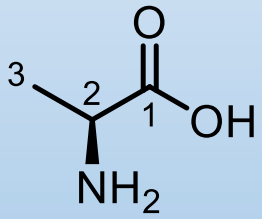
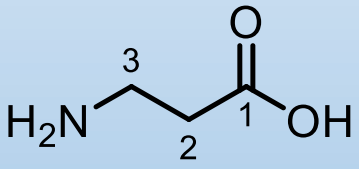
Aminokyseliny, Peptidy Proteiny

Aminokyseliny (AMK)

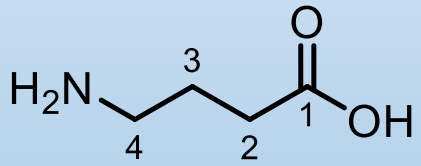
- Přirozeně vyskytující se aminokyseliny jsou převážně α-aminokyseliny (2-aminokarboxylové kyseliny) - jsou stavebními složkami peptidů
- Méně časté ß-aminokyseliny (ß-alanin) nebo γ-aminokyseliny (GABA) – různé funkce, složky biomolekul nebo neurotransmiterů



L-alanine



beta-alanine



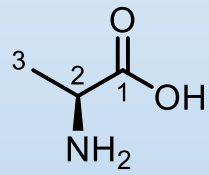
GABA

AMK - funkce

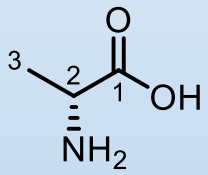
- Stavební složky: peptidů a fosfolipidů (geneticky kódováno asi 20 z nich – pouze tyto proteinogenní AMK se nacházejí pravidelně ve všech bílkovinách)
- Prekursory pro syntézu: oxokyselin, biogenních aminů, glukózy, hemu, nukleotidů, hormonů a neurotransmiterů
- Neurotransmitery: glutamát, aspartát, glycin

Enantiomery

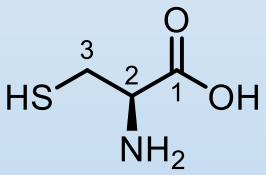
- Uhlík C-2 u α-aminokyselin nese stereogenní centrum



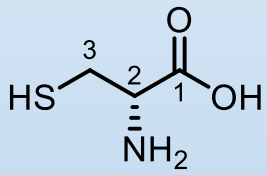
L-alanin



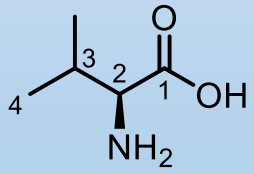
D-alanin



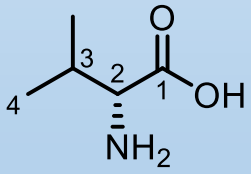
L-cystein



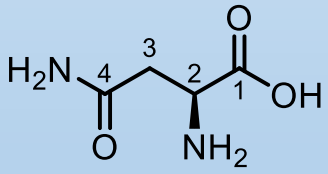
D-cystein



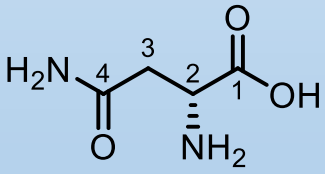
L-valin



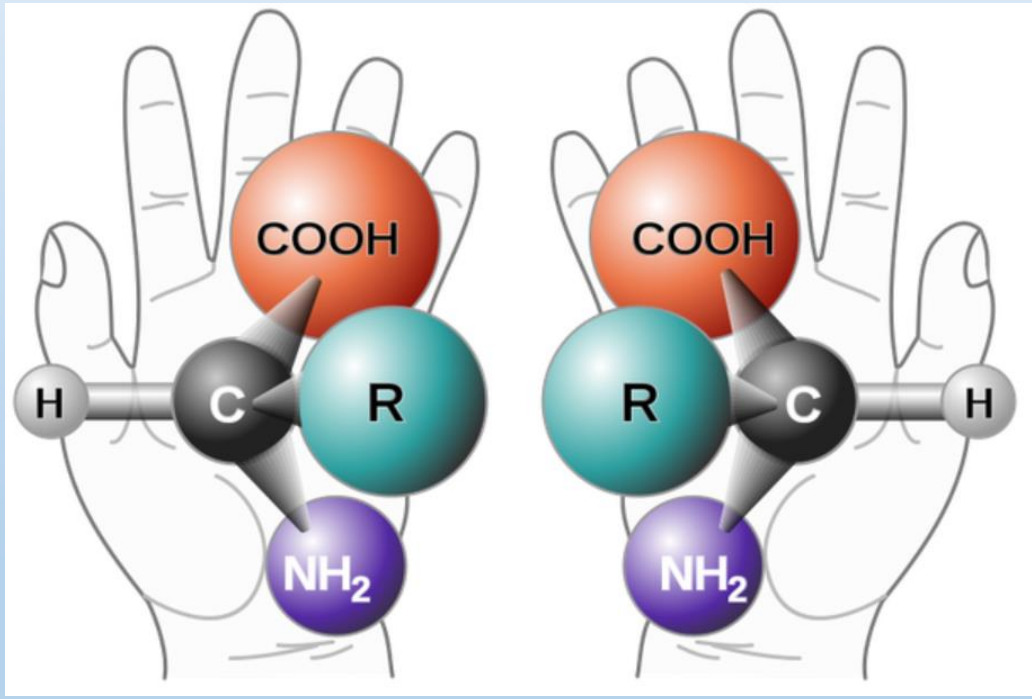
D-valin



L-asparagin

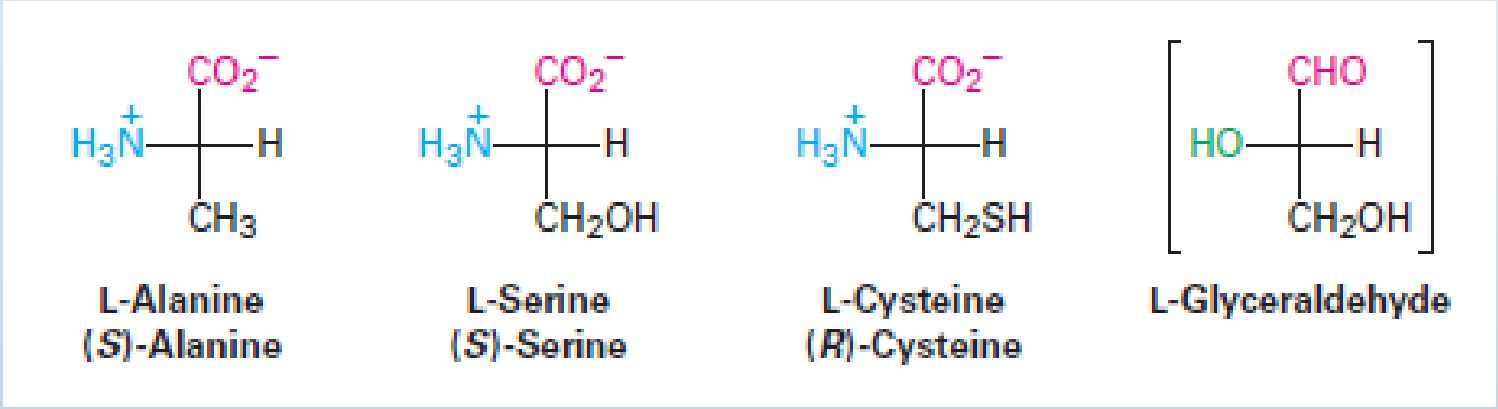


D-asparagin

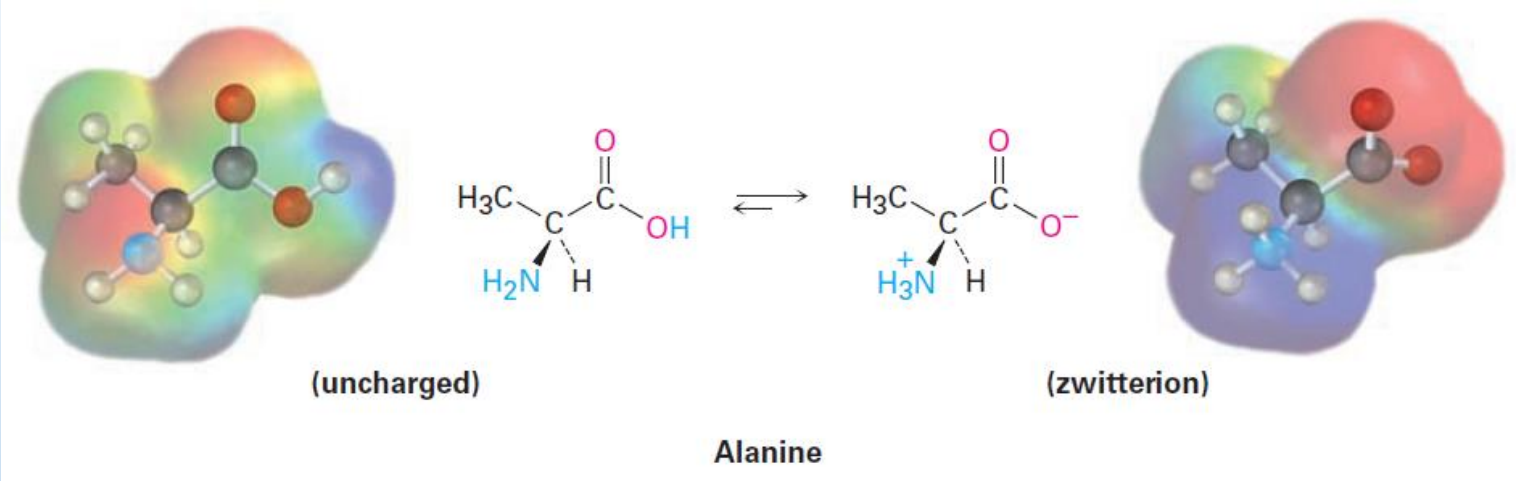


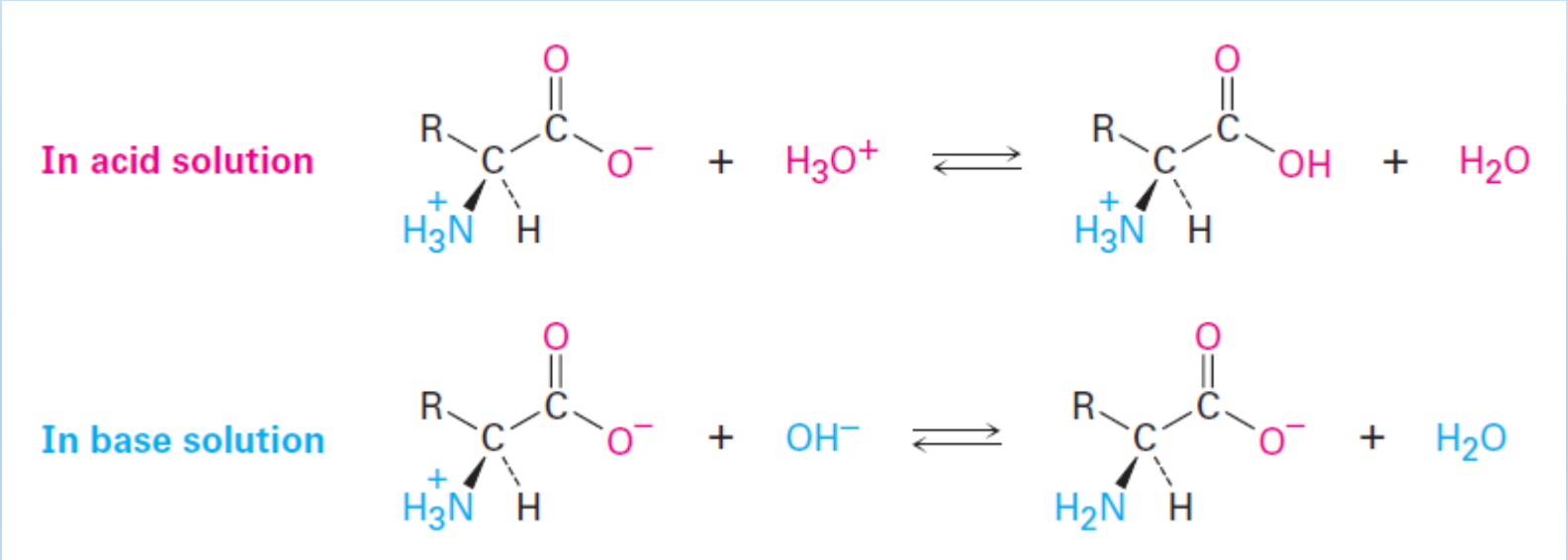
https://www.quora.com/Do-D-amino-acids-illicit-a-pathogen-recognition-response-What-advantage-does-a-bacterium-have-in-using-D-amino-acids-instead-of-L

Fischerova projekce



Struktura a chování AMK





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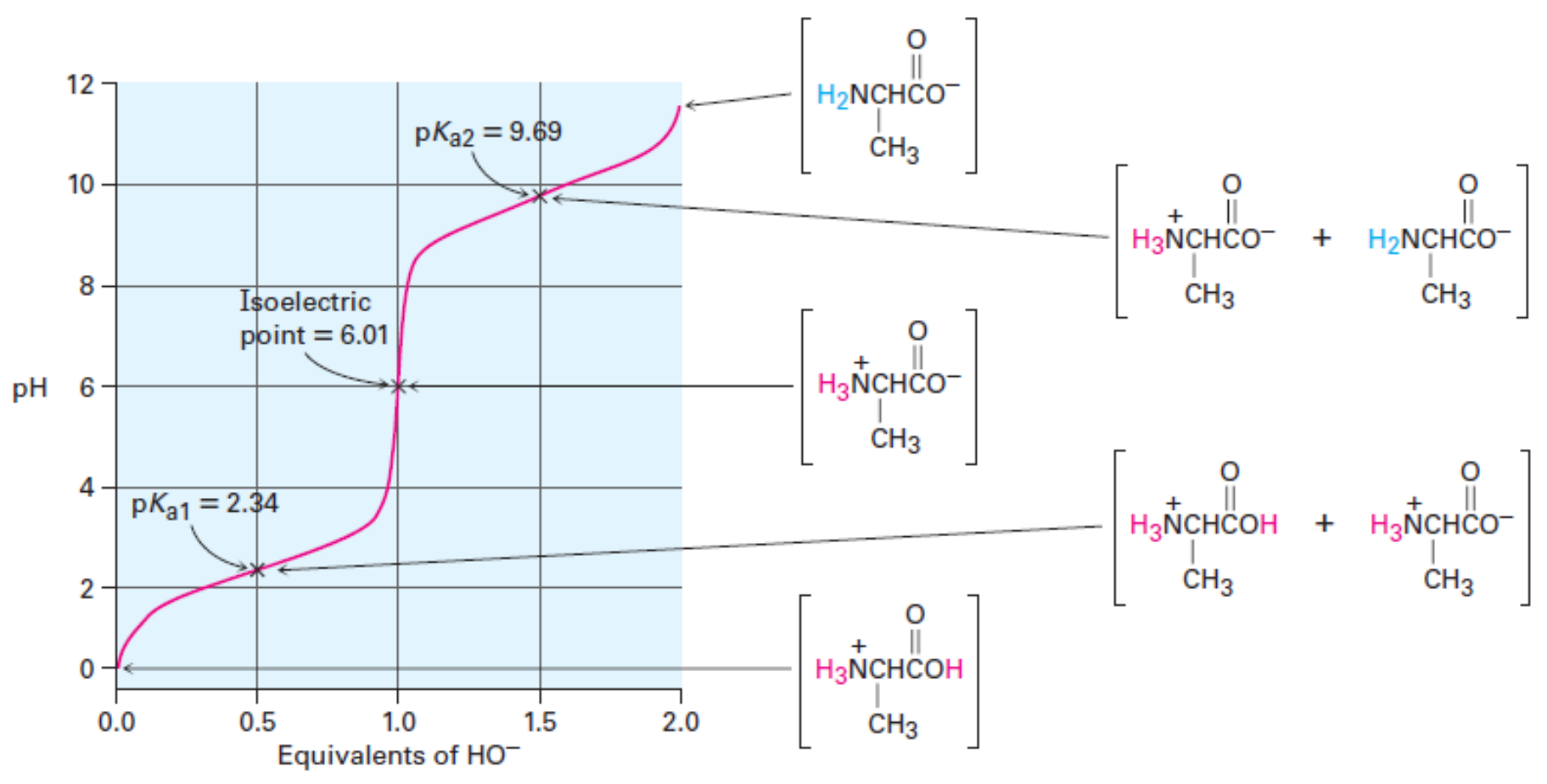
Titrační křivka alaninu



