



evropský  
sociální  
fond v ČR



EVROPSKÁ UNIE



MINISTERSTVO ŠKOLSTVÍ,  
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání  
pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

# Therapeutic peptides

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# Classification of therapeutic peptides

## **1. Hormones**

**1.1 Liberins and statins („releasing“ & „inhibiting“)**

**1.2. Soma(tot)ropin**

**1.3 Oxytocin, vasopressin and their analogues**

**1.4 Insulines, glucagon and GLP-1 analogues**

**1.5 Calcitonin**

**2. Blood factors of erythropoietine type**

**3. Colony stimulating factors**

**4. Non-specific antibodies**

# One- and three-letter symbols of L- $\alpha$ -amino acid rests

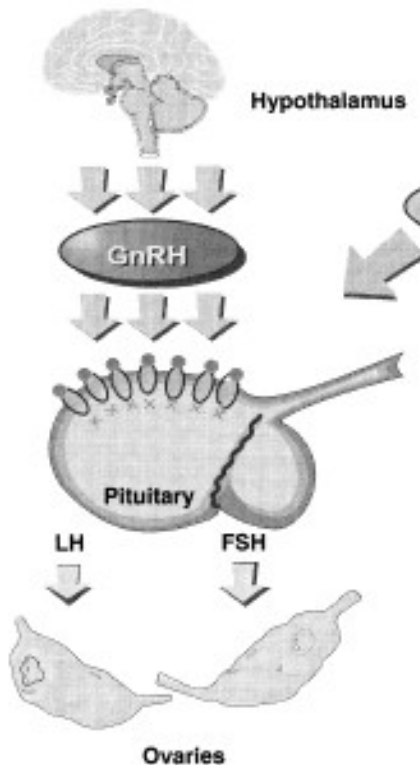
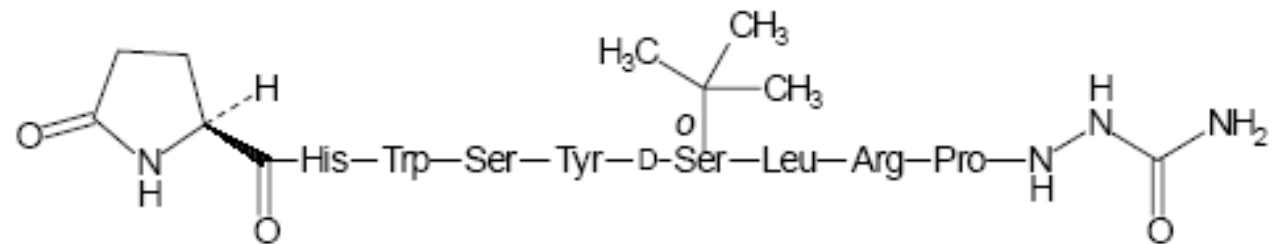
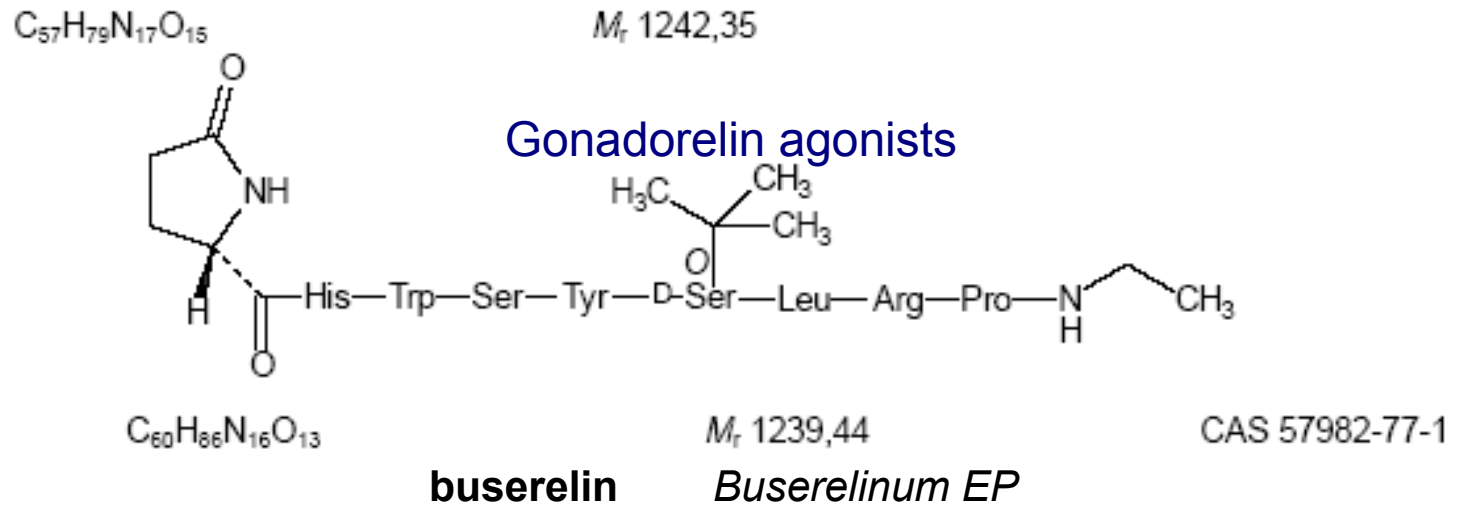
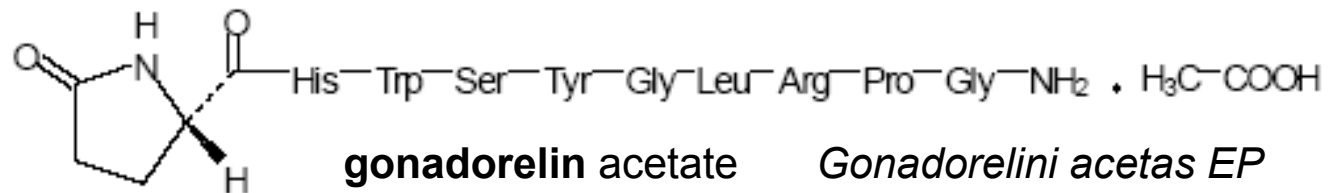
One-letter	Three-letter	
A	Ala	alanine
B	Asx	asparaginic acid or asparagine
C	Cys	cysteine
D	Asp	asparaginic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
Q	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine
U	Sec	selenocysteine
V	Val	valine
W	Trp	tryptofane
X	Xaa	unknown or „other“ amino acid
Y	Tyr	thyrosine
Z	Glx	glutamic acid or glutamine (or compounds such as 4-carboxyglutamic acid 5-oxoproline)

# 1. Hormones

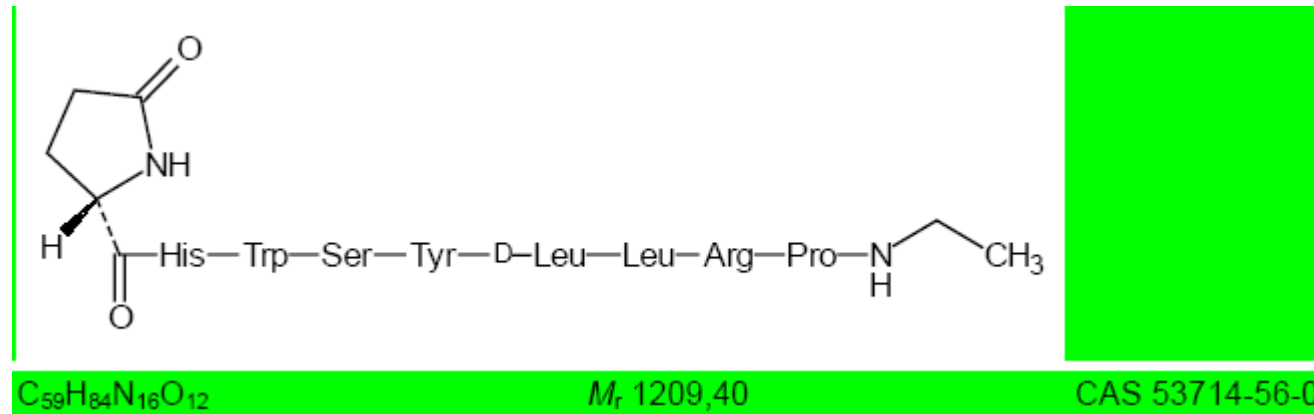
## 1.1 Liberins and statins („releasing“ & „inhibiting“)

### Gonadorelin (GnRH = LHRH) and its analogues

- hormone of hypothalamus
- stimulates releasing of follicles stimulating hormone (FSH) and luteinizing hormone (LH) from pituitary gland; GnRH receptors also in various non-reproductive tissues



## Gonadorelin and its analogues Agonists



**leuprorelin** (syn. leuprolide) *Leuprorelinum EP*

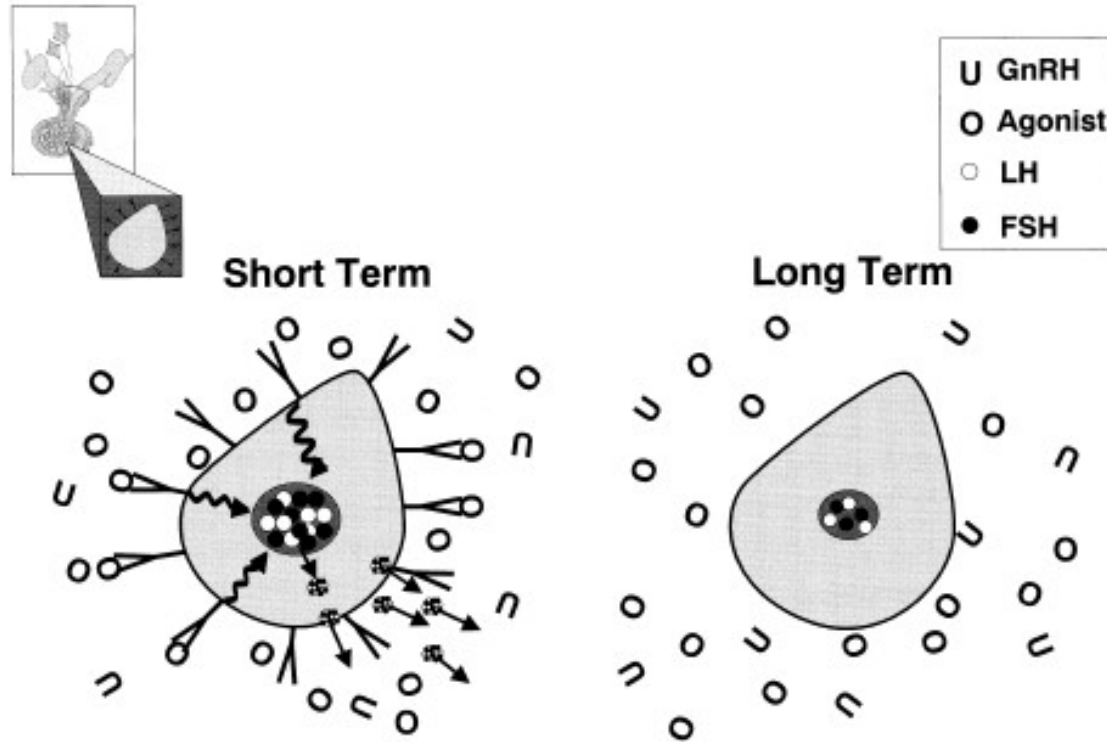
Eligard ®

- longer-term application lowers testosterone levels ⇒ treatment of prostate cancer  
⇒ treatment of sexual deviations



## Short- and long term action of gonadorelin agonists

Effects of GnRH agonist.

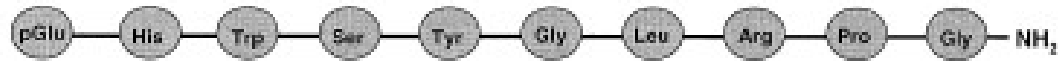


- long term action leads to receptors internalisation and stopping of the effect (due to decreasing LH and FSH levels and thus also levels of sexual hormones)

# Gonadorelin analogues Gonadorelin antagonists

The GnRH antagonists.

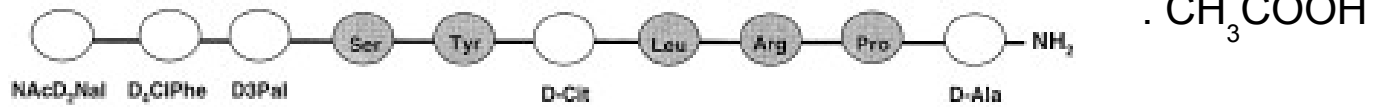
## GnRH



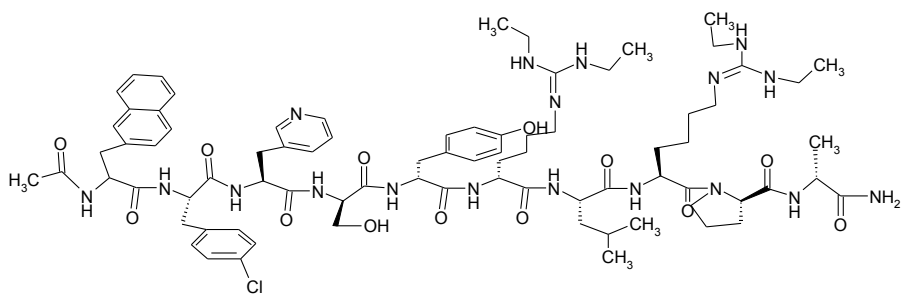
## Antagon™ (ganirelix acetate)



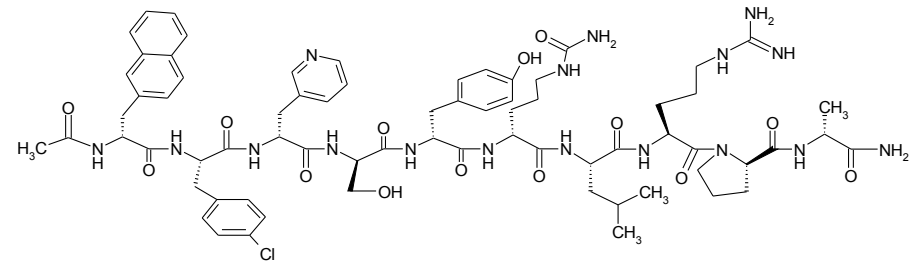
## Cetrotide® (cetrorelix acetate)



Amino Acid Number	1	2	3	4	5	6	7	8	9	10
	NAcD <sub>2</sub> Nal	D <sub>2</sub> CIPhe	D <sub>3</sub> Pal	Ser	Tyr	D-Cit	Leu	Arg	Pro	D-Ala



ganirelix



cetrorelix



## Gonadorelin and its analogues

- preparation: chemical synthesis
- usage: assisted reproduction, treatment of prostate cancer, sexual deviation ...
- advantages of analogues: significantly higher stability  $\Rightarrow$  longer elimination half-time  $\Rightarrow$   
 $\Rightarrow$  possibility of application in markedly longer intervals; a single injection of an agonist can replace a continuous infusion of gonadorelin

