8. Előadás
Peptidek

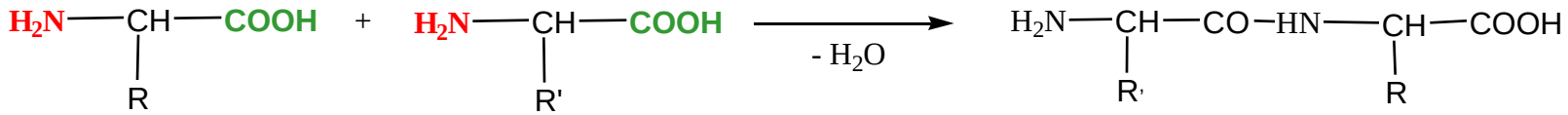
Jelentkeztek: 39 fő Jelen voltak: 27 fő

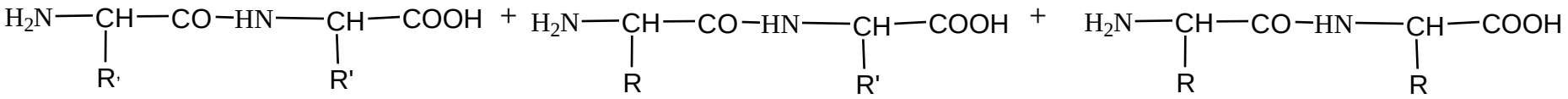
Név	Kód	%
Bödecs András	FM1BO9	71,8
Pozsgai Balázs	FSVV6K	94.9
Sági István	F937VV	76.9
Szigeti Szilvia	FD42VK	83.3

> 50 % : 6 fő

Peptid szintézis: a koncepció

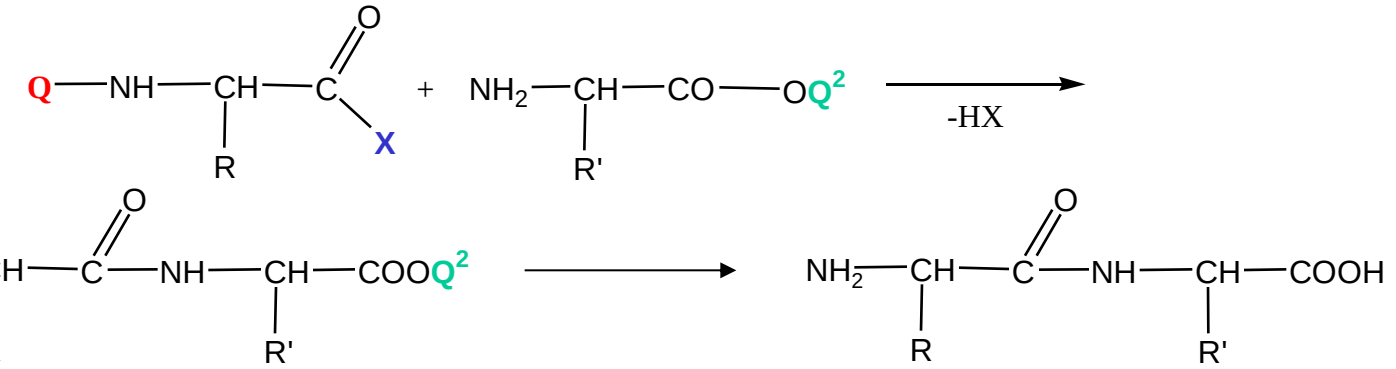
1. Probléma





Keverék, alacsony konverzió

2. Megoldás



Egyféle termék, magas konverzió

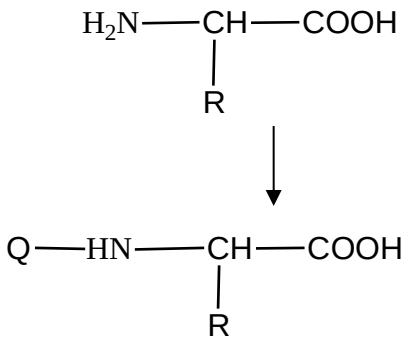
3. Követelmények

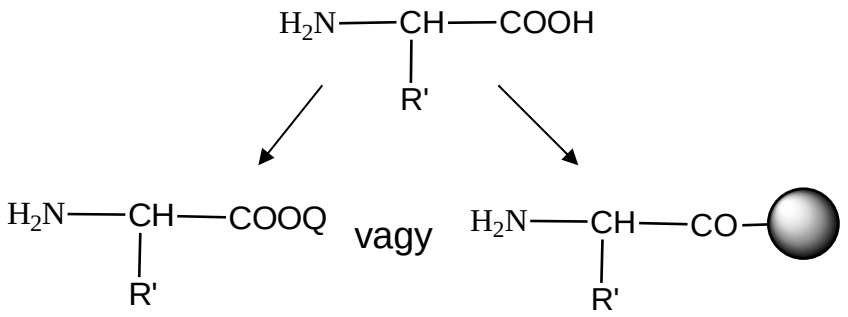
3.1. Védőcsoportok, egyszerű beépítés, egyszerű eltávolítás

3.2. Hatékony kapcsolási reakció

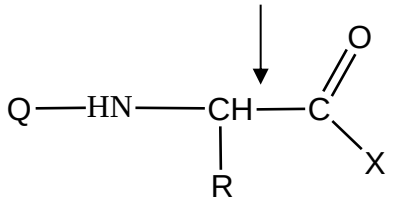
Négy lépés

4.1. Védett (N-, C- és oldallánc) aminosavszármazékok

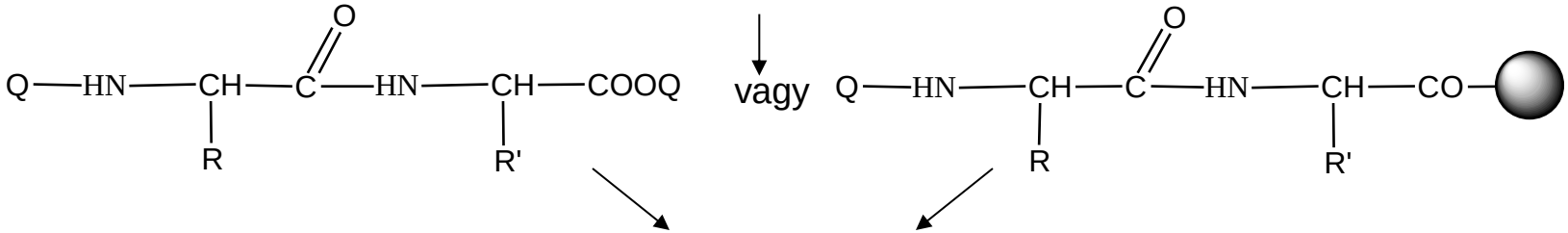




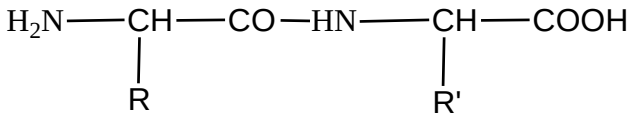
4.2. A C-terminálison „aktivált" aminosavszármazék szintézise



4.3. Kapcsolás



4.4. Védőcsoportok eltávolítása



1. Védőcsoportok

„All modern work in Peptide synthesis employs the following approach: the amino group of one amino acid is first stabilized by the introduction of a protecting group R, the carboxy group is modified so that it is capable of coupling, and finally the R group is removed to produce the finished peptide. The difficulty lies in finding a suitable group R, which can be removed so gently that the peptide is not significantly attacked."

Bergman, M., Zervas, L.: Berichte Chem. 65, 1192 (1932)

Csoportosítás

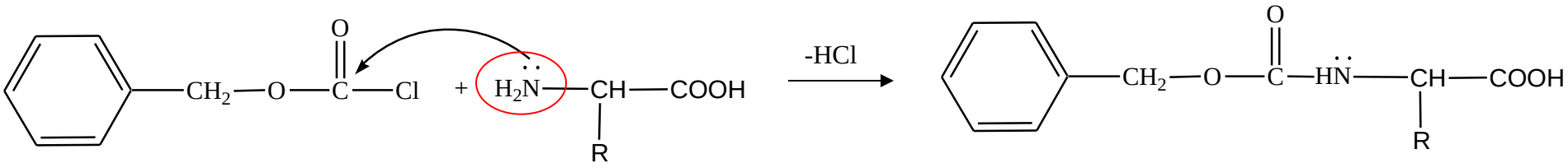
1. N^{α} - védőcsoport
2. C^{α} - védőcsoport
3. Oldallánc védőcsoport

1.1. N^{α}-Amino csoport védelme

1.1.1. Benziloxi-karbonil (Z)

Bergman, M., Zervas, L. : Berichte Chem. 65,1192 (1932)

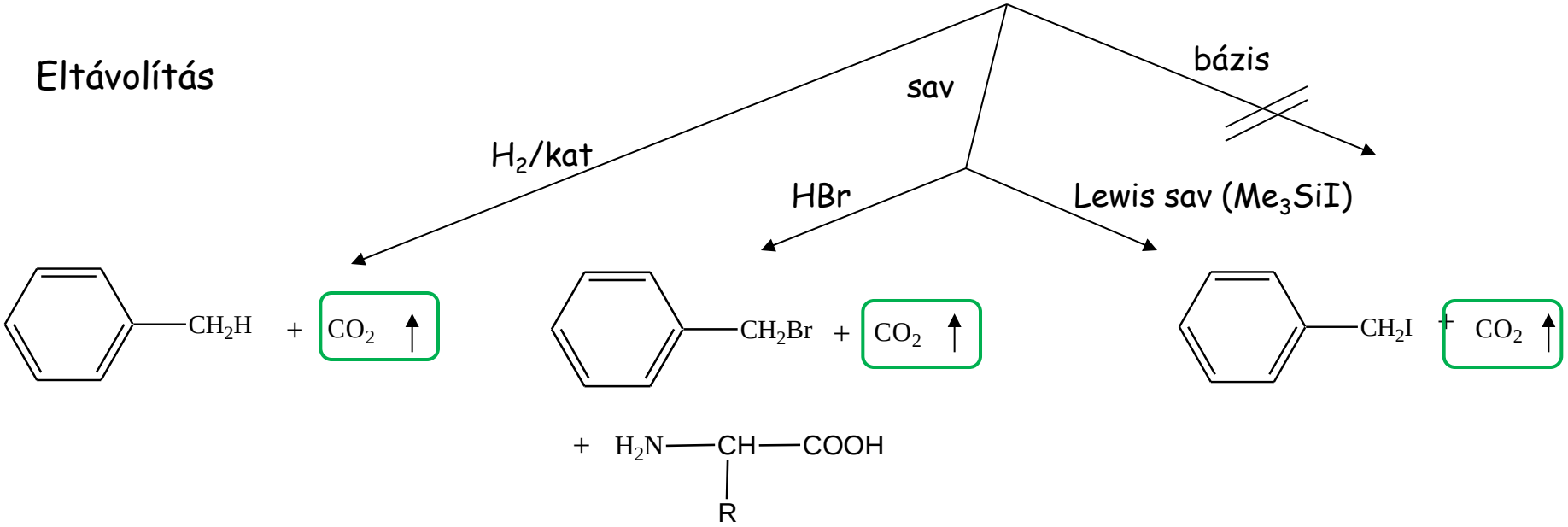
Beépítés



Benziloxikarbonil klorid

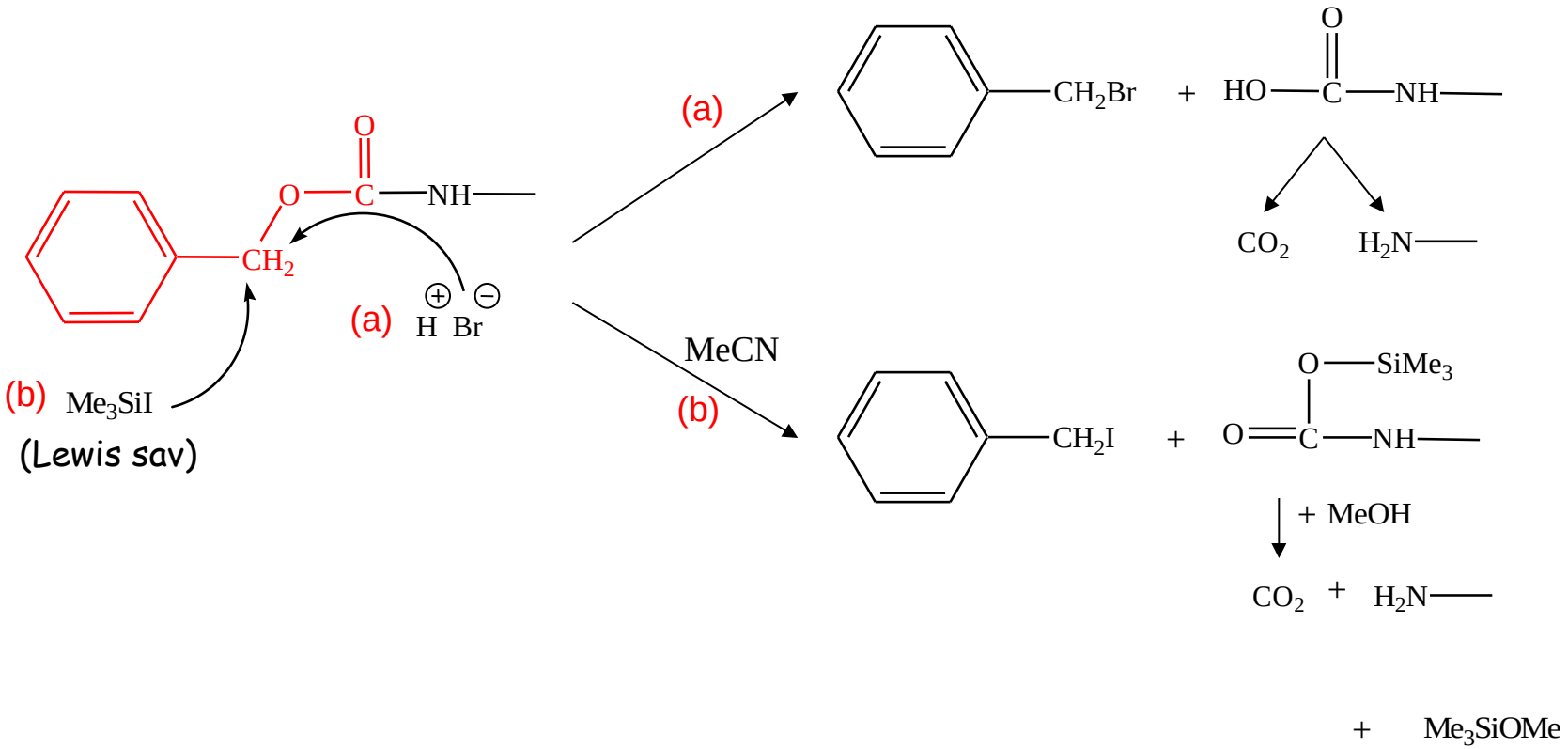
Benziloxikarbonil aminosav

Eltávolítás



Hasítási mechanizmus

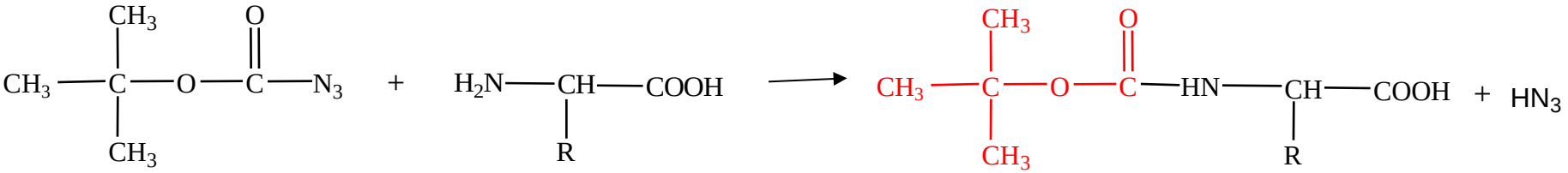
Benziloxi-karbonil



1.1.2. *t*-Butiloxi-karbonil (Boc)

Carpino, L.A. et al. JACS (1957)