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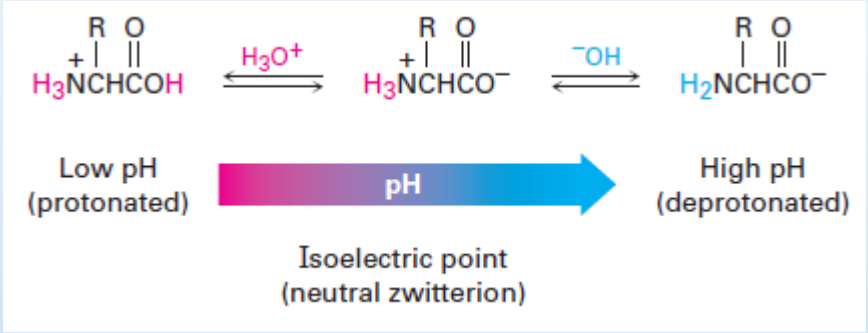

R O
+
H3NCHCOH
H3O+
R O
+
H3NCHCO−
−OH
R O
H2NCHCO−
Low pH
(protonated)
pH
High pH
(deprotonated)
Isoelectric point
(neutral zwitterion)

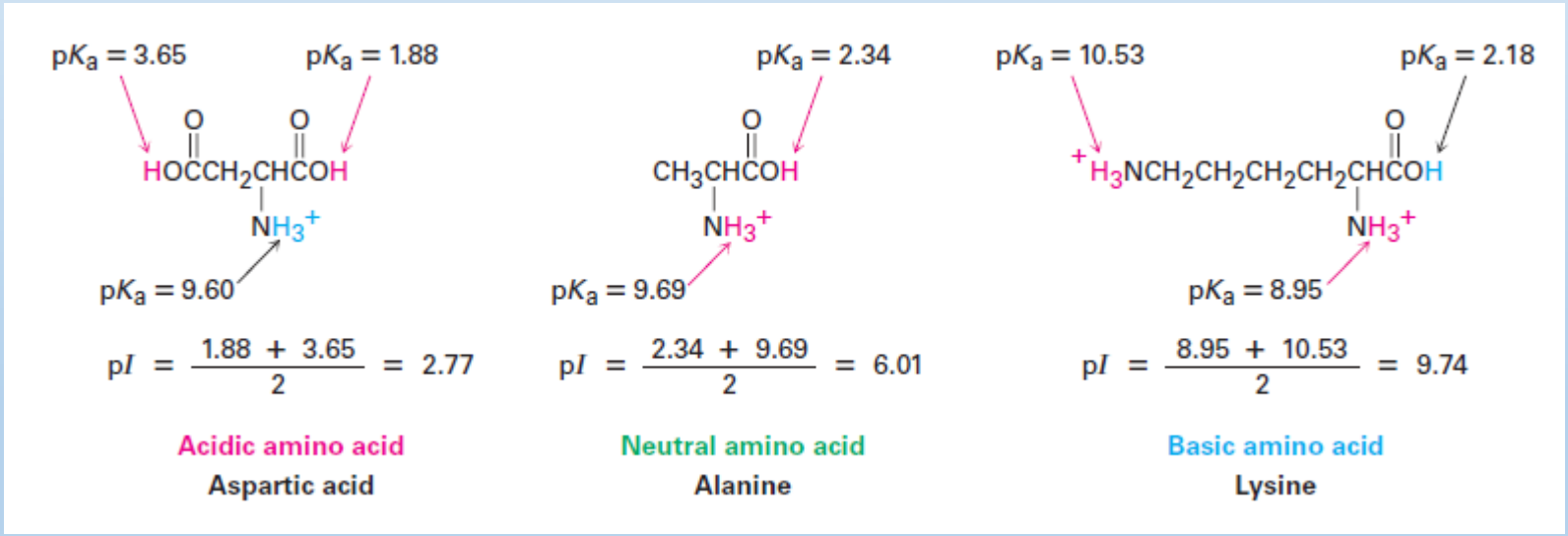

pKa = 3.65
pKa = 1.88
HOCCH2CHCOH
NH3+
pKa = 9.60
pI = 1.88 + 3.65 / 2 = 2.77
Acidic amino acid
Aspartic acid
pKa = 2.34
CH3CHCOH
NH3+
pKa = 9.69
pI = 2.34 + 9.69 / 2 = 6.01
Neutral amino acid
Alanine
pKa = 10.53
pKa = 2.18
+H3NCH2CH2CH2CH2CHCOH
NH3+
pKa = 8.95
pI = 8.95 + 10.53 / 2 = 9.74
Basic amino acid
Lysine

Table 26.1 The 20 Common Amino Acids in Proteins

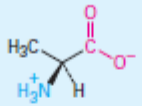
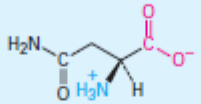
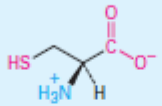
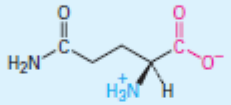
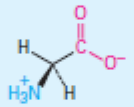
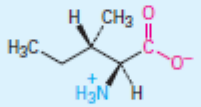
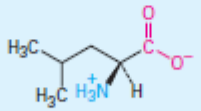
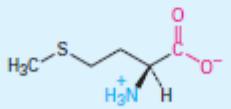
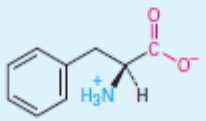
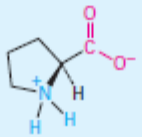
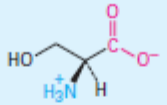
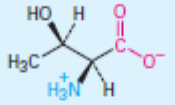
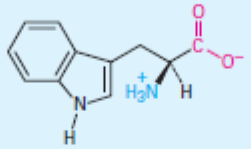
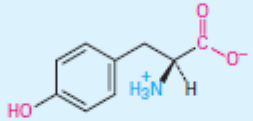
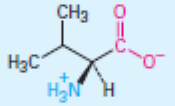
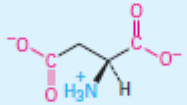
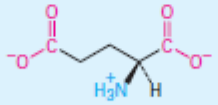
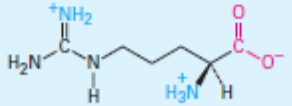
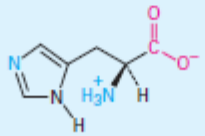
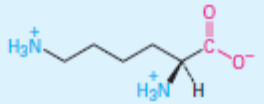
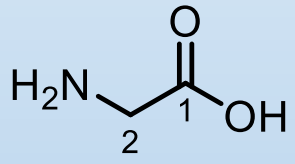
Name	Abbreviations		MW	Stucture	pK_a α-CO_2H	pK_a α-NH_3^+	pK_a side chain	pI
Neutral Amino Acids								
Alanine	Ala	A	89		2.34	9.69	—	6.01
Asparagine	Asn	N	132		2.02	8.80	—	5.41
Cysteine	Cys	C	121		1.96	10.28	8.18	5.07
Glutamine	Gln	Q	146		2.17	9.13	—	5.65
Glycine	Gly	G	75		2.34	9.60	—	5.97
Isoleucine	Ile	I	131		2.36	9.60	—	6.02
Leucine	Leu	L	131		2.36	9.60	—	5.98
Methionine	Met	M	149		2.28	9.21	—	5.74
Phenylalanine	Phe	F	165		1.83	9.13	—	5.48
Proline	Pro	P	115		1.99	10.60	—	6.30

Table 26.1 The 20 Common Amino Acids in Proteins *(continued)*

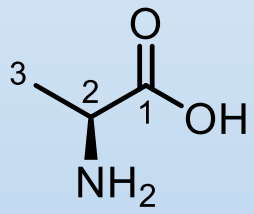
Name	Abbreviations		MW	Stucture	pK_a α-CO_2H	pK_a α-NH_3^+	pK_a side chain	pI
Neutral Amino Acids *(continued)*								
Serine	Ser	S	105		2.21	9.15	—	5.68
Threonine	Thr	T	119		2.09	9.10	—	5.60
Tryptophan	Trp	W	204		2.83	9.39	—	5.89
Tyrosine	Tyr	Y	181		2.20	9.11	10.07	5.66
Valine	Val	V	117		2.32	9.62	—	5.96
Acidic Amino Acids								
Aspartic acid	Asp	D	133		1.88	9.60	3.65	2.77
Glutamic acid	Glu	E	147		2.19	9.67	4.25	3.22
Basic Amino Acids								
Arginine	Arg	R	174		2.17	9.04	12.48	10.76
Histidine	His	H	155		1.82	9.17	6.00	7.59
Lysine	Lys	K	146		2.18	8.95	10.53	9.74

Proteogenní aminokyseliny

- 1) **Nepolární alifatické**: glycin (Gly, G), alanin (Ala, A), prolin (Pro, P), valin (Val, V), leucin (Leu, L), izoleucin (Ile, I), methionin (Met, M).
- Branched chain aminoacid – BCAA – Val, Leu, Ile, Met (esenciální)

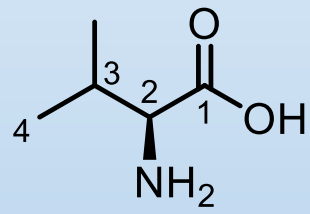


L-glycin

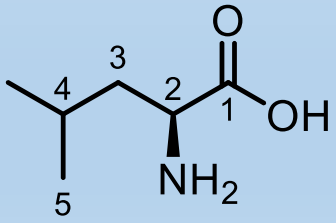


L-alanin

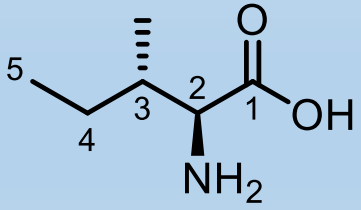
L-prolin



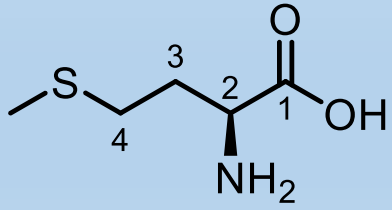
L-valin



L-leucin



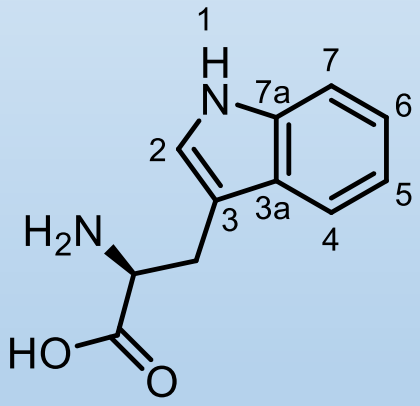
L-isoleucin



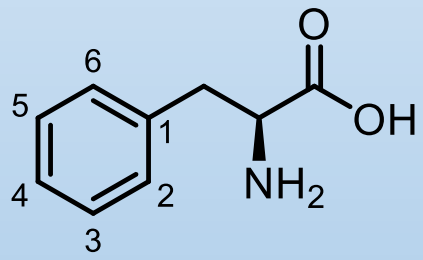
L-methionin

Proteogenní aminokyseliny

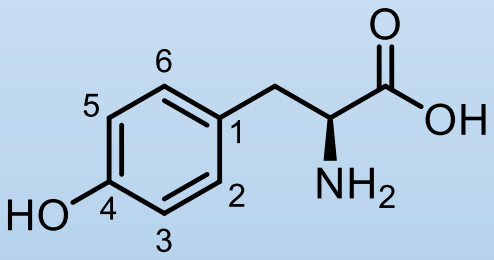
- 2) **Aromatické**: *nepolární* - tryptofan (Trp, W), fenylalanin (Phe, F), *polární* - tyrosin (Tyr, Y)



L-tryptofan



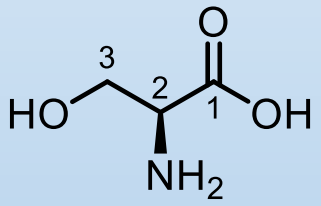
L-fenylalanin



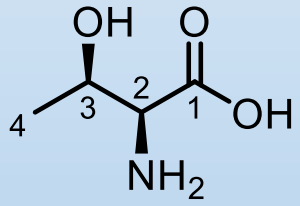
L-tyrosin

Proteogenní aminokyseliny

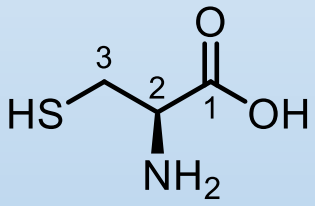
- 3) **Polární nenabité (neutrální)**: serin (Ser, S), threonin (Thr, T), cystein (Cys, C), asparagin (Asn, N), glutamin (Gln, Q), + 1 speciální AMK – selenocystein.



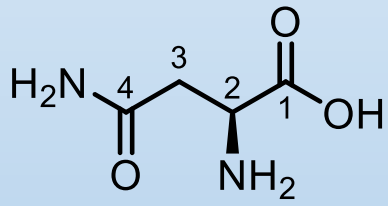
L-serin



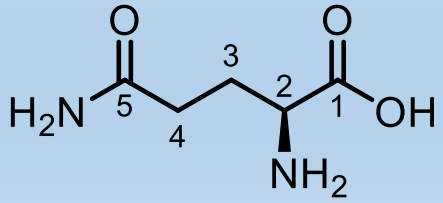
L-threonin



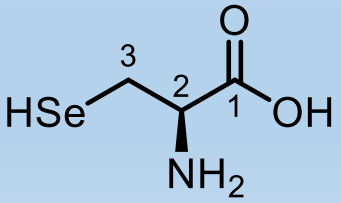
L-cystein



L-asparagin



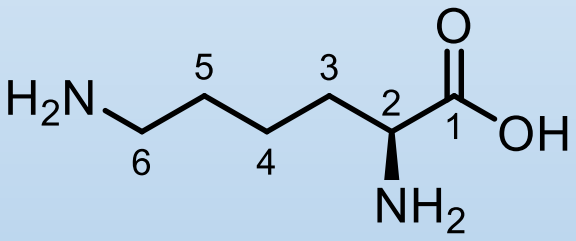
L-glutamin



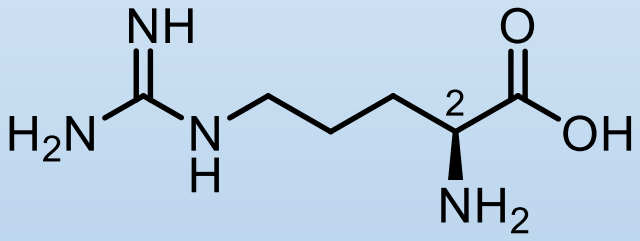
L-selenocystein

Proteogenní aminokyseliny

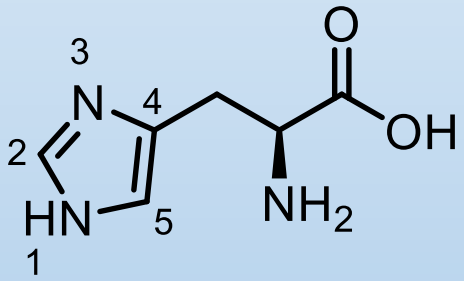
- 4) **Polární bazické**: lysin (Lys, K), arginin (Arg, R), histidin (His, H).



L-lysin



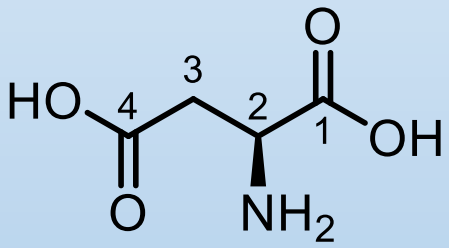
L-arginin



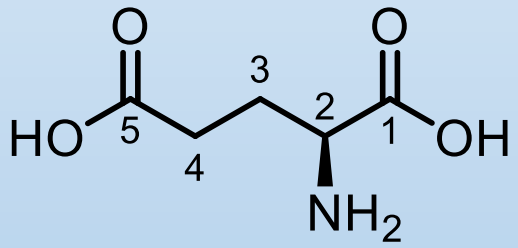
L-histidin

Proteogenní aminokyseliny

- 5) **Polární kyselé**: asparagová kyselina (Asp, D), glutamová kyselina (Glu, E).