### Structure – activity relationships (SAR)

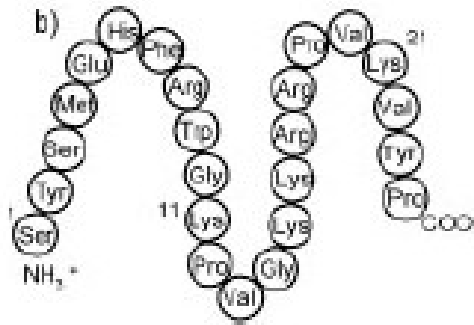
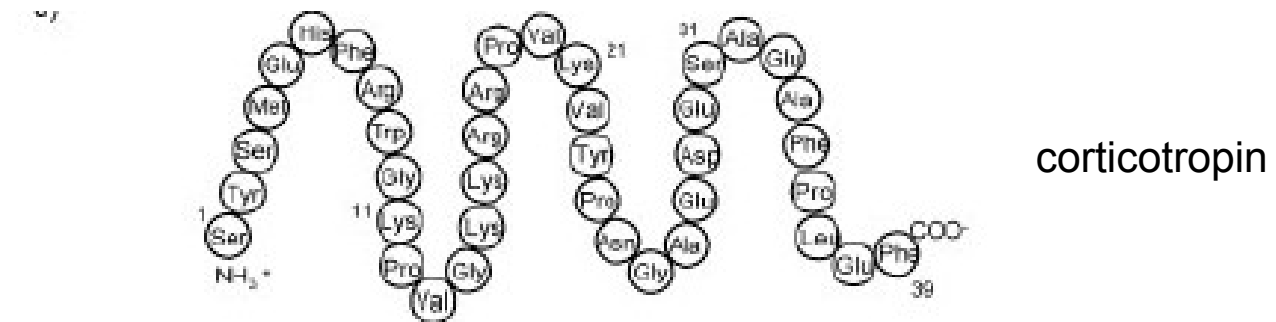
- replacement of Gly in position 6 with a more bulky amino acid leads to stability increase
- the sequence of the first three amino acids is needed for receptor binding and is kept in agonists
- antagonists have Trp in position 3 replaced with a non-physiologic amino acid, they bind to GnRH and avoid its action on receptors

## Corticotropin and its analogues

Corticotropin = Adrenocorticotrophic hormone (ACTH); an anterior pituitary hormone that stimulates the adrenal cortex and

its production of both gluco- and mineralocorticoids and growth of adrenal glands

- polypeptide of 39 amino acids; N-terminal 24 identical in all species
- N-terminal 24 AA are responsible for biologic activity; C-terminal 15 AA for immunospecificity

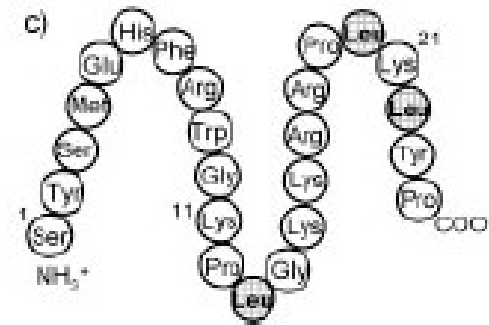


### tetraacosactide

syn. cosyntropin [USAN]

*Tetraacosactidum EP*

Synacten®

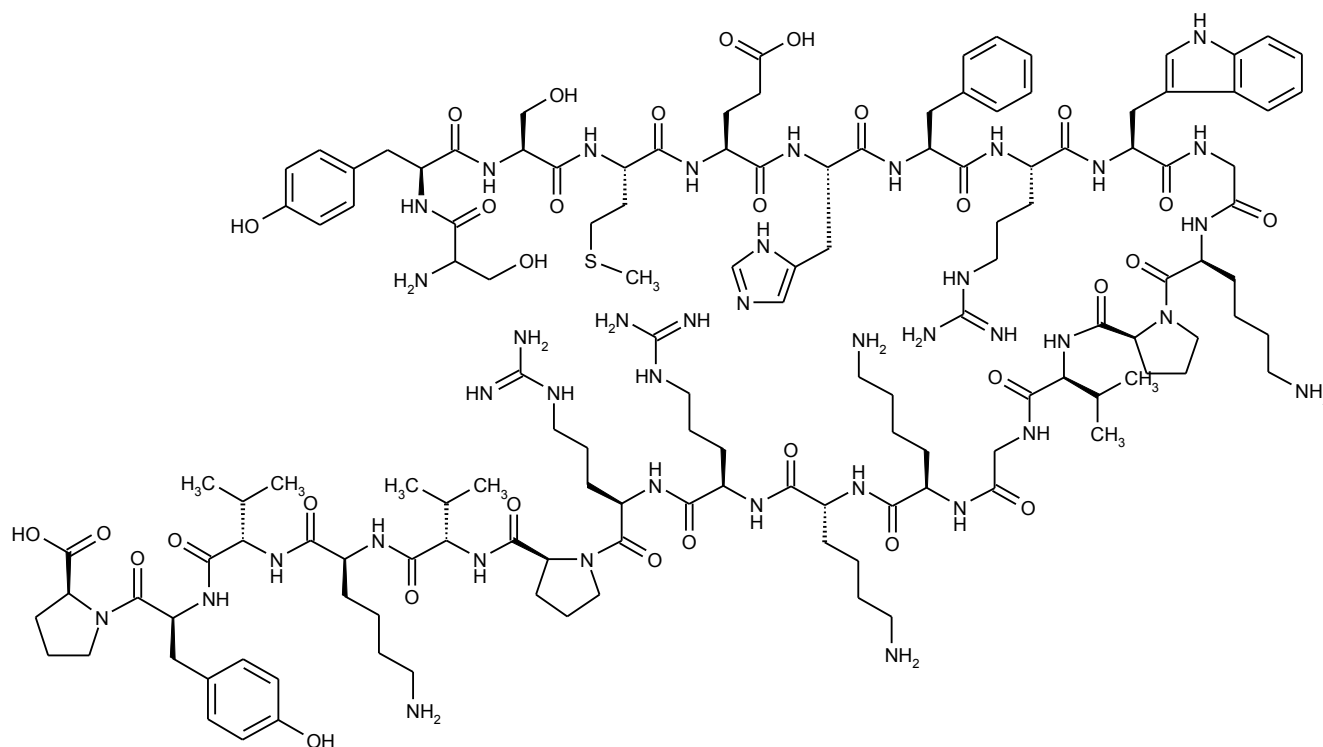


### SynVL

• compound used as a standard for determination of tetraacosactide by mass spectrometry

## Usage of corticotropin and tetracosactide

- diagnosis of adrenal glands function
- substitution treatment in lack of glucocorticoids
- substitution of depot administration of glucocorticoids in a long-term treatment

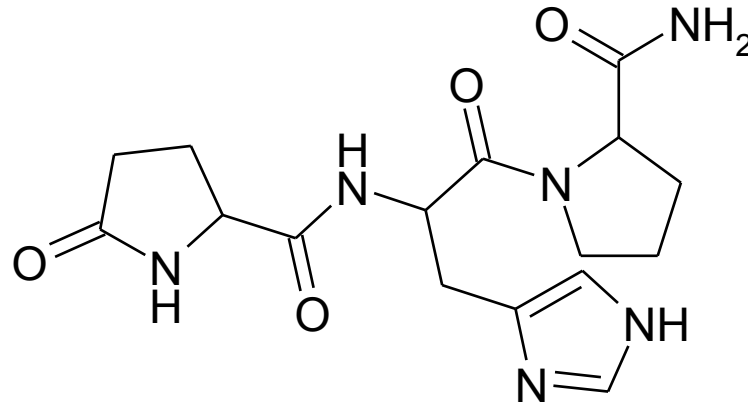


### **tetracosactide**

- used since 1961
- prepared by synthesis
- misused for doping in sport

## Protirelin – synthetic thyrotropin-releasing hormone (TRH)

- a hormone synthesized in paraventricular nucleus of hypothalamus, stimulating release of thyrotropin and prolactin from the anterior pituitary gland
- also neurotransmitter in CNS, takes part in food intake regulation, control of energy metabolism etc.



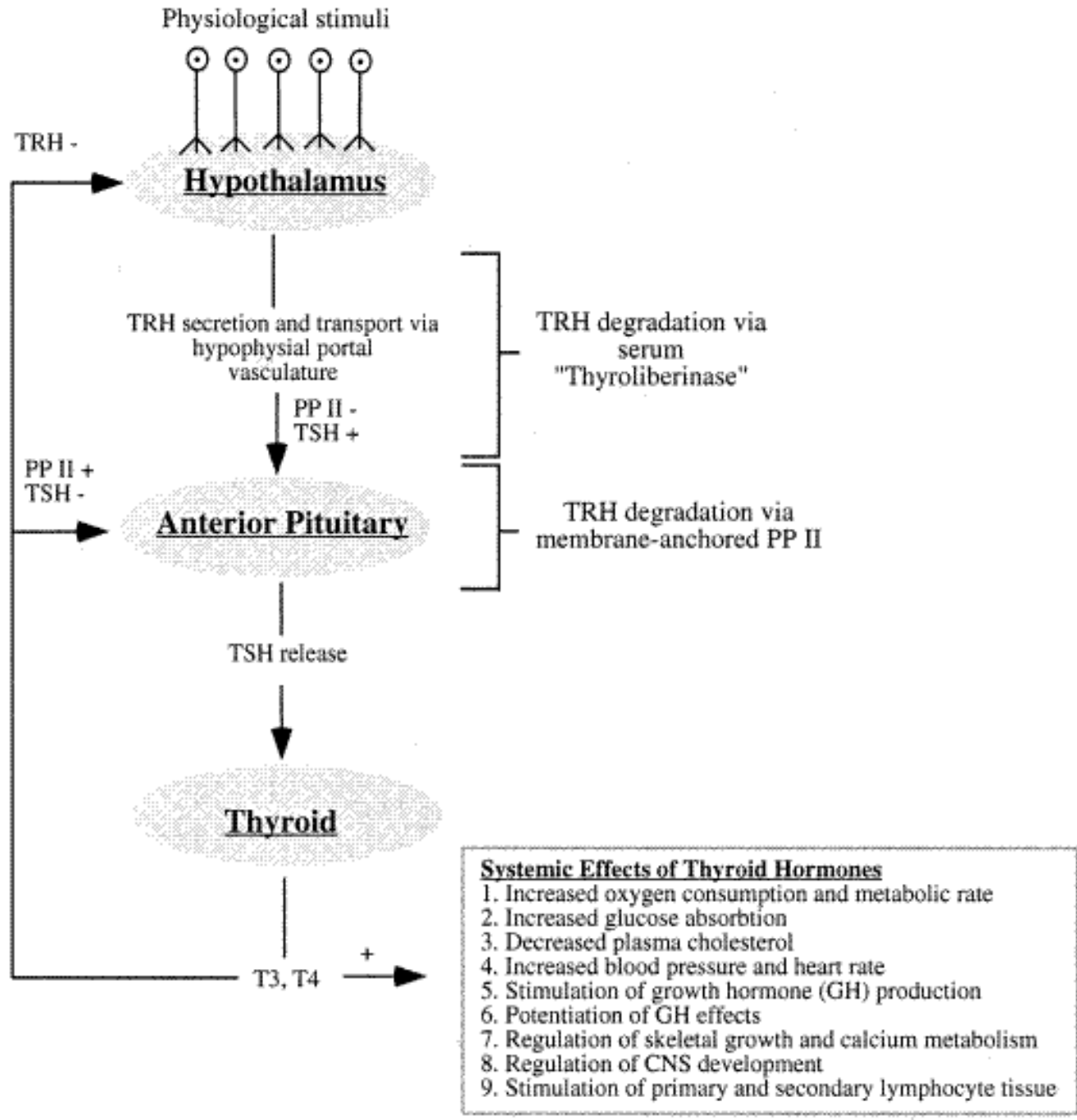
### protirelin

5-oxopropyl-histidyl-prolinamide

*Protirelinum EP*

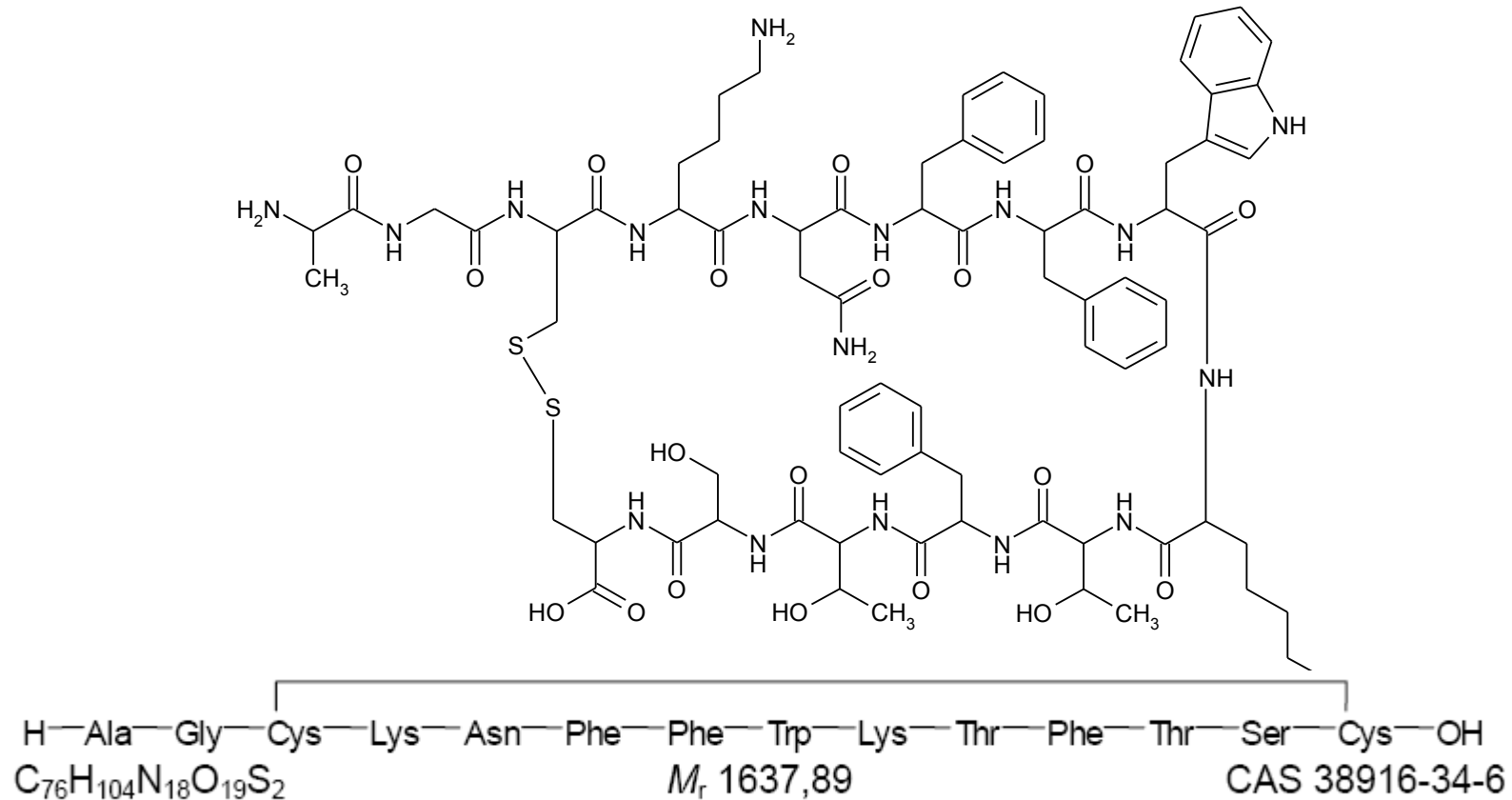
- structure elucidated 1969, used approx. 1976 – 1991, then abandoned
- administered *p.o.*
- used as cognitive functions enhancer for treatment of post-traumatic conditions in injuries of brain and spinal cord and of neurodegeneration diseases (Alzheimer, Parkinson, motoric neuronal disease etc.)