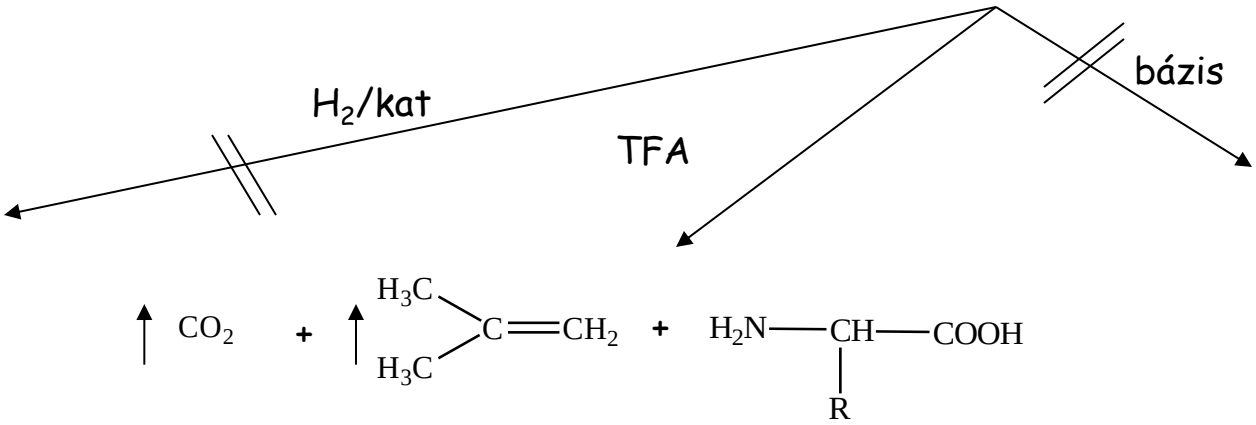
Beépítés



terc-Butiloxikarbonil azid

terc-Butiloxikarbonil aminosav

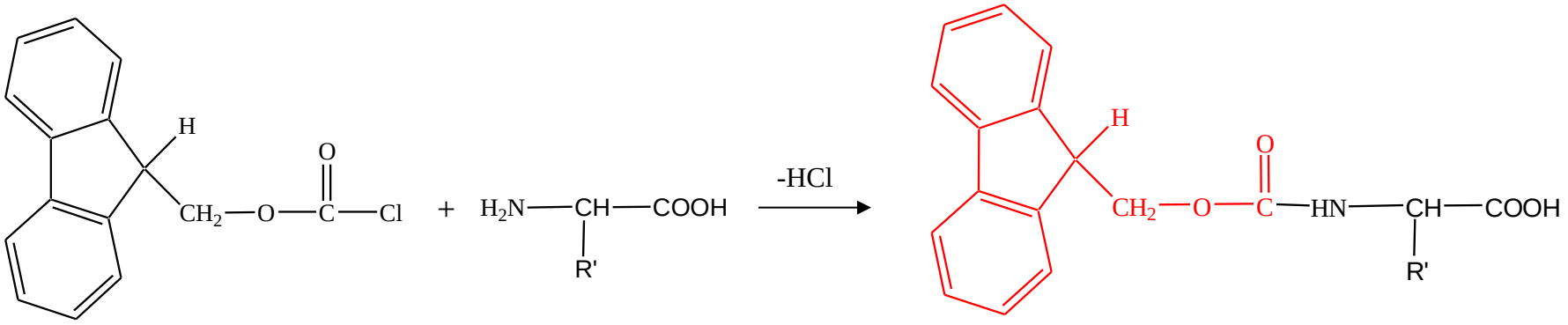
Eltávolítás



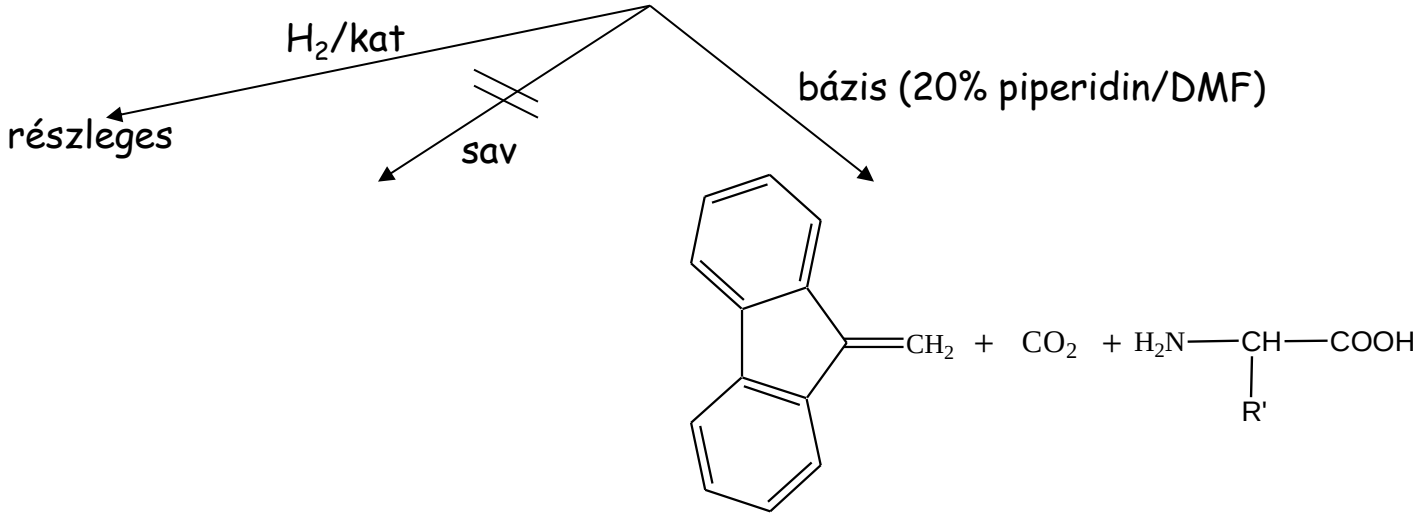
1.1.3. 9-Fluorenilmetoxi-karbonil

Carpino, L.A. et al. JACS 92, 5748 (1970)

Beépítés

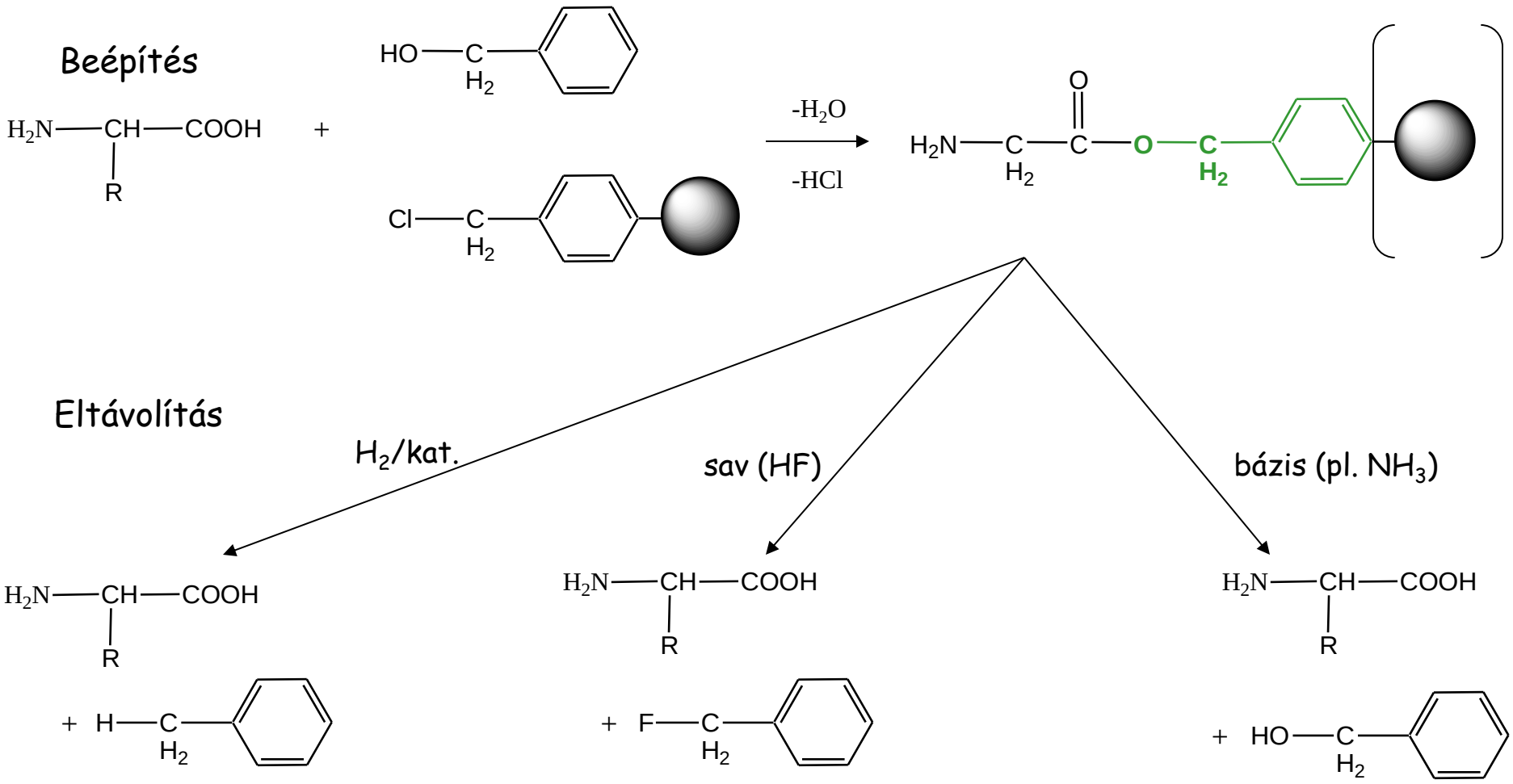


Eltávolítás



1.2. Karboxi védelem

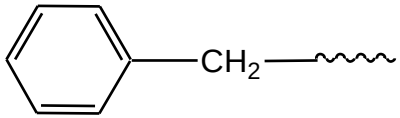
Észter kötés kialakítása



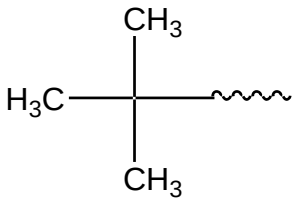
1.3. Oldallánc védelem

1.3.1. Glu, Asp
1.3.2. Lys
1.3.3. Ser, Thr, Tyr
1.3.4. Arg
1.3.5. His
1.3.6. Trp
1.3.7. Cys

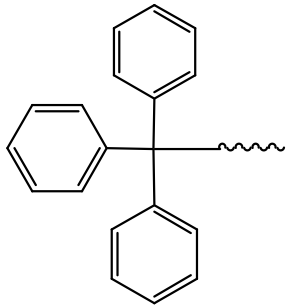
-OH védőcsoport



Benzil (éter)
(Bzl)

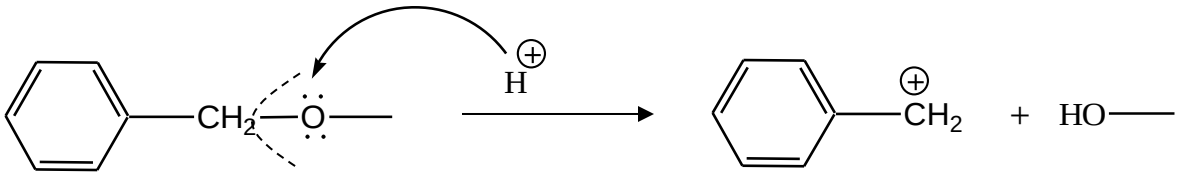


terc-Butil (éter)
(t-Bu)



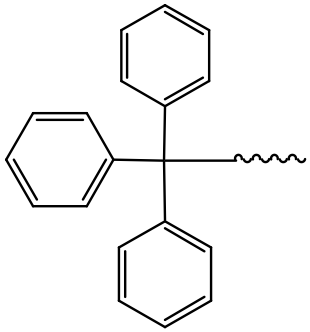
Tritil*
(Trt)

Eltávolítás



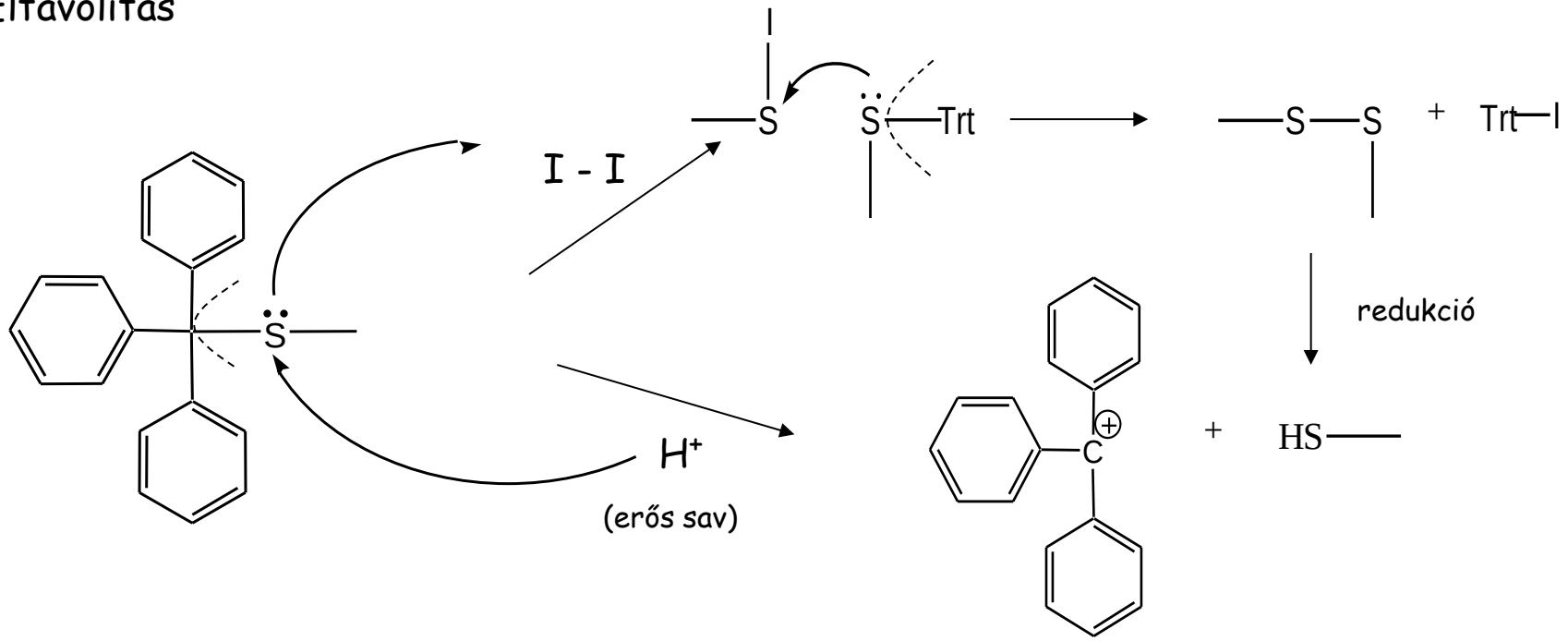
* csak Ser/Thr

-SH védőcsoport

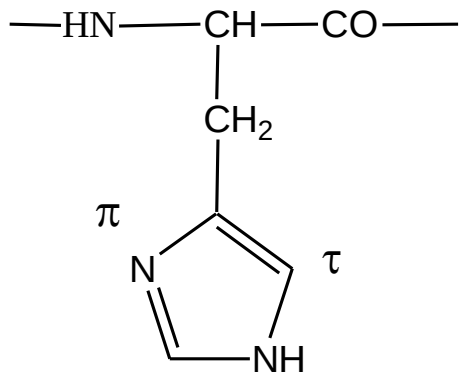


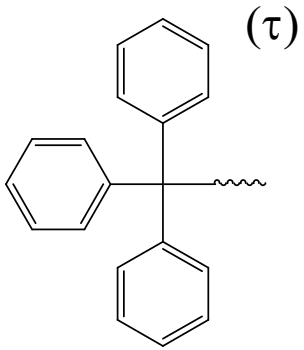
Tritil
(**Trt**)

Eltávolítás

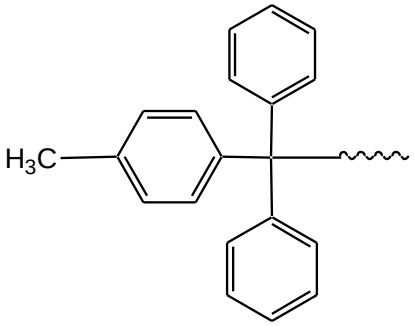


Imidazol védőcsoport

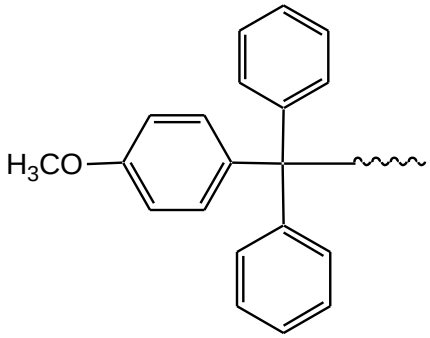




Tritil
(Trt)

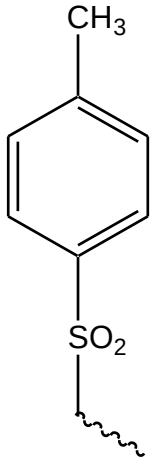


4-Metiltritil
(Mtt)



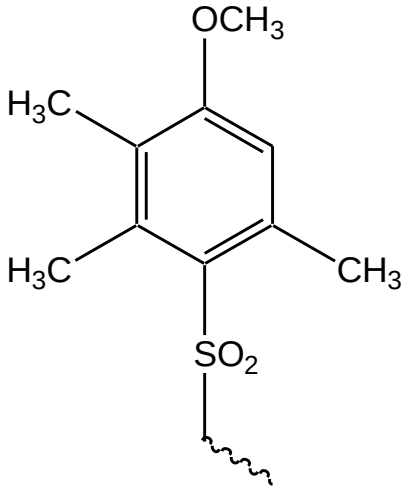
4-Metoxitritil
(Mmt)

Guanidino védőcsoport



4-Toluolszulfonil*
(Tos)

* HF vagy Na/NH_3



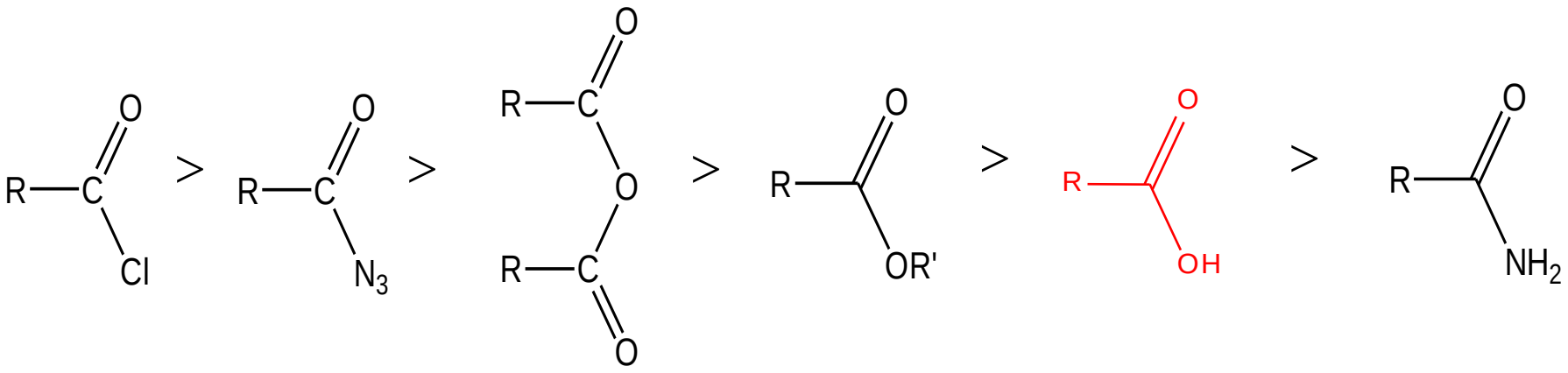
4-Metoxi-2,3,6-trimetilbenzolszulfonil
(Mtr)

Az optimális védőcsoport – összegzés

1. Könnyű és hatékony beépülés.
2. A peptidkötés érintetlensége a beépítés/eltávolítás alatt.
3. Több mint egyféle eltávolítási lehetőség.
4. Nincs racemizáció.
5. A melléktermékek egyszerű és könnyű eltávolítása.
6. A reakciók szobahőmérsékleten menjenek végbe.