L-kys. asparagová



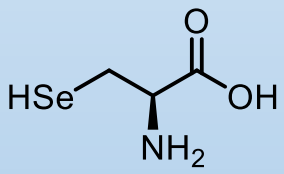
L-kys. glutamová

Proteogenní aminokyseliny

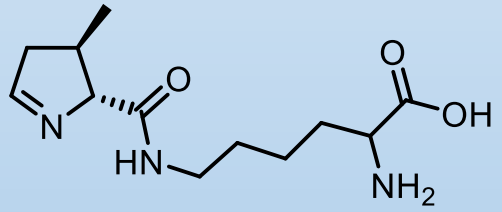
- Téměř polovinu esenciálních AMK si tělo nedokáže samo vyrobit a musí je přijímat z potravy – esenciální aminokyseliny – Val, Leu, Ile, Met, Phe, Trp, Thr, His, Lys.
- Pro kojence zvláště významné cystein a arginin
- Prolin – vytváření ohybů v peptidech
- Glycin – bez chirálního centra
- Thr a Ile – 2 chirální centra – ale v organismu se exlusivně vyskytuje jen (2S, 3R)-threonin, (2S, 3S)-isoleucin

Další aminokyseliny

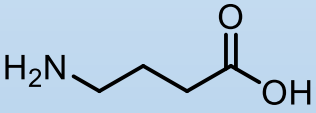
- Kromě 20-ti proteogenních AMK (AMK nacházených v proteinech) existuje několik dalších AMK.
- Selenocystein (geneticky kódován) – glutathion peroxidáza (odbourávání hydroxyperoxidů v lipidech), dejodáza (přeměna tyroxinu na trijodtyronin), thioredoxin reduktáza (antioxidační ochrana, syntéza deoxyribonukleotidů).
- Pyrrolysin (geneticky kódován) – stop codon UAG, biosytéza proteinů v methanobakteriích a archeách.
- GABA neurotransmiter v mozku
- Homocystein v krvi – spojován s onemocněními srdce (metabolit methioninu)
- Thyroxin – hormon štítné žlázy
- L-DOPA – syntéza katecholaminů
- Ornitin, citrulin – močovinový cyklus



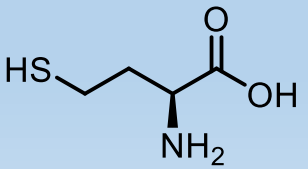
L-selenocystein



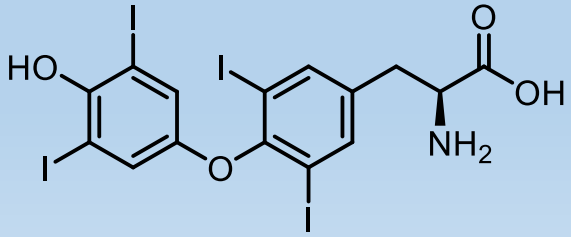
L-pyrrolysine



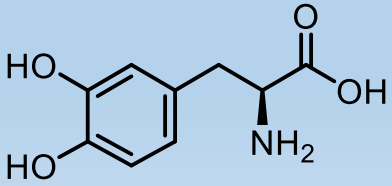
GABA



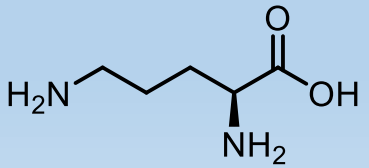
homocystein



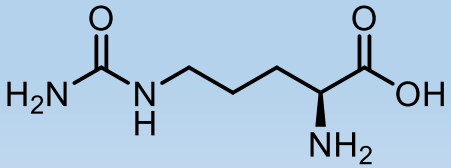
thyroxin



L-dopa



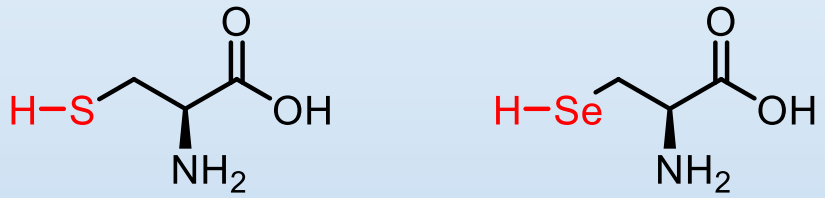
ornithin

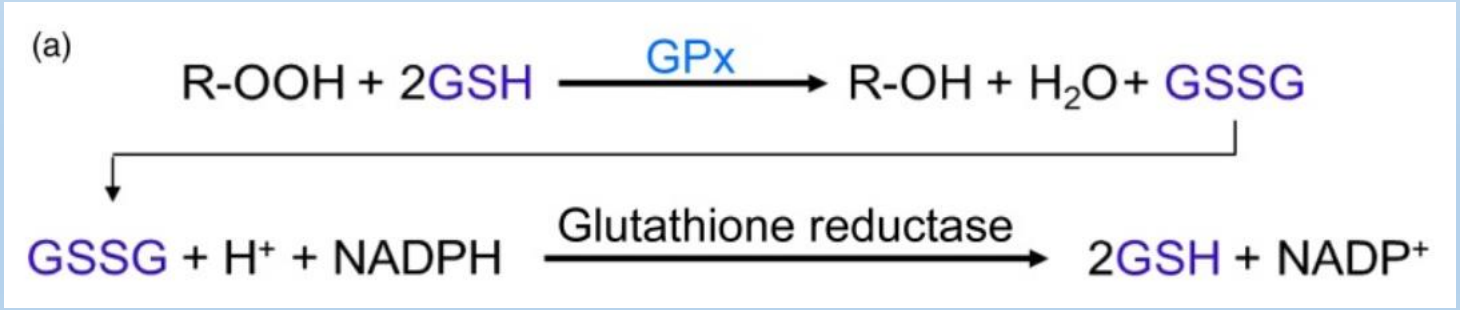


citrulin

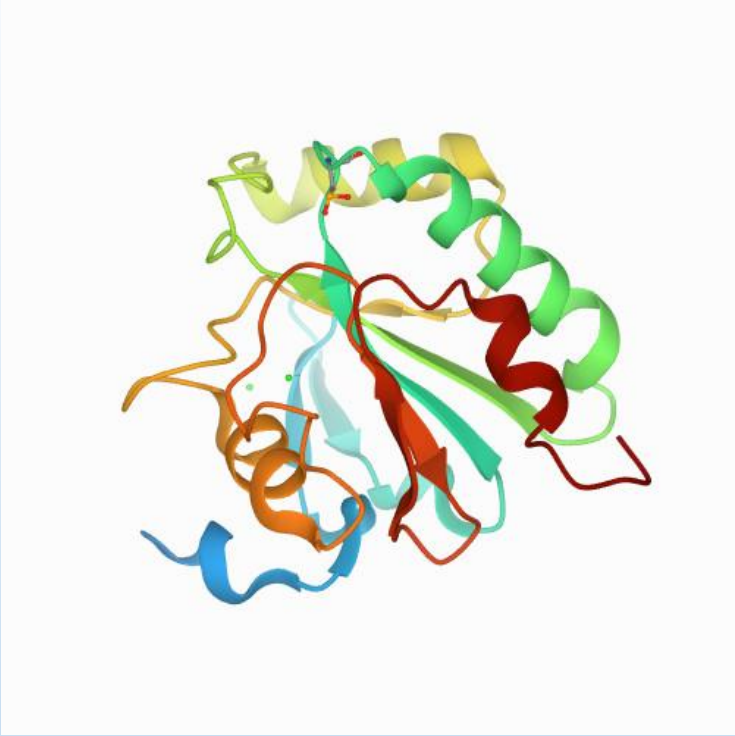
Cys vs. Sec

- Glutathion peroxidáza





DOI: 10.1017/jns.2013.17



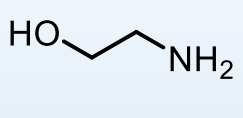
High resolution structure of selenocysteine containing human GPX4
DOI: 10.2210/pdb6ELW/pdb

Classification: OXIDOREDUCTASE
Organism(s): Homo sapiens
Expression System: Escherichia coli BL21(DE3)

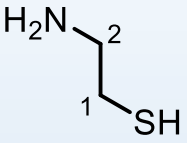
(Protein databank, https://www.rcsb.org/structure/6ELW)

Biogenní aminy

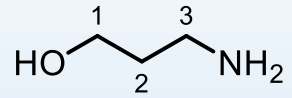
- Při odbourávání AMK dekarboxylázami aminokyselin (dekarboxylace) vznikají různé „biogenní aminy“
- Serin → ethanolamin → součást lipidů (membránové lipidy)
- Systein → cysteinamin → součást koenzymu A
- Threonin → aminopropanol → B12
- Aspartát → beta-Ala → koenzym A (také odbouráváním uracilu)
- Ornithin → putrescin → syntéza polyaminů
- Glutamát → γ-aminobutyrát → GABA (neurotransmiter)
- Histidin → histamin → mediátor (imunitní odpověď, alergická reakce), neurotransmiter
- DOPA → dopamin → neurotransmiter (porucha syntézy → Parkinsonova nemoc)
- 5-OH-tryptofan → serotonin → mediátor, neurotransmiter, neurohormon, krevní tlak
- Arginin → agmatin → neuromodulátor
- Inaktivace biogenních aminů probíhá pomocí monoanimooxidázy (MAO, regulace, NH_2 → COOH).



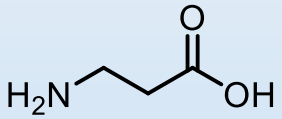
ethanolamin



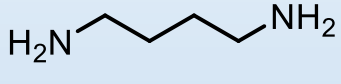
cysteinamin



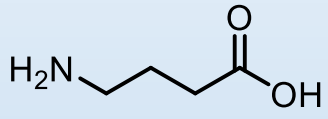
aminopropanol



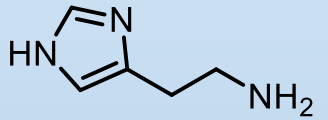
beta ala



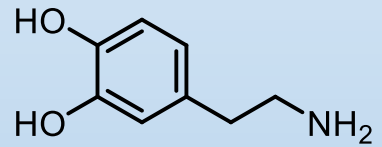
putrescin



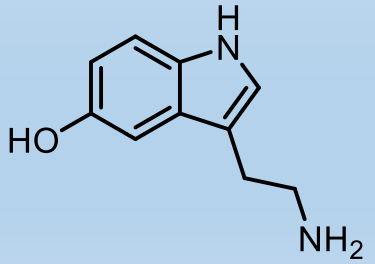
GABA



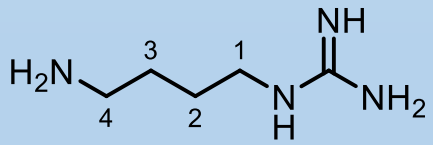
histamin



dopamin



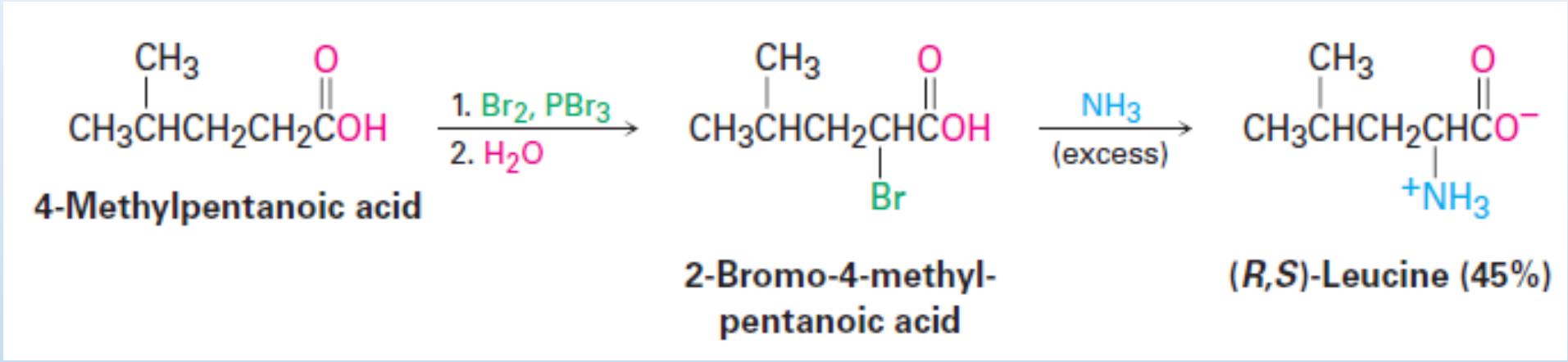
serotonin



agmatin

Chemická syntéza AMK

Hell-Volhard-Zelinski pak Sn2

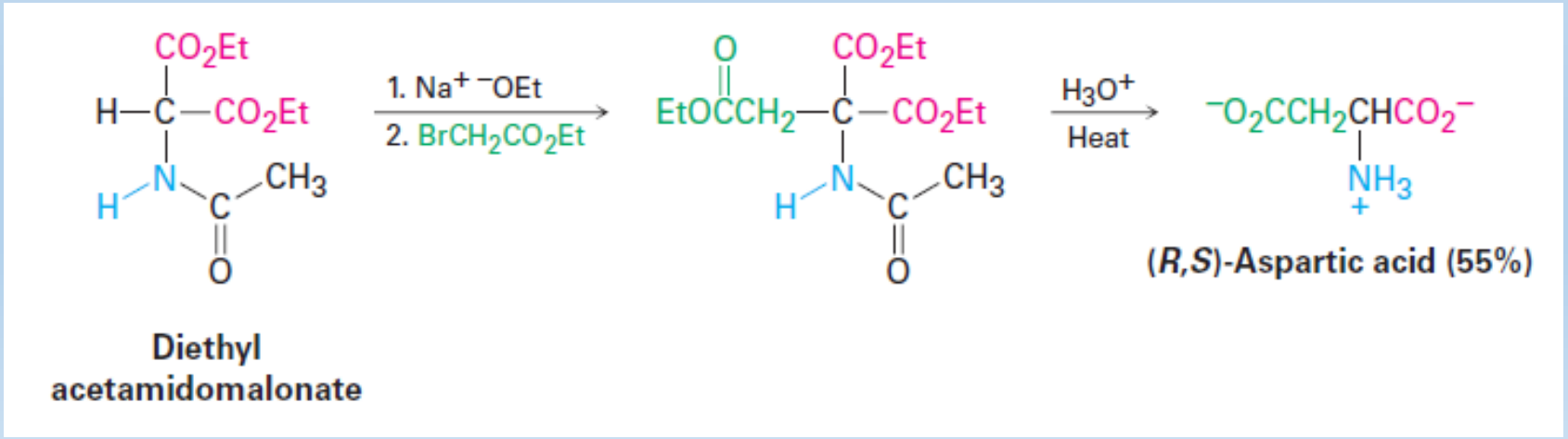


4-Methylpentanoic acid

2-Bromo-4-methyl-pentanoic acid

(*R,S*)-Leucine (45%)

Amidomalonátová syntéza

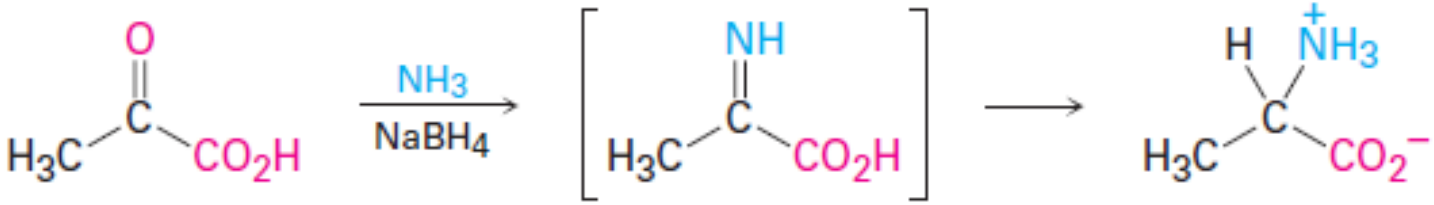


Diethyl acetamidomalonate

(*R,S*)-Aspartic acid (55%)