# Metabolism of TRH and its regulation



# Somatostatin

- cyclic tetradecapeptide formed namely in hypothalamus, but also in peripheral nervous
- system, the gut, and other organs
- inhibits pituitary growth hormone (somatotropin) release and probably also release of TRH, prolactin, insulin and glucagon
- has impact to functions of kidneys, pancreas and GIT
- also acts as neurotransmitter in CNS („neuropeptide“)



## somatostatin

*Somatostatinum EP*

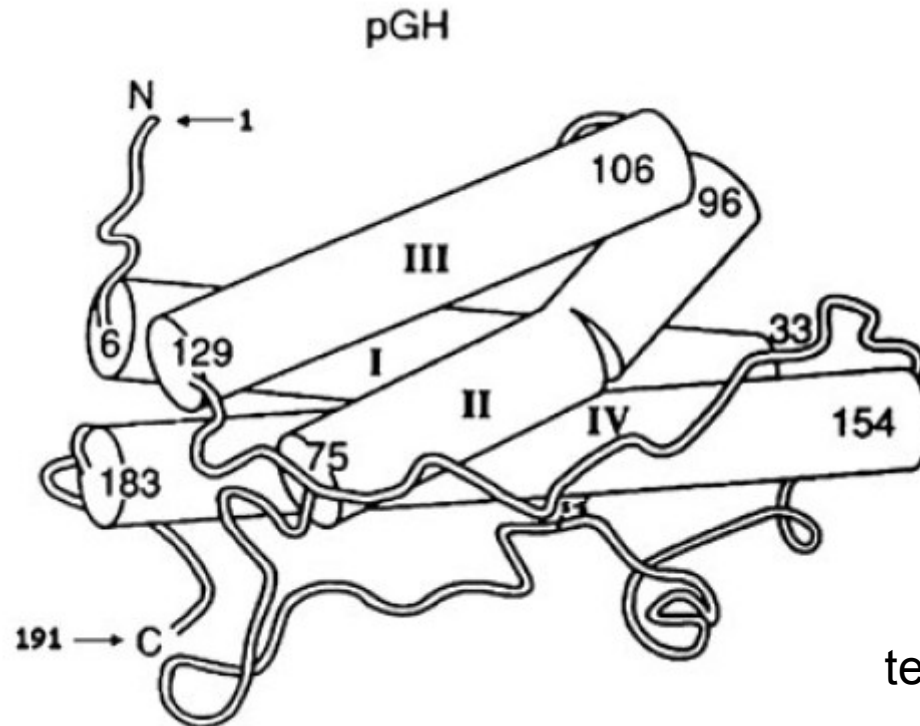
Somatostatin Eumedica<sup>®</sup> inf.

- prepared by synthesis
- treatment of acromegaly

## 1.2 Soma(to)tropin

= growth hormone (GH)

- peptide consisted of 191 AA secreted from anterior pituitary gland
- stimulates mitosis, growth and differentiation of cells of some tissues
- influences expression of genes and metabolism
- sequence of AA known since 1972, nucleotide sequence of the encoding gene since 1977



### somatropin

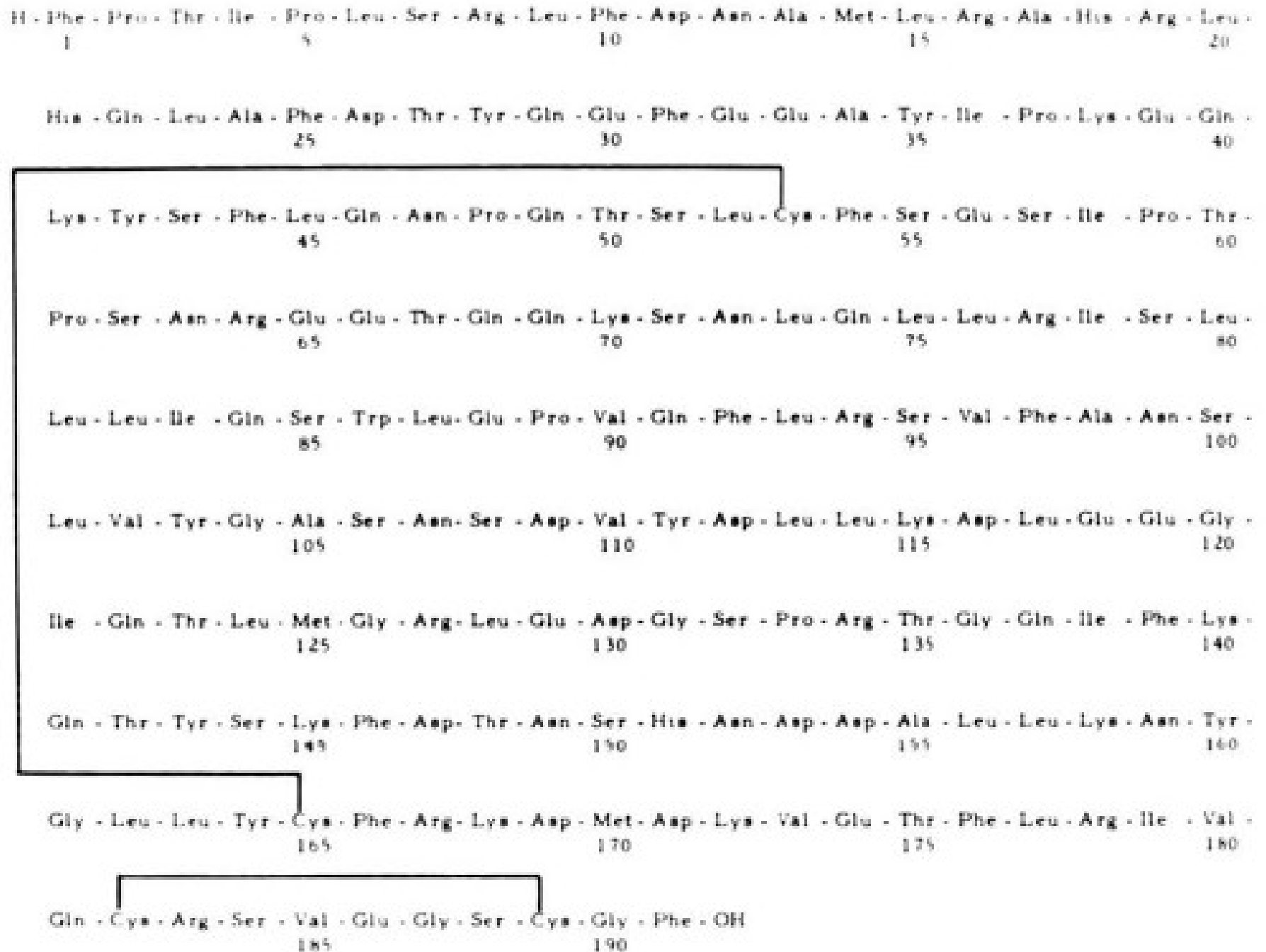
*Somatropinum EP*

- human, prepared by recombinant technology, used since 1985
- substitution treatment of natural GH deficiency

Genotropin ® , Humatrope ® , Nutropinaq ® , Omnitrope ® ...

1 MATGSRTSLL LAFGLLCLPW LQEGSAFPTI PLSRLFDNAM LRAHRLHQLA FDTYQEFEEA YIPKEQKYSF LQNPQTSLCF SESIPTPSNR EETQQKSNLE 100  
101 LLRISLLLIQ SWLEPVQFLR SVFANSLVYG ASDSNVYDLL KDLEEGIQLT MGRLEDGSPR TGQIFKQTYS KFDNTSHNDD ALLKNYGLLY CFRKDMDKVE 200  
201 TFLRIVQCRS VEGSCGF

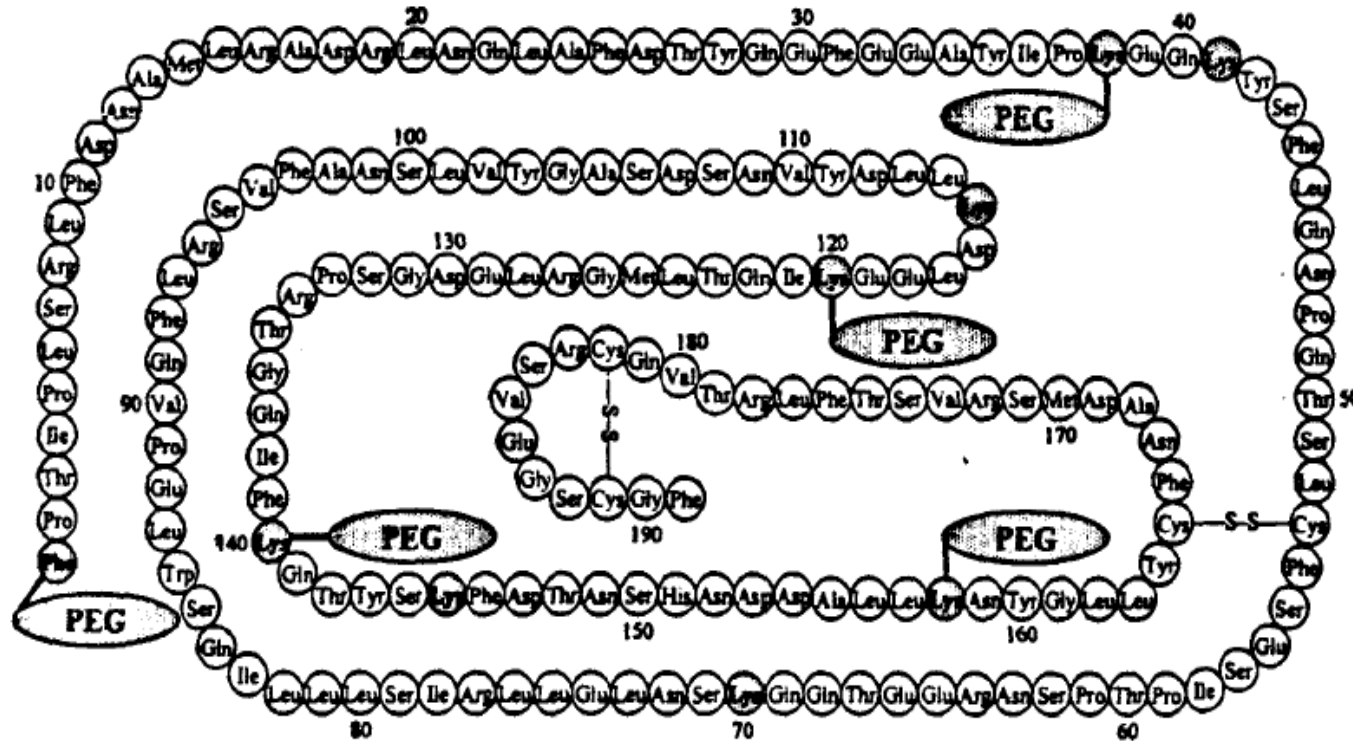
# Primary structure of human somatotropin





# Somatropin (GH) analogues

## Amino Acid Sequence of Pegvisomant Protein



\* Stippled residues indicate PEG attachment sites (Phe<sub>1</sub>, Lys<sub>38</sub>, Lys<sub>41</sub>, Lys<sub>70</sub>, Lys<sub>115</sub>, Lys<sub>120</sub>, Lys<sub>140</sub>, Lys<sub>145</sub>, Lys<sub>158</sub>)

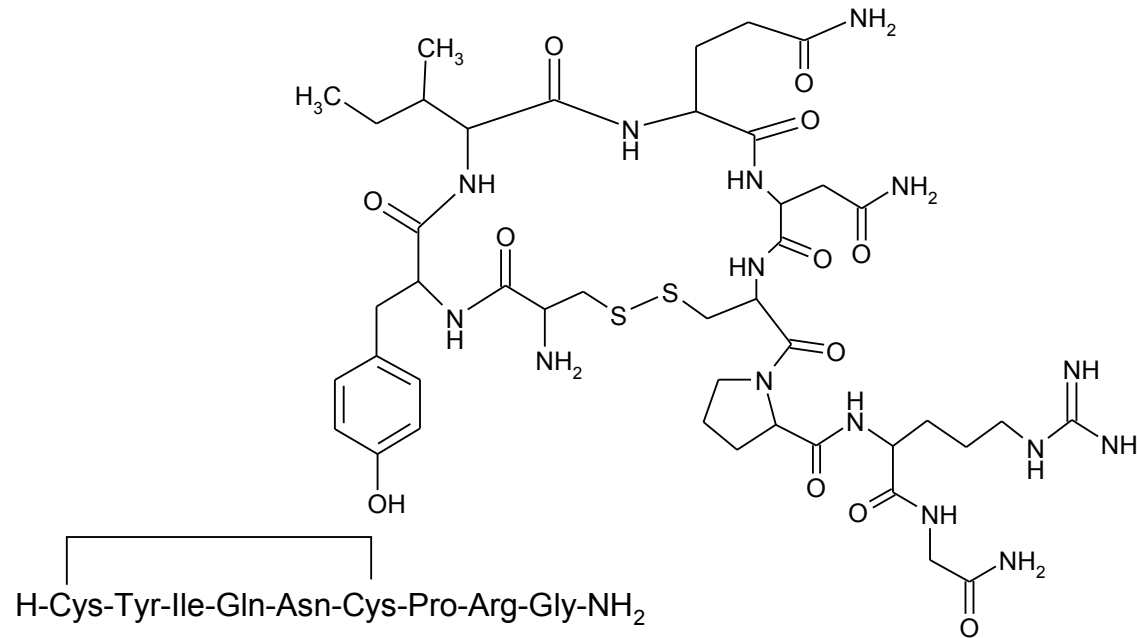
### pegvisomant

- analogue – antagonist of human GH, in which 9 AA are changed; which enables it to block binding of native GH to its receptor by means of preventing receptor dimerisation
- pegylation is performed on 4 – 5 sites randomly selected from Phe<sub>1</sub> and various 8 Lys residues
- prepared by the recombinant technology followed by a controlled reaction with oxiran (polyaddition) which results to covalent binding of 4 – 5 polyoxoethylene chains of M<sub>r</sub> ~ 500
- pegylation lowers antigenicity and prolongs the biologic half-time
- using: treatment of acromegaly

