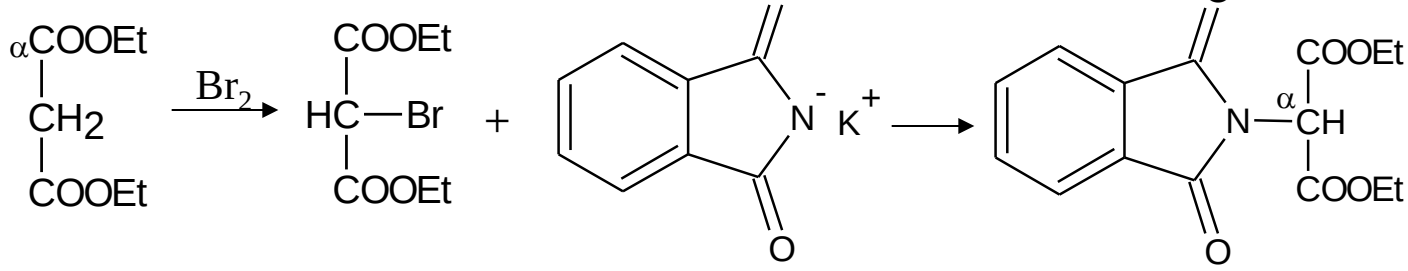
Példa:




(D,L)-metionin (racém)

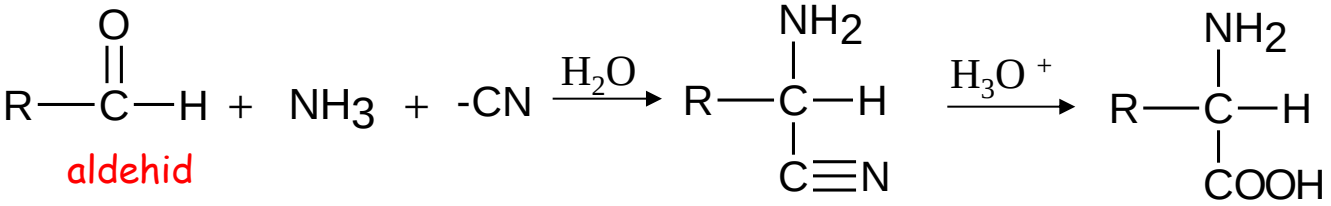
---

### * Reagens előállítása

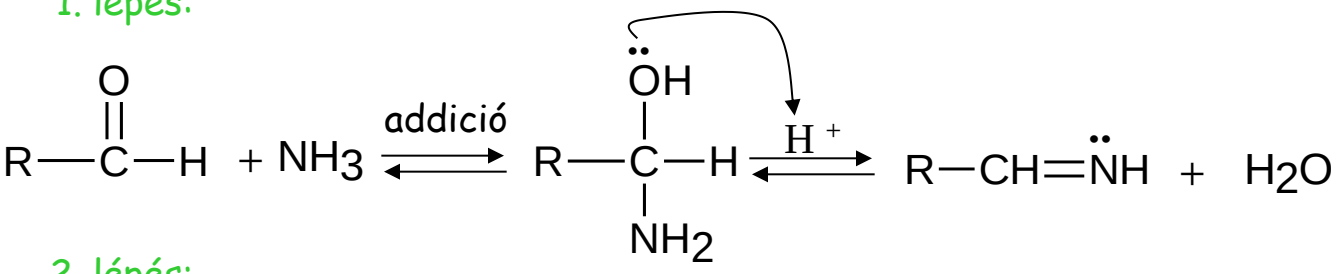



ftálimid kálium

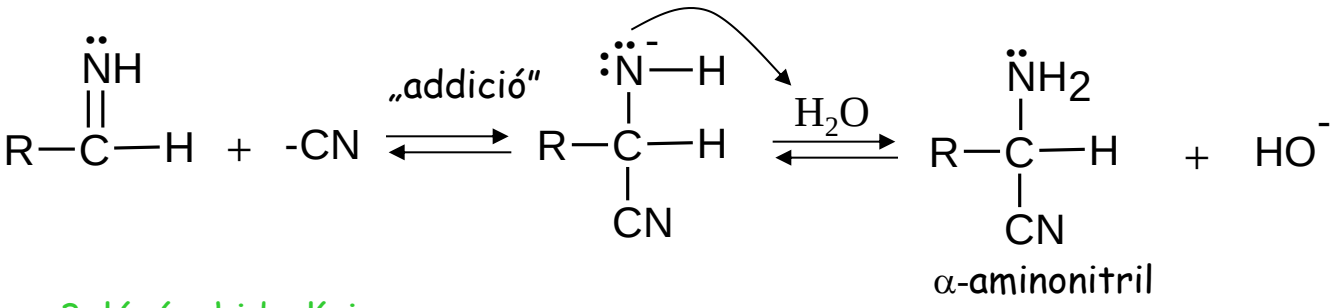
## 5. Strecker-szintézis (Ala, Phe)




1. lépés:

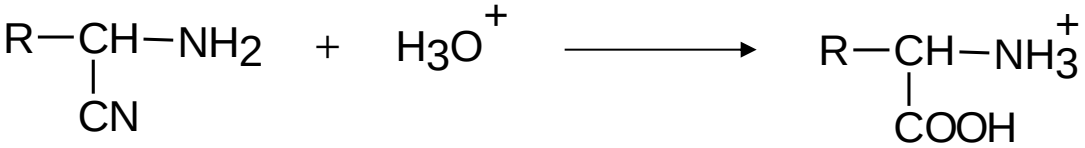



2. lépés:

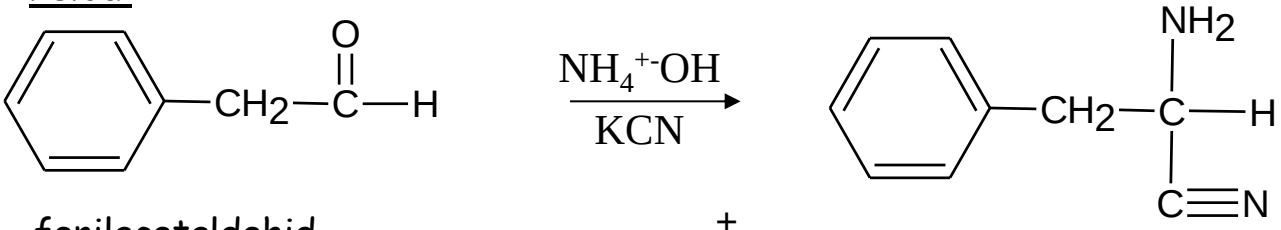



α-aminonitril

3. lépés: hidrolízis

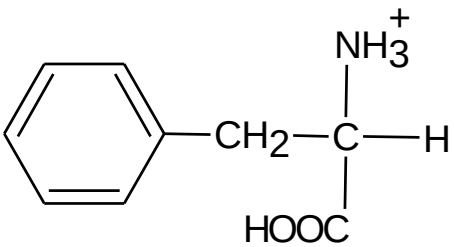


Példa:



fenilacetaldehid

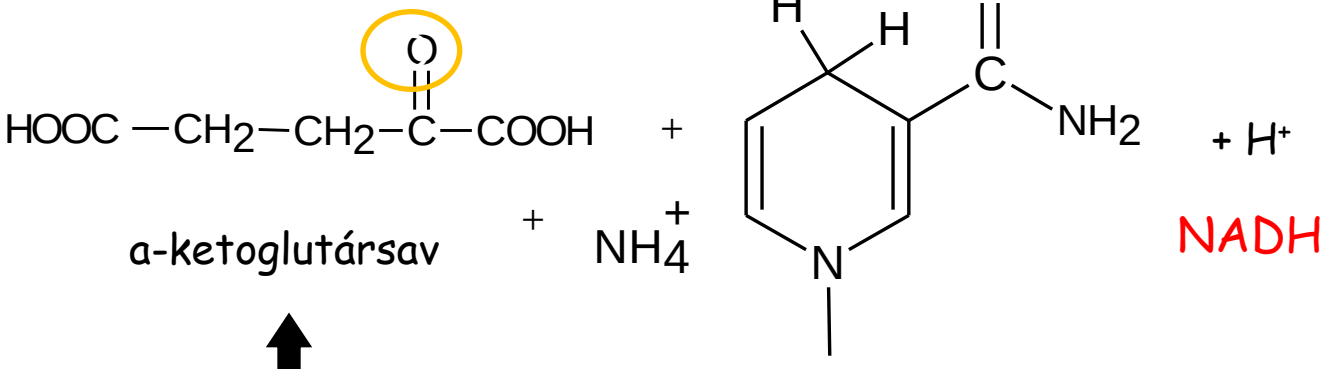
α-aminonitril



(D,L)-Phe (70%) (racém)