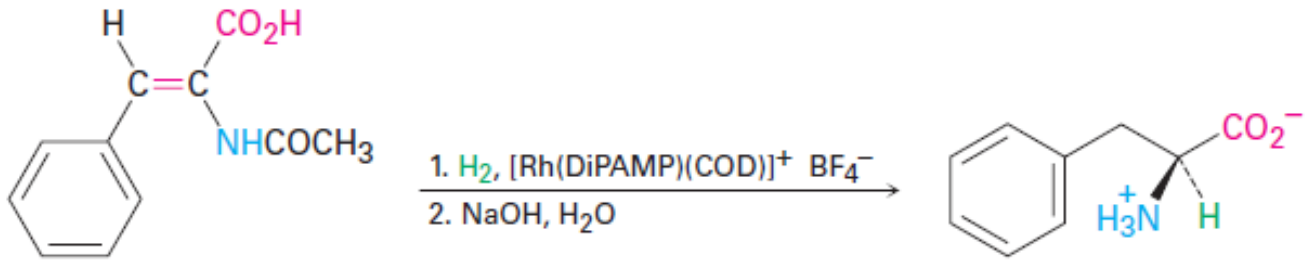
Chemická syntéza AMK

Reduktivní aminace α-ketokyselin

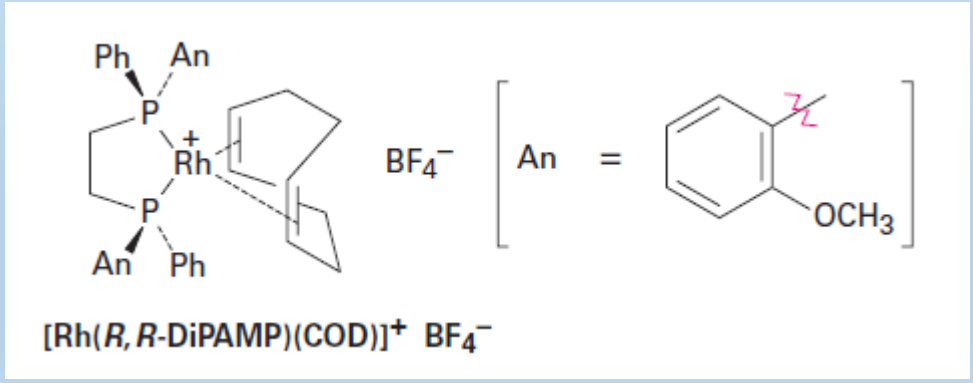


Pyruvic acid | Imine intermediate | (*R*,*S*)-Alanine

Katalytická hydrogenace (rhodium(I) komplex, Knowles 2001 Nobel price)



A (*Z*) enamido acid | (*S*)-Phenylalanine



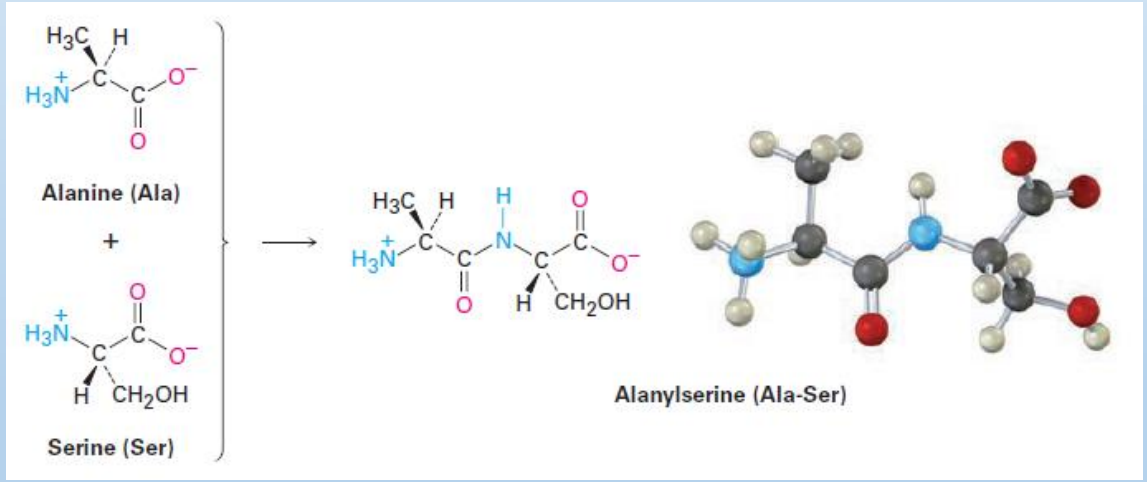
[Rh(*R*,*R*-DiPAMP)(COD)]+ BF_4^-

Peptidy a Proteiny

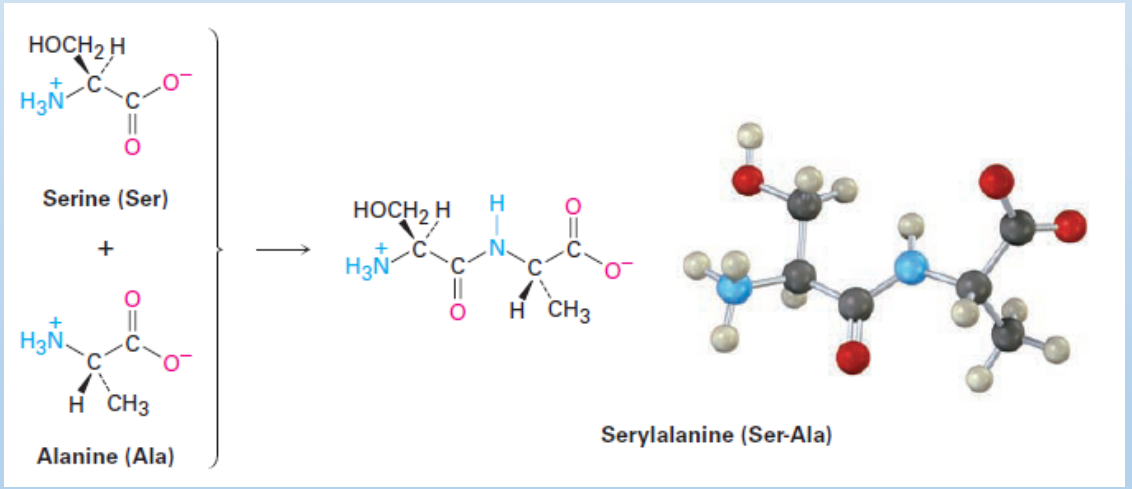
(příprava, analýza, struktura a funkce)

Peptidy a proteiny

- Proteiny a peptidy jsou polymery vzniklé spojením jednotlivých aminokyselin, skrze peptidovou vazbu. (aminoskupina jedné AMK vytvoří amidovou vazbu s karboxylovou kyselinou druhé AMK)
- Peptidy asi tak do 100 AMK
- Proteiny – delší než 100 AMK (bílkoviny)



@



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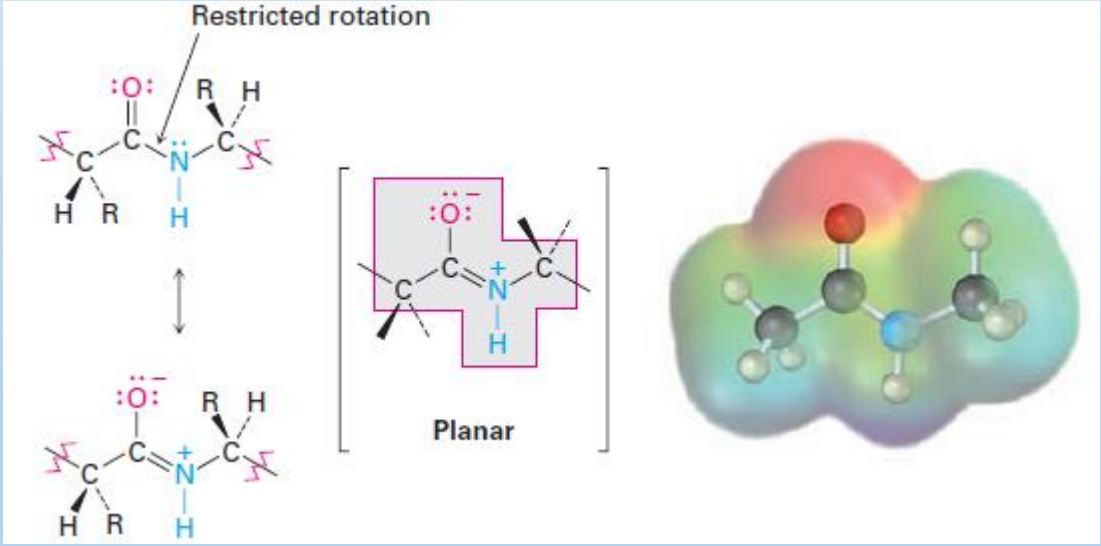
Peptidová vazba

- **Je planární** – omezená rotace kolem C-N vazby, která má částečný charakter dvojné vazby (volný el. pár na dusíku je delokalizovaný interakcí s karbonylovou skupinou a p-orbital dusíku se částečně překrývá p-orbitalem karbonylu)



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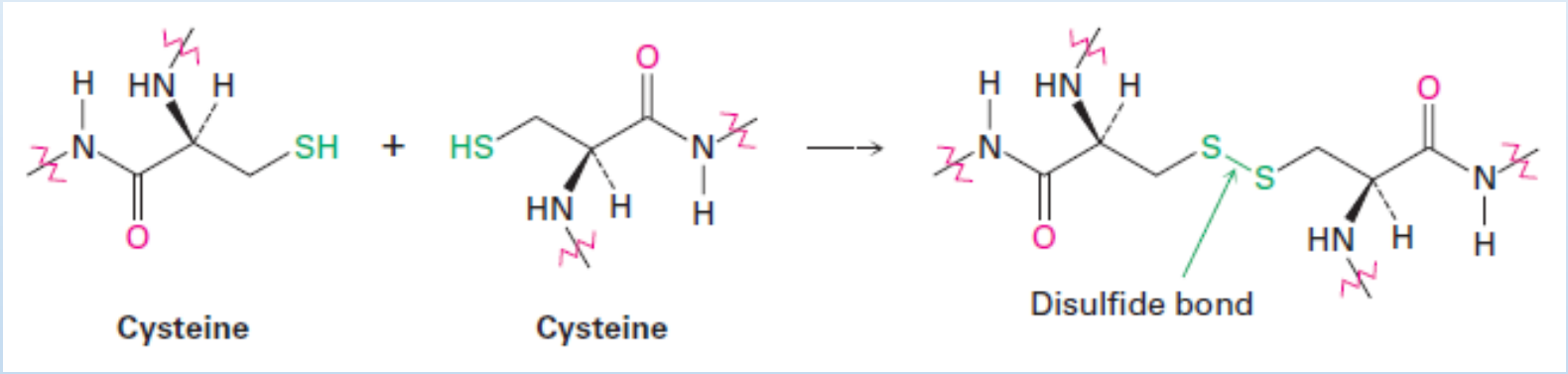
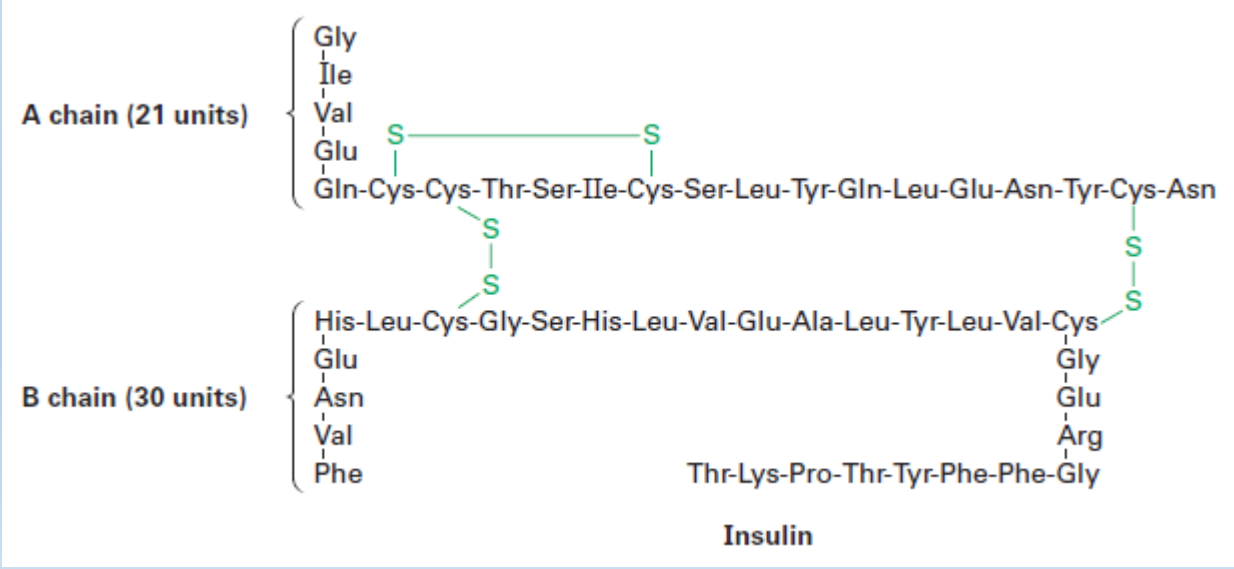
Disulfidový můstek

- Vzniká reakcí mezi dvěma cysteinovými AMK (cystin = 2 x cystein, oxidace thiolů)
- stabilizující efekt ve struktuře peptidů a proteinů





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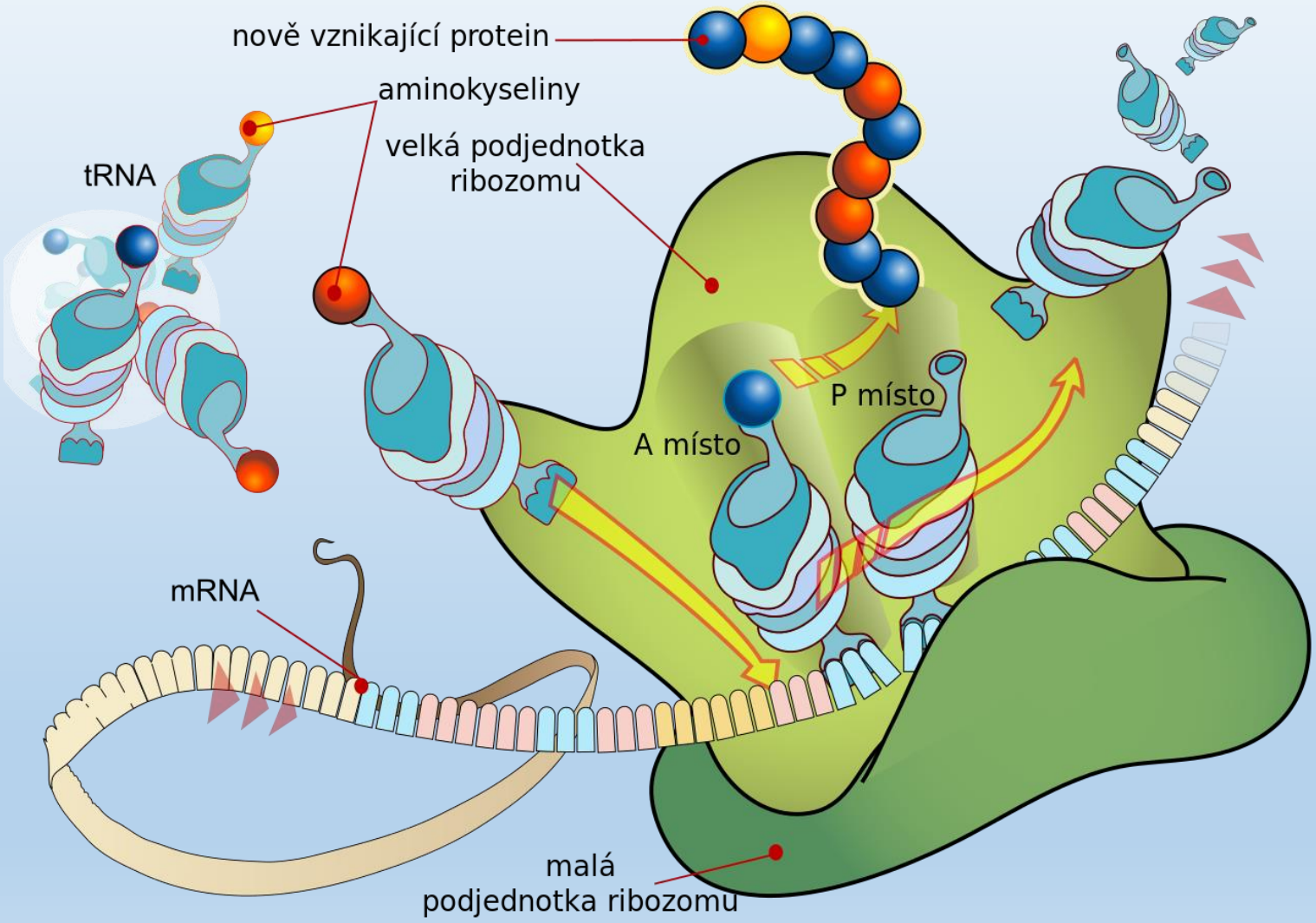
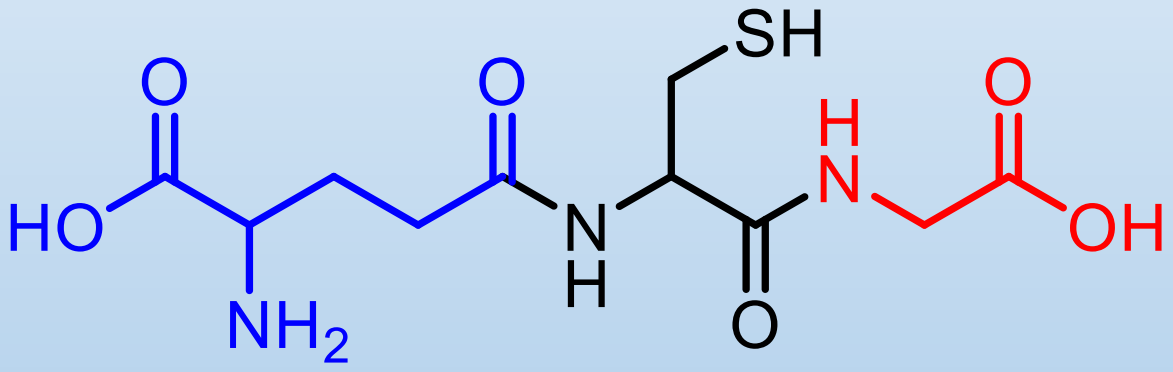
Vznik (biosyntéza) peptidů - Ribosom