## 1.3 Oxytocin, vasopressins and their analogues

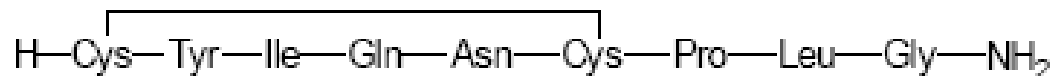
### Vasotocin

= phylogenetic precursor of oxytocin and vasopressins in organisms lower than mammals



### Oxytocin

- a cyclic nonapeptide released from the posterior pituitary gland (neurohypophysis)
- acts on smooth muscle cells, such as causing uterine contractions and milk ejection



C<sub>43</sub>H<sub>66</sub>N<sub>12</sub>O<sub>12</sub>S<sub>2</sub>

M<sub>r</sub> 1007,19

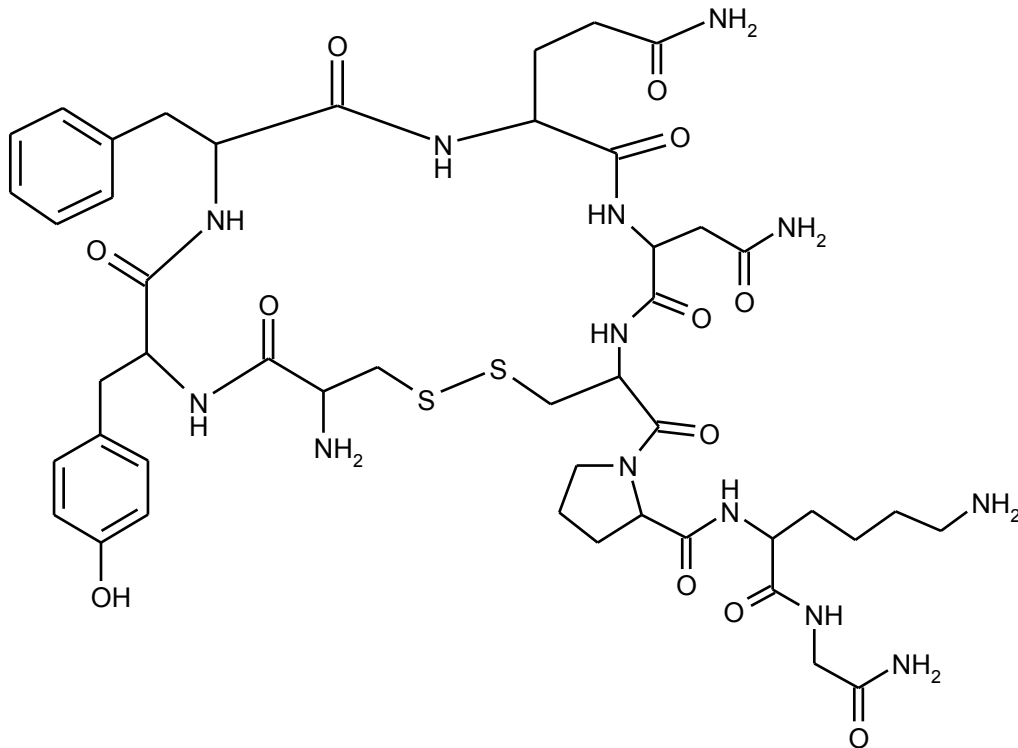
CAS 50-56-6

- prepared by synthesis
- used for triggering of the birth and enhancing of uterine contractions  
*Oxytocinum EP*; Oxytocin Ferring-Léčiva ® inj. sol.

# Vasopressin(s)

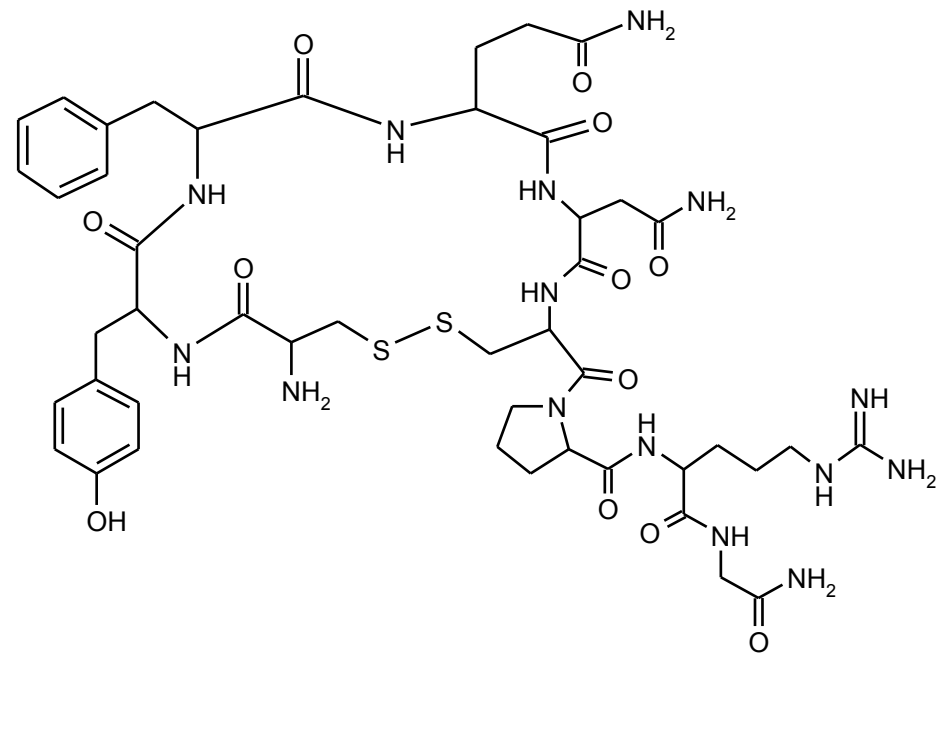
=antidiuretic hormone(s) (ADH)

- octapeptides released from the neurohypophysis of all vertebrates (precursor synthesized in hypothalamus)
- control body water content (regulation of kidneys, lungs etc.)
- potential neurotransmitters
- semi-synthetic derivatives used predominantly



lysine-vasopressin  
**lypressin**

•*Suidae* family only

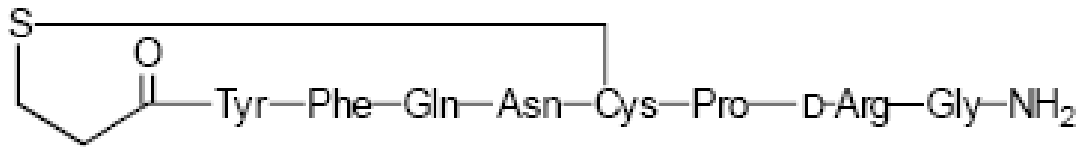


arginine-vasopressin  
**argipressin**

•predominant form of mammalian ADH

•treatment of *diabetes insipidus* and low blood pressure

Vasopressin analogues  
**Desmopressin**



$C_{46}H_{64}N_{14}O_{12}S_2$

$M_r$  1069,22

CAS 16679-58-6

*Desmopressinum EP*

- cyclic pseudononapeptide
- prepared by synthesis
- antidiuretic (*enuresis nocturna*, ...)

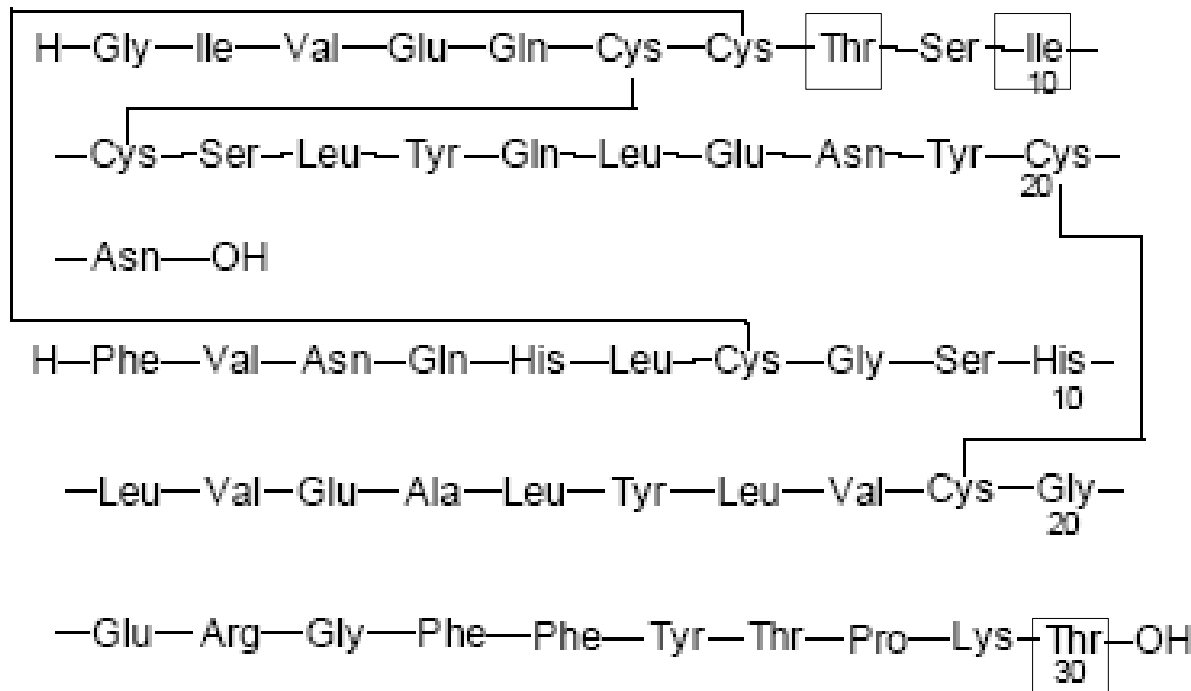
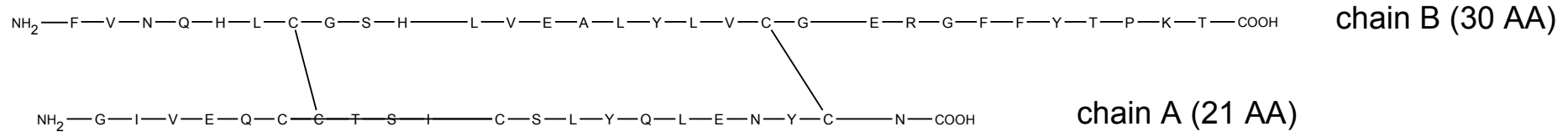


## 1.4 Insulines, glucagon and GLP-1 analogues

### Insuline

- Secreted mostly by  $\beta$ -cells of Langerhans islets of pancreas
- Enables utilisation of glucose by cells of body
- First isolated by Banting and Best from dog's pancreas in 1921

### Human insuline



C<sub>257</sub>H<sub>383</sub>N<sub>65</sub>O<sub>77</sub>S<sub>6</sub>

M<sub>r</sub> 5807,60

CAS 11061-68-0

- formed from its precursor proinsuline consisted of 110 AA

10                  20                  30                  40                  50                  60  
MALWMRLLPL LALLALWGPD PAAAFVNQHL CGSHLVEALY LVCGERGFFY TPKTRREAED

70                  80                  90                  100                  110  
LQVGQVELGG GPGAGSLQPL ALEGLQKRG IVEQCCTSIC SLYQLENYCN

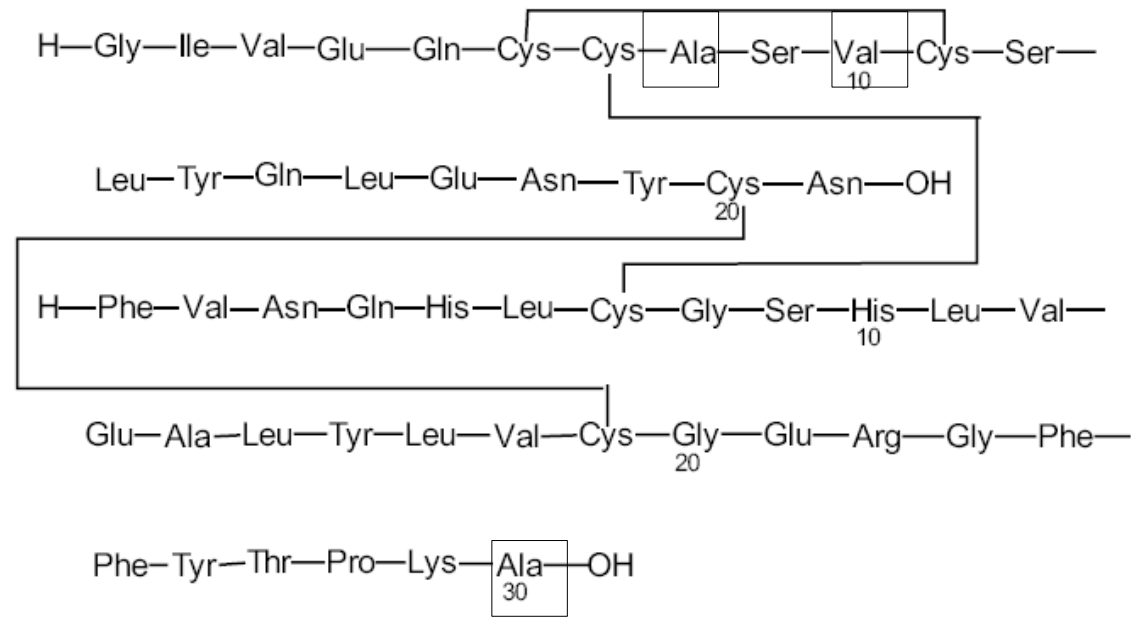
1-24 signal sequence; 25-54 chain B; 57-87 peptide C; 90-110 chain A

- today produced by recombinant technology, or by partial synthesis from the porcine one