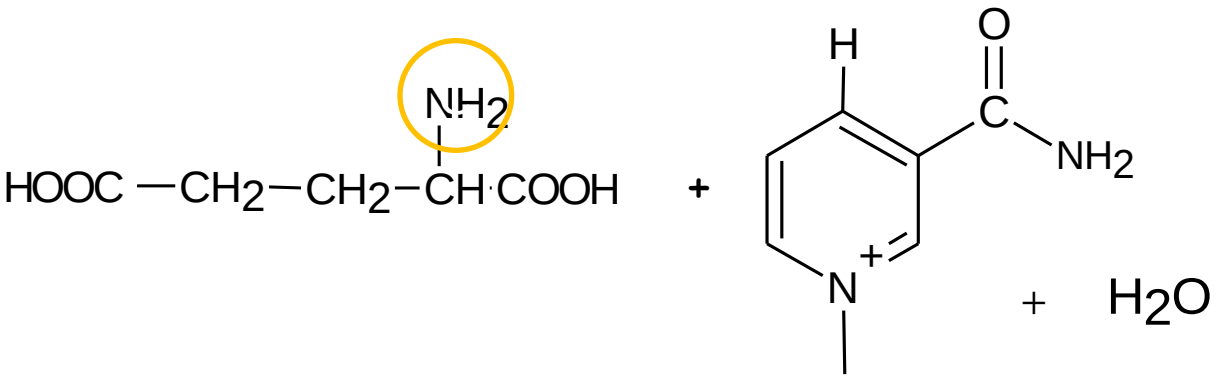
*Bioszintézis*

1. Reduktív aminálás




Szénhidrát metabolizmus

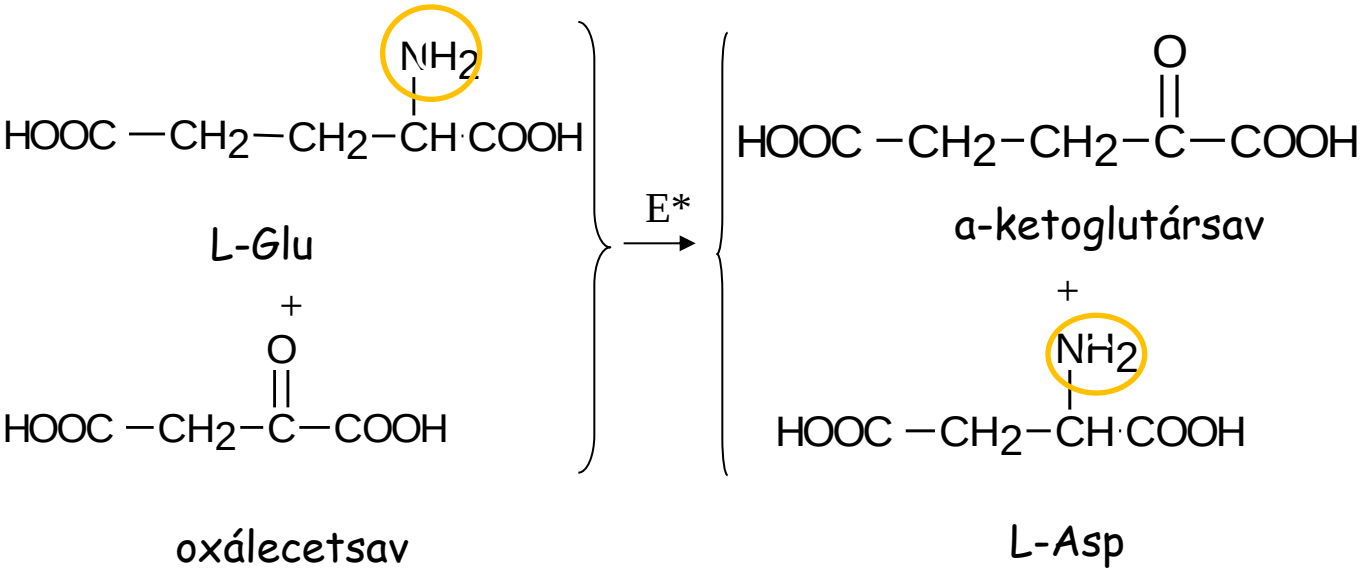
Enzim



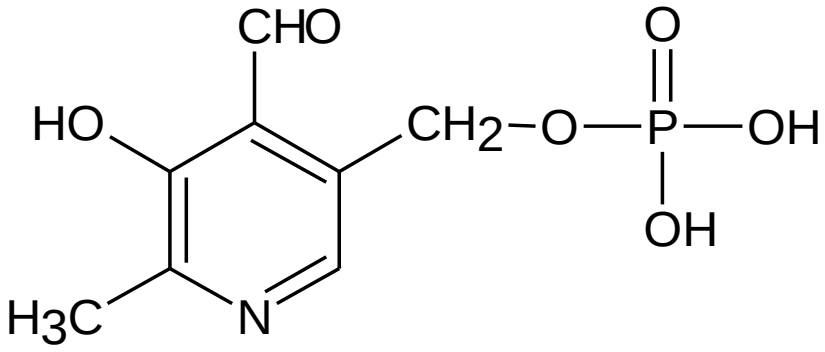

L-glutaminsav

NAD+

## 2. Transzaminálás

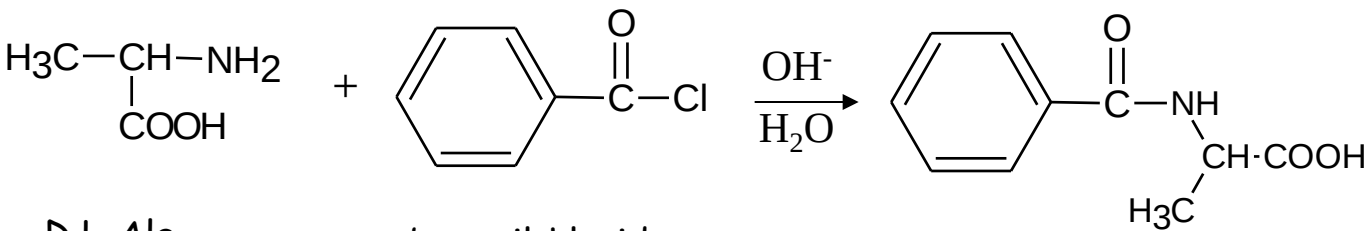



E* = Transzamináz, koenzim = piridoxál-5-foszfát



# *Rezolválás*

## 1. Diasztereomer vegyületpár képzés




D,L-Ala benzoil-klorid *N*-benzoil-alanin + HCl