Ribosome mRNA translation es.svg: https://commons.wikimedia.org/w/index.php?curid=32110356

Biologicky významné peptidy

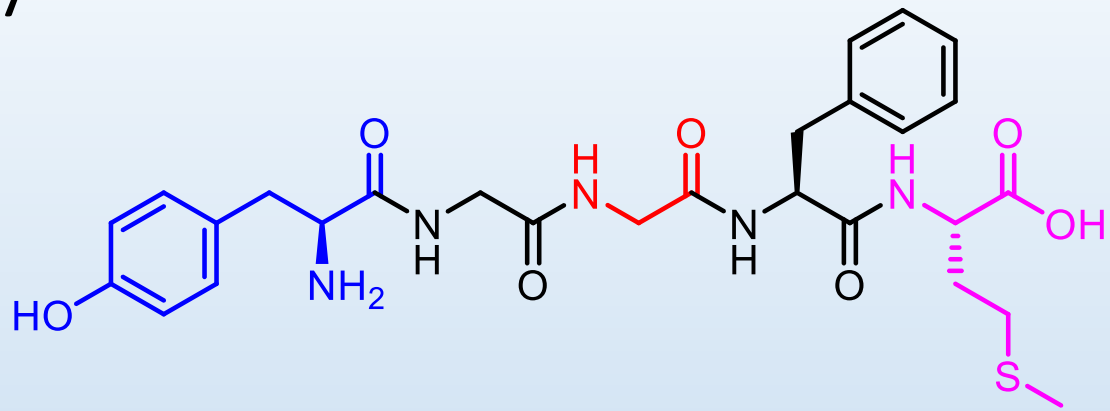
glutathion - redukovaná forma



Glu-Cys-Gly

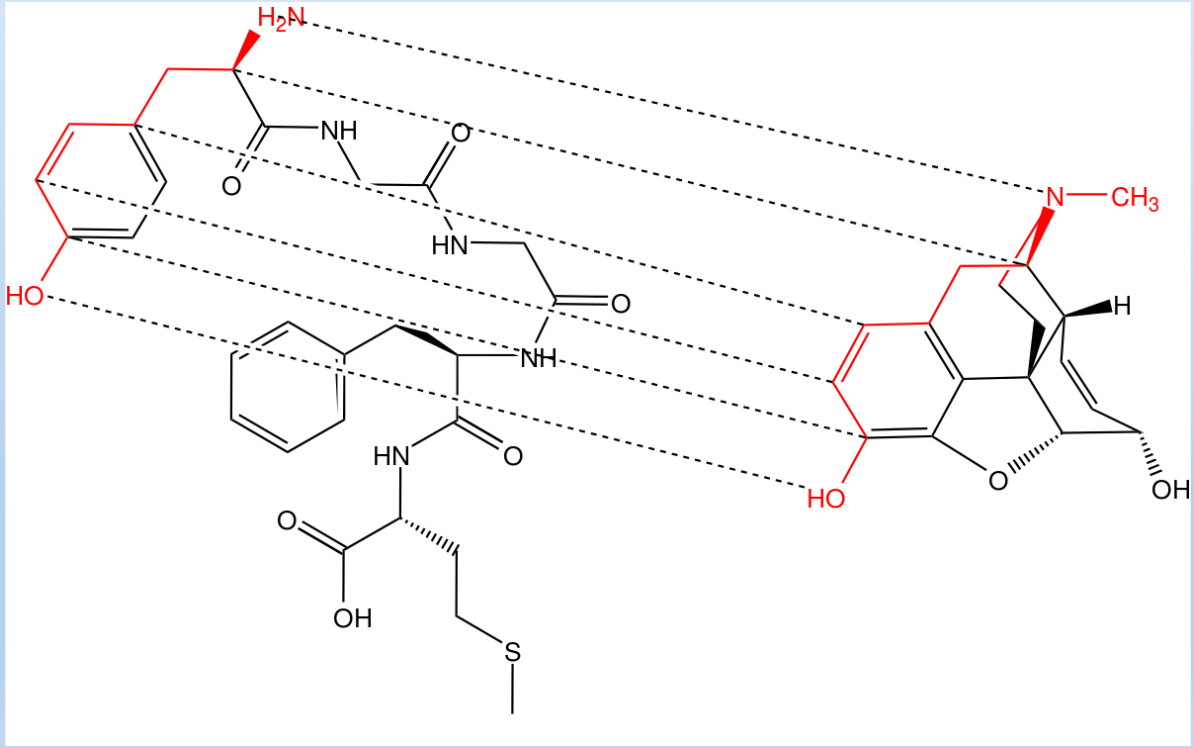
Biologicky významné peptidy

- Peptidové hormony – **methionin enkefalin**



tyr-gly-gly-phe-met

Met-enkefalin Morfin

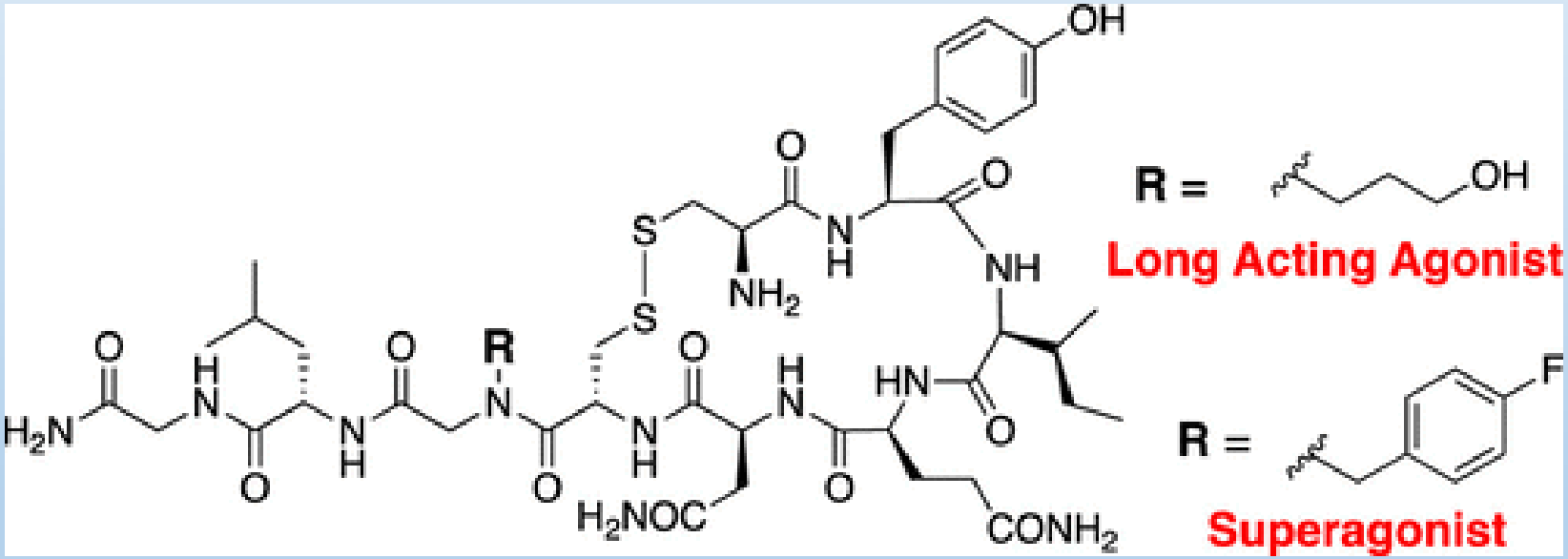


Porovnání vazebných motivů methioninu-enkefalinu a morfinu (PheAla se váže na stejné místo jako morfin v opioidních receptorech)

https://commons.wikimedia.org/

Biologicky významné peptidy

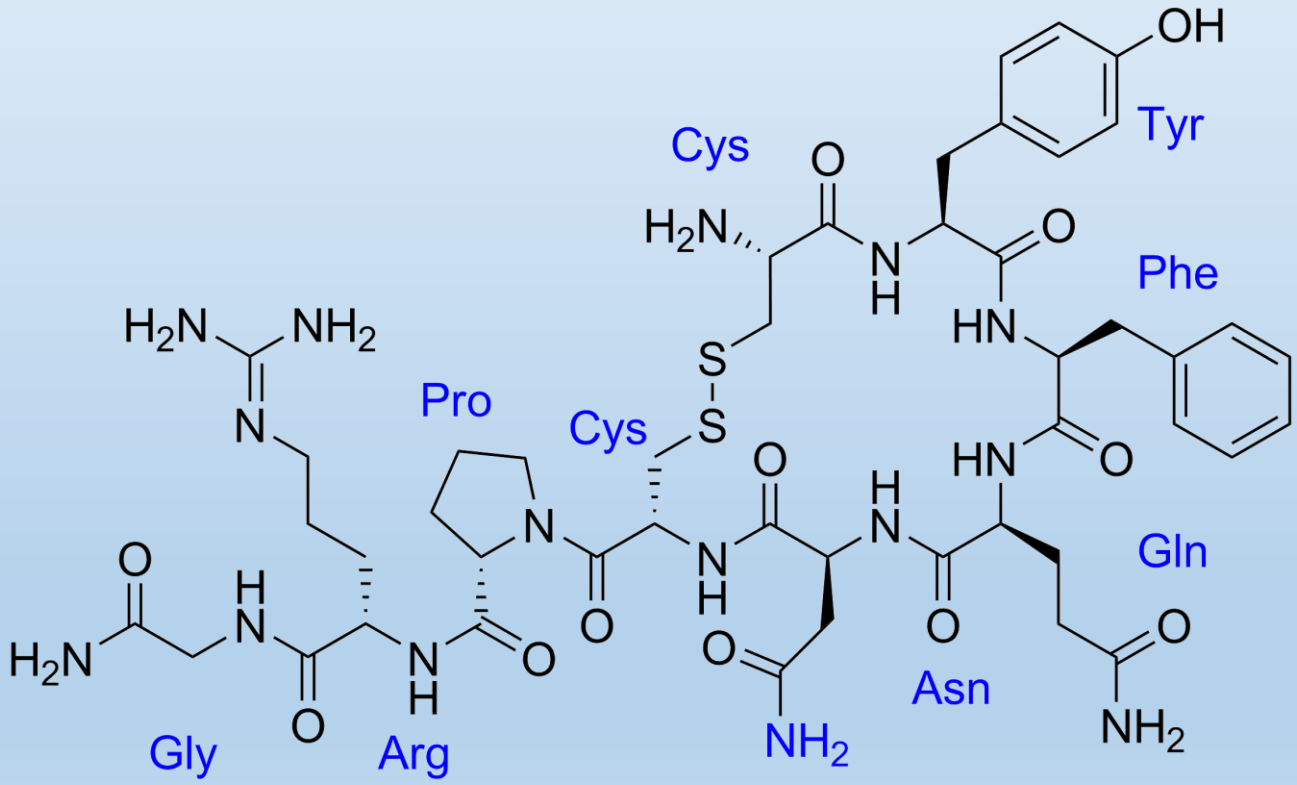
- **Oxytocin** – hormon (neuropeptid), hraje důležitou úlohu při vytváření sociálních vazeb, rozmnožování a porodu



Wataru, I. Et. al.: J. Med. Chem. 2019, 62, 7, 3297–3310.

Biologicky významné peptidy

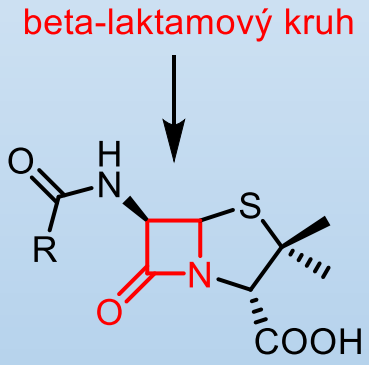
- **Vasopressin** (nebo také antidiuretický hormon ADH) – zodpovědný za osmotickou rovnováhu v těle



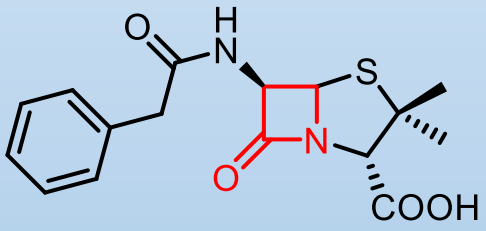
https://en.wikipedia.org/wiki/Vasopressin

β-laktamová antibiotika

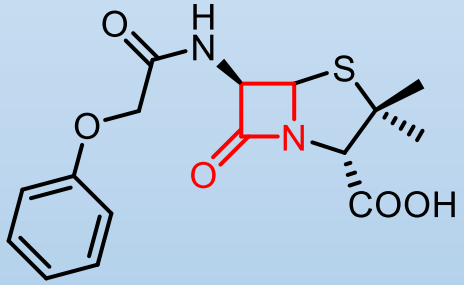
- 1928 – Alexander Fleming (St. Mary's Hospital London) – objev penicilinu (plíseň - *Penicillinum sp*.)