*Insulinum humanum PhEur*

- syn. humuline

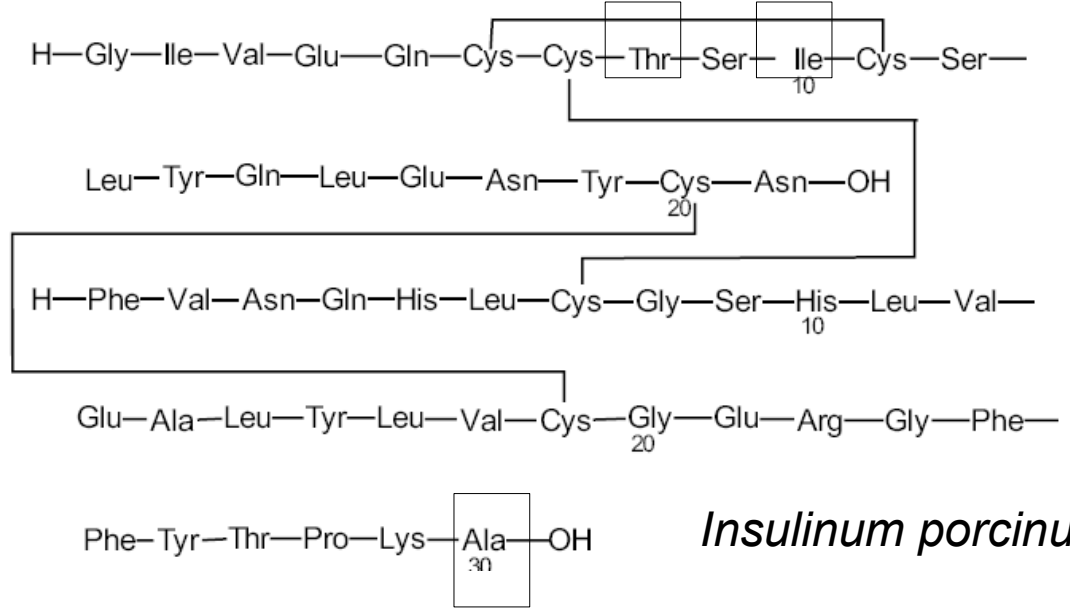
## Bovine (cow's) insuline



### *Insulinum bovinum PhEur*

- isolation from beef pancreases

## Porcine (swine) insuline

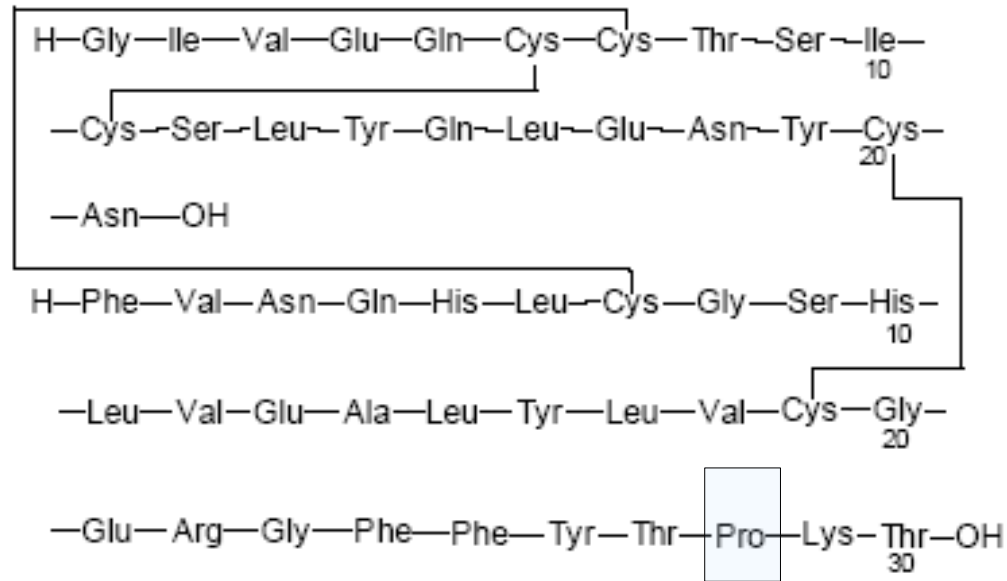


### *Insulinum porcinum PhEur*



# Insuline analogues

human

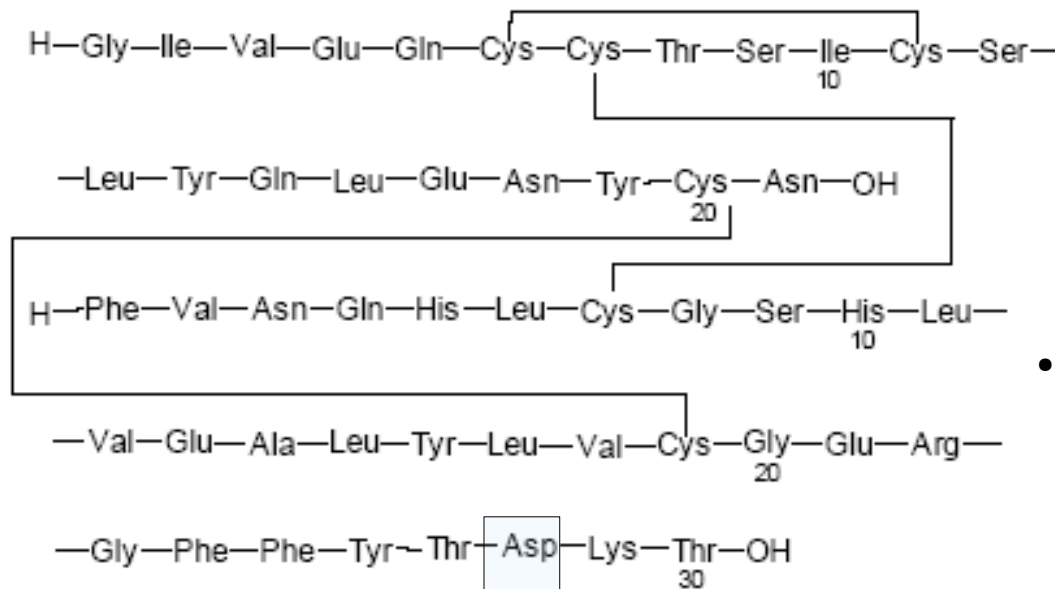


$C_{257}H_{383}N_{65}O_{77}S_6$

$M_r$  5807,60

CAS 11061-68-0

aspart  
*Insulinum aspartum*  
*PhEur*  
 Novorapid®

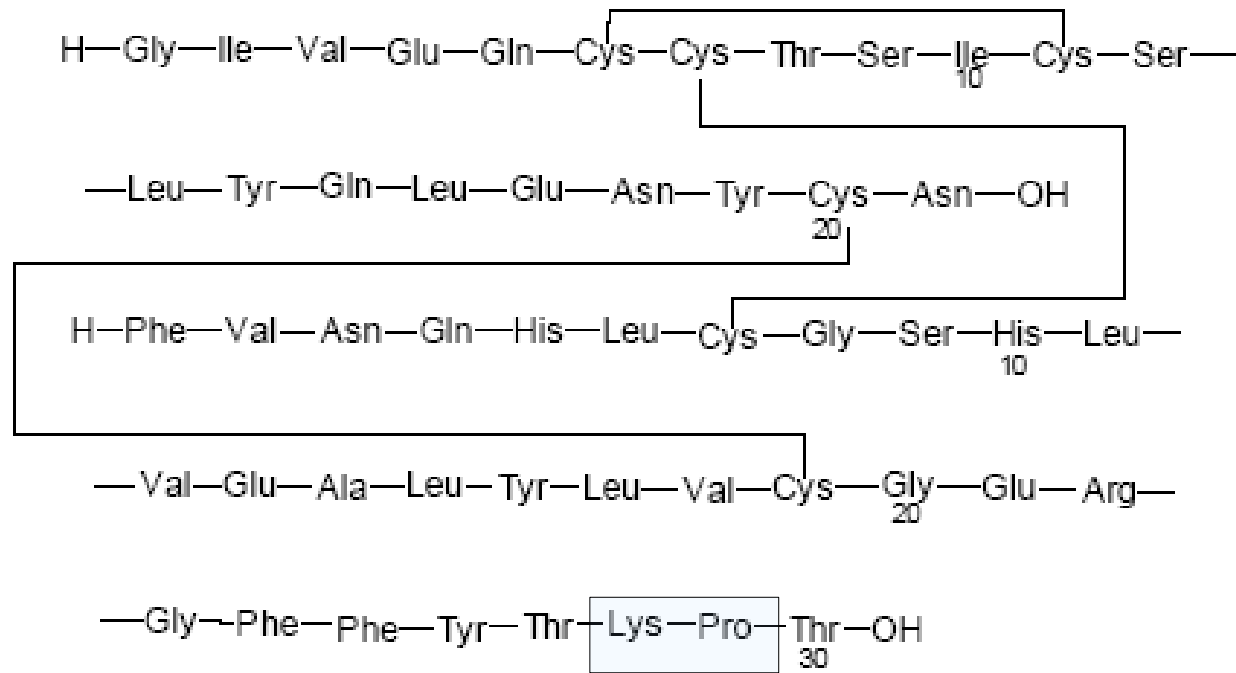


•recombinant technology

$C_{256}H_{381}N_{65}O_{79}S_6$

$M_r$  5825,58

CAS 116094-23-6



$C_{257}H_{383}N_{65}O_{77}S_6$

$M_r$  5807,61

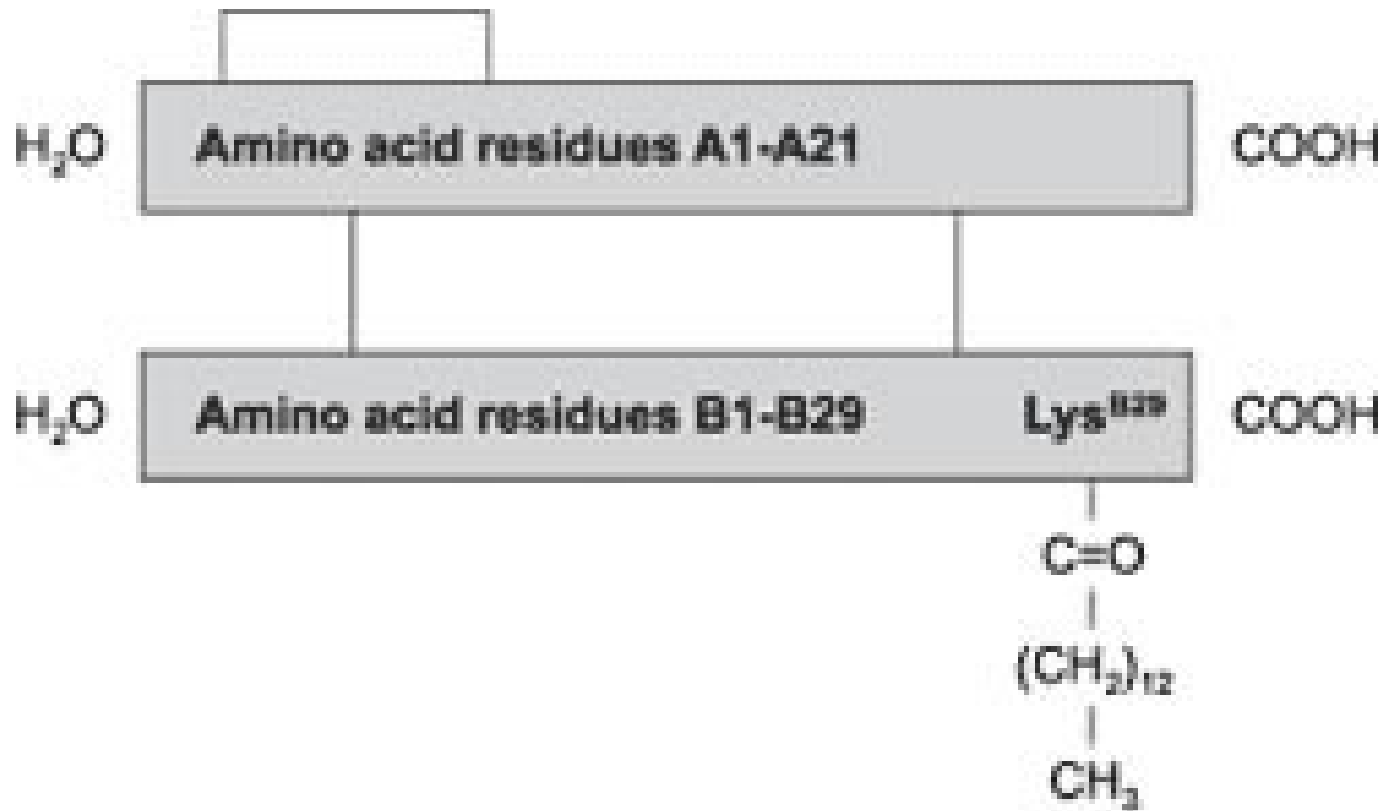
CAS 133107-64-9

insulin-lispro

*Insulinum lisprum PhEur*

•recombinant

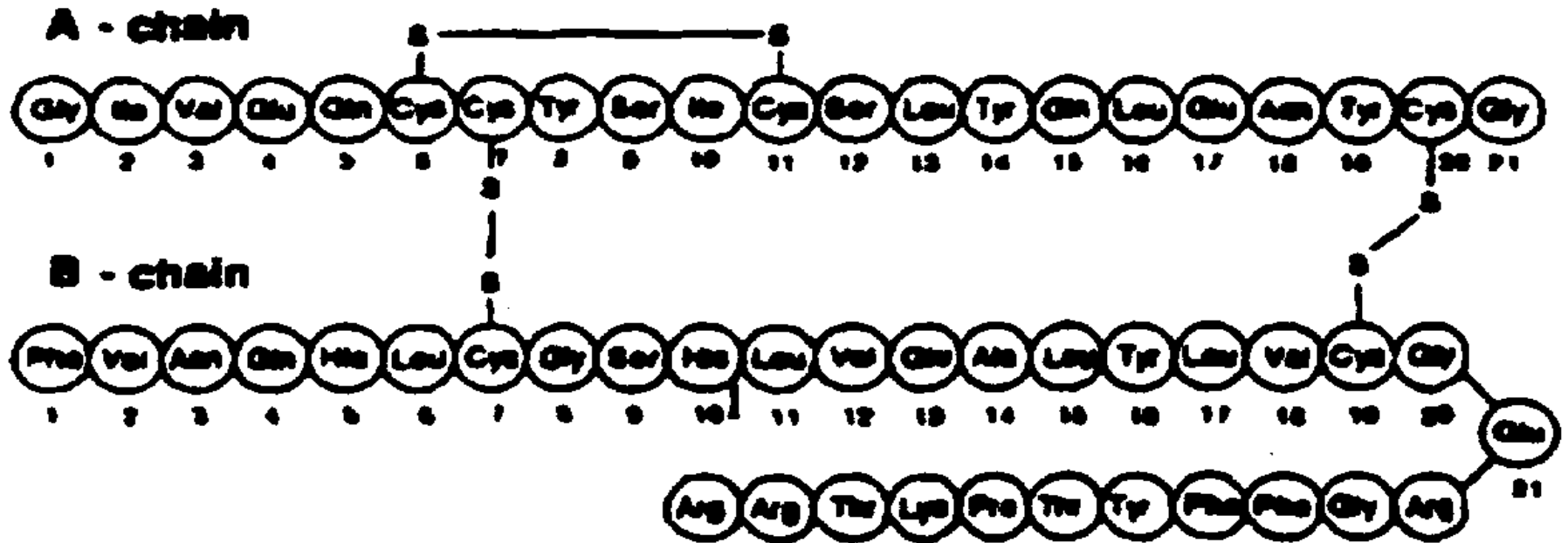
Humalog ®, Liprolog ®



insulin-detemir

- chain B has only 29 AA, tetradecanoyl (myristoyl) attached to Lys<sup>B29</sup>
- recombinant-semi synthetic