D,L-Ala

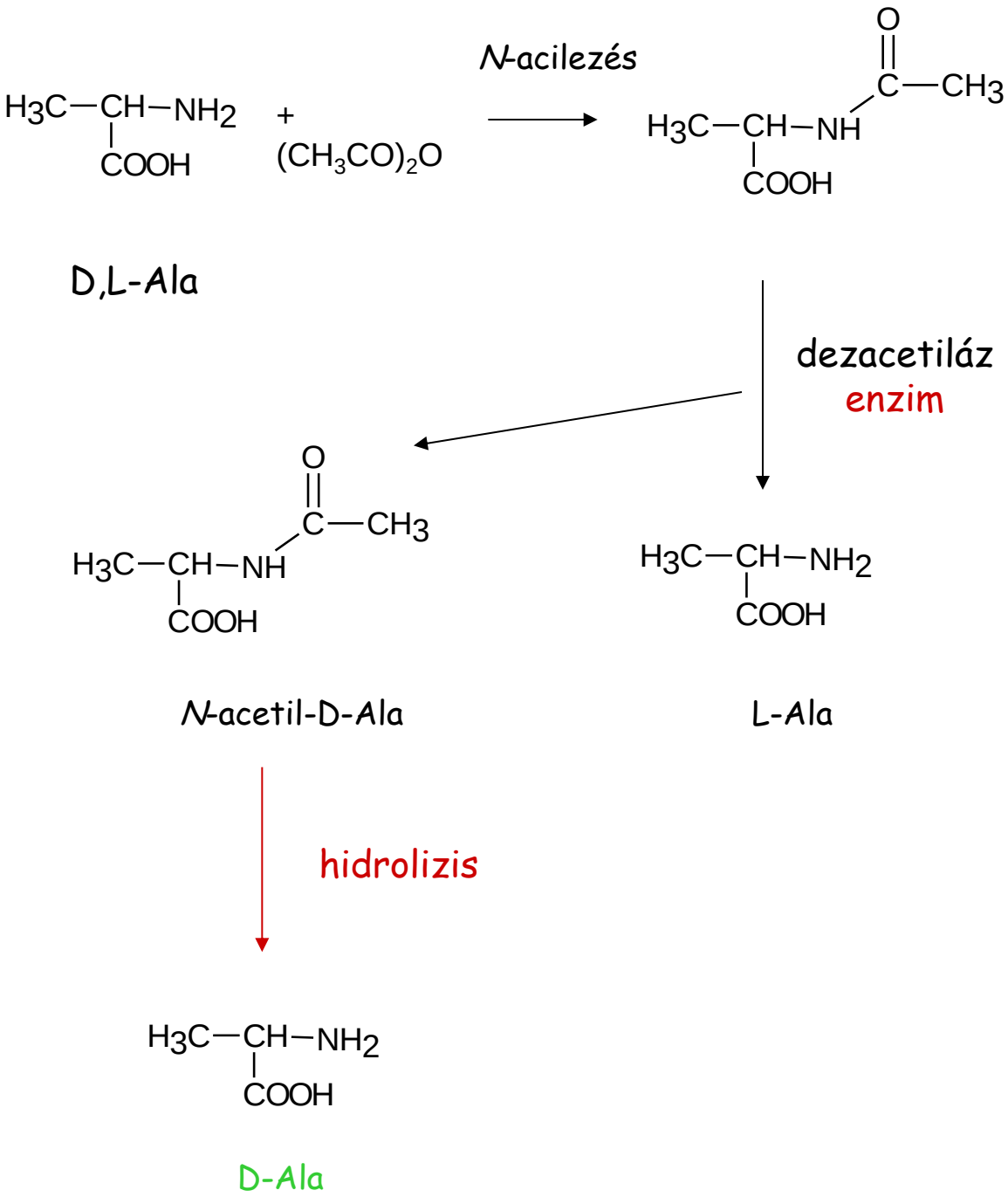
↓

N-benzoil-alanin

↓ Brucin/stcrichnin

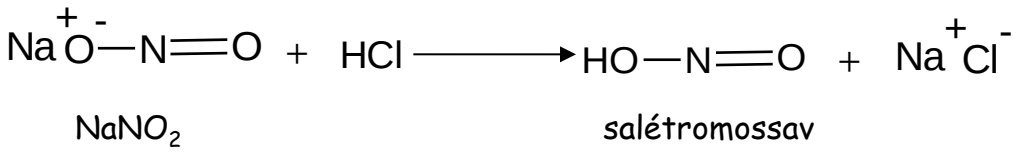
| N-benzoil-D-Ala | N-benzoil-L-Ala |
|---|---|
| brucin sója | brucin sója |
| „oldhatatlan" | „oldható" |
| ↓ $H^+/H_2O$ | ↓ $H^+/H_2O$ |
| N-benzoil-D-Ala | N-benzoil-L-Ala |
| ↓ 1.) $OH^-/H_2O$, D<br>2.) $H^+/H_2O$ | ↓ 1.) $OH^-/H_2O$, D<br>2.) $H^+/H_2O$ |
| D-Ala | L-Ala |