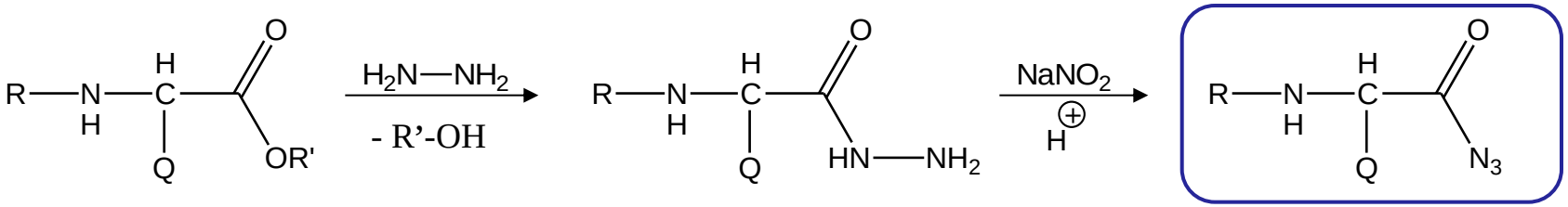
2. lépés: Az aminosav „aktiválása”

2.1. A karbonsav származékok aktivitása

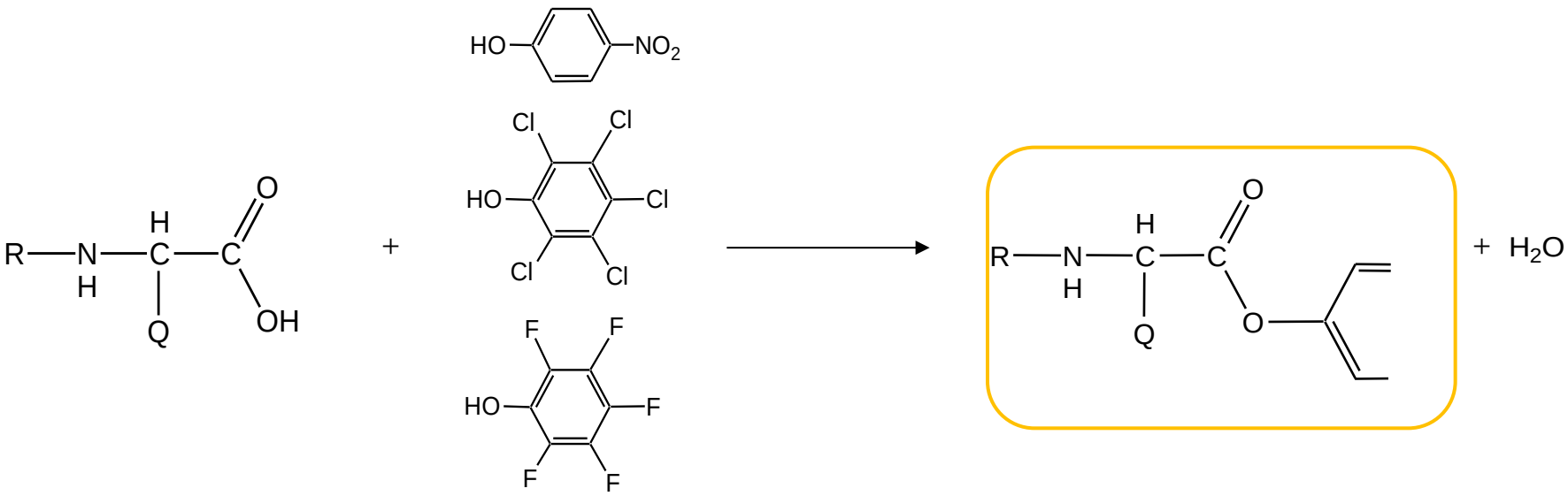


2.2. A karbonsav származékok aktiválása

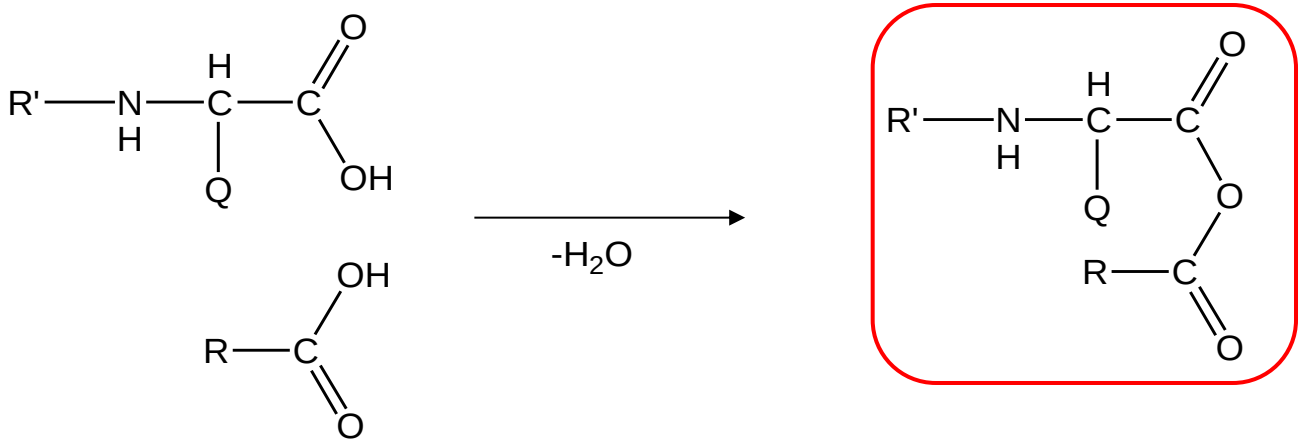
2.2.1. Acil azid (T. Curtius, Berichte Chem. 35, 3226,1902)



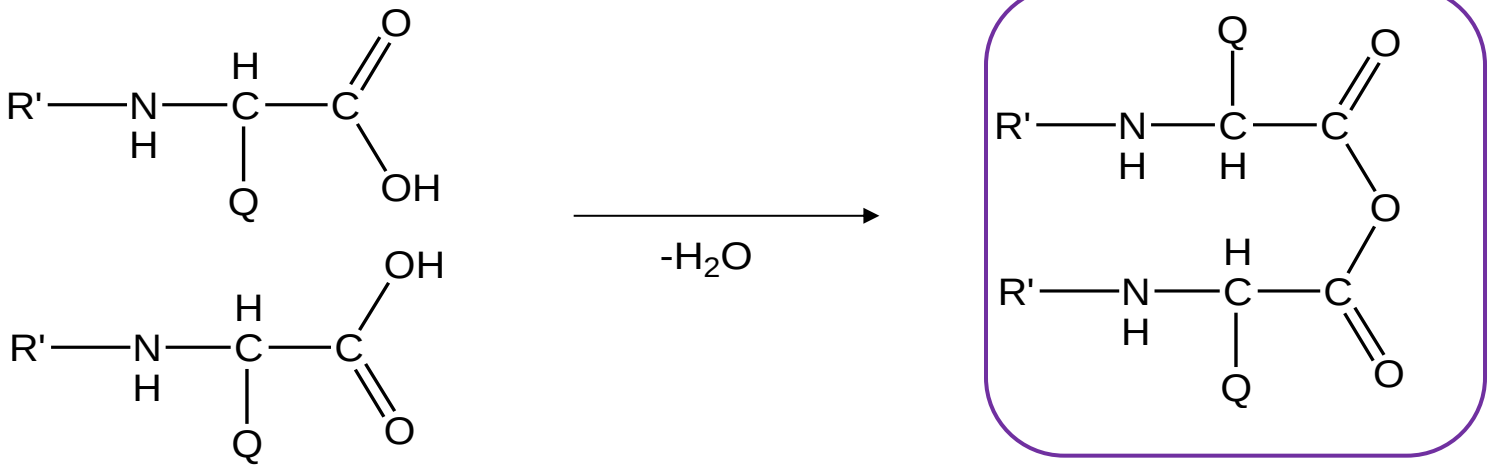
2.2.2. Actív észter (M. Bodanszky, Nature, 175, 685, 1955; J. Kovacs, JACS, 89, 183, 1967; L. Kisfaludy, Liebigs Ann. 1421, 1973)



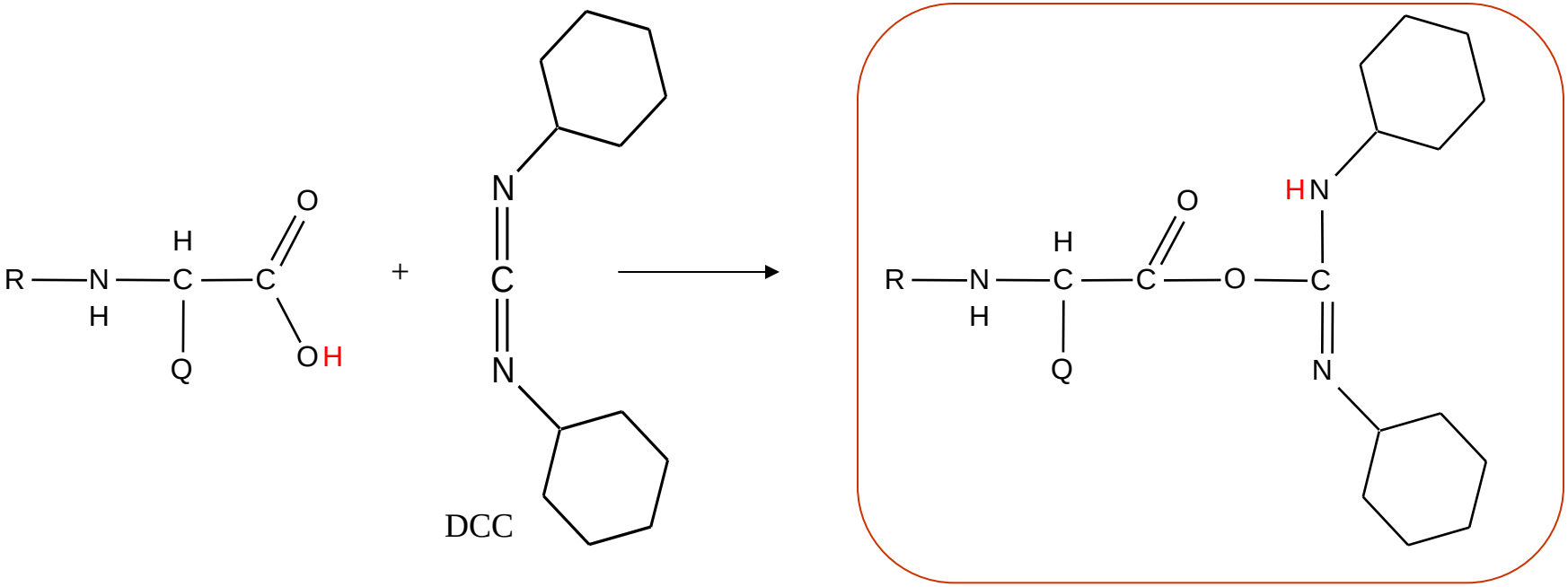
2.2.3. Anhidrid (vegyes) (E. Fischer, Berichte Chem. 36, 2094,1903; T. Wieland, Liebigs Ann. 569, 122, 1950)