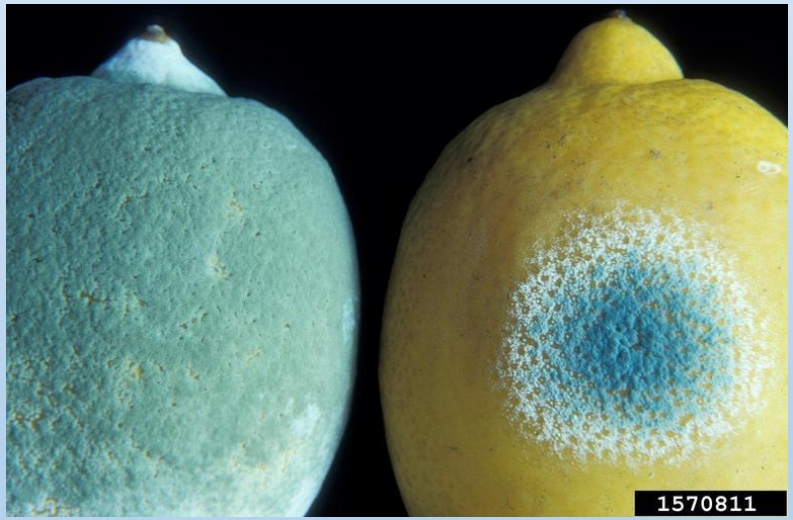
Penicillin



Penicillin G



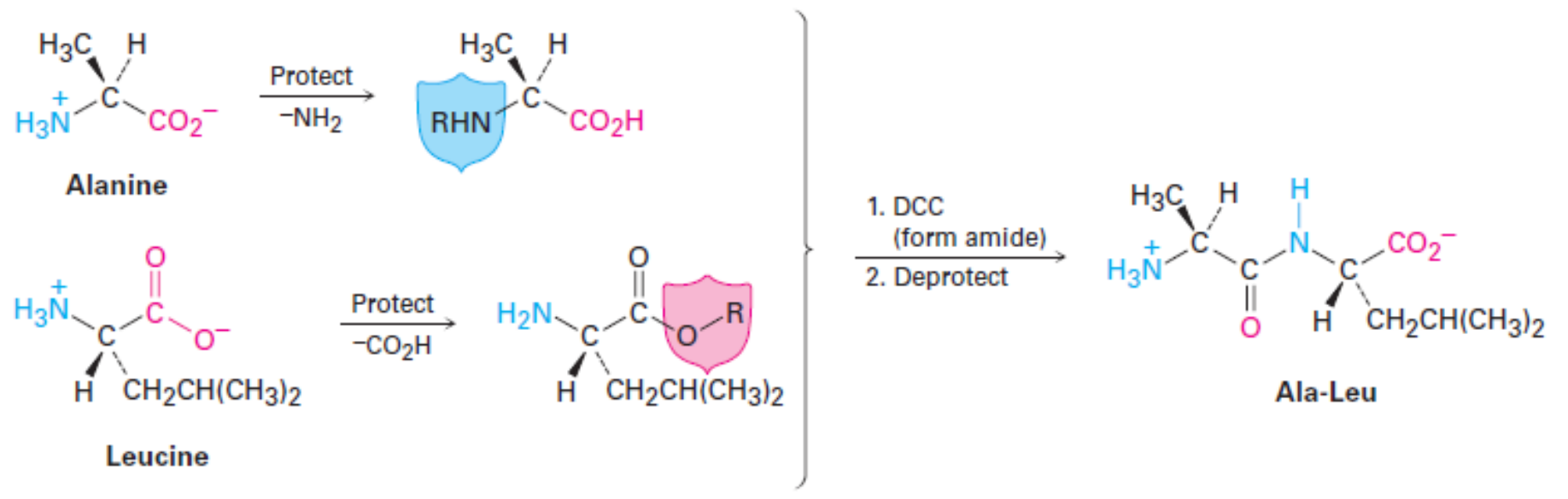
Pennicilin V



www.invasive.org (penicillinum)

Syntéza peptidů

- **strategie**: 1) ochránit NH_2 skupinu první aminokyseliny, 2) ochránit COOH skupinu druhé AMK, 3) tvorba peptidové vazby, 4) odchránění chránicích skupin



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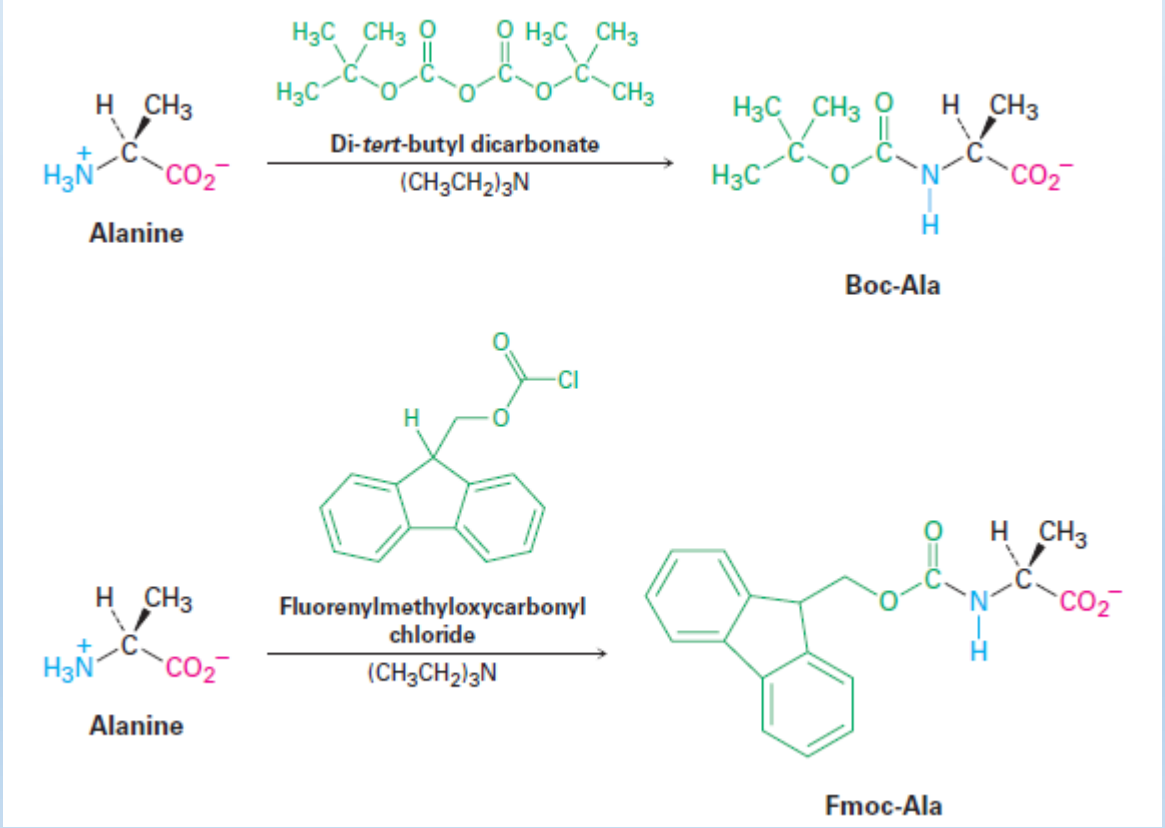
Syntéza peptidů

- **Chránění aminoskupiny**: existuje velké množství chránicích skupin, ale je potřeba aby byly přiměřeně stabilní pro syntézu peptidů, a hlavně aby při odchránění nevyžadovaly drastické podmínky – nepoužívanější Boc (odchránění CF_3COOH) a Fmoc (odchránění piperidin).



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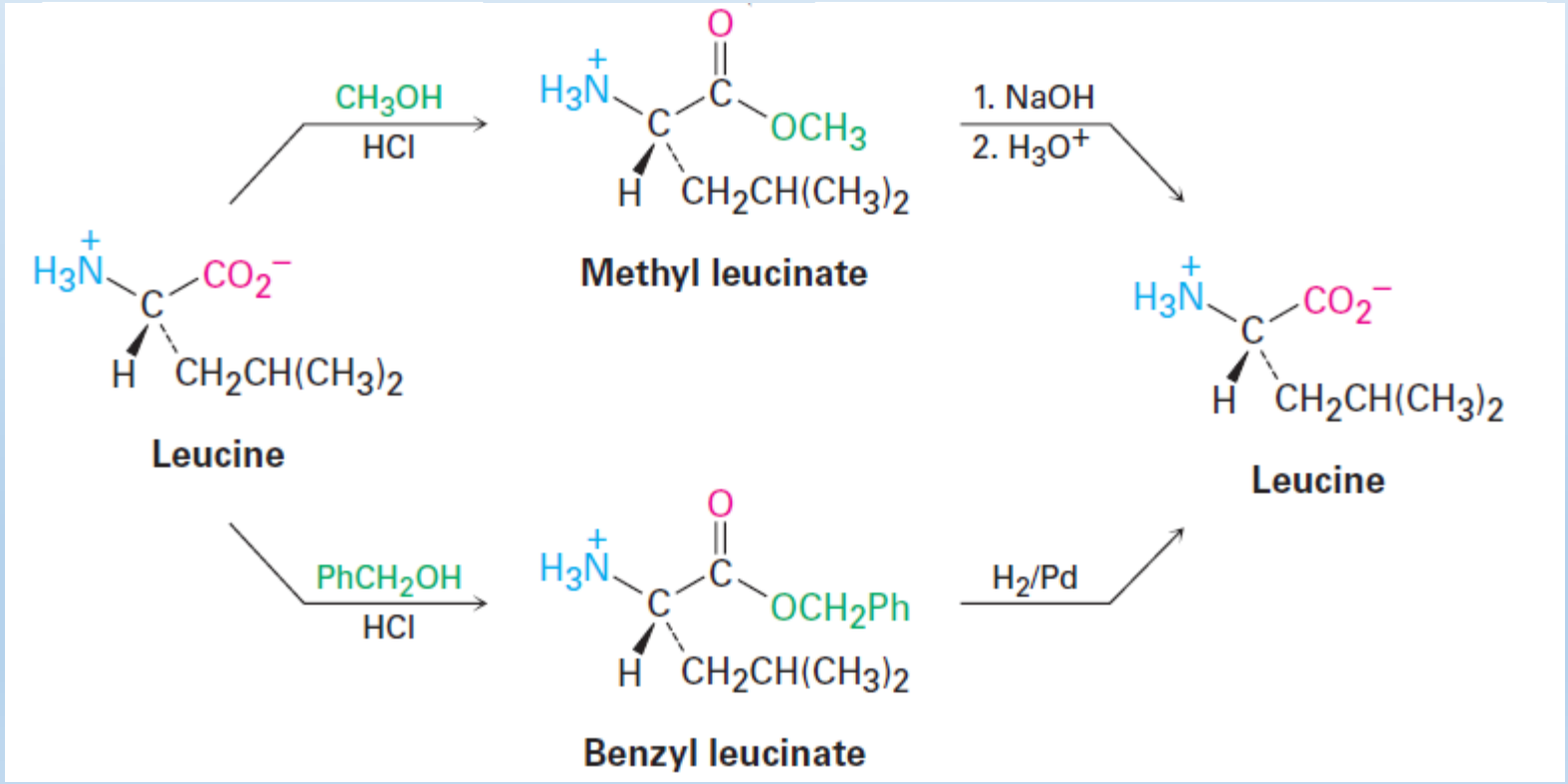
Syntéza peptidů

- **Chránění karboxylové funkce**: formou esterové vazby nejčastěji methyl (odchránění NaOH za mírných podmínek) či benzyl ester (odchránění hydrogenolýzou).



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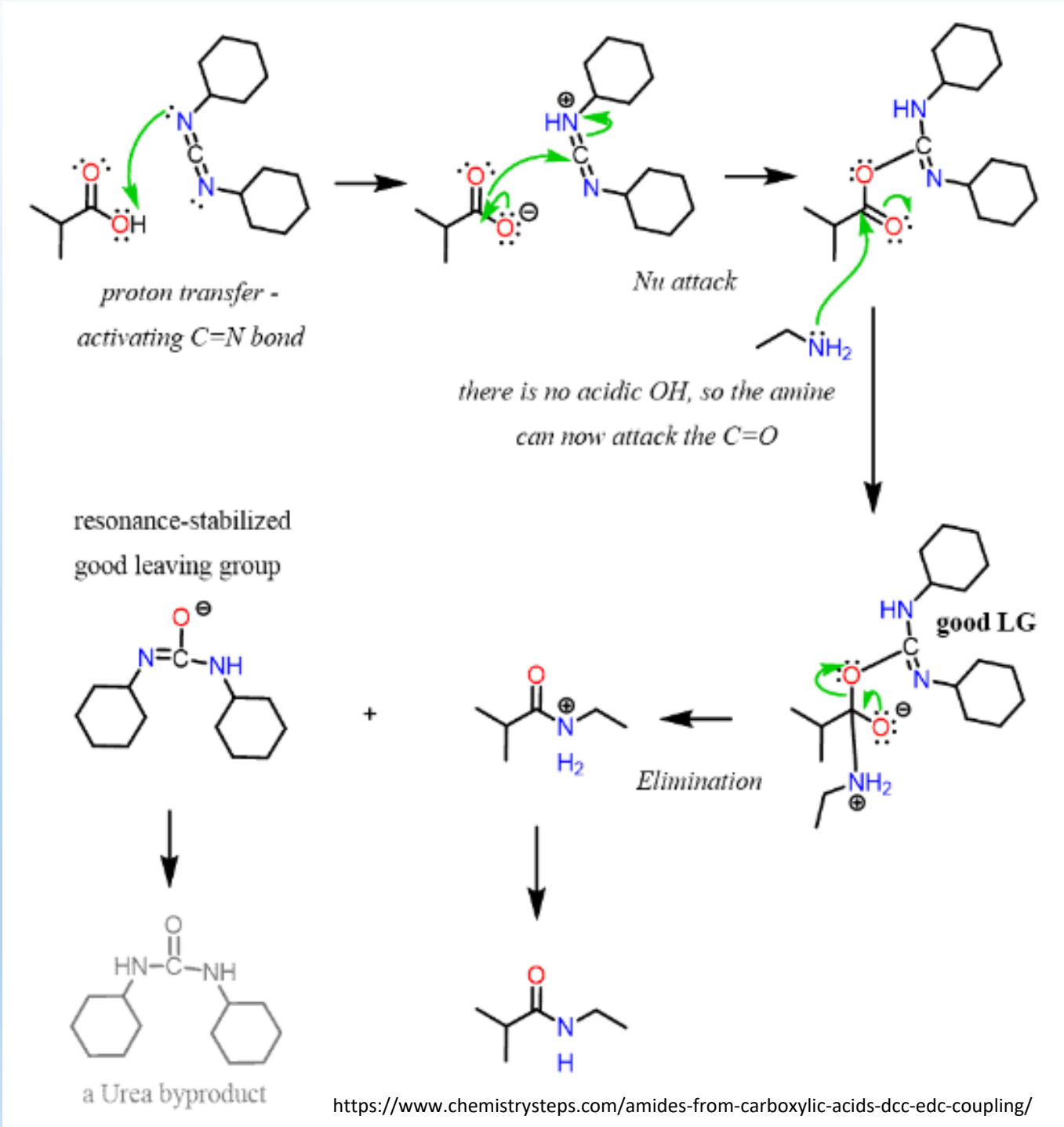
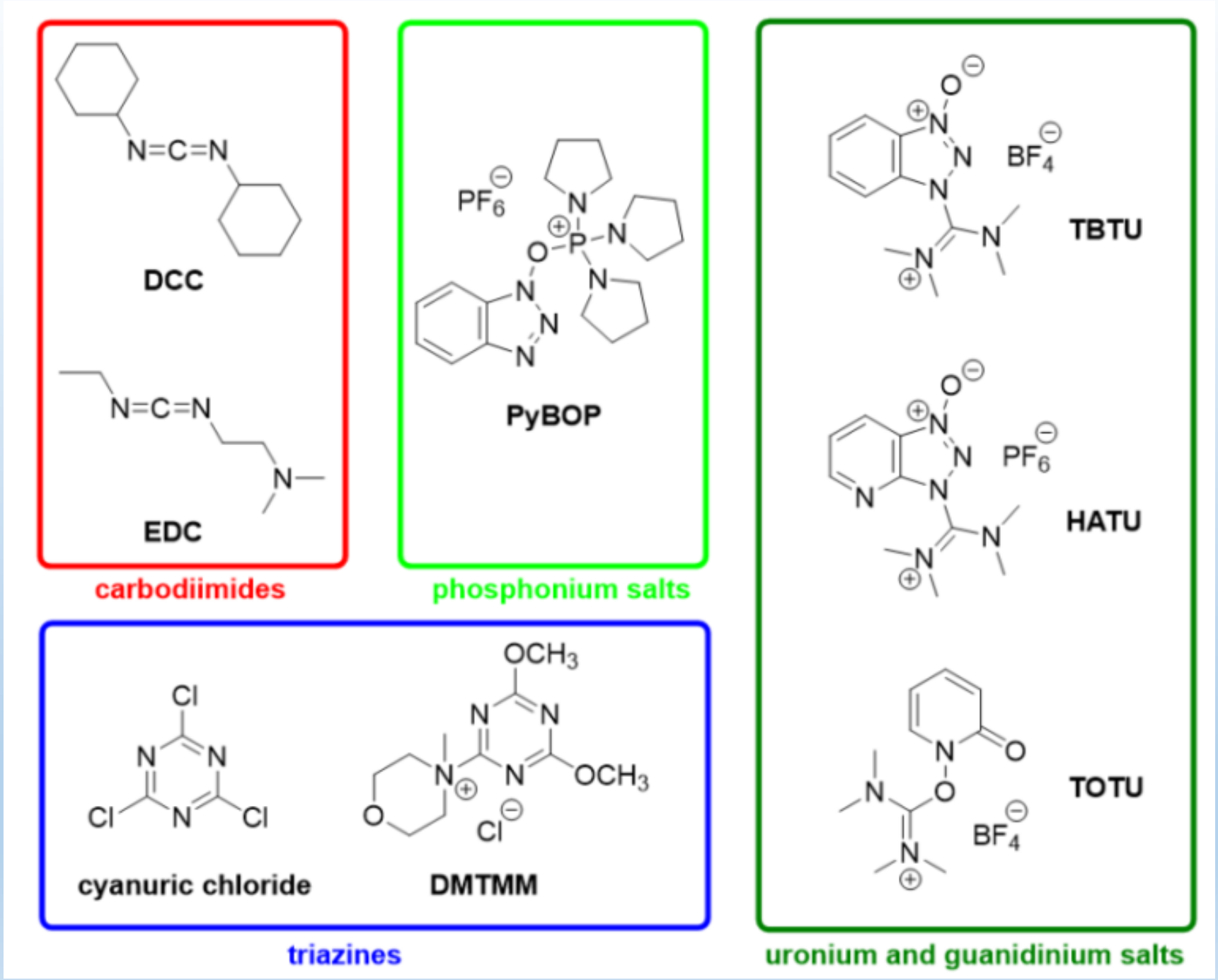
DCC peptide coupling