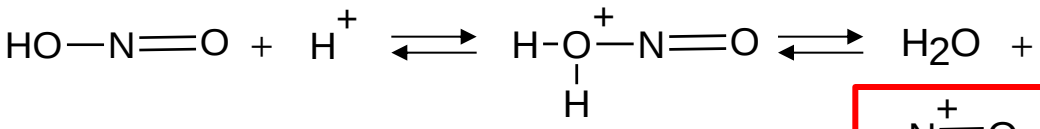
## 2. Enzimatikus módszer

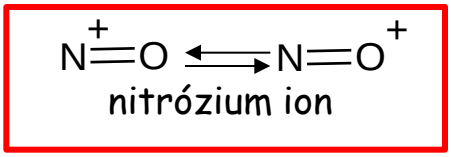


# Aminosavak kimutatása

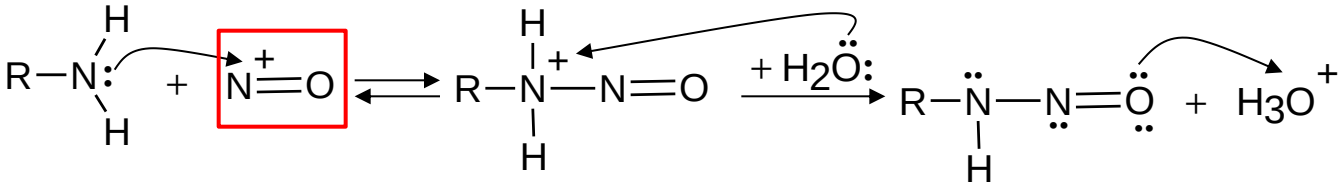
## 1. Reakció salétromos savval



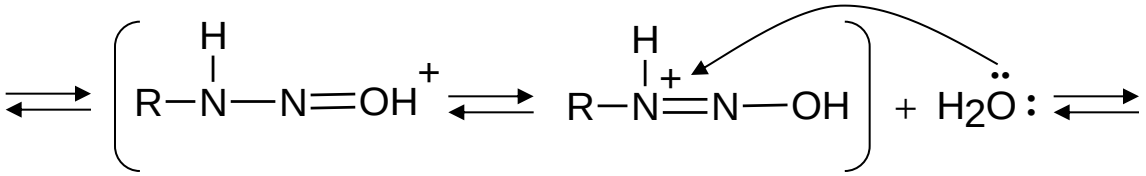



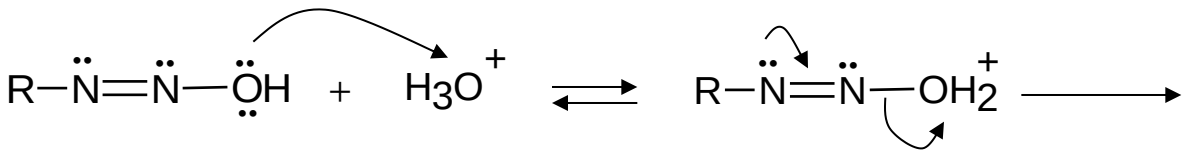



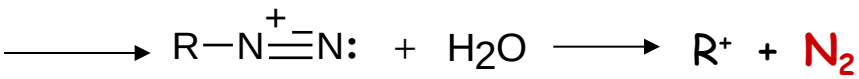

## Primer amin




N-nitrozo amin

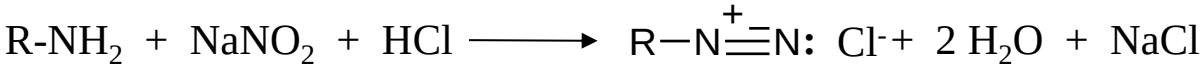


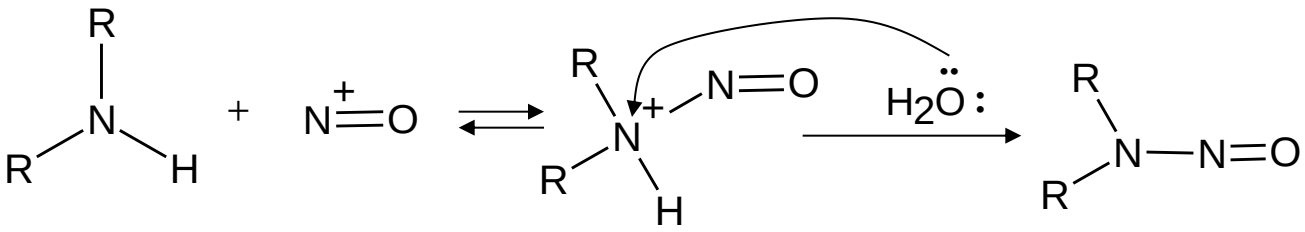




diazónium kation

### Összegzés

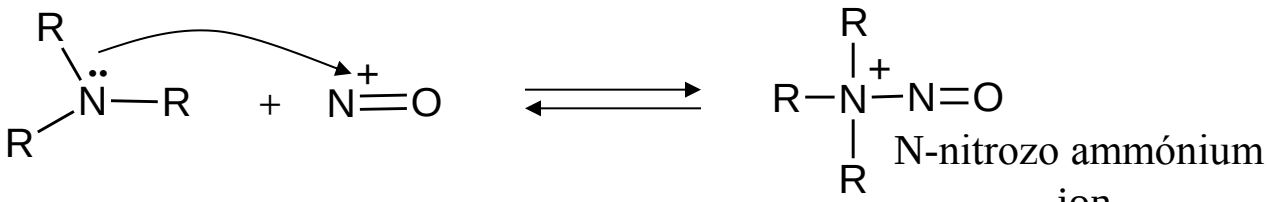


## Szekunder amin



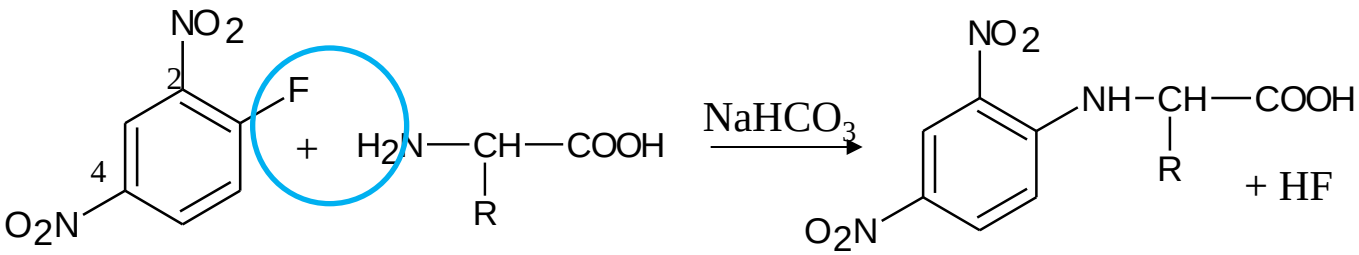
N-nitrozo amin

## Tercier amin



N-nitrozo ammónium ion

## 2. Sanger reakció

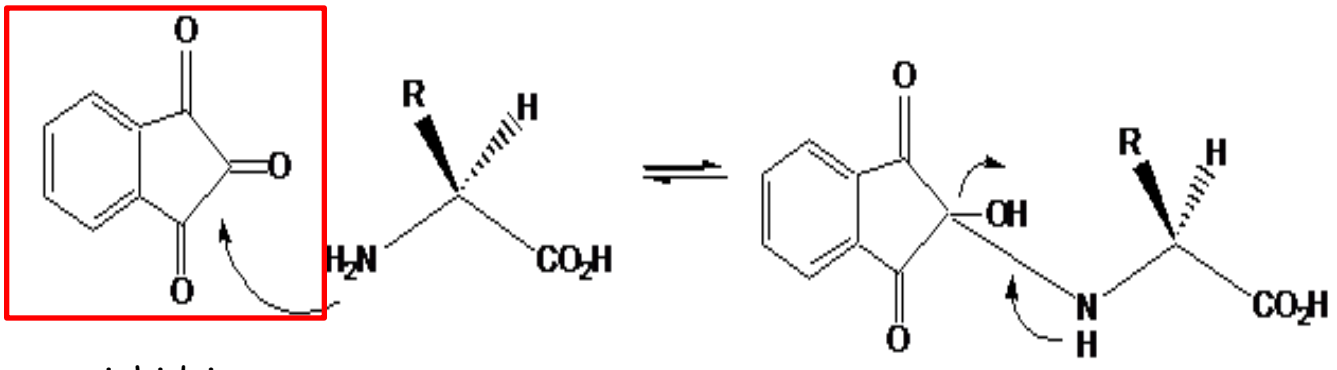


1-fluor-2,4-dinitro benzol
(Sanger, F. 1954)