2.2.4. Anhidrid (szimmetrikus) (F. Weygand et al., Z. Naturf. 22, 1084, 1967)

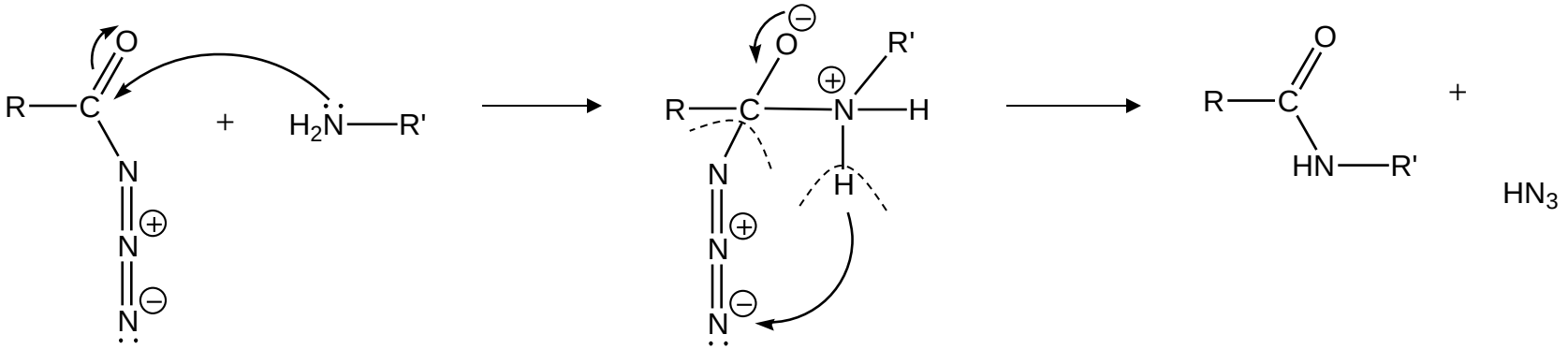


2.2.5. Karbodiimid (J.C. Sheenan, G.P. Hess, JACS, 77, 1067, 1955)

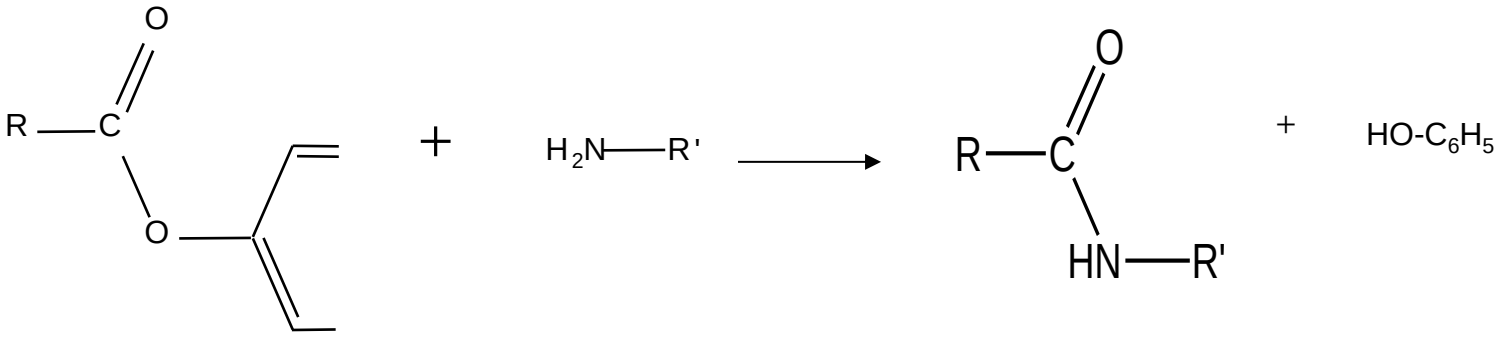


3. lépés: A peptidkötés kialakítása

3.1. Acil azid (nincs racemizáció)

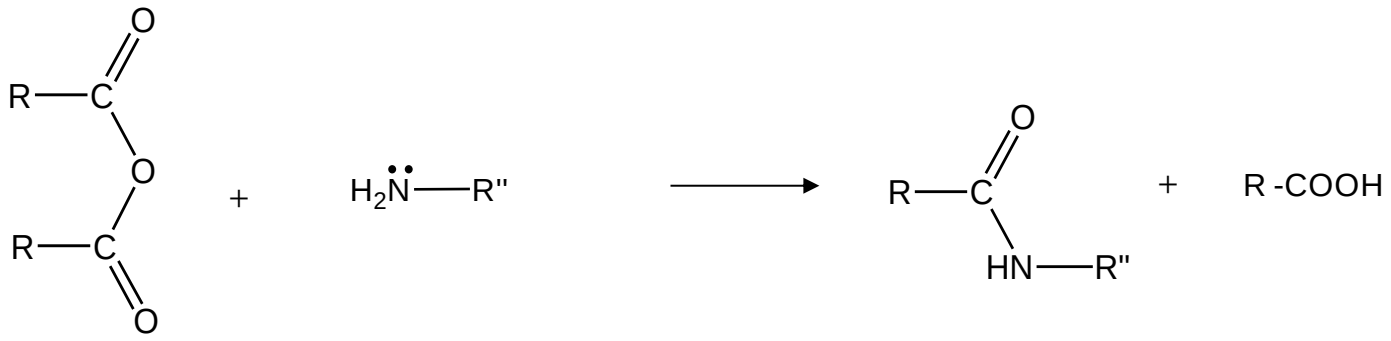


3.2. Aktív észter (S_N)

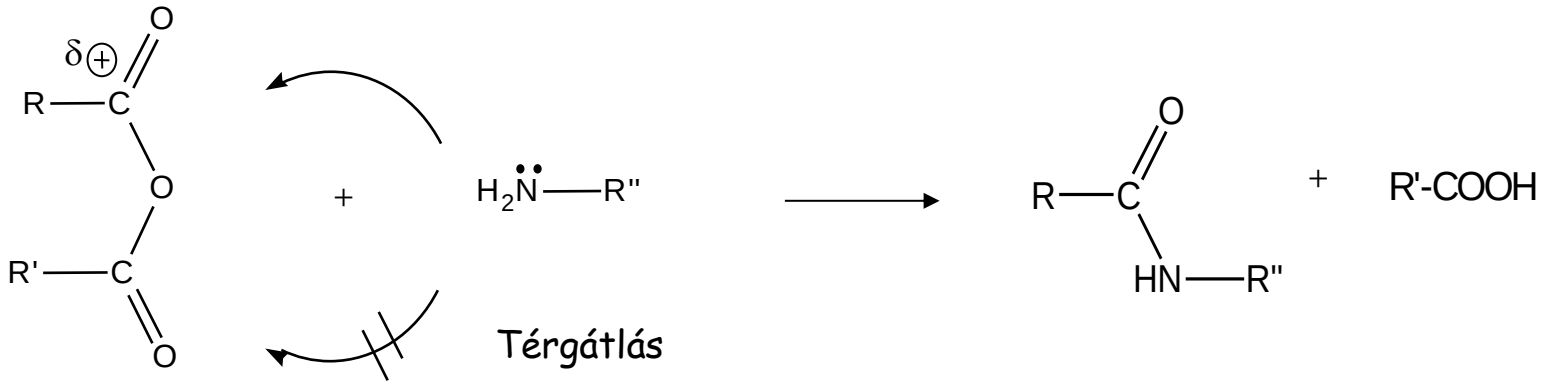


3.3. Anhidrid

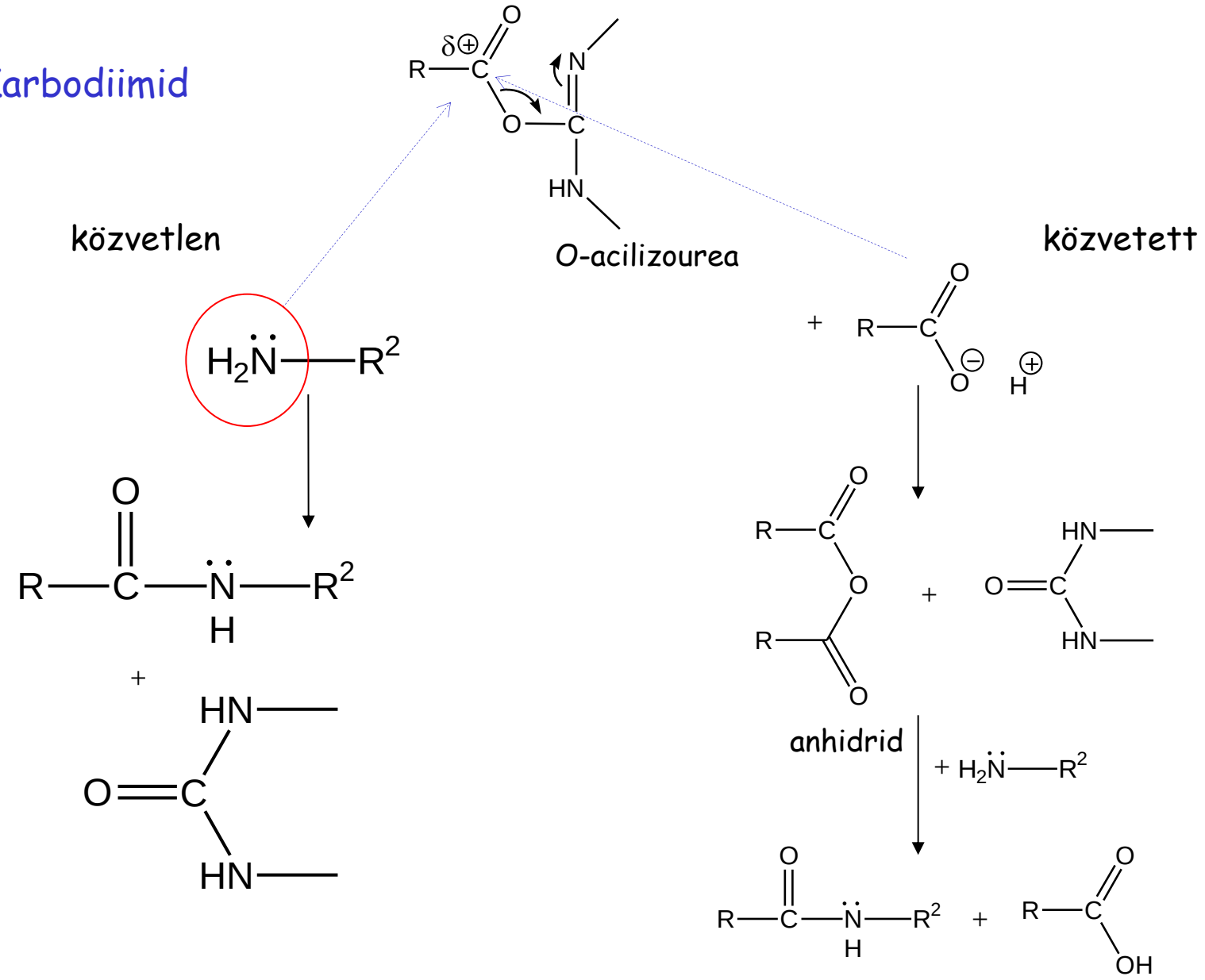
szimmetrikus



aszimmetrikus

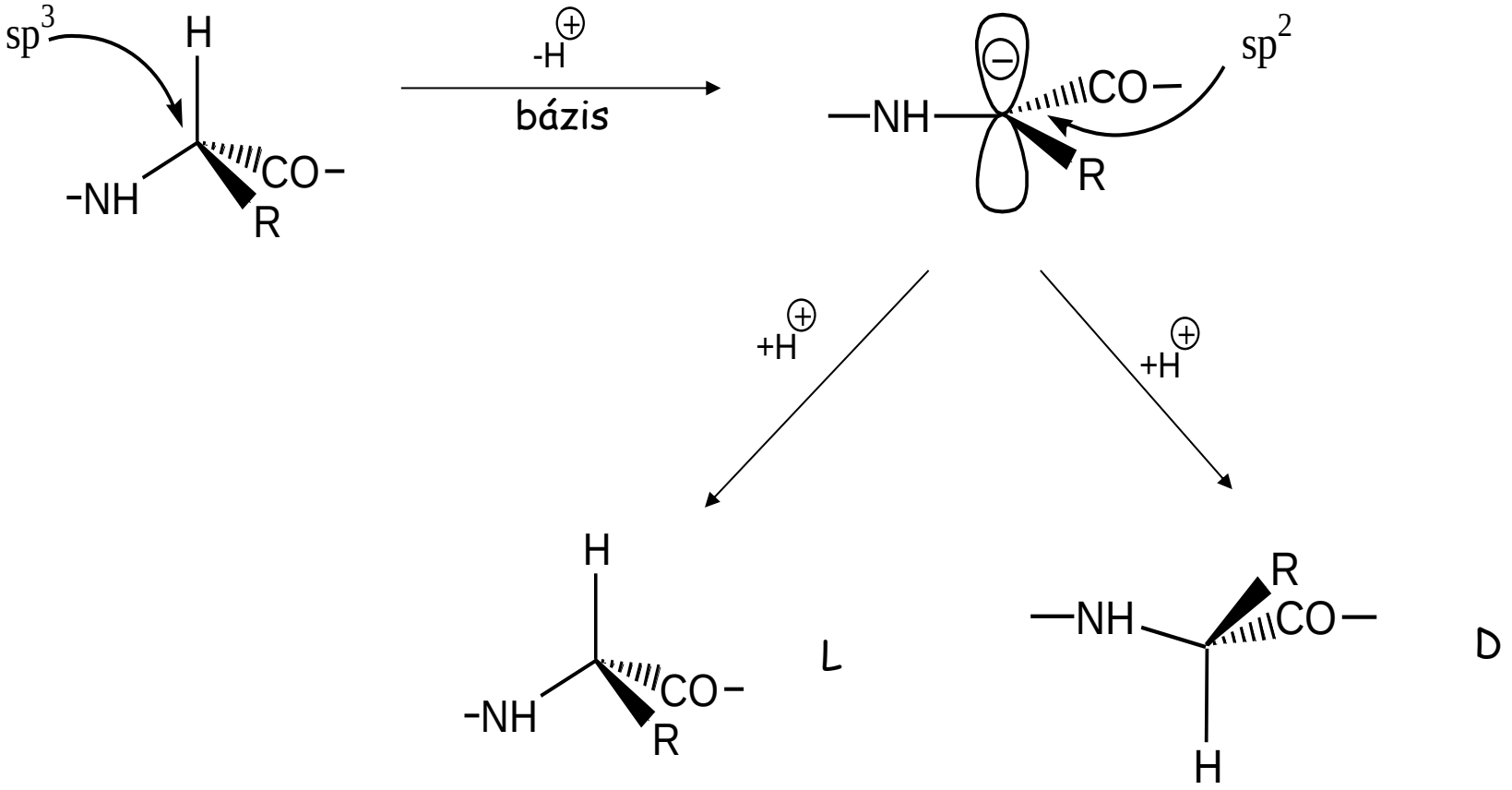


3.4. Karbodiimid



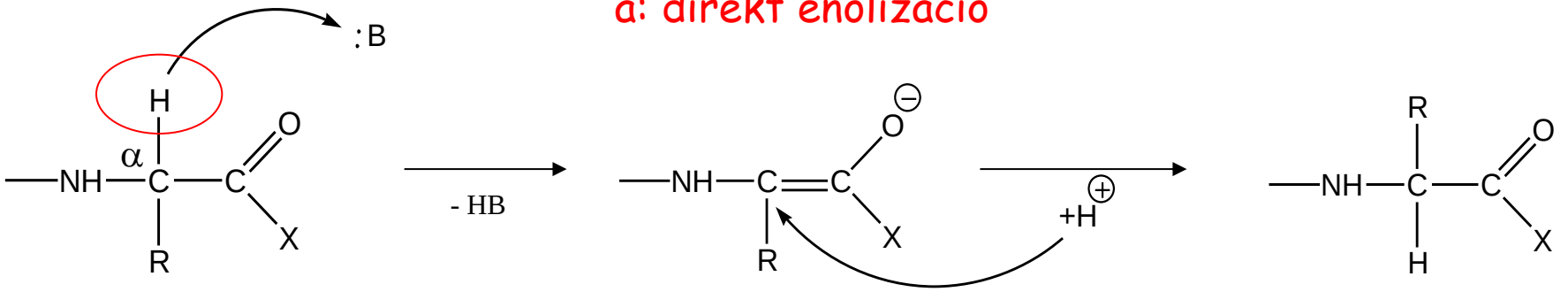
Racemizáció

Az enantiomer átalakulása enantiomer keverékké

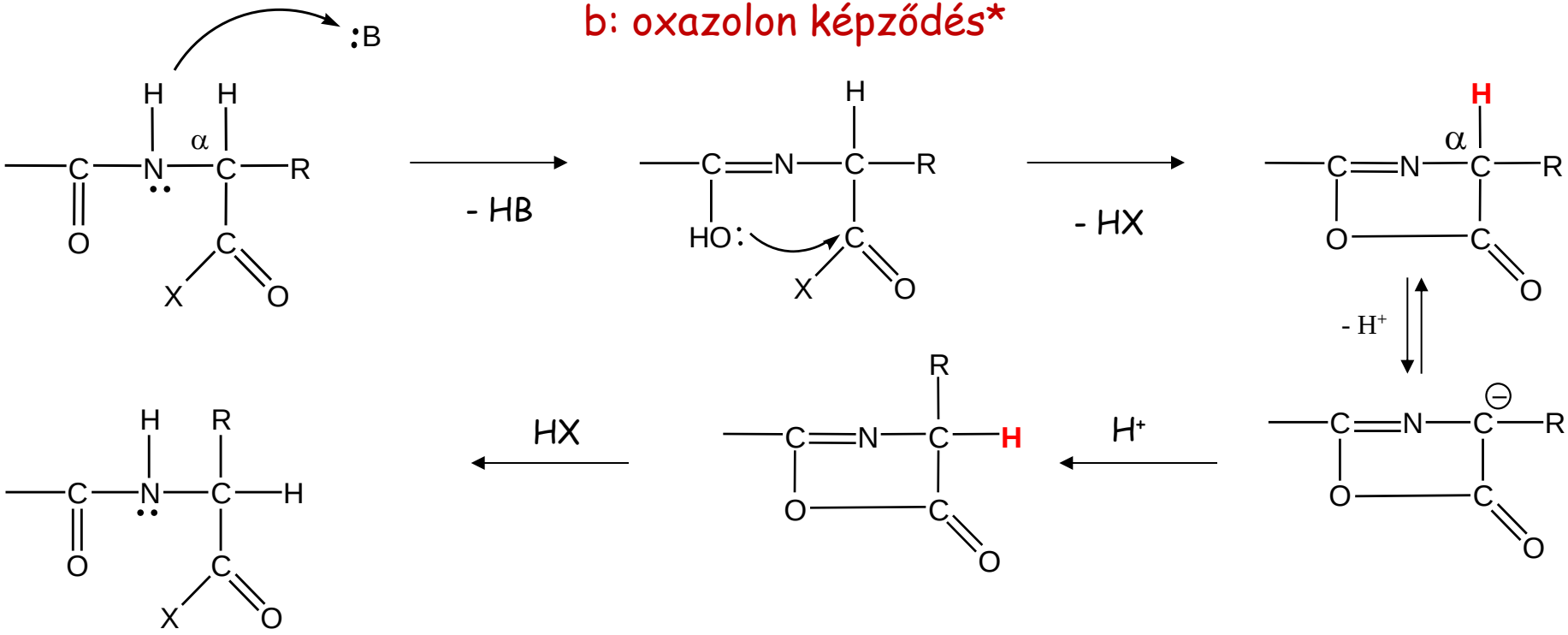


A racemizáció mechanizmusa

a: direkt enolizáció



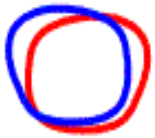
b: oxazolon képződés*

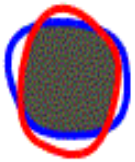


* :B nélkül :N iniciálja a reakciót

Poláros fény

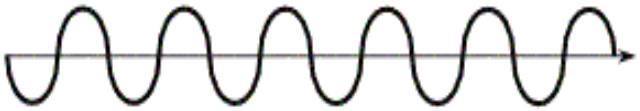
Looking at two polarized lenses at the same time, by rotating one of the lenses, we can either let light through, or we can stop the light all together.





How does it work?

Visible Light is a form of electromagnetic Radiation. So we know that visible light acts like a wave. The wave motion forces light to vibrate sideways. So, when light moves forward, it is also vibrating sideways.



In the light that we see, some light vibrates up and down.

Some light that we see vibrates right and left.

If we were to look at enough light photons, we w find that each photon vibrate in a different direc So in a beam of light different light photons vibrate in all directions.

Experiment 1

Before light enters the first lens, we find that the photons vibrate in all directions.

The first lens only lets the light that vibrates up and down, through the lens.

Only the light that vibrates up and down remains. All the rest of the light has been eliminated.

The second lens also lets the light that vibrates up and down, through the lens.

You can still see light, since both lenses allow the light that vibrates up and down to pass through.

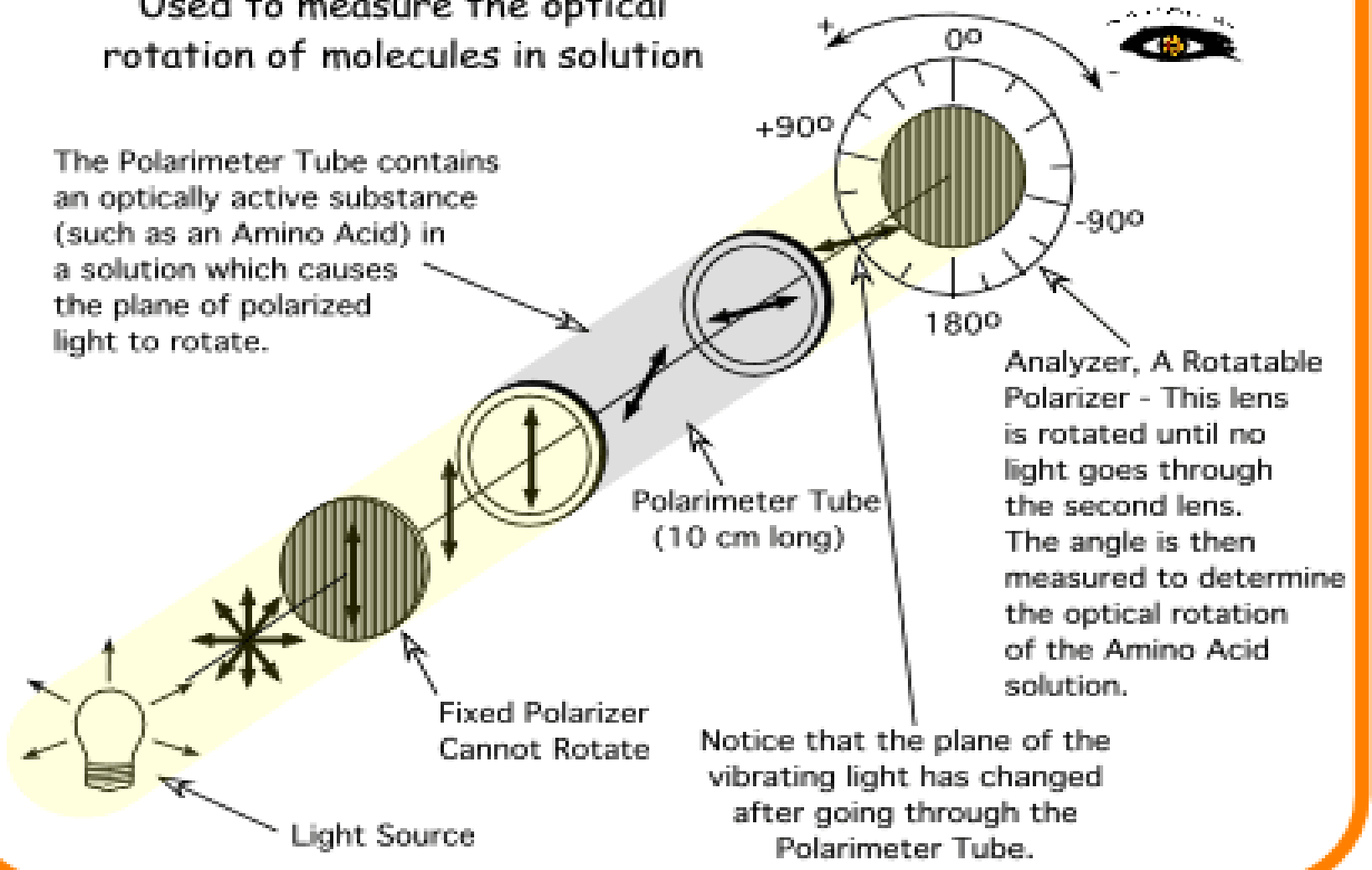
Experiment 2

The second lens only lets the light that vibrates right and left, through the lens.