Levemir ®

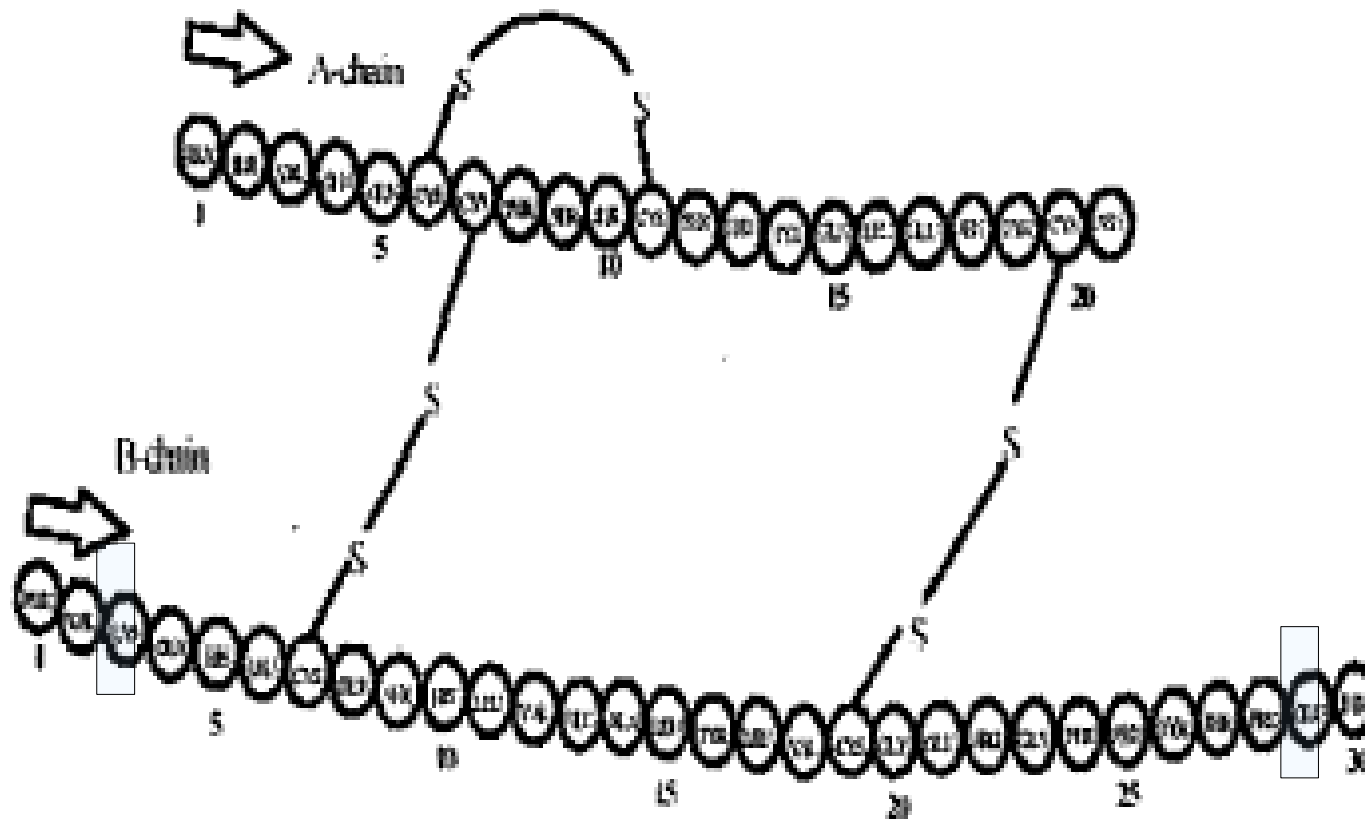


## insulin-glargin

Gly<sup>21A</sup>-L-Arg<sup>30B</sup>-L-Arg<sup>31B</sup>-insulin

Lantus<sup>®</sup>, Optisulin<sup>®</sup>

- insulin of 1<sup>st</sup> choice in diabetes of 2<sup>nd</sup> type when oral antidiabetics are not satisfactory
- long  $T_{1/2}$ , typically administered 1x daily s.c. before sleeping



Chemical name: 3βLys-29βGlu-human insulin

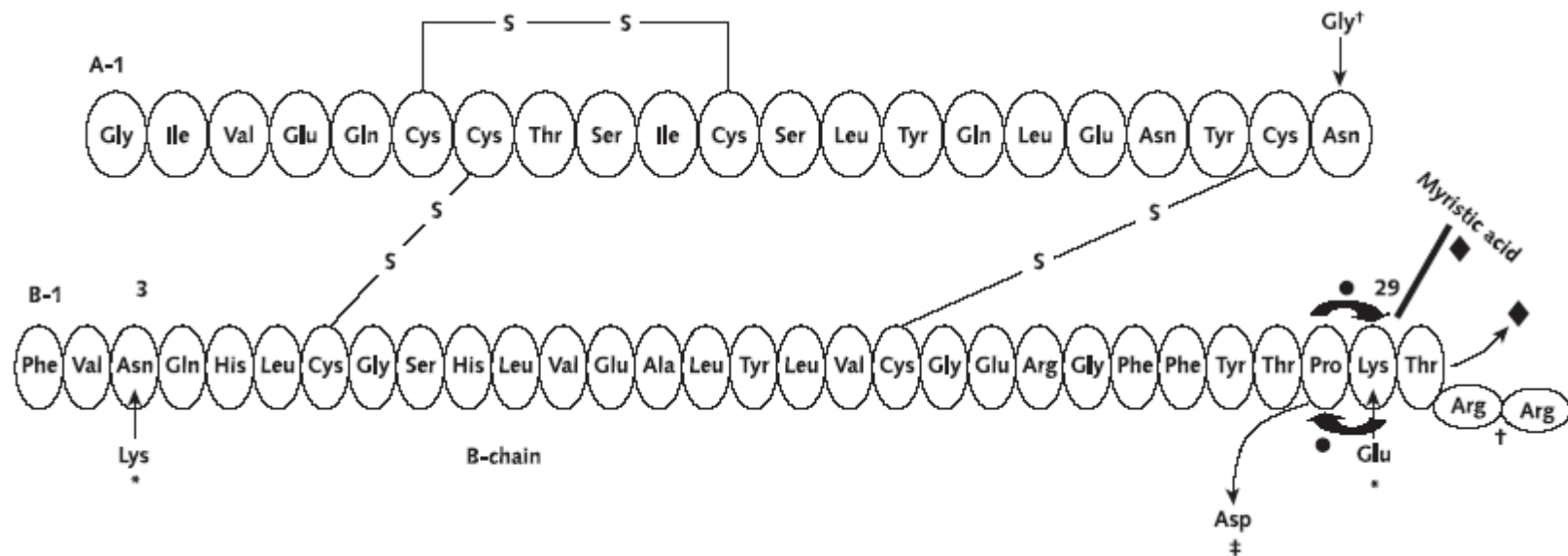
CAS registry number: 207748-29-6

Molecular formula/molecular weight:  $C_{258}H_{384}O_{78}N_{64}S_6/5823$

**insulin-glulisin**

Apidra ®

## Summary of the used insuline analogues



● = Insulin lispro differs from human insulin by the substitution of proline with lysine at position 28 and the substitution of lysine with proline at position 29 of the insulin  $\beta$  chain.

‡ = Insulin aspart is designed with the single replacement of the amino acid proline by aspartic acid at position 28 of the human insulin  $\beta$  chain.

\* = Insulin glulisine is designed with the substitution of the amino acid lysine with asparagine at position 3 of the human insulin  $\beta$  chain and by substitution of the amino acid lysine at position 29 with glutamine.

† = Insulin glargine differs from human insulin in that the amino acid asparagine at position A21 is replaced by glycine and 2 arginines are added to the C-terminus of the  $\beta$  chain.

◆ = Insulin detemir is designed to bind albumin in plasma after absorption. Threonine is omitted from position 30 of the insulin  $\beta$  chain and replaced by myristic acid, a C14 fatty acid chain.

Figure reprinted with permission from reference 2: Oiknine R, Bernbaum M, Mooradian AD. A critical appraisal of the role of insulin analogues in the management of diabetes mellitus. *Drugs*. 2005;65:325-40. [PMID: 15669878]

## Glucagone

- peptid consisted of 29 AA from pancreas supporting cleavage of liver glycogene and increasing glycaemia
- causes relaxation of smooth gastric muscles similarly to cholinergics

H—His—Ser—Gln—Gly—Thr—Phe—Thr—Ser—Asp—Tyr—

10

Ser—Lys—Tyr—Leu—Asp—Ser—Arg—Arg—Ala—Gln—

20

Asp—Phe—Val—Gln—Trp—Leu—Met—Asn—Thr—OH

C<sub>153</sub>H<sub>225</sub>N<sub>43</sub>O<sub>49</sub>S

M<sub>r</sub> 3482,78

CAS 16941-32-5

### *Glucagonum PhEur*

- isolated from porcine or bovine pancreases

### *Glucagonum humanum PhEur*

- produced by recombinant technology; AA sequence is identical
- usage: treatment of serious hypoglycaemia, X-ray GIT diagnostic etc.

## GLP-1 analogues

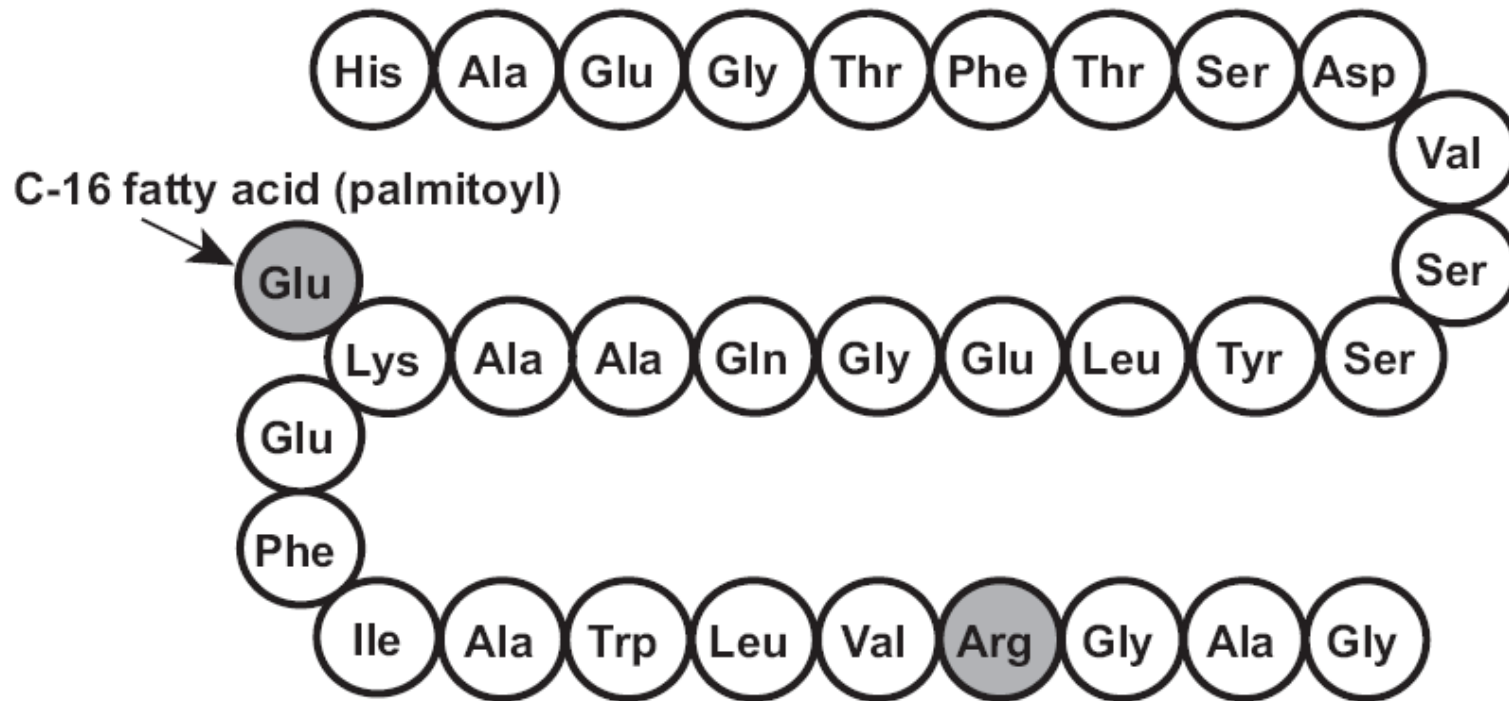
- GLP-1: Glucagon-like peptide 1 = an intestinal hormone, which together with glucose-dependent insulinotropic polypeptide (GIP)\* potentiates insulin secretion induced by food
- potentiates all steps of insulin biosynthesis; has positive impact to function and surviving of  $\beta$ -cells
  - decreases redundant glucose production in liver, slows down stomach emptying leading to postprandial hypoglycaemia, its central effect leads to appetite decrease ( $\Rightarrow$  body weight loss), probably also positive effects to cardiovascular system
  - disadvantages of GLP-1 as a drug: necessity of administration in a continual infusion, extremely short biological half-time  $T_{1/2} = 2 - 3$  min (fast decomposition by peptidases)  $\Rightarrow$  need of more stable analogues

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\*Both are known also as **incretins**.



## GLP-1 analogues



### **liraglutide**

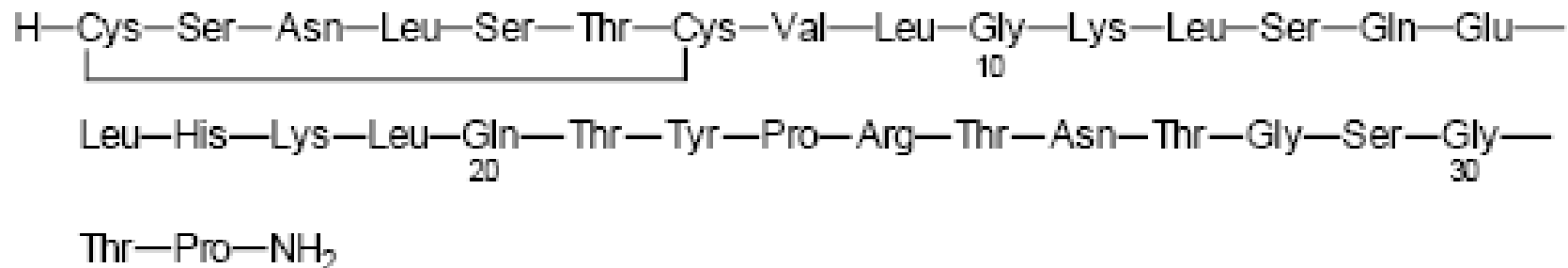
Victoza® inj. sol.