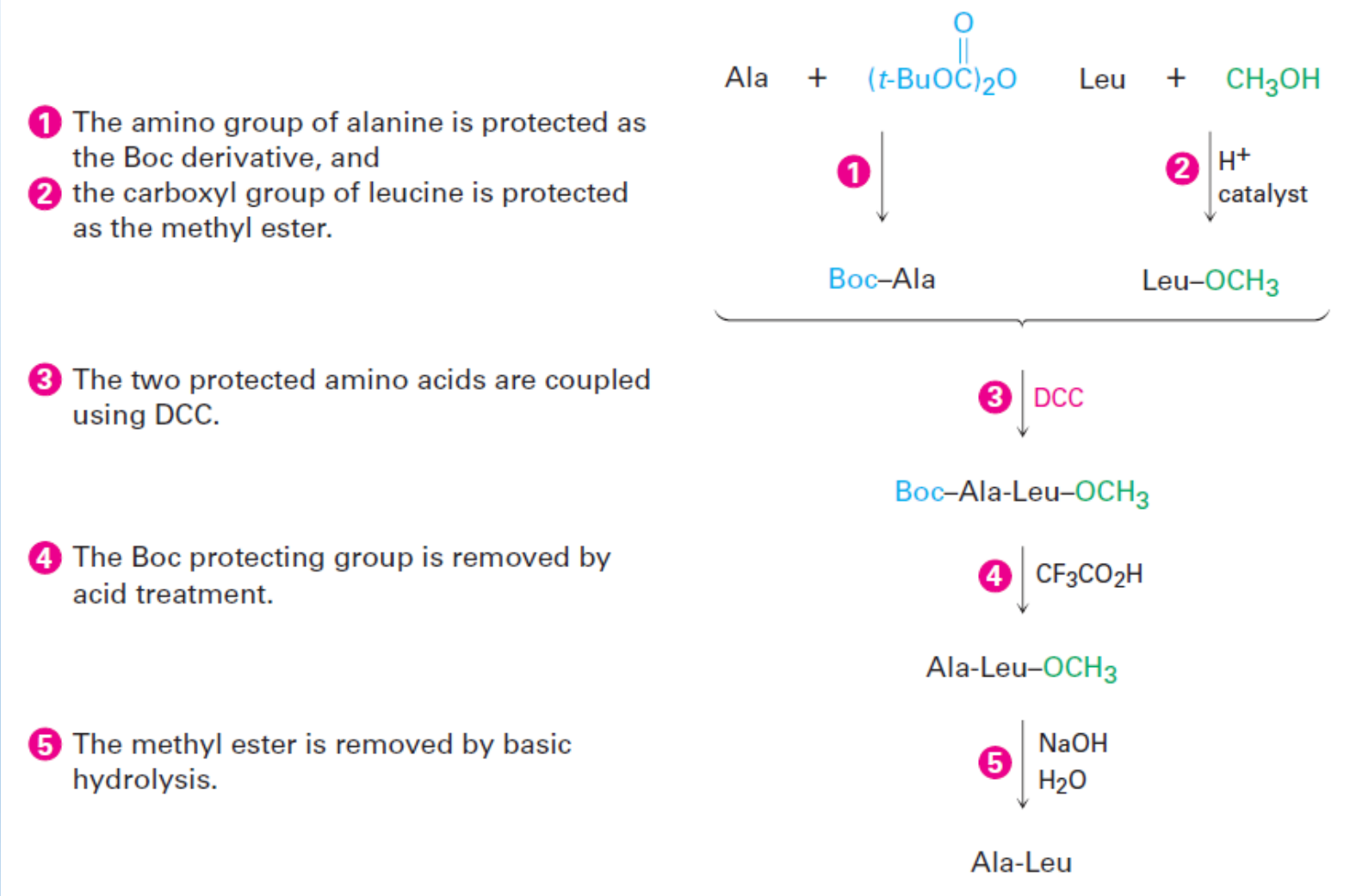
https://www.chemistrysteps.com/amides-from-carboxylic-acids-dcc-edc-coupling/

Další činidla pro tvorbu peptidové vazby



https://www.santiago-lab.com/reagent-of-the-month-march-dmtmm/

Syntéza peptidů



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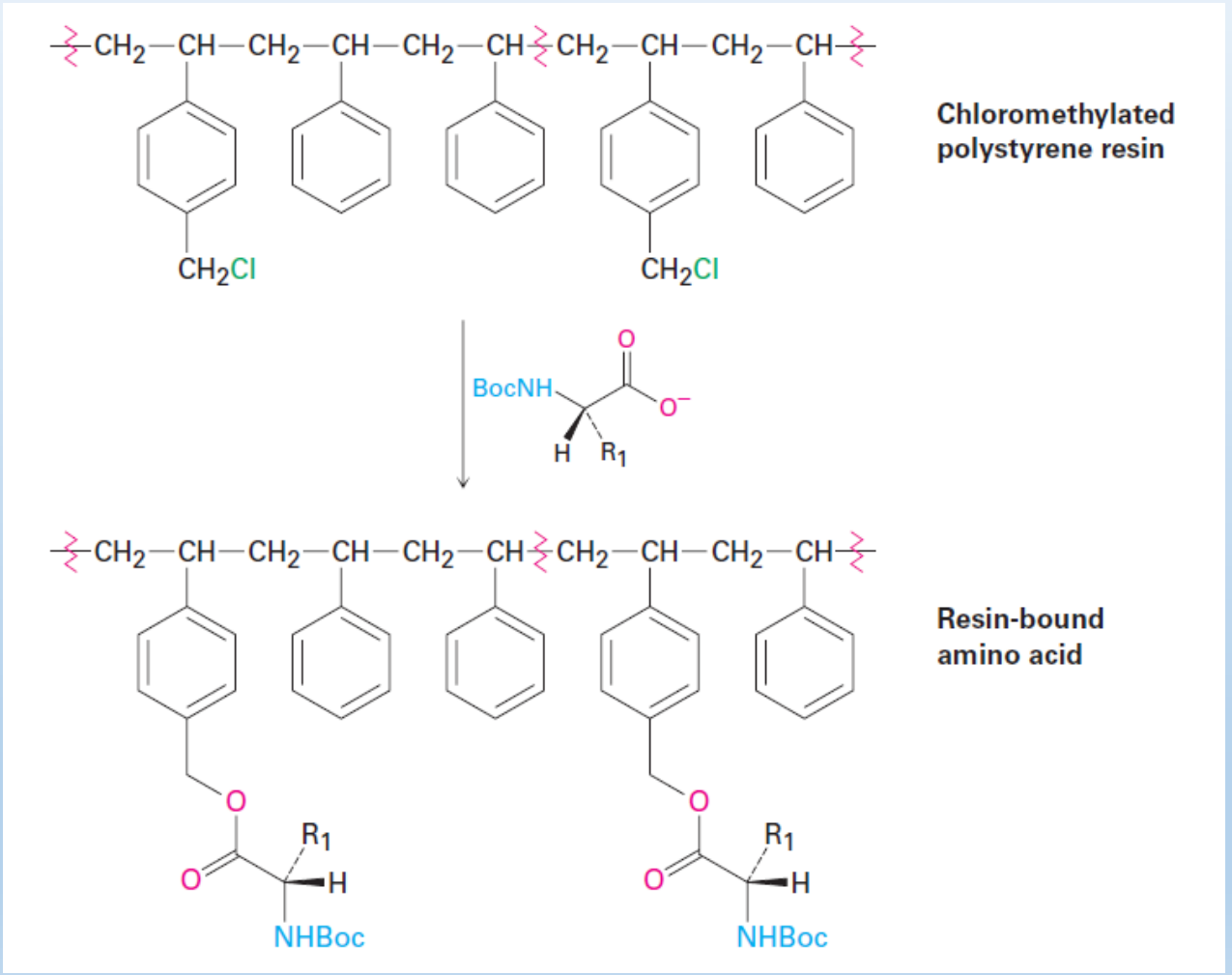
Automatizovaná syntéza peptidů

Solid phase synthesis – syntéza na pevné fázi.
Principem je ukotvení první AMK na reaktivní polystyrenovou pryskyřici a následné prodlužování peptidového řetězce.

http://beta.cemx.com/beta2018/en/liberty-blue-ht12



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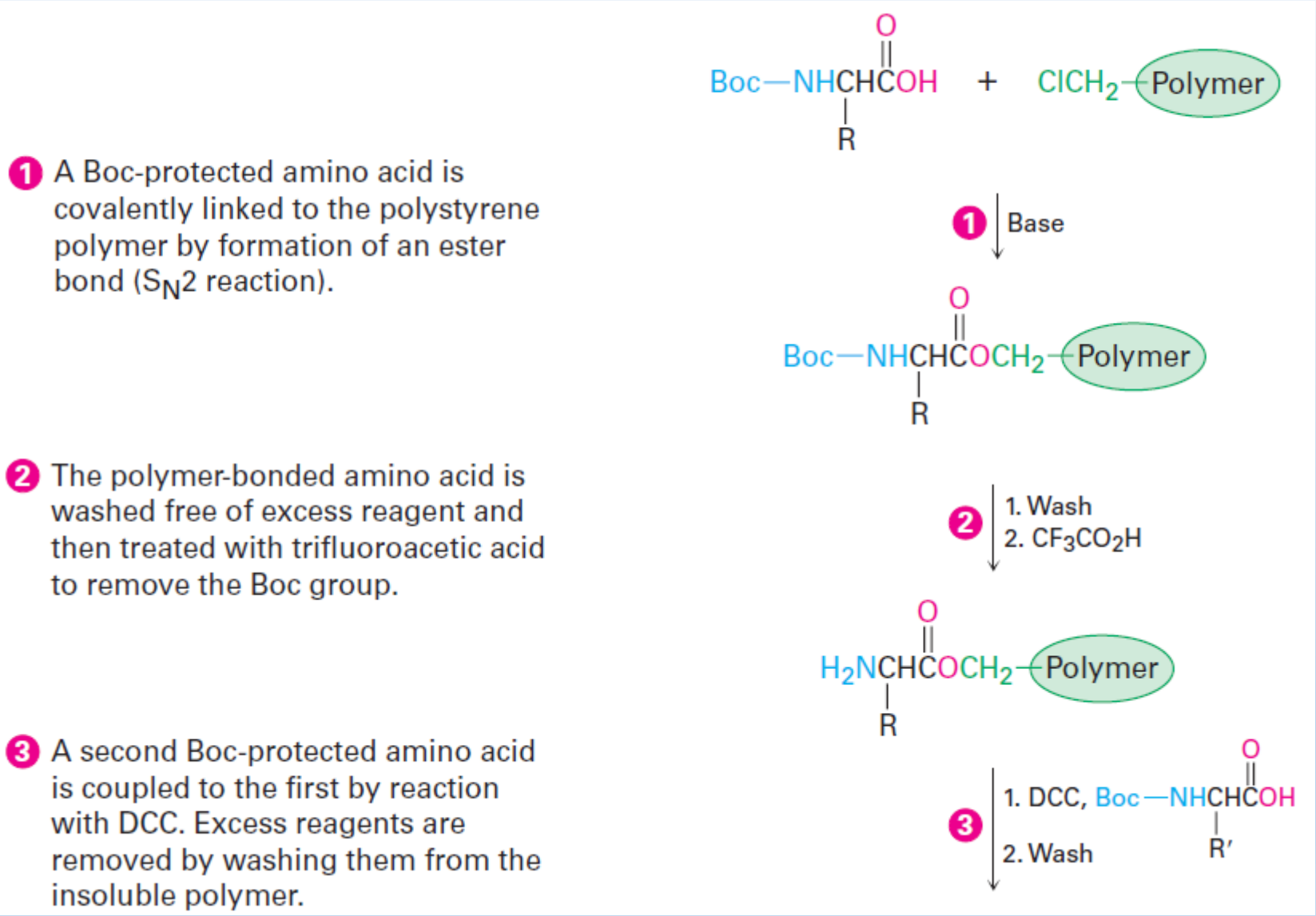
Automatizovaná syntéza peptidů

System can build 164-amino-acid-long proteins in hours instead of weeks or months

https://cen.acs.org/synthesis/Automated-synthesizer-make-long-protein/98/i21
Science 2020, DOI: 10.1126/science.abb2491

Automatizovaná syntéza peptidů



1. A Boc-protected amino acid is covalently linked to the polystyrene polymer by formation of an ester bond (S_N2 reaction).

2. The polymer-bonded amino acid is washed free of excess reagent and then treated with trifluoroacetic acid to remove the Boc group.

3. A second Boc-protected amino acid is coupled to the first by reaction with DCC. Excess reagents are removed by washing them from the insoluble polymer.

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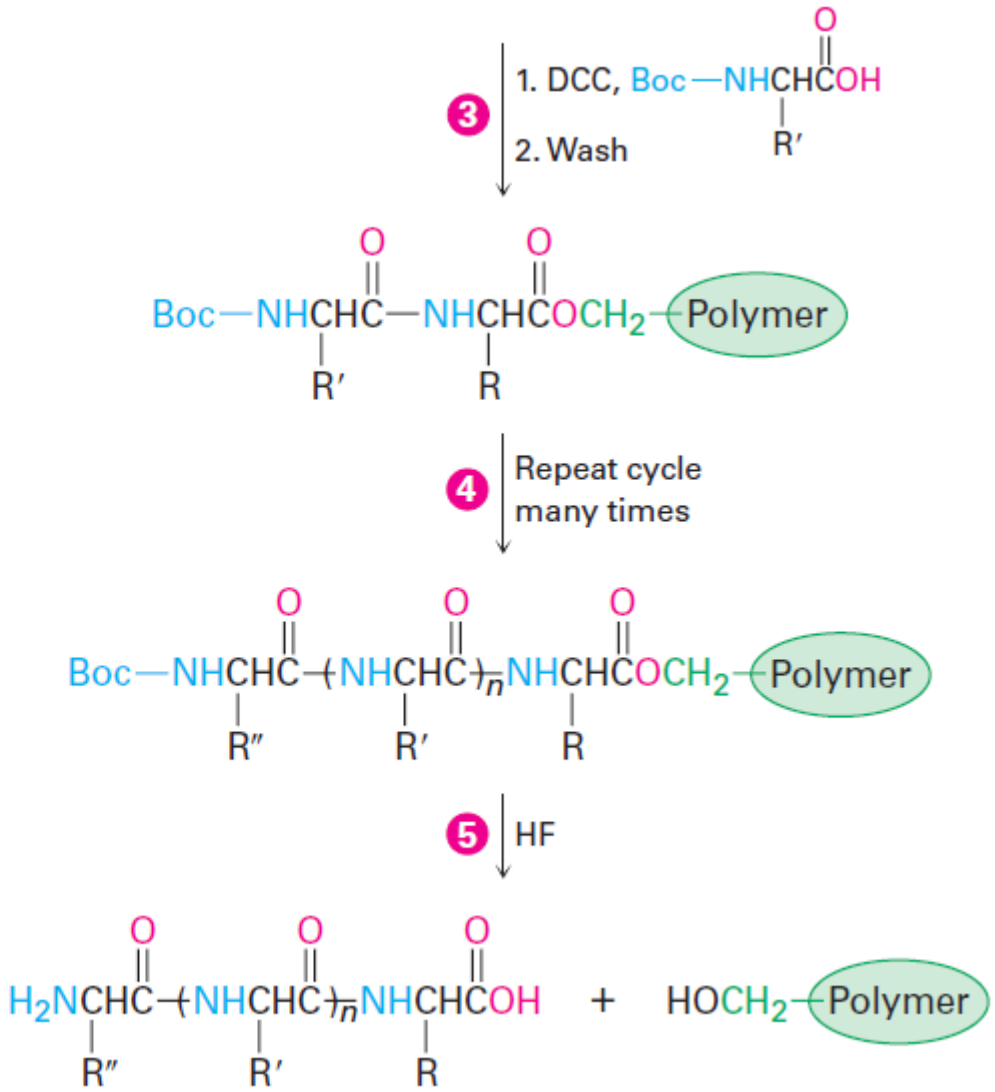
Automatizovaná syntéza peptidů

3. A second Boc-protected amino acid is coupled to the first by reaction with DCC. Excess reagents are removed by washing them from the insoluble polymer.