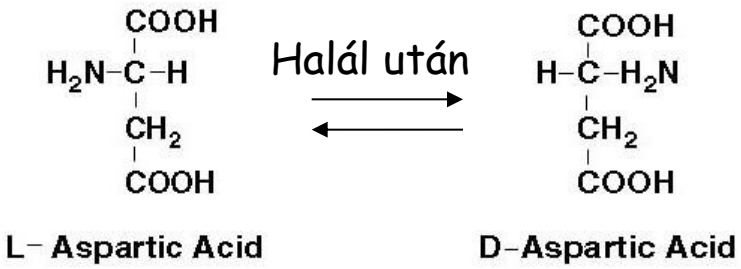
No Light Comes out of the 2nd Polarized Lens!

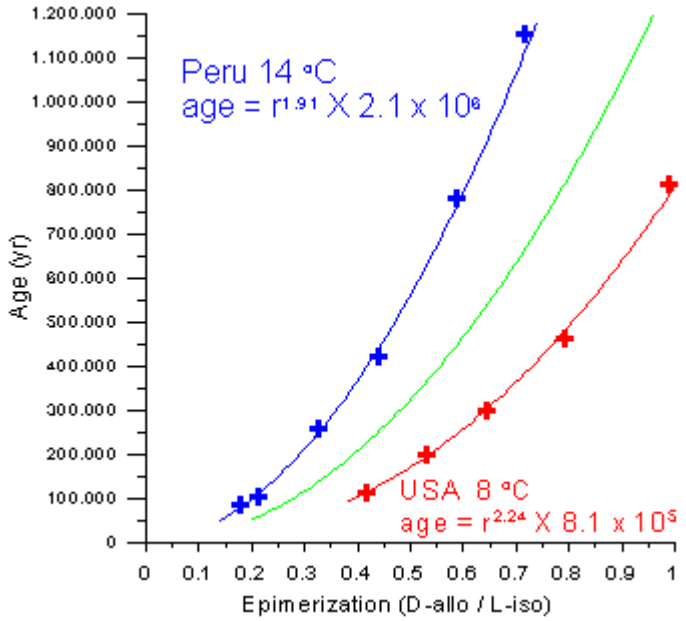
Polarimeter

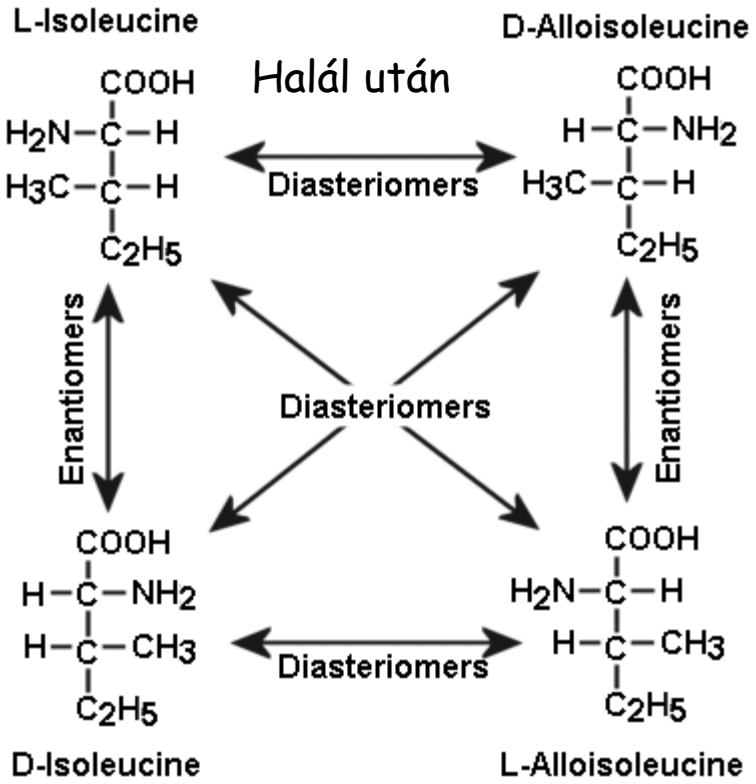
Used to measure the optical rotation of molecules in solution



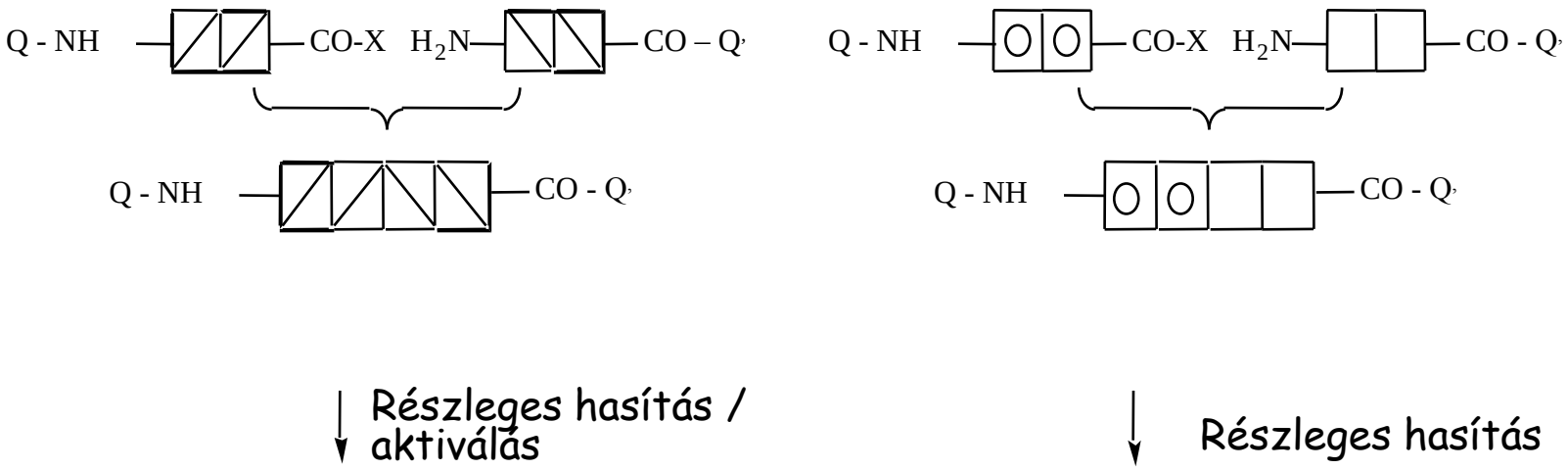
A racemizáció és a kormeghatározás





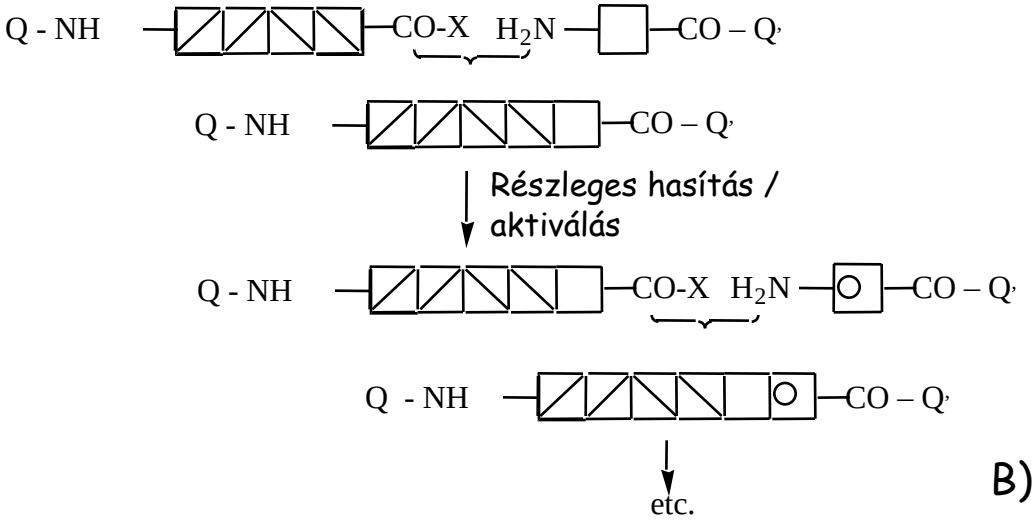


Stratégiák: Szegmens kondenzáció

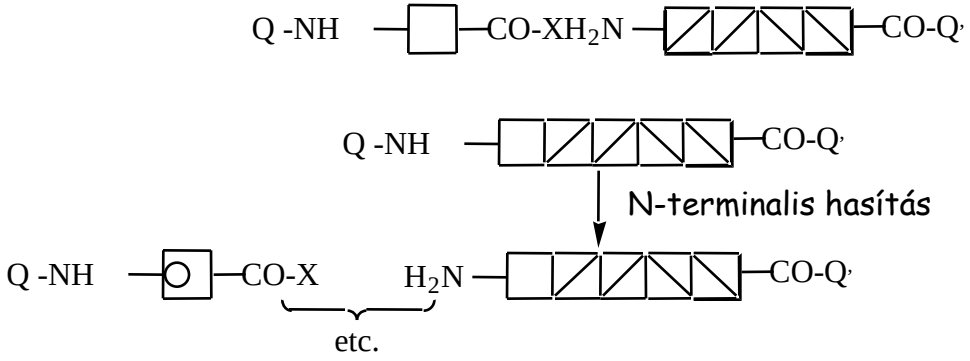


Stratégiák: Lépésenkénti hosszabbítás

A) C-terminálison



B) N-terminálison



Bruce R. Merrifield

There is a need for rapid, quantitative, automatic method for the synthesis of long peptides.

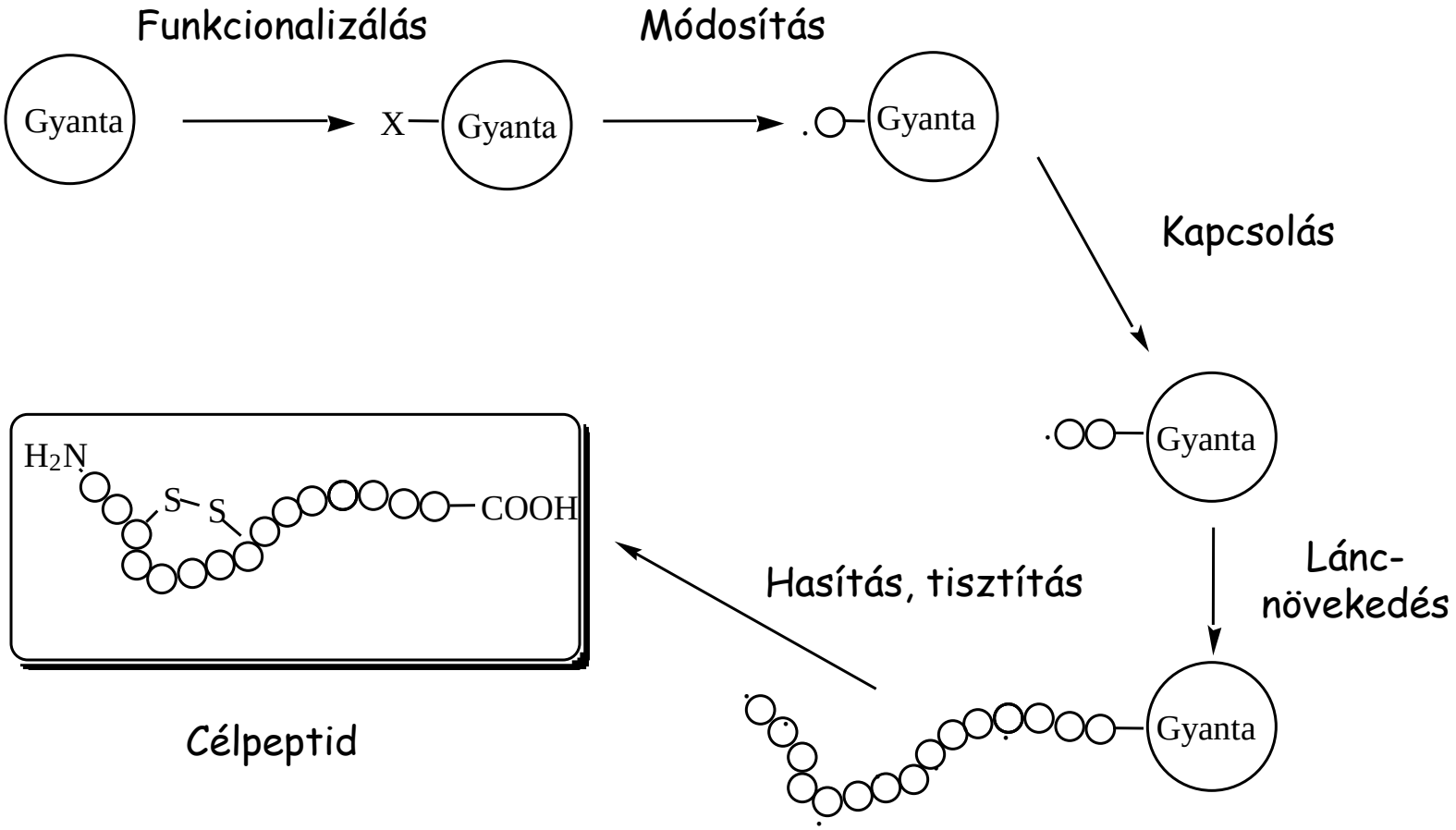
A possible approach may be the use of chromatographic columns, where the peptide is attached to the polymer packing and added to an activated amino acid followed by removal of protecting group, with repetition of the process until the desire peptide is built up.

Finally the peptide must be removed from the supporting medium.

R.B.Merrifield Laboratory note book (1959)

JACS 85, 2149 (1963)

A szilárd fázisú peptidszintézis elve



A szilárd fázisú peptid szintézis főbb szempontjai

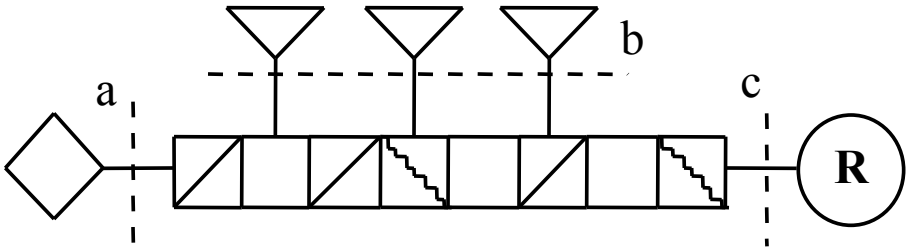
A. A szilárd felszín

1. Tartalmazzon reaktív csoportokat funkcionalizálásra
2. Peptid-polimer kötés hasítható legyen
3. Stabil legyen a szintézis körülményei között
4. A peptidlánc hozzáférhető legyen az oldószer és a reagensek számára

B. Védőcsoport kombinációk

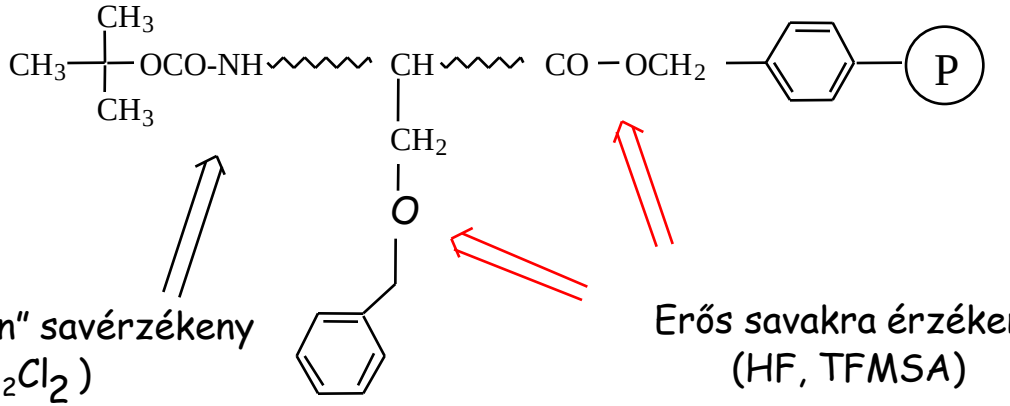
1. Peptid-polimer kötés stabil legyen a szintézis alatt
2. „Átmeneti" védőcsoport az α-amino csoporton
3. „Állandó" védőcsoport az oldalláncokon
4. Hatékony hasítás.