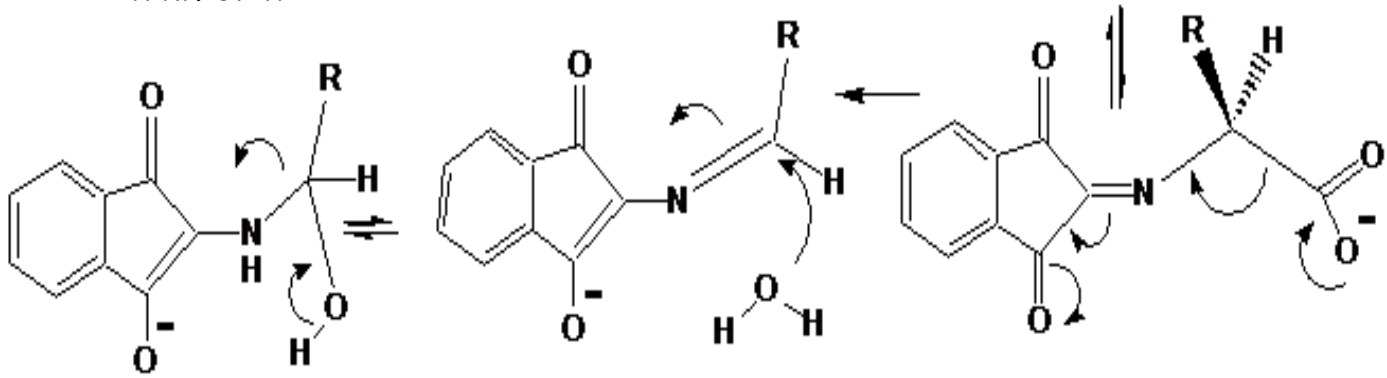
2,4-dinitrofenil aminosav
(Dnp-aminosav)