4. The cycle of deprotection, coupling, and washing is repeated as many times as desired to add amino acid units to the growing chain.

5. After the desired peptide has been made, treatment with anhydrous HF removes the final Boc group and cleaves the ester bond to the polymer, yielding the free peptide.



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Moderní produkce peptidů (rekombinantní proteiny)

rekombinantní inzulin: polypeptid – řetězec A 21 AMK a řetězec B 30 AMK, spojeny disulfidickými můstky

1) Umělá syntéza genu (DNA) pro lidský insulin nebo izolace z β-buněk Lagerhansových ostrůvků (pankreas)
2) Vložení genu do plasmidu
3) Zavedení plasmidu do E. Coli
4) Kultivace E. Coli (fermentor)
5) Extrakce a čištění rekombinantního genu pomocí afinitní chromatografie

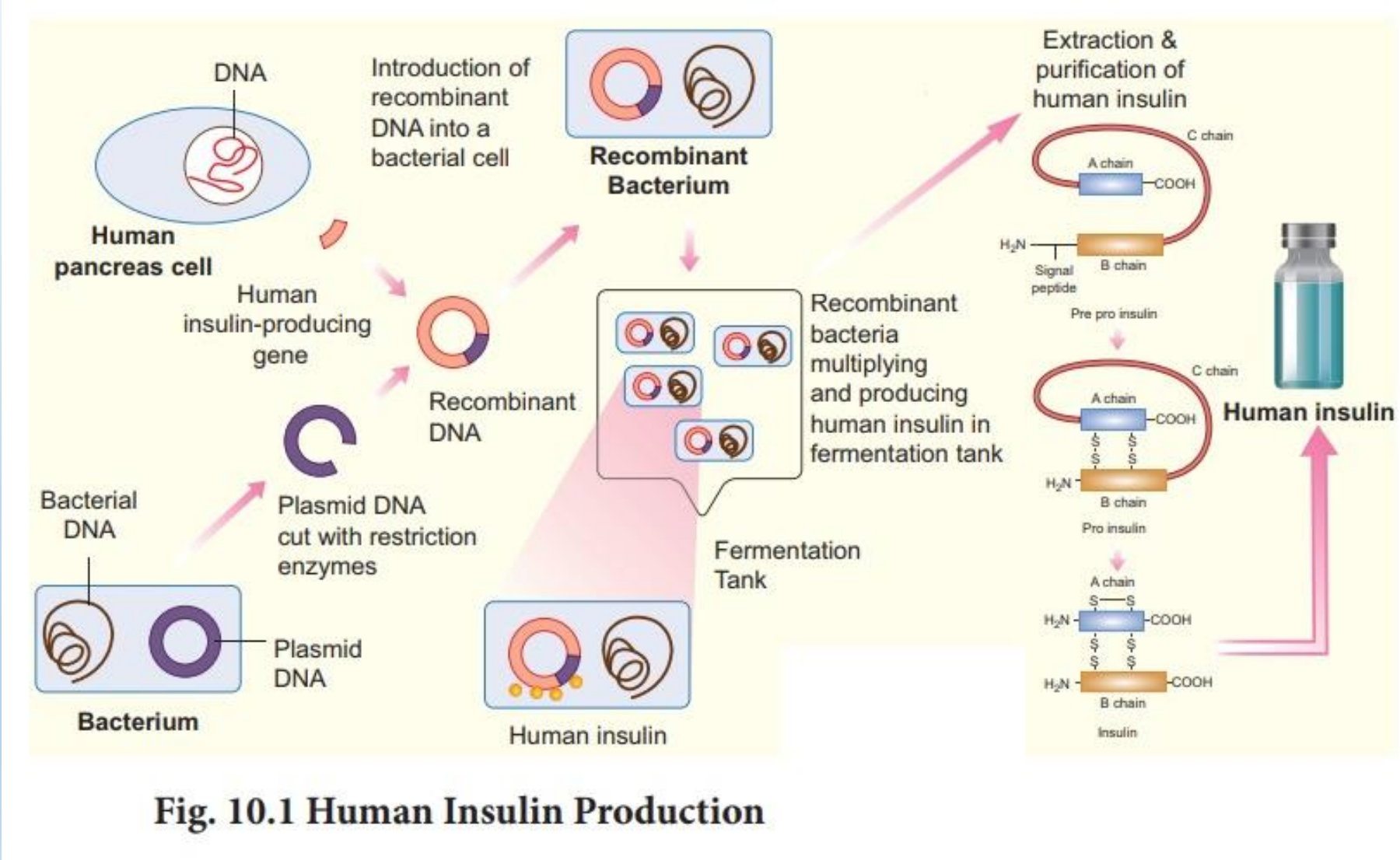
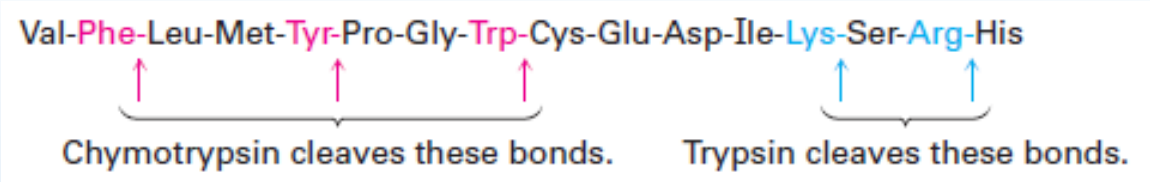


Fig. 10.1 Human Insulin Production

https://www.brainkart.com/article/Applications-of-biotechnology-in-Medicine_38121/

Analýza peptidů



- Dnes částečná hydrolýza peptidů pomocí enzymů (endopeptidázy = trypsin, chymotrypsin) → vznik specifických fragmentů → analýza pomocí MS (Maldi, TOF)

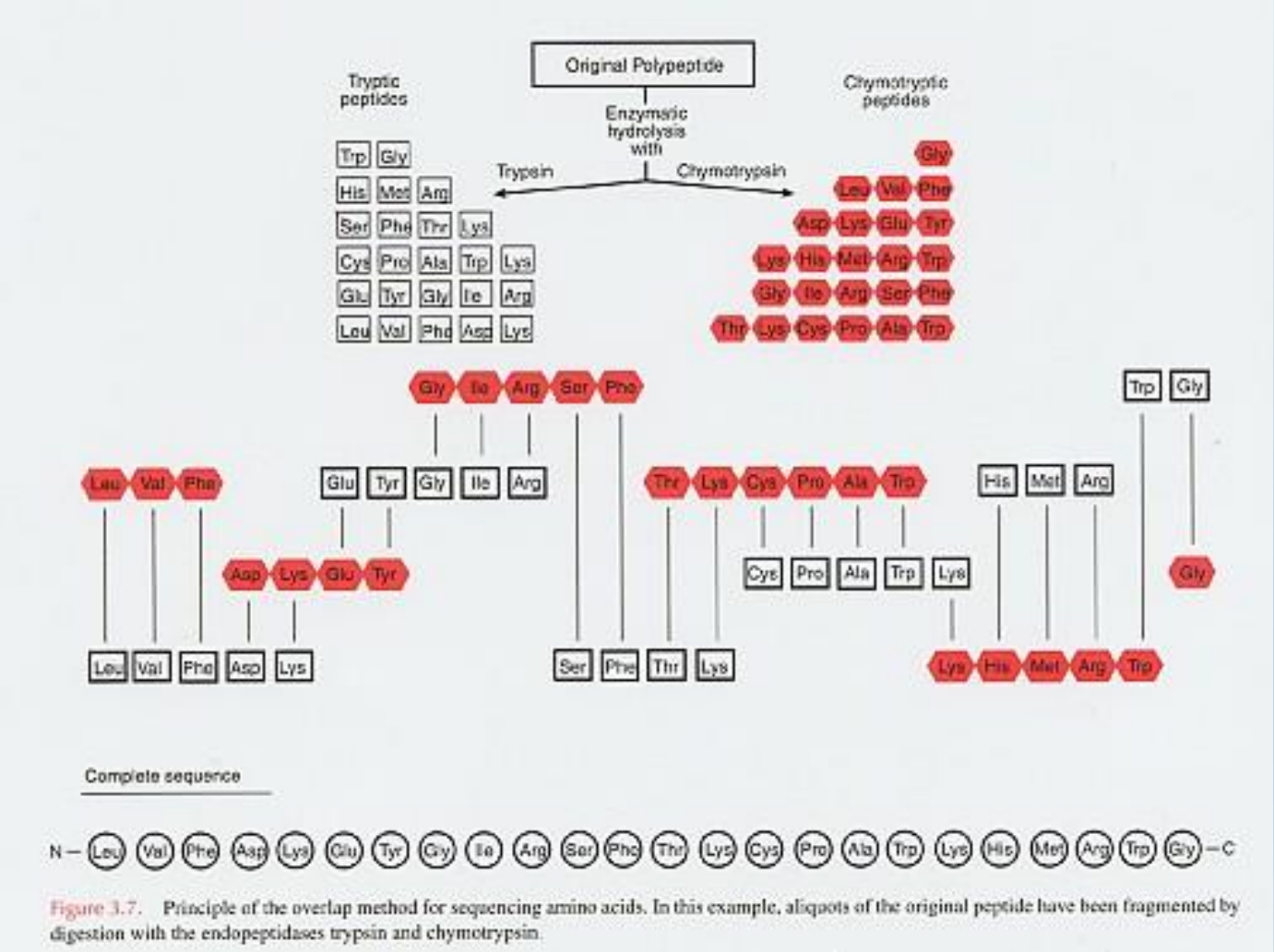
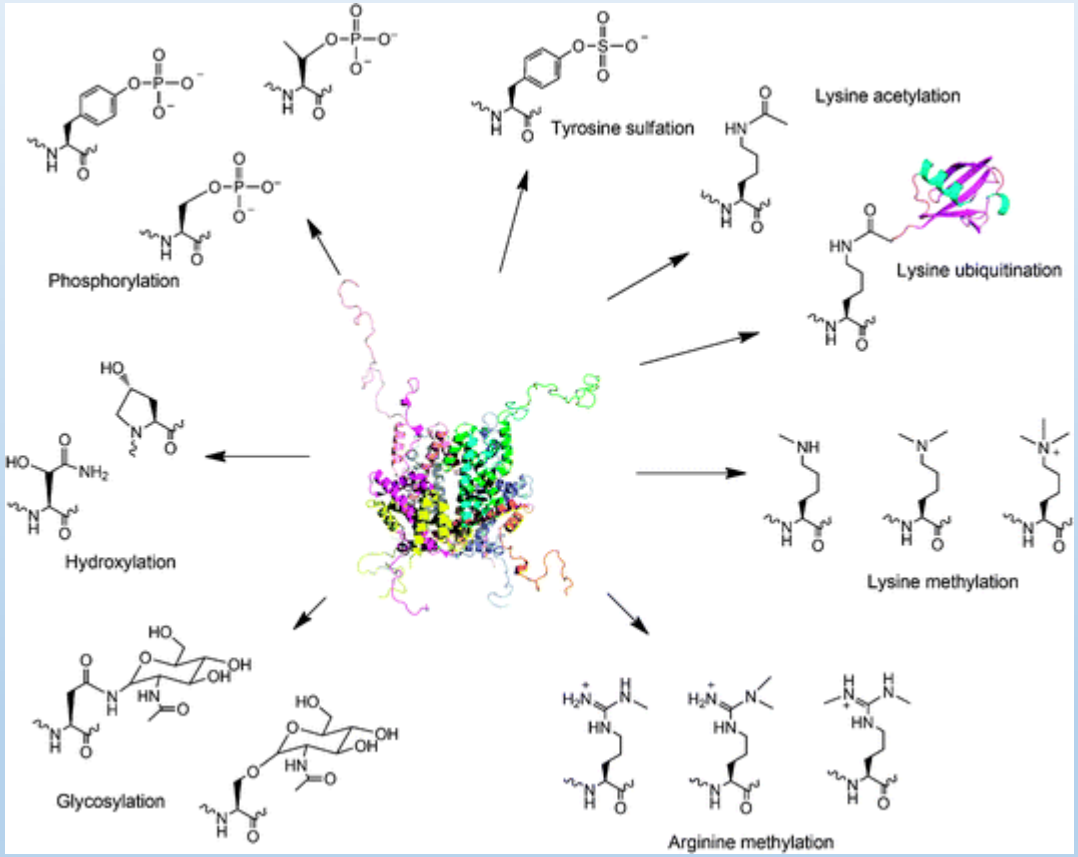
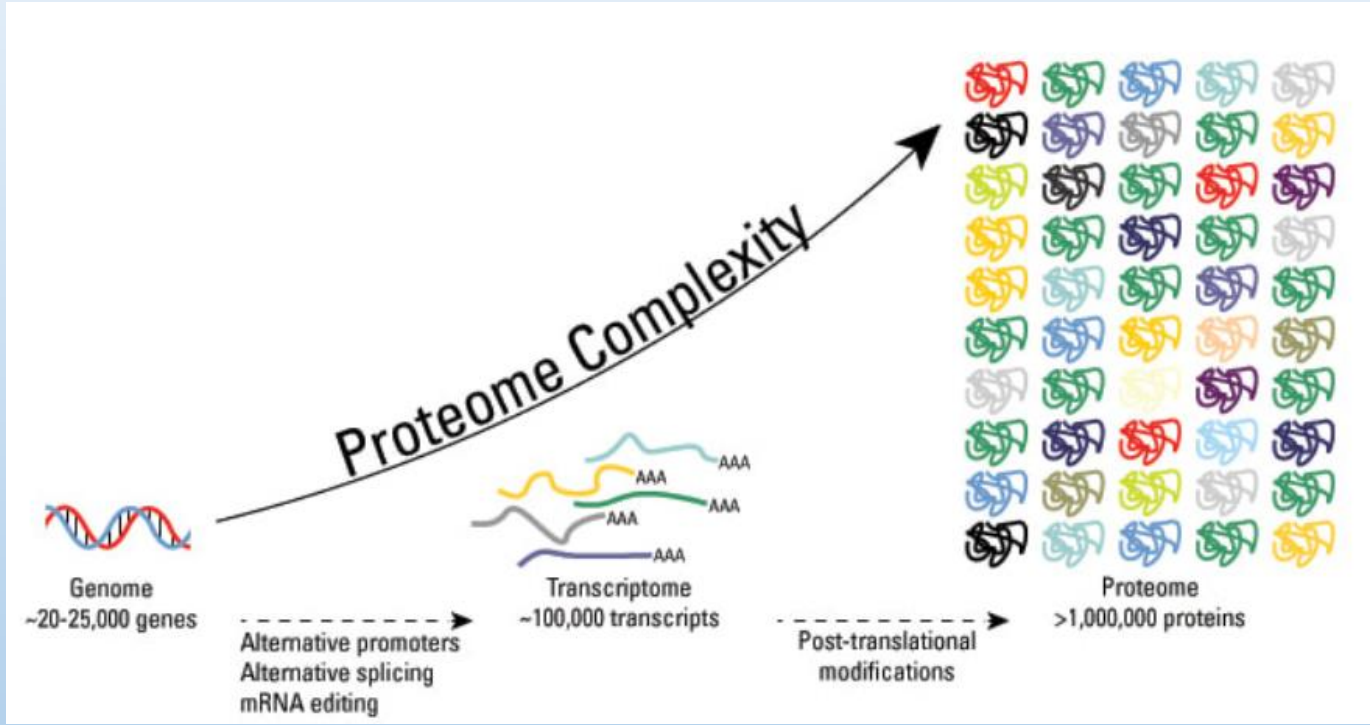


Figure 3.7. Principle of the overlap method for sequencing amino acids. In this example, aliquots of the original peptide have been fragmented by digestion with the endopeptidases trypsin and chymotrypsin.

Posttranslační modifikace bílkovin



Mol. BioSyst., 2011, **7**, 38-47



https://www.thermofisher.com/content/dam/LifeTech/Images/integration/Proteome-Complexity-Figure-650px.jpg