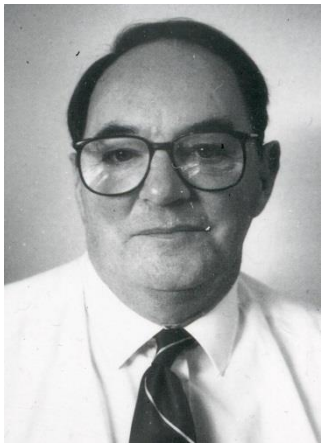
Védőcsoport típusok a szilárd fázisú szintézisben



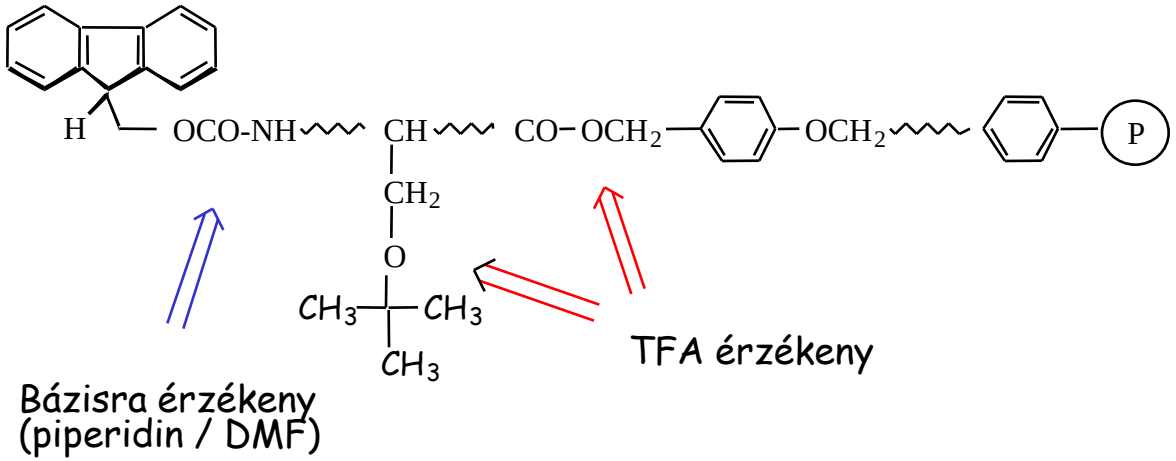
a. N^{α}- védőcsoport („átmeneti")
b. Oldallánc védőcsoportok („állandó")
c. Gyanta-peptid kötés

Boc/benzil stratégia - Merrifield módszer

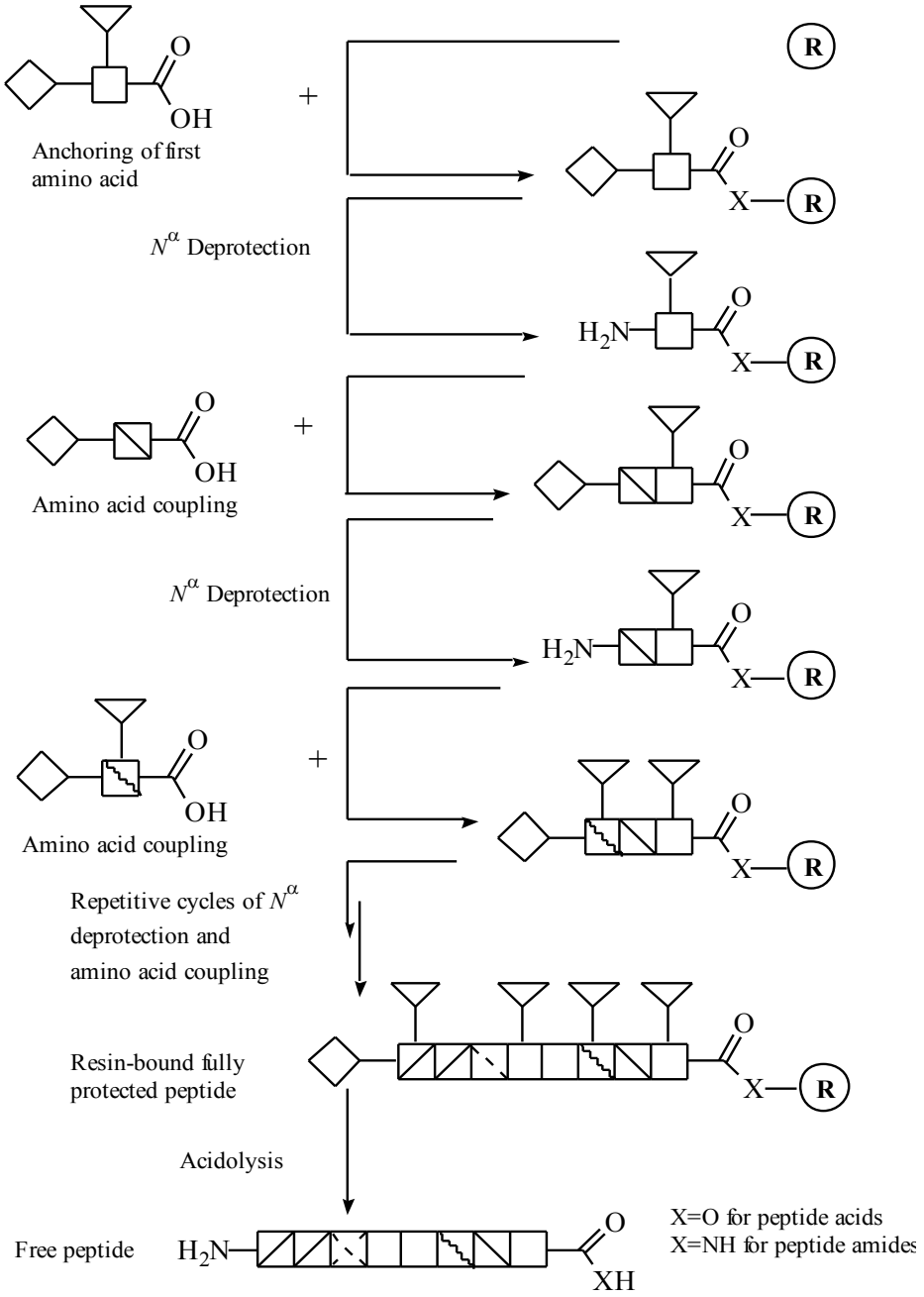




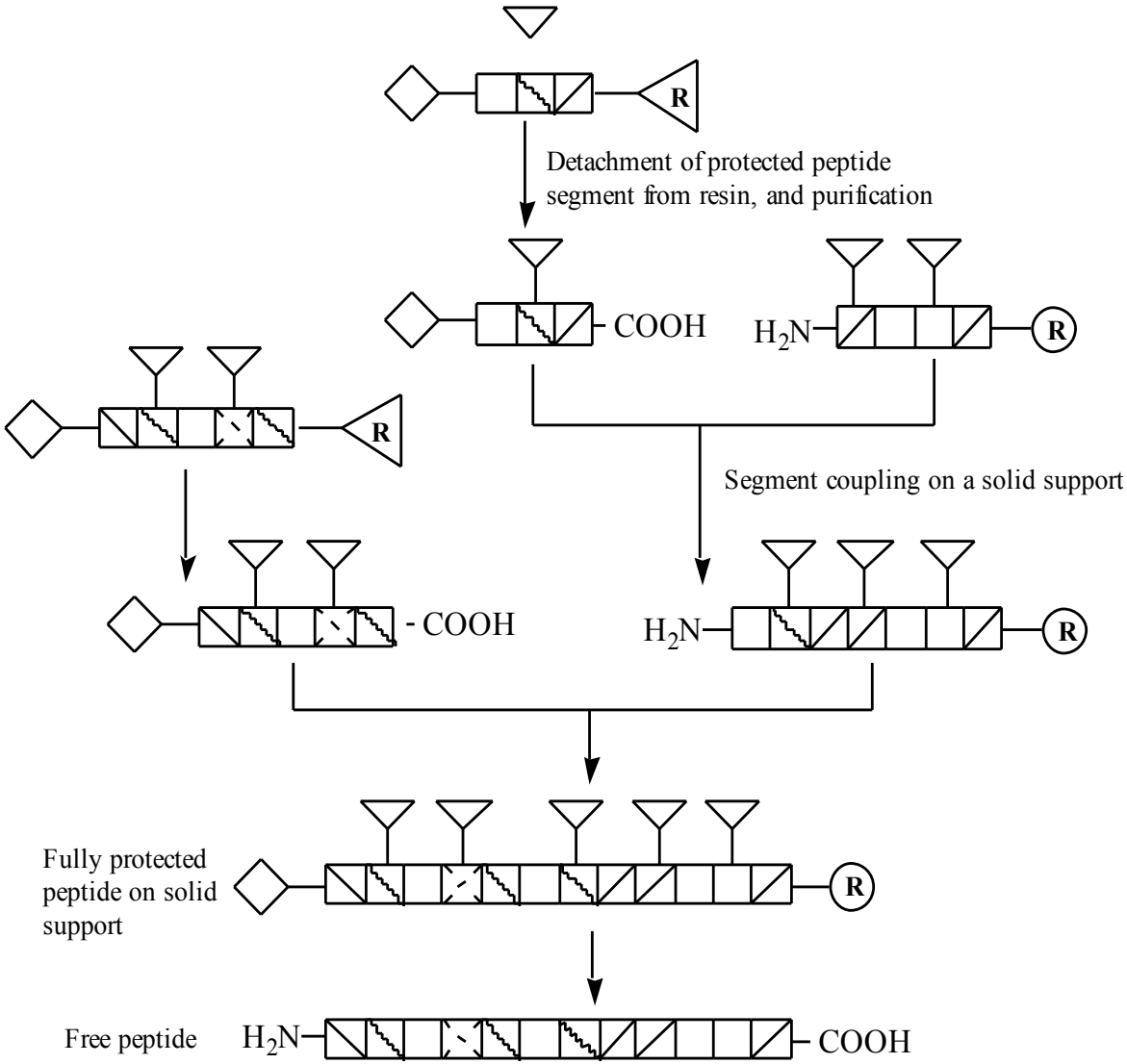
Fmoc/t-butil stratégia - Sheppard módszer



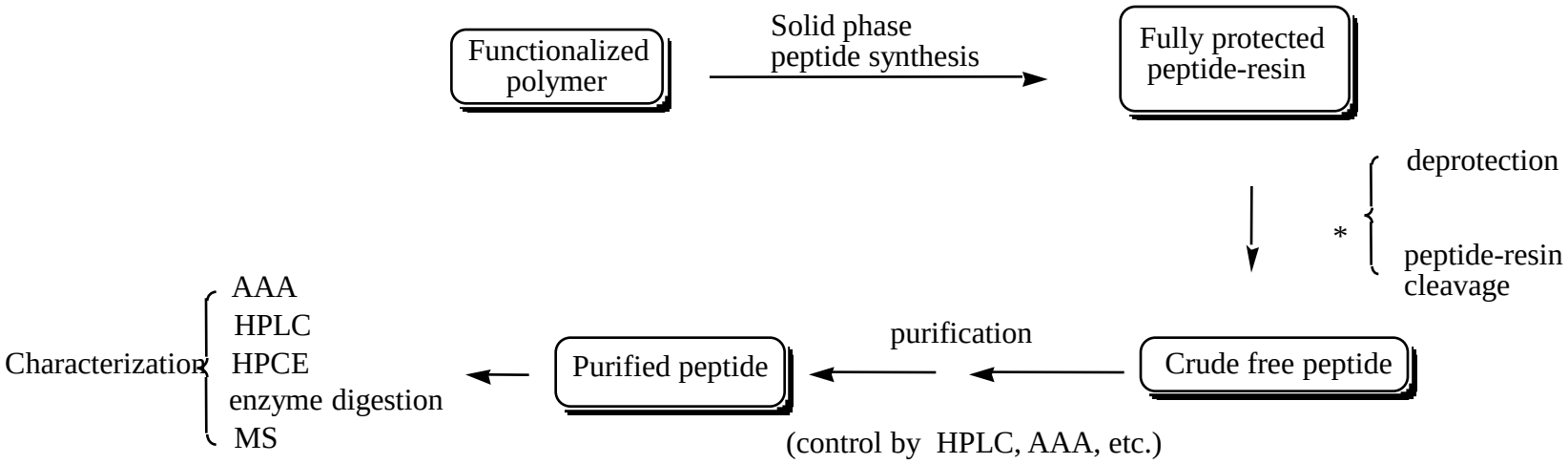
Lineáris szintézis



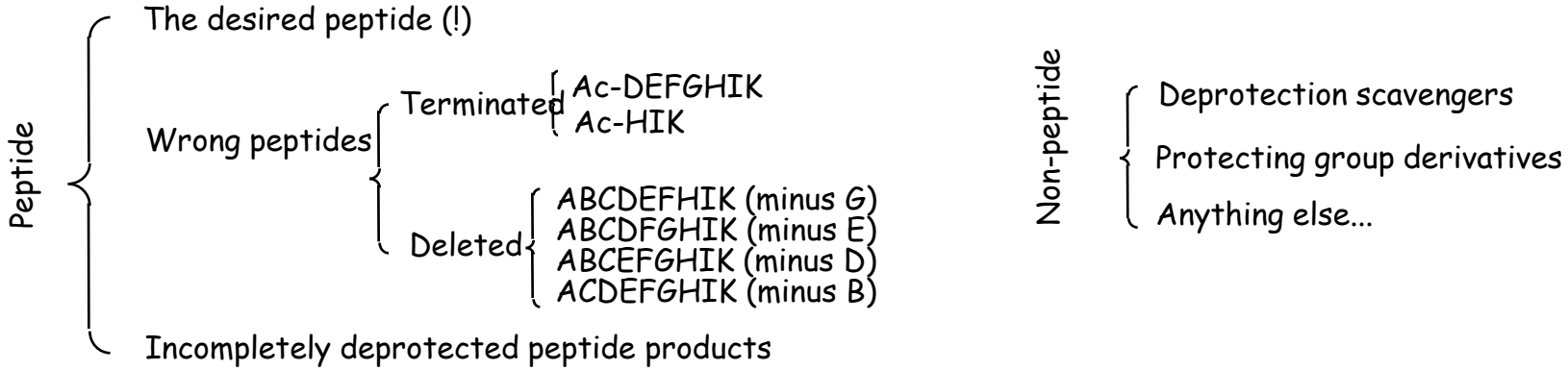
Konvergens szintézis



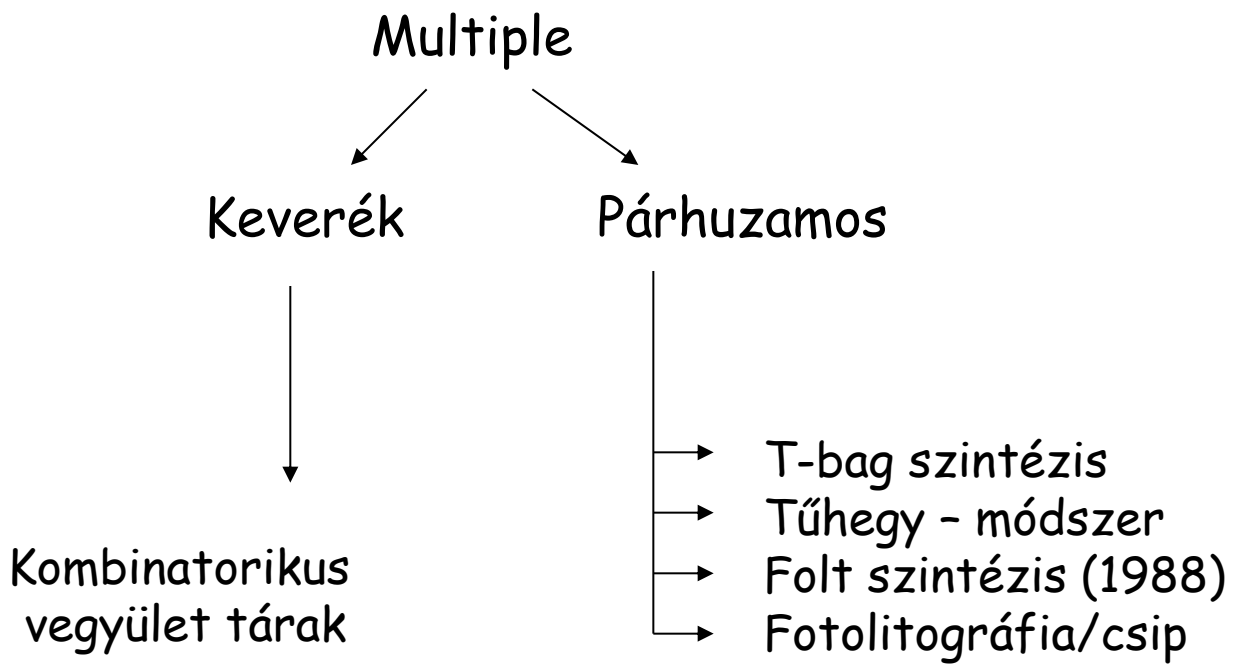
Szilárd fázisú peptid szintézis: Összegzés



Mit találhatunk a szintézis „nyers" termékében ?