λ=254 nm

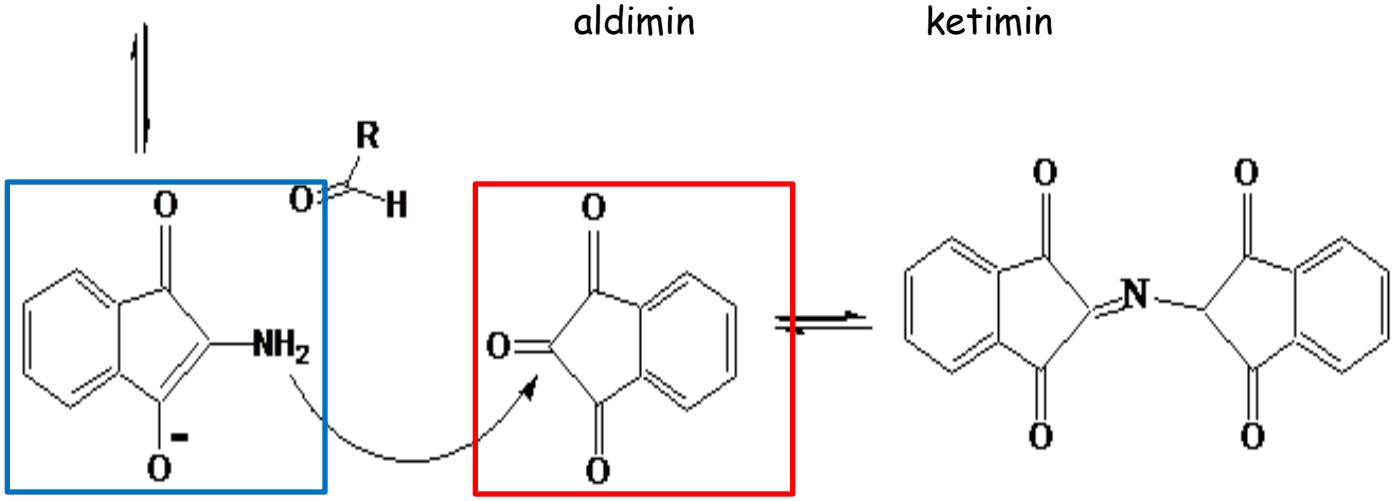
## 3. Ninhidrin reakció



ninhidrin



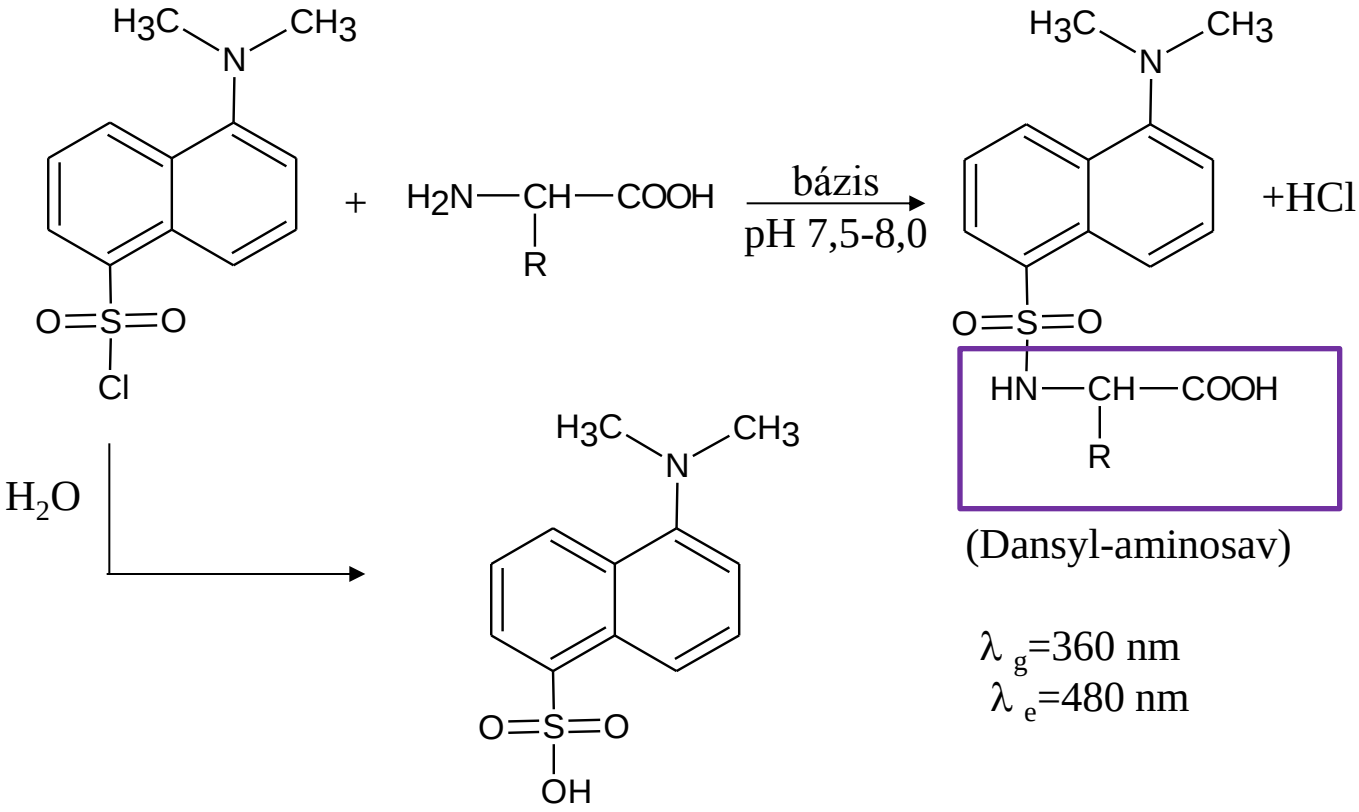
aldimin ketimin



ninhidrin

Ruheman, 1909

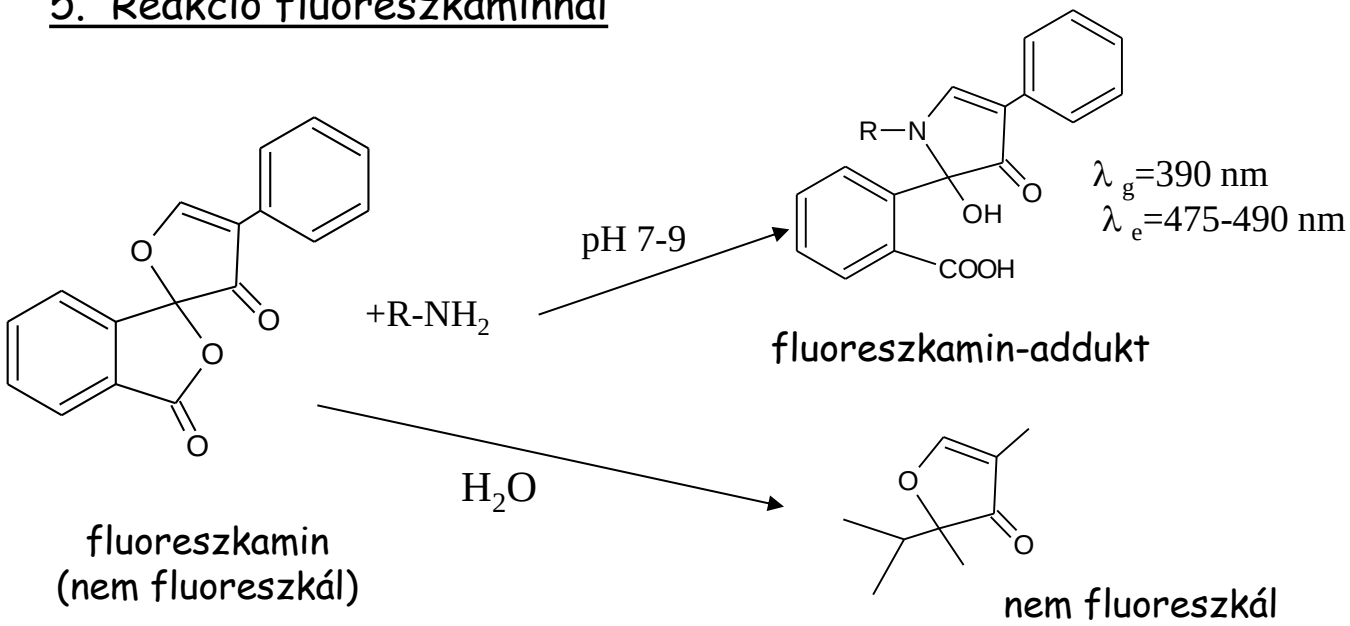
## 4. Reakció 1-dimetilamino-naftalin-5-szulfonil kloriddal (Dansyl-klorid, Hartley, 1963)




(Dansyl-aminosav)