$\gamma$ -L-glutamoyl(N- $\alpha$ -hexadecanoyl)-Lys<sup>26</sup>, Arg<sup>34</sup>-GLP-1(7-37)

- sequence of amino acid rests shares 97 % identity with the fragment 7-37 of the native GLP-1
- strong binding to serum albumin, mutual association of molecules, does not come under glomerular filtration  $\Rightarrow T_{1/2} = 12.5$  hours after s.c. injection
- improves functions of both  $\alpha$  and  $\beta$  cells

## Calcitonin

- released from thyroidal C-cells ( = parafollicular cells – Baber 1876), in lower vertebrates from ultimobranchial bodies, originated from 5<sup>th</sup> branchial fissure
- peptide from 32 amino acid residues (salmon's – *Onchorhyncus kisutch*; human has 139 AA)
- receptors on osteoclasts (also in kidneys and brain)
- ↓ excretion of  $\text{Ca}^{2+}$  from the bone ( $\Rightarrow$  ↓ calcaemia)
- ↓ osteoclasts formation
- used together with  $\text{Ca}^{2+}$  for treatment of osteoporosis

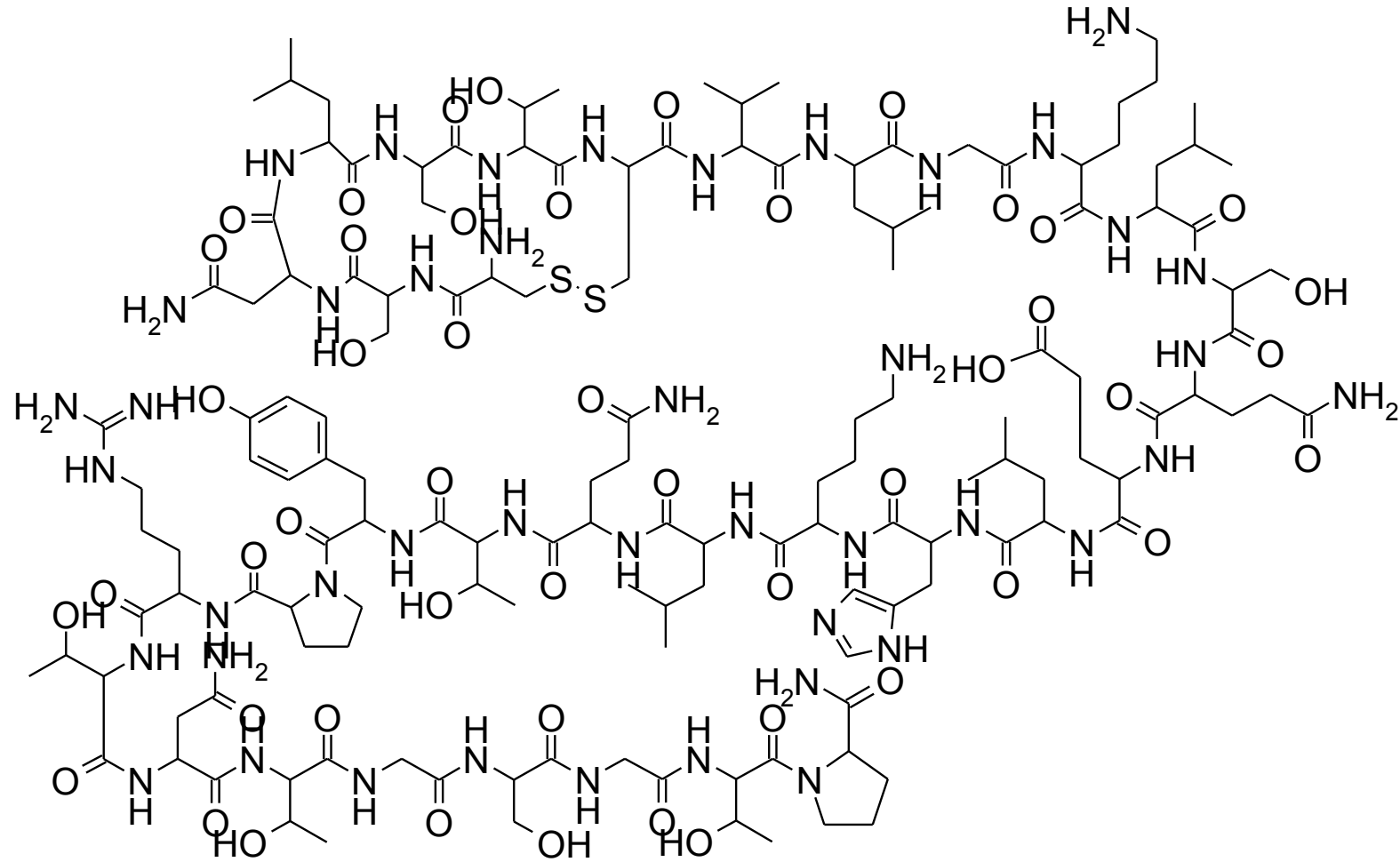


$\text{C}_{145}\text{H}_{240}\text{N}_{44}\text{O}_{48}\text{S}_2$

$M_r$  3431,88

CAS 47931-85-1

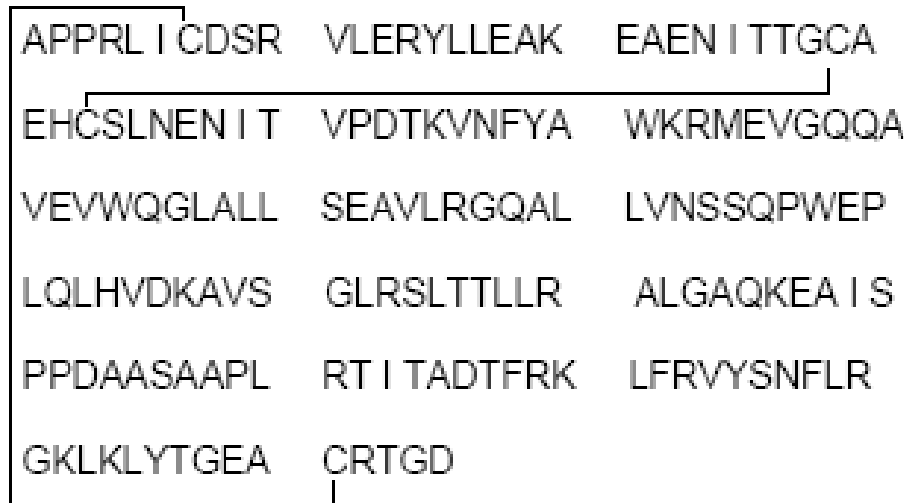
# Calcitonin



*Calcitoninum salmonis EP* = **calcitonin salmon** (synthetic; AA sequence corresponds with salmon hormon)

Miacalcic® inj., nasal; Osteodon®; Tonocalcin®

## 2. Blood factors of erythropoietine type



$M_r$  about 30 600

CAS 113427-24-0

### erythropoietin

= glycosylated protein from 165 AA

*Erythropoietini solutio concentrata EP*

= a solution containing a group of closely related glycoproteins, which are not to distinguish from the natural human erythropoietin (urine erythropoietin) from the point of view of 165 amino acids sequence and their average profile of glycosylation

- naturally released from kidneys of adults and in liver of foetus
- stimulates stem cells of bone marrow to proliferation and differentiation
- produced *in vitro* in rodent cell lines by a method based on the recombinant DNA technology
- treatment of haematopoietic disorders, misused for doping

### 3. Colony stimulating factors

APARSPSPST QPWEHVNAIQ EARRLLNLSR  
DTAAEMNETV EWISEMFDLQ EPTCLQTRLE  
LYKQGLRGSL TKLKGPLTMM ASHYKQHCPP  
TPETSCATQI ITFESFKENL KDFLLVIPFD  
CWEPVQE

C<sub>639</sub>H<sub>1007</sub>N<sub>171</sub>O<sub>196</sub>S<sub>3</sub>

M<sub>r</sub> 14 477,49

CAS 99283-10-0

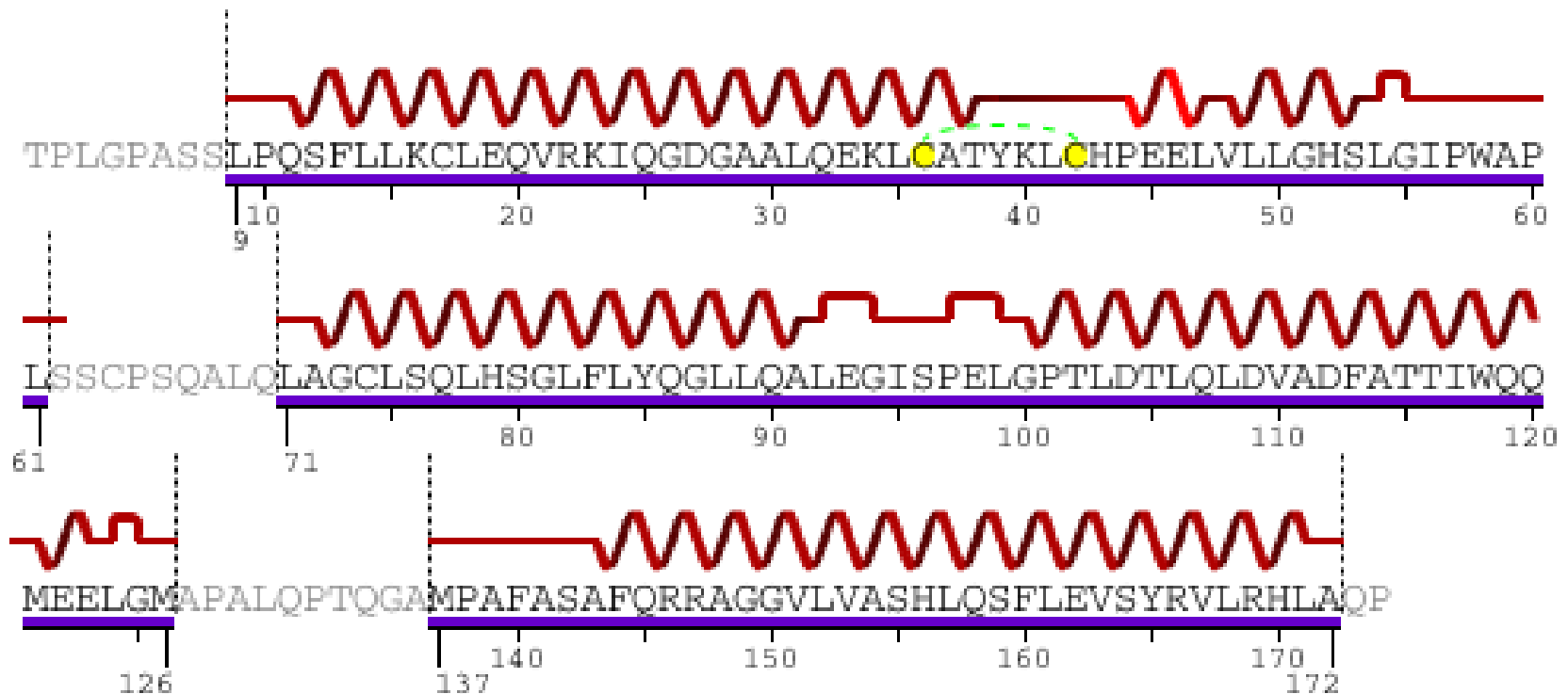
#### **molgramostim**

= a factor stimulating granulocytes and macrophages colonies released from various kinds of blood cells

- not glycosylated
- stimulates differentiation and proliferation of leukocyte pluripotent stem cells into matured granulocytes and macrophages
- production by a recombinant technology using bacteria as host cells
- treatment of leukopenia in cancer chemotherapy or HIV infections

## Filgrastim and pegfilgrastim

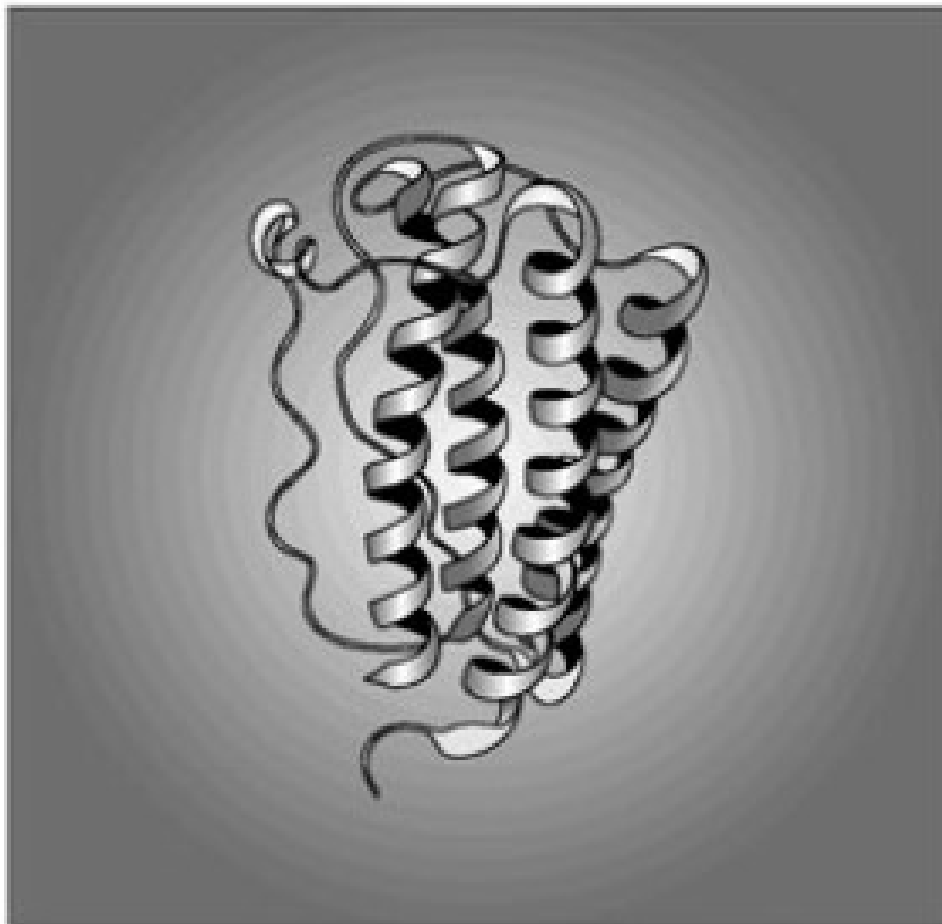
Filgrastim = human granulocytes colony-stimulating factor (G-CSF); glycosylated, 174 AA  
Sequence of filgrastim precursor



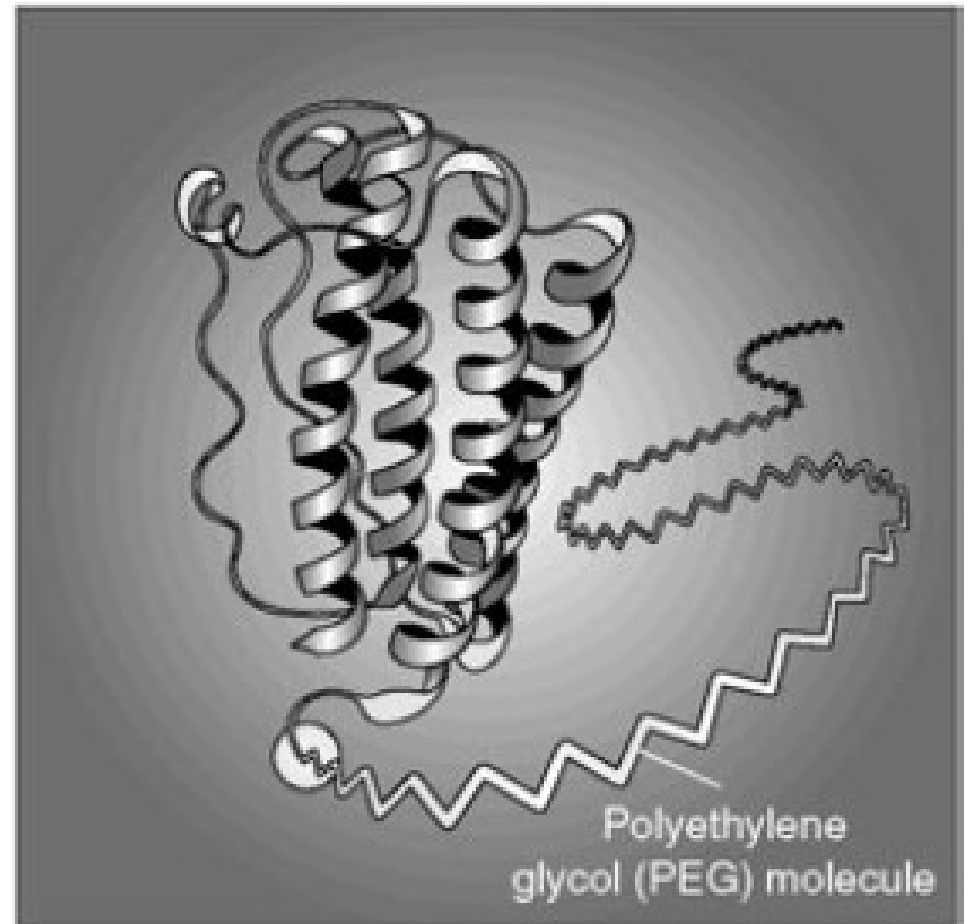
- treatment of neutropenia in cancer chemotherapy and in AIDS