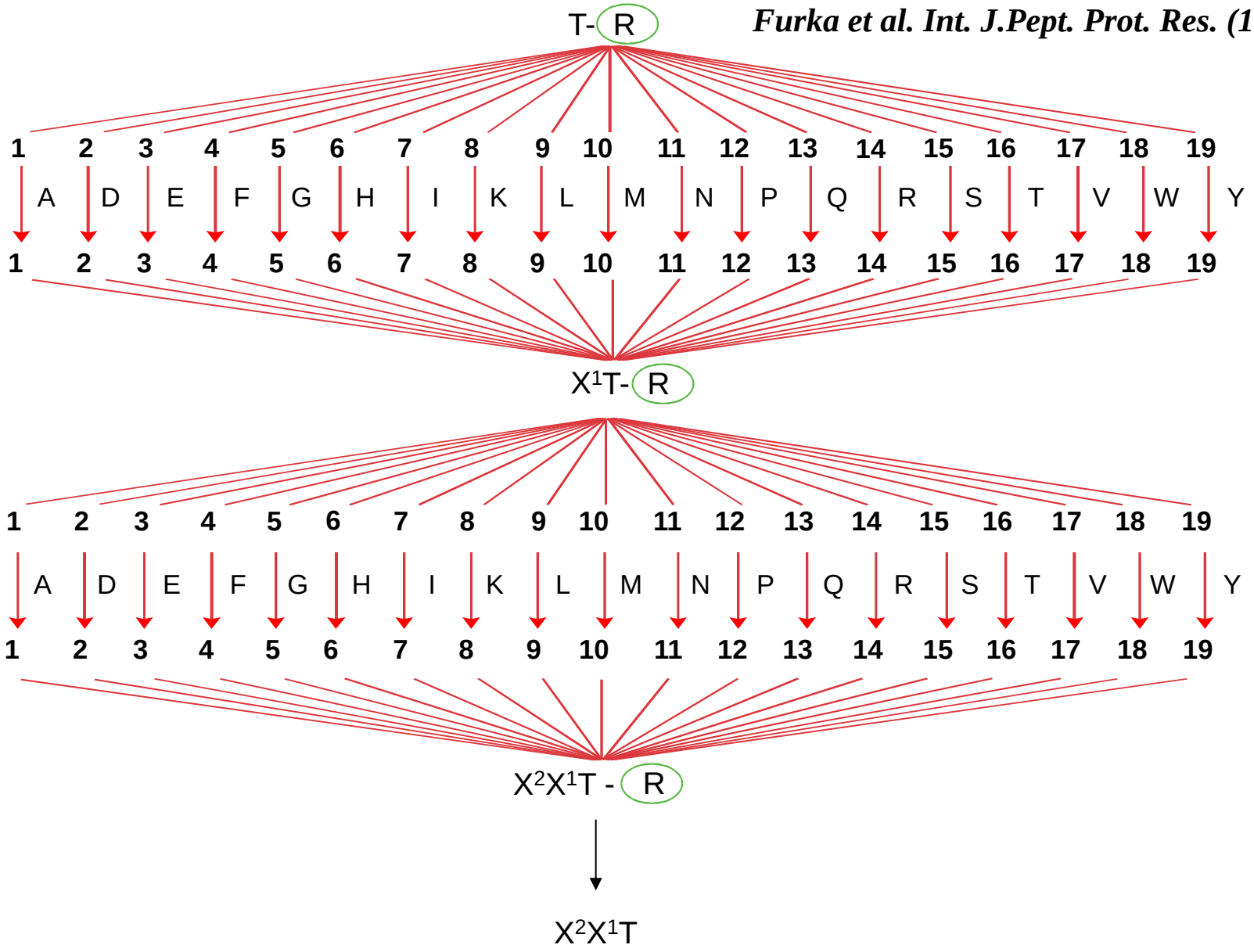


Szintézis „formák”

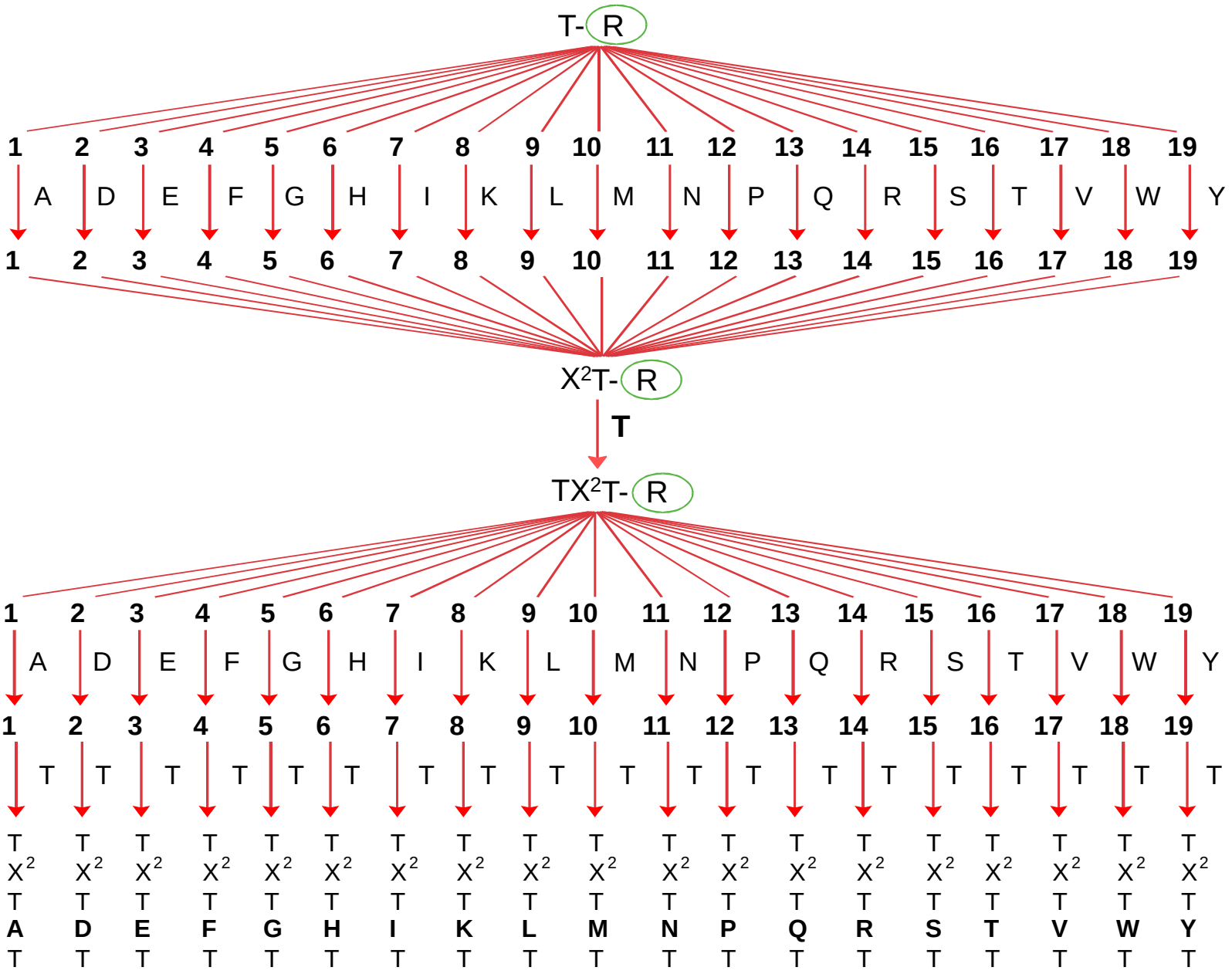


Kombinatorikus szintézis: keverés-osztás elv

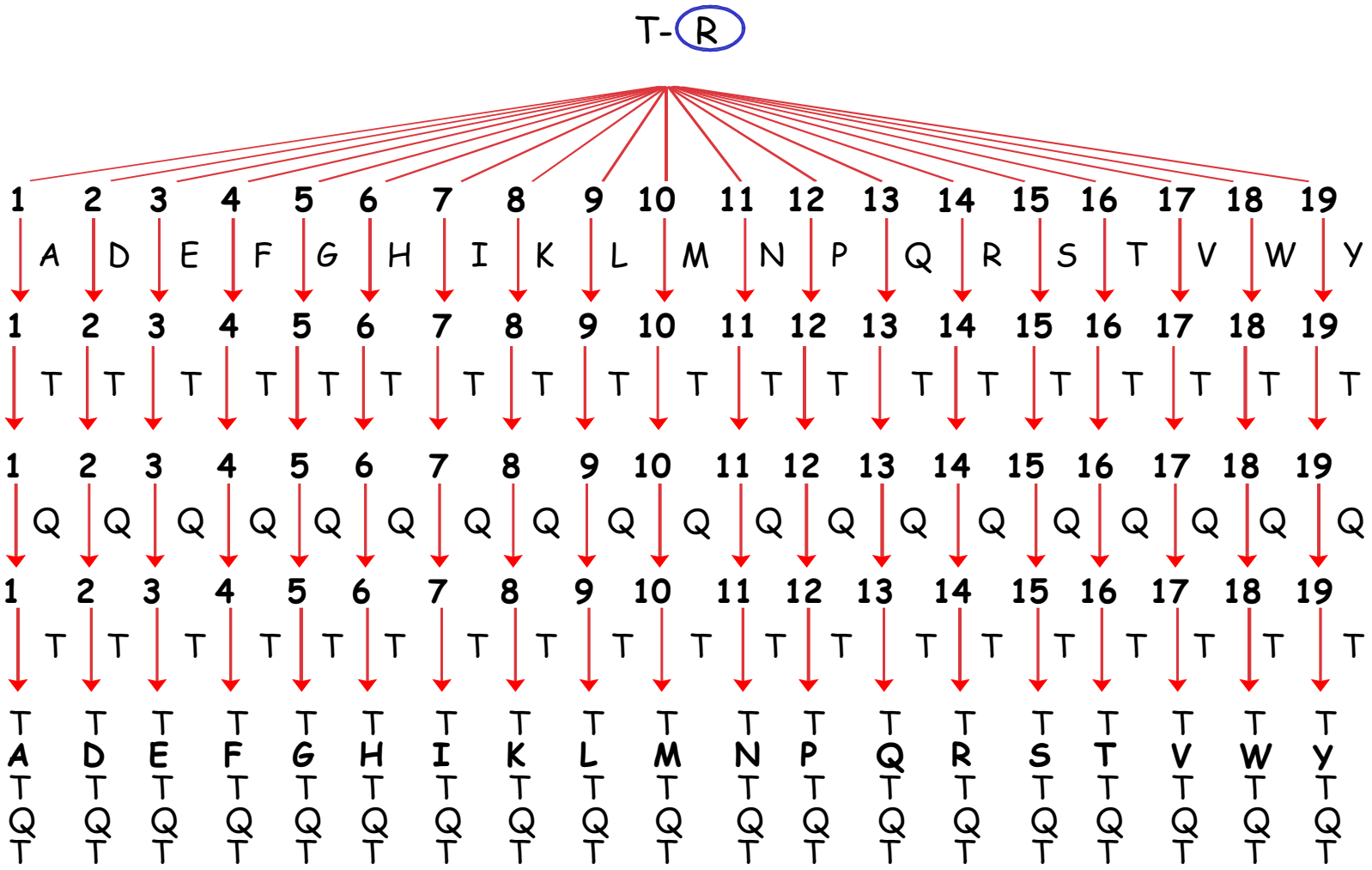
Furka et al. Int. J.Pept. Prot. Res. (1991)

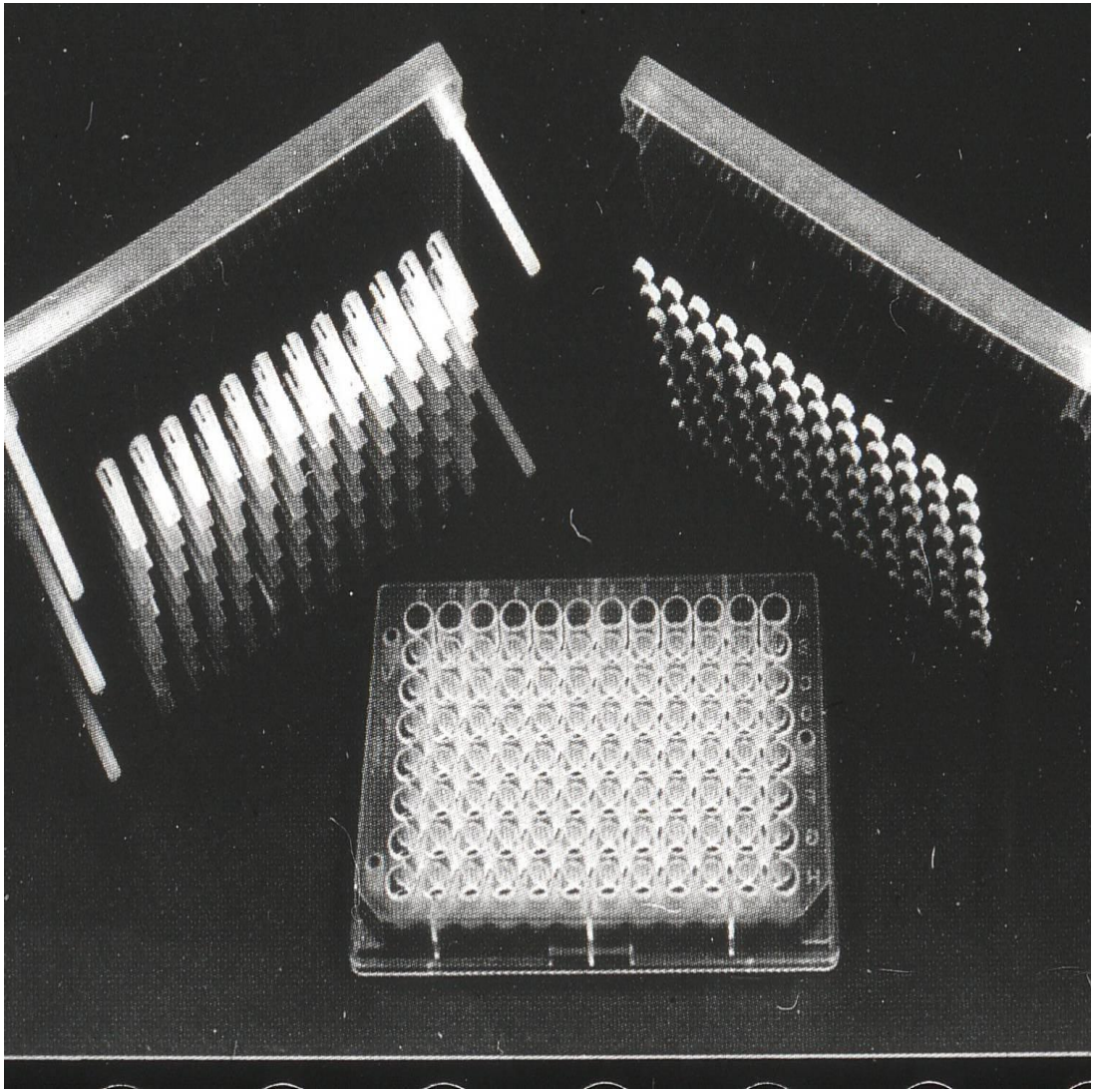


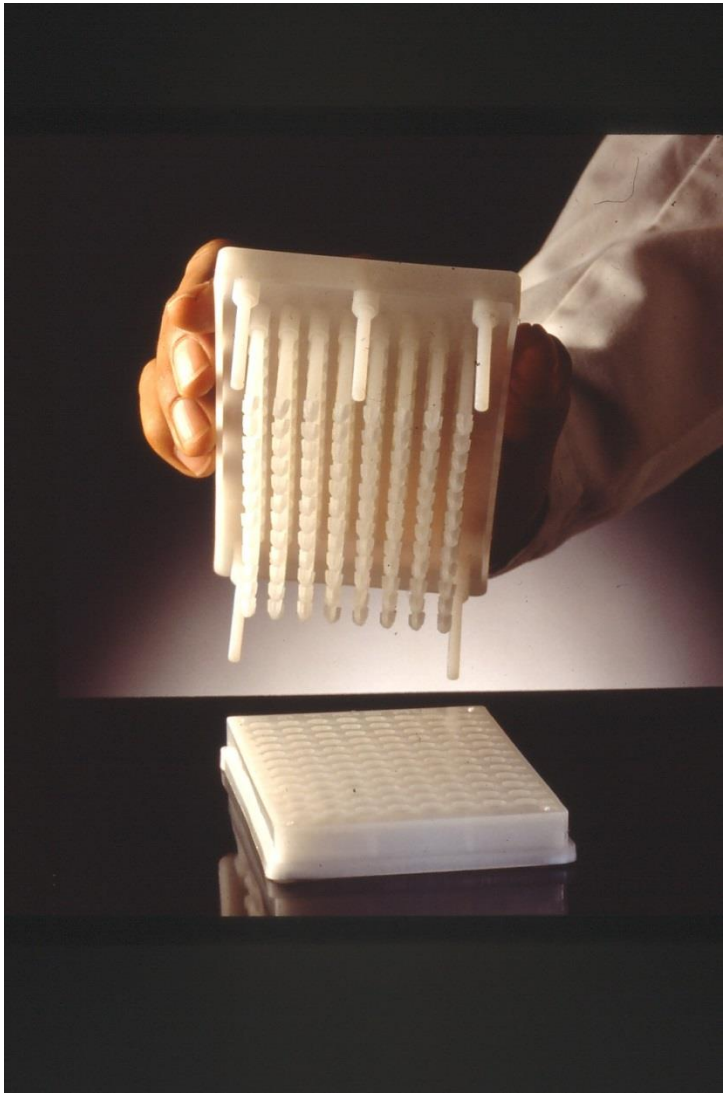
A TX^1TX^2T peptidtár kombinatorikus szintézise