$\lambda_g$=360 nm
$\lambda_e$=480 nm

## 5. Reakció fluoreszkaminnal




$\lambda_g$=390 nm
$\lambda_e$=475-490 nm

fluoreszkamin-addukt

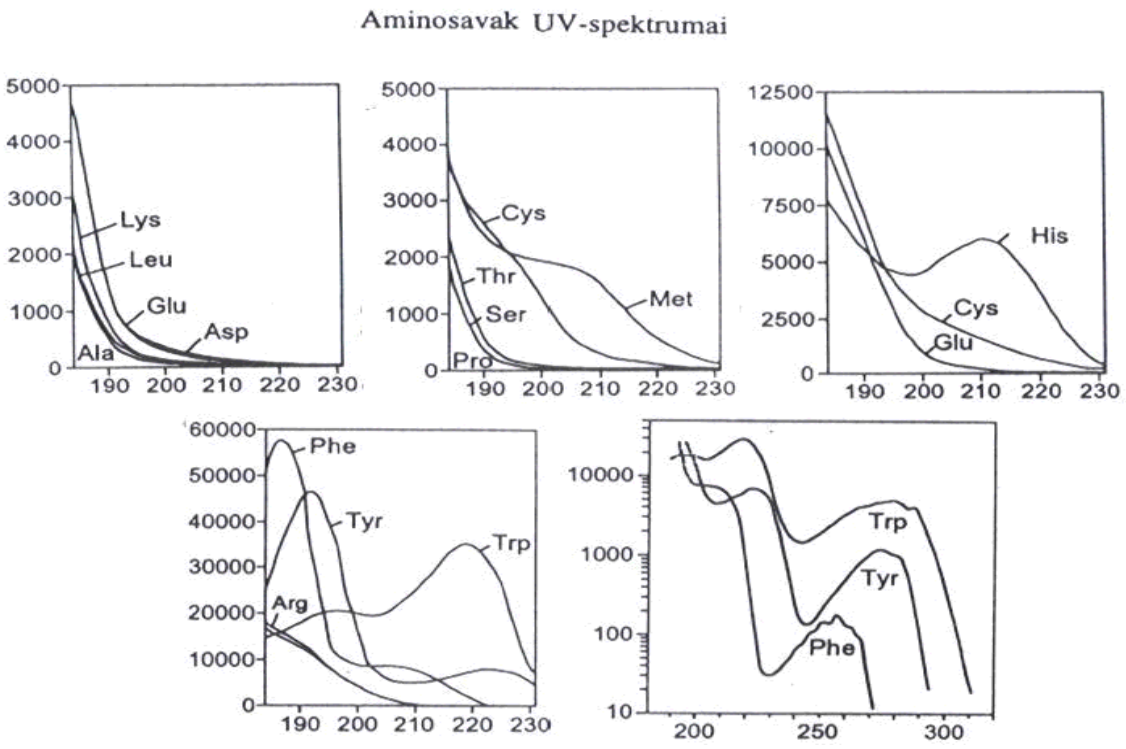
fluoreszkamin
(nem fluoreszkál)

nem fluoreszkál

# 5. Spektroszkópia

| | $\lambda_{max}$ (nm) | $\varepsilon$ ($M^{-1}cm^{-1}$ | $\lambda_{max}$ (nm) | kvantum hatásfok |
|---|---|---|---|---|
| Phe | 257.4 | 197 | 282 | 0.04 |
| Tyr | 274.6 | 1420 | 303 | 0.21 |
| Trp | 279.8 | 5600 | 348 | 0.20 |

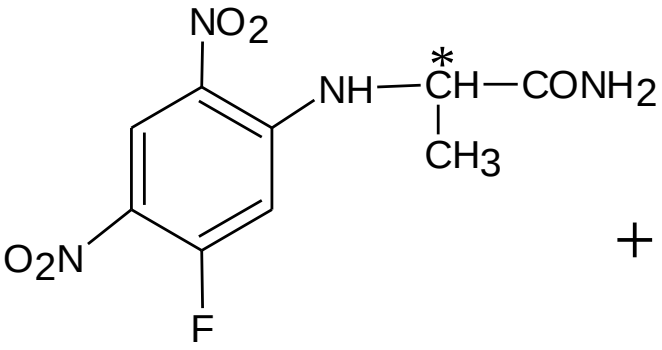
| Módszer | kromofór | Érzékenység (mg/ml | szerkezeti hatás |
|---|---|---|---|
| $A_{280}$ | Trp/Tyr | 200-3000 | erős |
| $A_{220}$ | peptid kötés | 1-100 | nincs |
| fluoreszcencia | Trp | 5-50 | erős |




vizes oldat
(pH 5-6, ill. pH 3 (Cys))

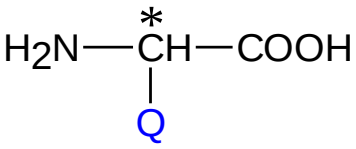
Hullámhossz (nm)

## 6. Kovalens származékképzés enatiomer összetétel meghatározására (Marfey reagens)



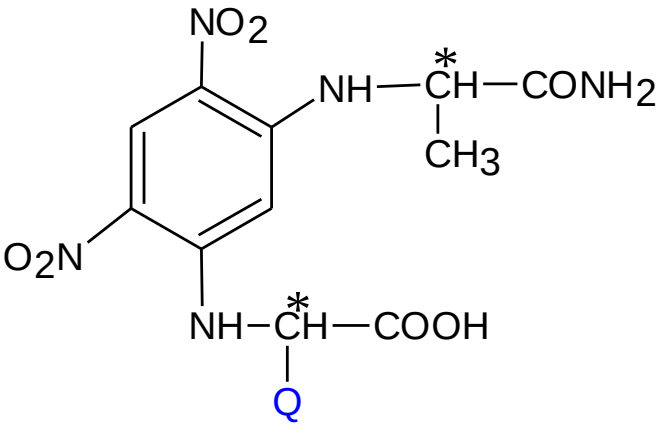
1-fluor-2,4-dinitrofenil-
5-L-Ala-amid
(FDAA)

+

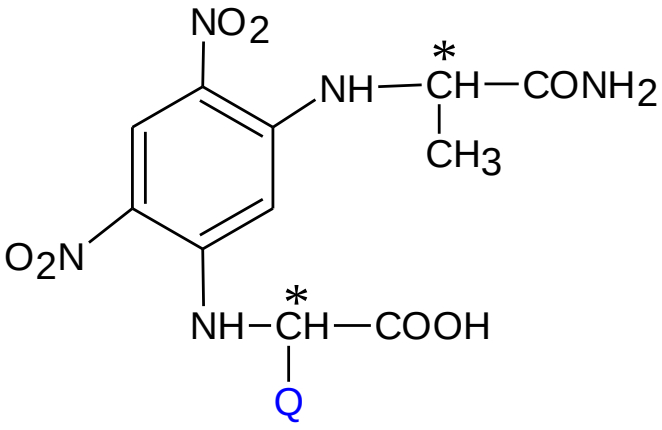


(D,L)

pH 9,0; 10 perc



D,L-származék



L,L-származék

HPLC elválasztás