**Pegfilgrastim** has covalently attached PEG chain of  $M_r$  cca 20 000 on N-terminus

- longer elimination half-time
- recombinant and semi-synthetic production

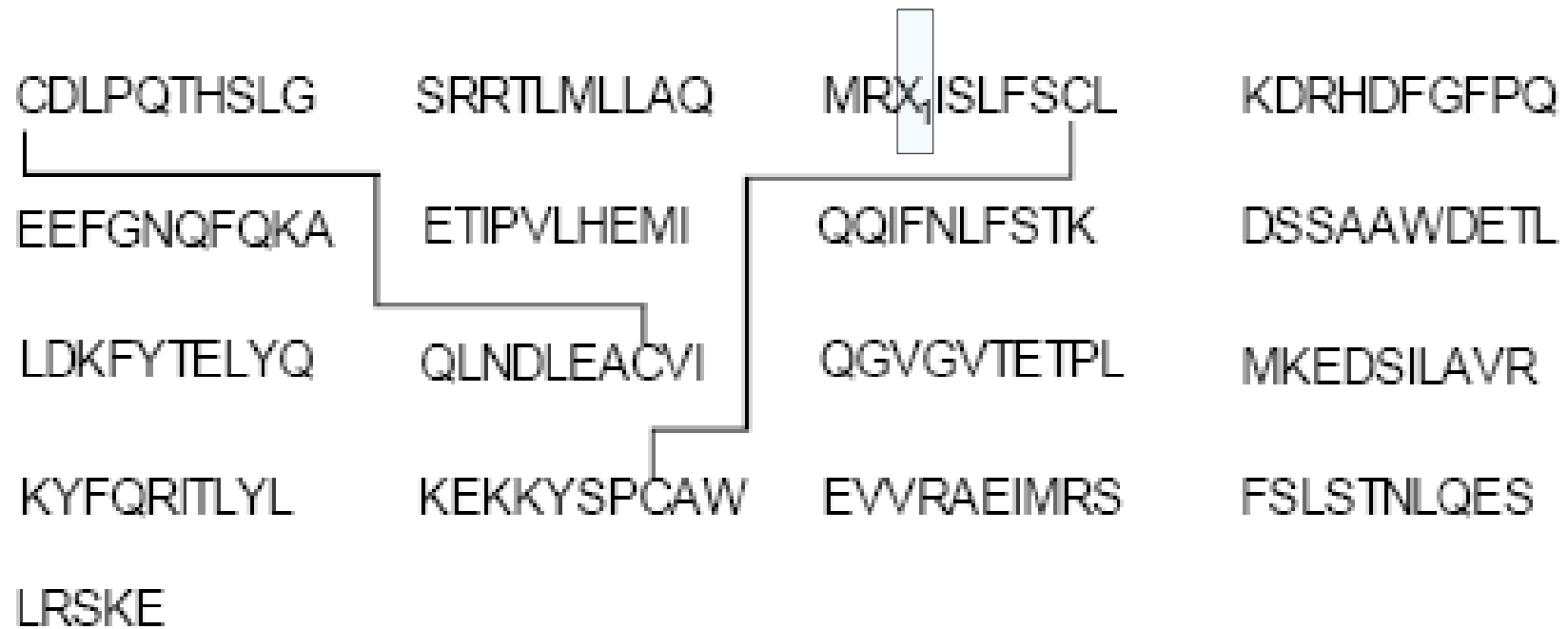


**Filgrastim**



**Pegfilgrastim**

## 4. Non-specific antibodies - interferons



### interferon $\alpha_2$

*Interferoni alfa-2 solutio concentrata EP*

X1 = Lys  $\alpha_{2a}$

X1 = Arg  $\alpha_{2b}$

- antiviral activity during viral RNA and protein syntheses
- antiproliferation activity
- produced by a recombinant technology on bacteria

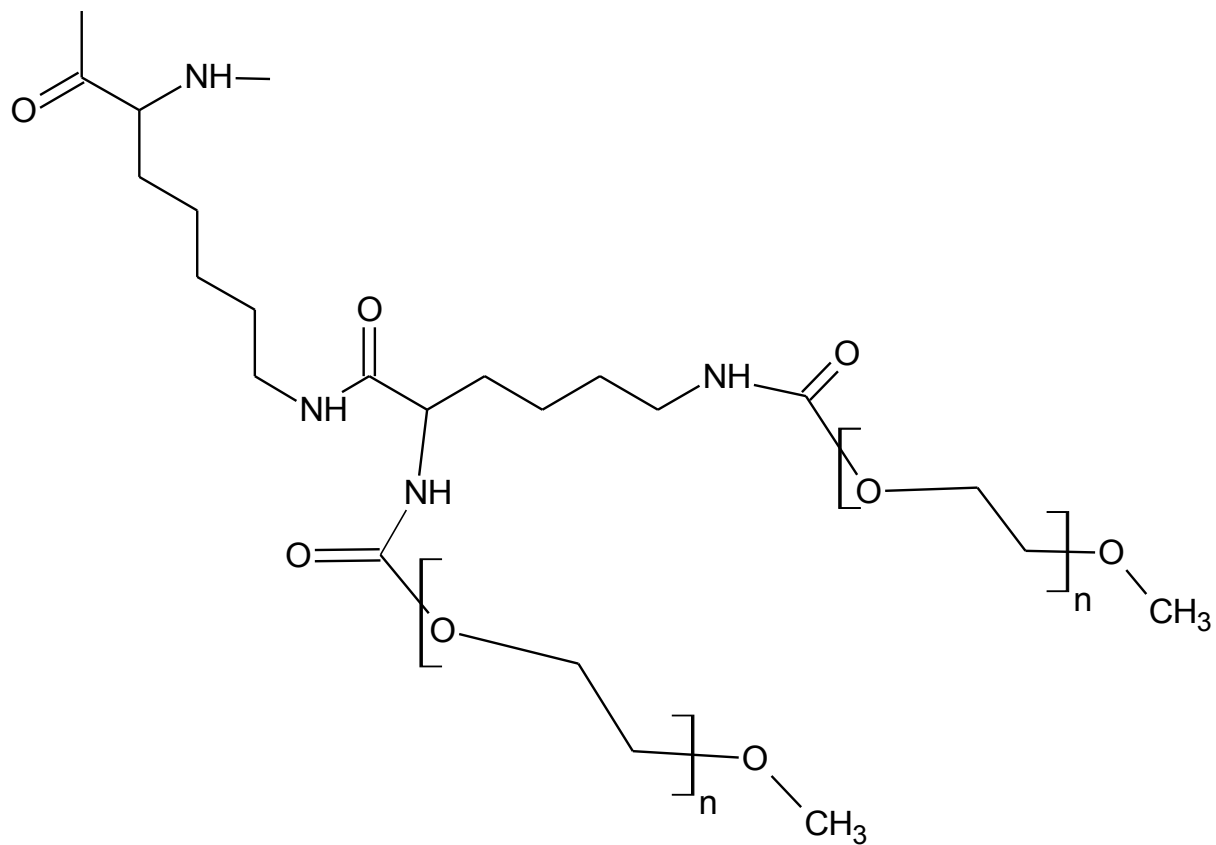


## Pegylated interferons $\alpha$

- **peginterferon  $\alpha_{2a}$**  (Pegasys®) - on some Lys residues attached N<sup>2</sup>, N<sup>6</sup>-dicarboxy-Lys esterified with PEG-monomethylether of M<sub>r</sub> about 20 000
  - substitution is stable, free interferon is not released
  - peginterferon  $\alpha_{2a}$  interacts directly with receptors on surface of the infected cell
  - lowered activity (only 7 % of free interferon  $\alpha_{2a}$ ) is counterbalanced by much longer half-time
  - treatment of hepatitis B and C combined with ribavirin
- **peginterferon  $\alpha_{2b}$**  (Pegintron®) - only one PEG chain of M<sub>r</sub> about 12 000 attached via urethane linker to a His, most frequently to His<sub>34</sub>
  - urethane moiety is labile, free interferon  $\alpha_{2b}$  is released into the circulation and directly interacts with receptors
  - treatment of hepatitis C

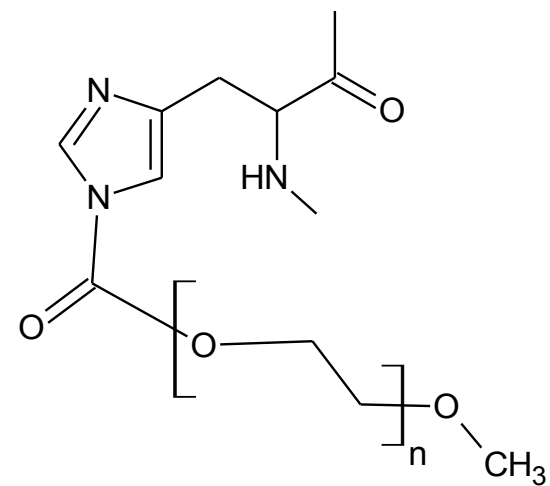
# Pegylated interferons $\alpha$

## Differences in their substitutions



Lys

$\alpha_{2a}$



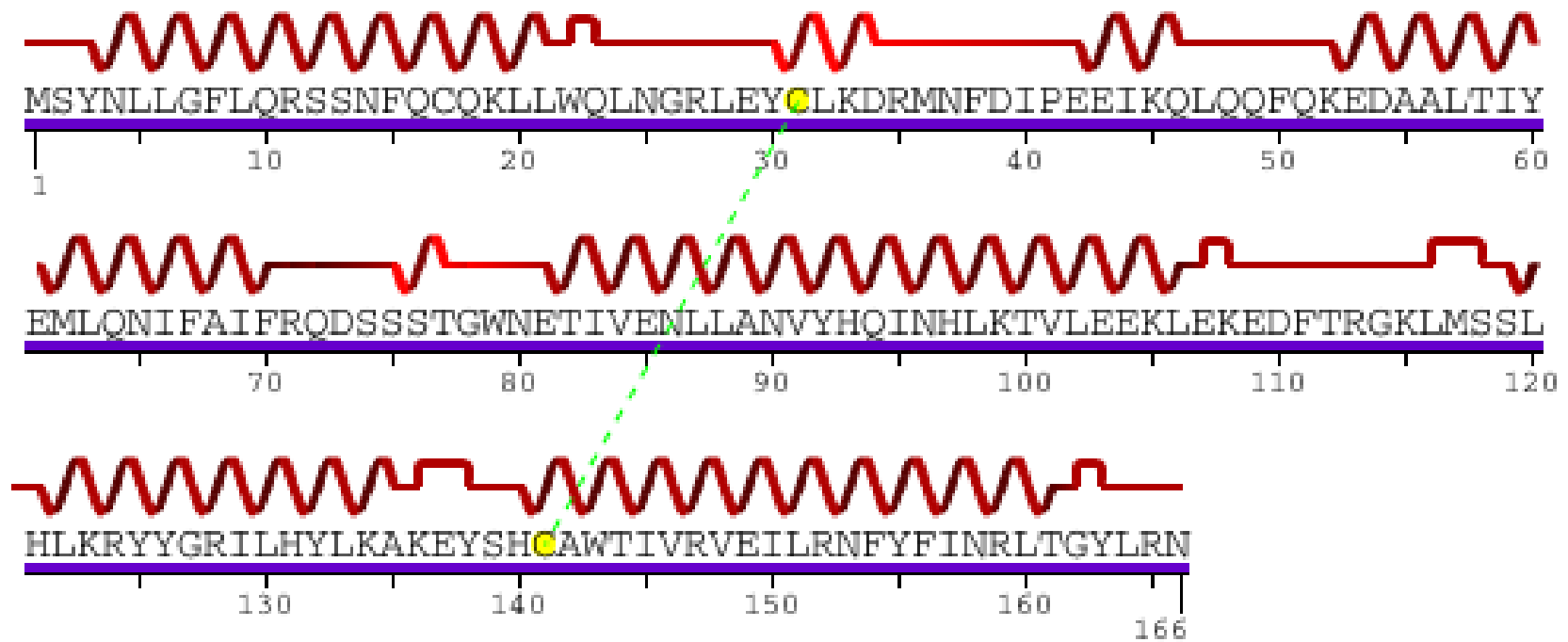
His<sup>34</sup>

$\alpha_{2b}$

## interferon $\beta$

= a glycosylated peptide consisted of 166 AA

- produced by fibroblasts in response to stimulation by a living or inactivated virus or double-strained RNA



- treatment of multiple sclerosis

## Variants of interferon $\beta$

- $\beta_{1a}$  (Avonex<sup>®</sup> , Betaferon<sup>®</sup> , Rebif<sup>®</sup> )
  - $M_r$  cca 20 000
  - prepared by a recombinant technology on Chinese hamster ovary cell lines
  - preparations are not equally active probably due to different glycosylation
  - recommended *i.m.* application once weekly
    - injected s.c. is much more painful than  $\beta_{1b}$
- $\beta_{1b}$  (Extavia<sup>®</sup> )
  - Cys<sub>17</sub> changed to Ser
  - recombinant technology on *E. coli*
  - s.c. application every other day

## interferon $\gamma_{1b}$

- released by human T-lymfocytes in response to viral infections and other agents
- imunomodulatory effects
- non-covalent dimer of 2 identicas monomers consisted of 141 AA

Sequence of the monomer:

M

QDPYVKEAEN LKKYFNAGHS DVADNGTLFL GILKNWKEES  
DRKIMQSQIV SFYFKLFKNF KDDQSIQKSV ETIKEDMNVK  
FFNSNKKKRD DFEKLTNYSV TDLNVQRKAI HELIQVMAEL  
SPAAKTGKRK RSQMLFRGR

$C_{734}H_{1166}N_{204}O_{216}S_5$

$M_r$  16 464,76

- production by recombinant technology on bacteria
- supporting treatment of idiopatic lung fibrosis; only increases the hope of patients live to see lungs transplantation