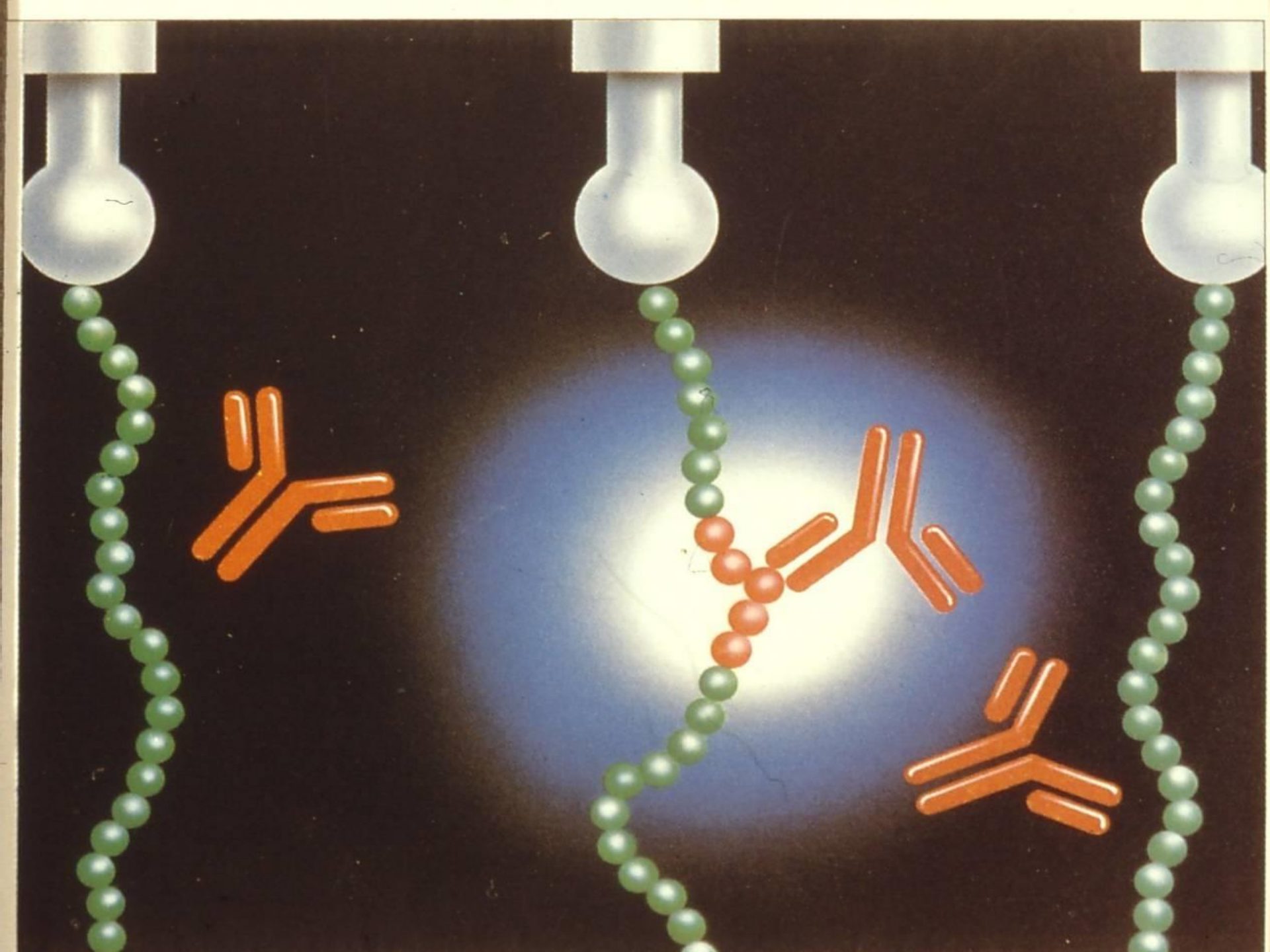


A TQTX²T alpeptid tárak szintézise

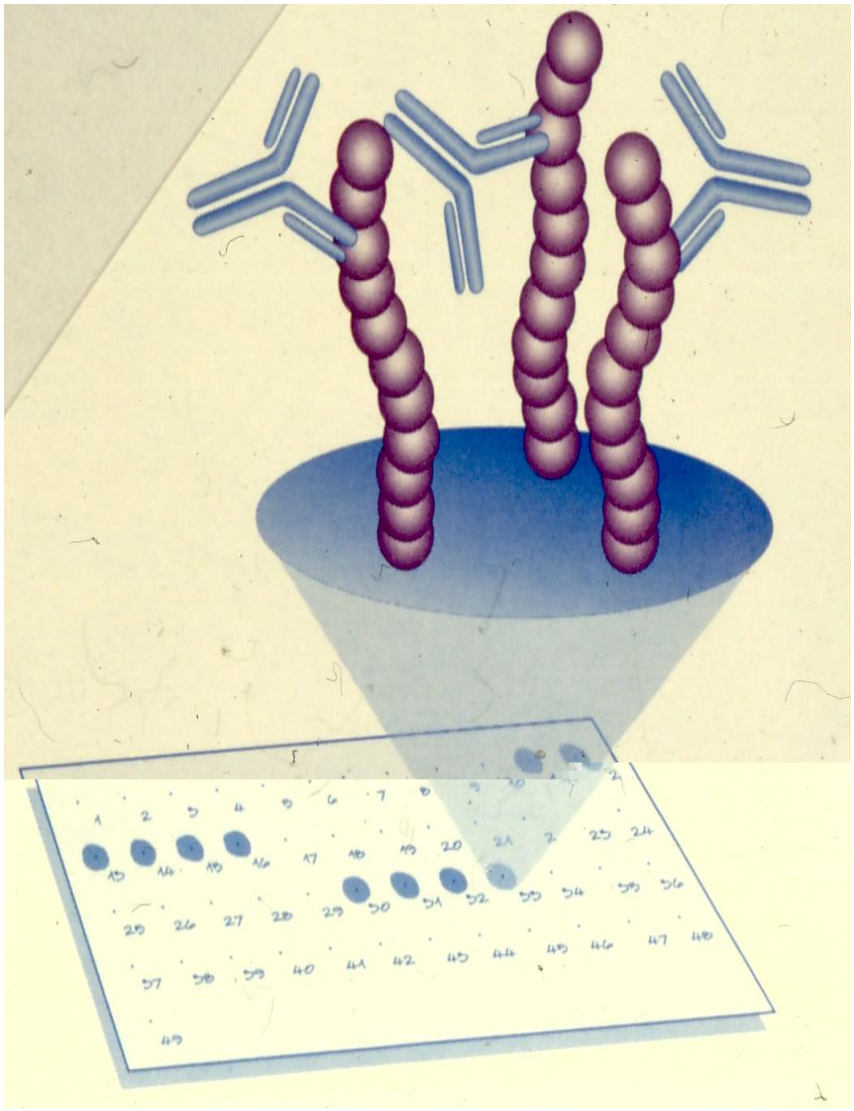


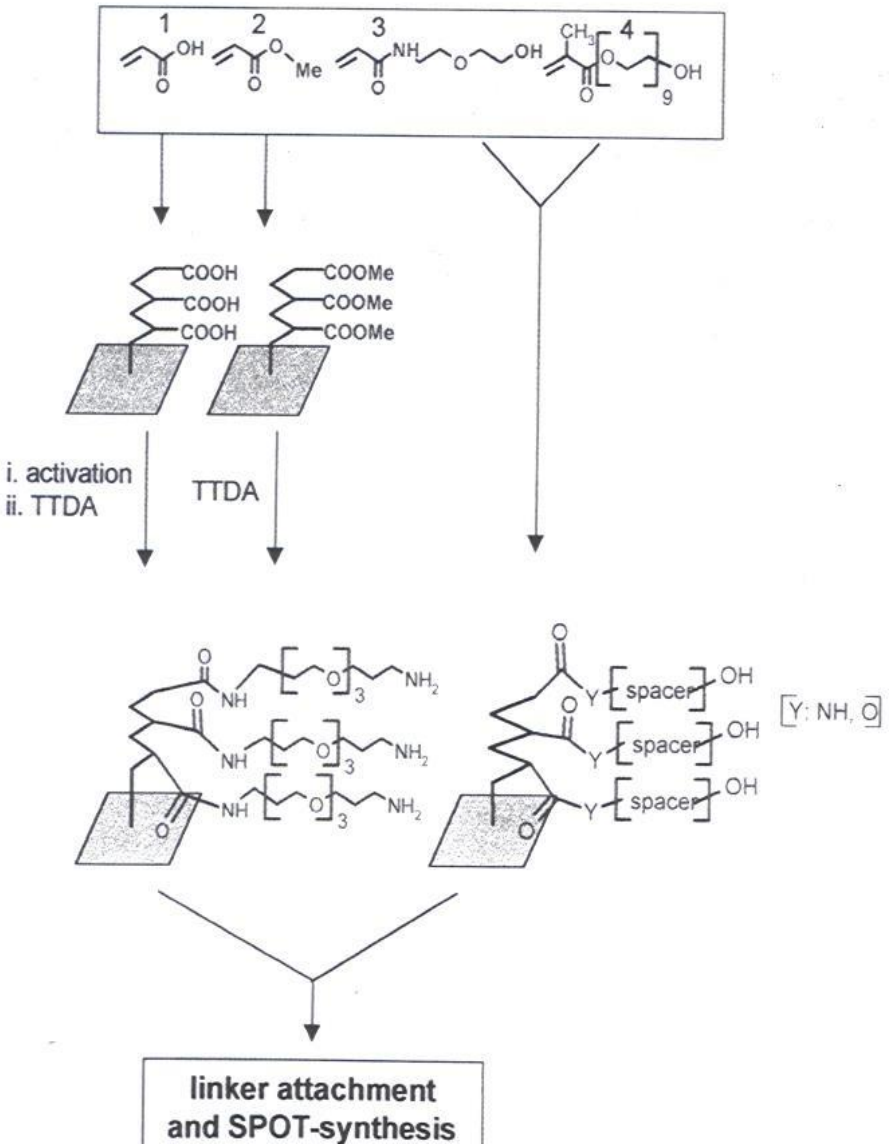




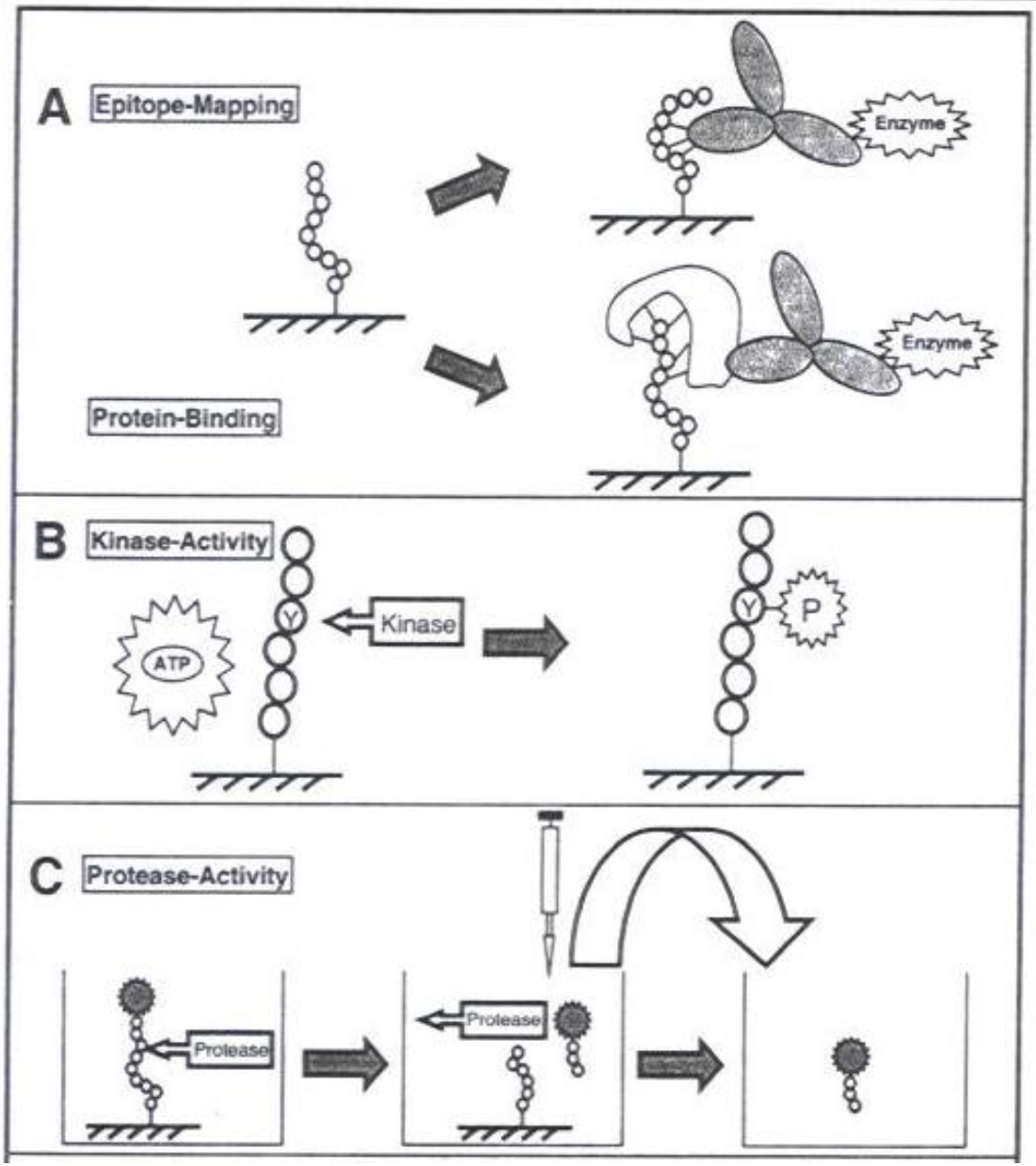


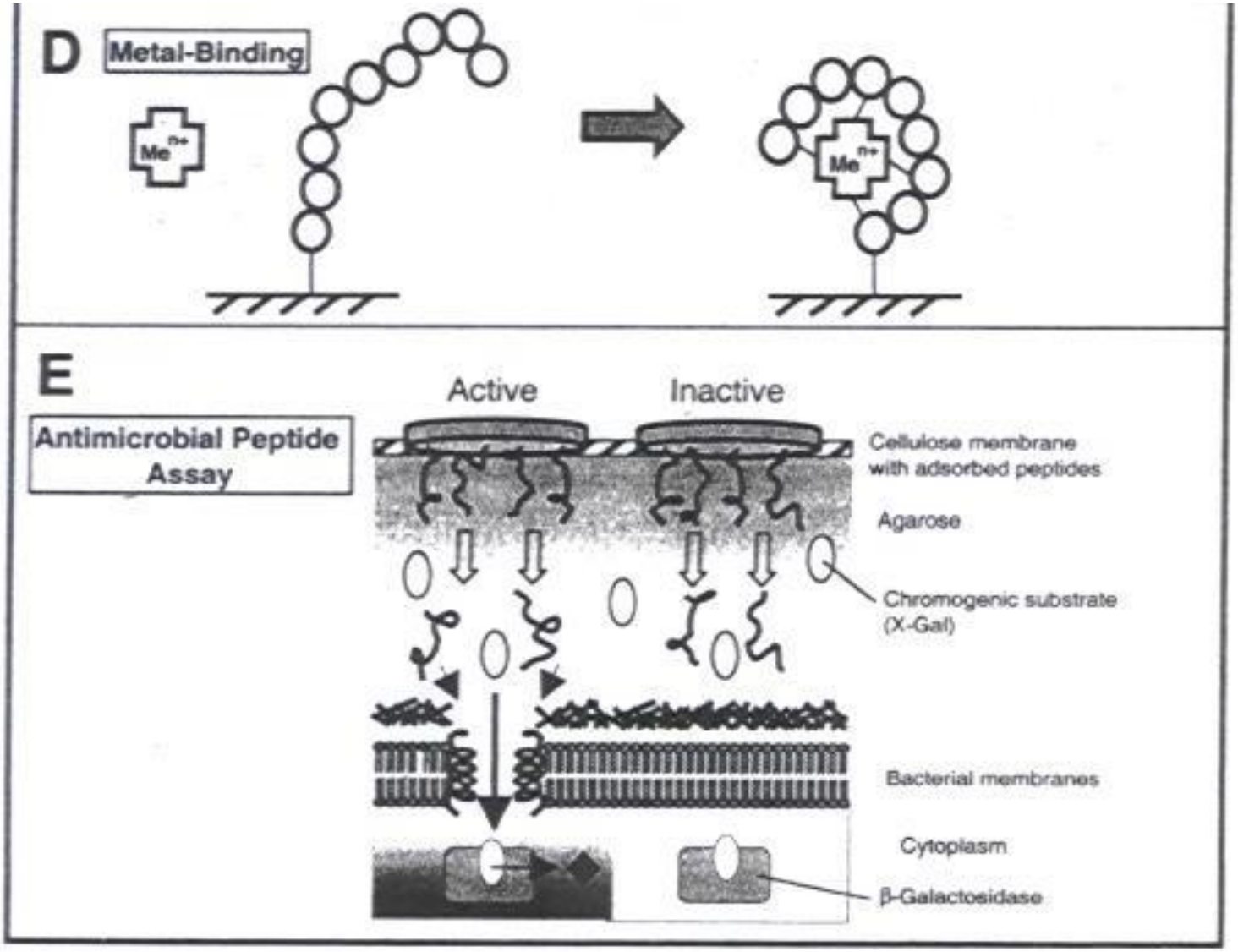
Folt (spot) peptidszintézis



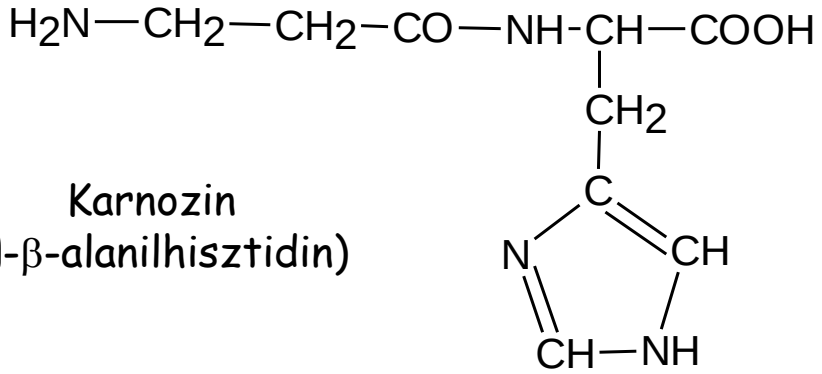


Alkalmazások




D
Metal-Binding
Me n+
Me n+
E
Antimicrobial Peptide Assay
Active
Inactive
Cellulose membrane with adsorbed peptides
Agarose
Chromogenic substrate (X-Gal)
Bacterial membranes
Cytoplasm
β-Galactosidase

Néhány „fontos” peptid



Karnozin
(N-β-alanilhisztidin)

Glutation

Glu
|
Cys-Gly

Izolálás: 1921 Hopkins
Szerkezet: 1930
Szintézis: 1935

Cisztin
↓
Cisztein

γ-L-glutamil-L-ciszteinil-glicin

Oxitocin

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

Peptidhormon: tejelválasztás, uterus kontrakció
Szerkezet: 1953 Duvigneaud (Nobel díj 1955)
Szintézis: 1954 Duvigneau

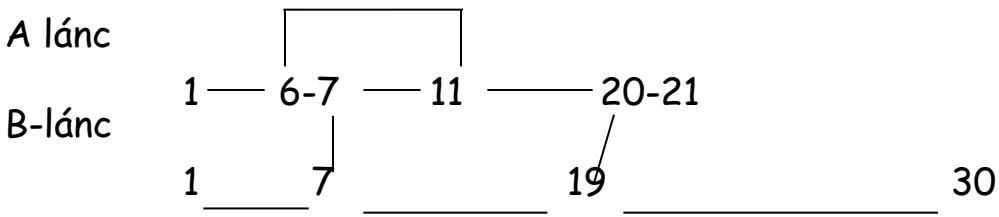
Vazopresszin

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH_2

Vérnyomás szabályozása, Duvigneau

Inzulin

Hasnyálmirigy hormonja



Izolálás: 1922 Banting Primer
Szerkezet: 1953 Sanger
Szintézis: 1969 Zahn, Wang, Katsoyannis
Térszerkezet: 1965 Hodgkin

ATCH

39 aminosav
Sertés: szintézis Schwitzer, 1963
Humán: szintézis Bajusz, Kisfaludy, Medzihradszky 1971