

Therapeutic peptides

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Classification of therapeutic peptides

1. Hormones

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

1.2. Soma(to)tropin

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- α -amino acid rests

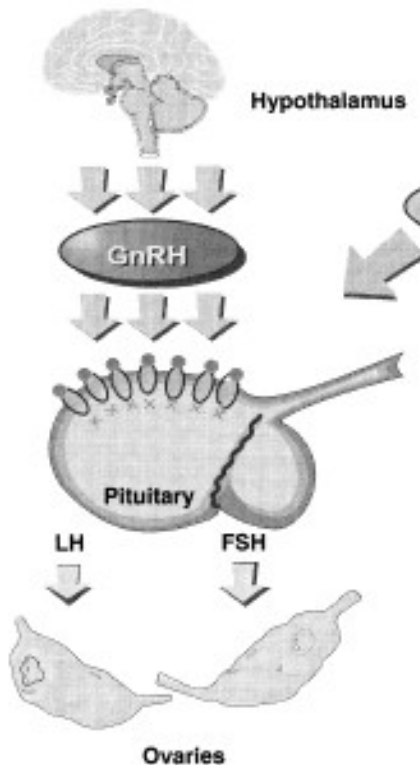
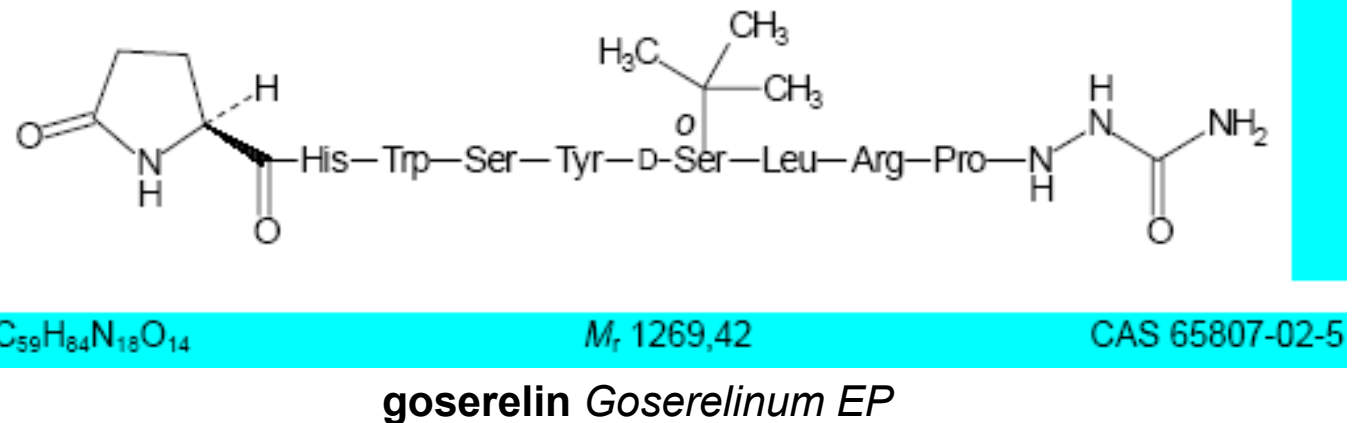
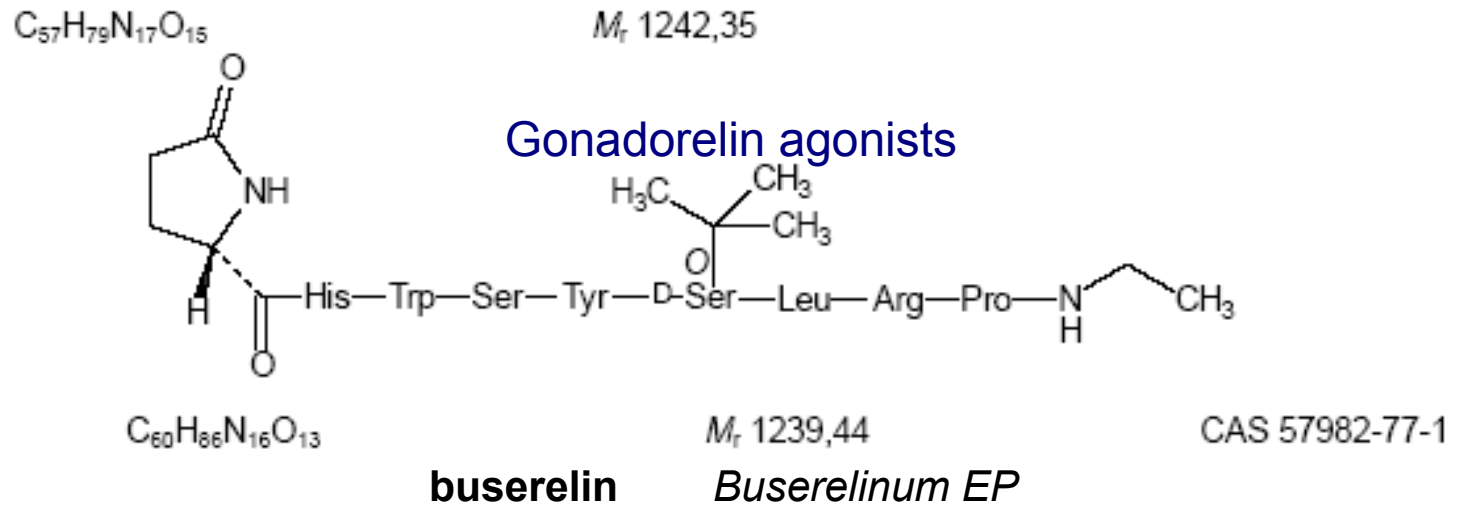
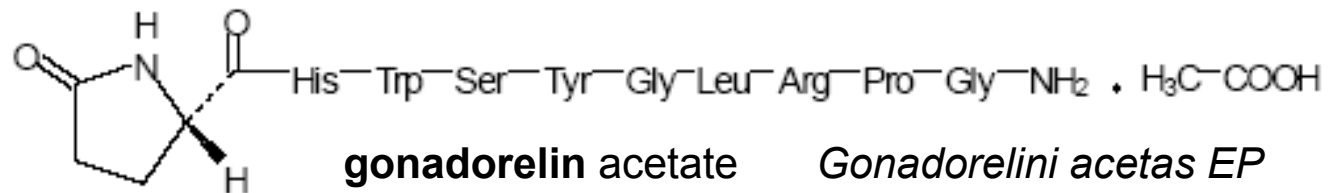
One-letter	Three-letter	
A	Ala	alanine
B	Asx	asparaginic acid or asparagine
C	Cys	cysteine
D	Asp	asparaginic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
Q	Gln	glutamine
R	Arg	arginine
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

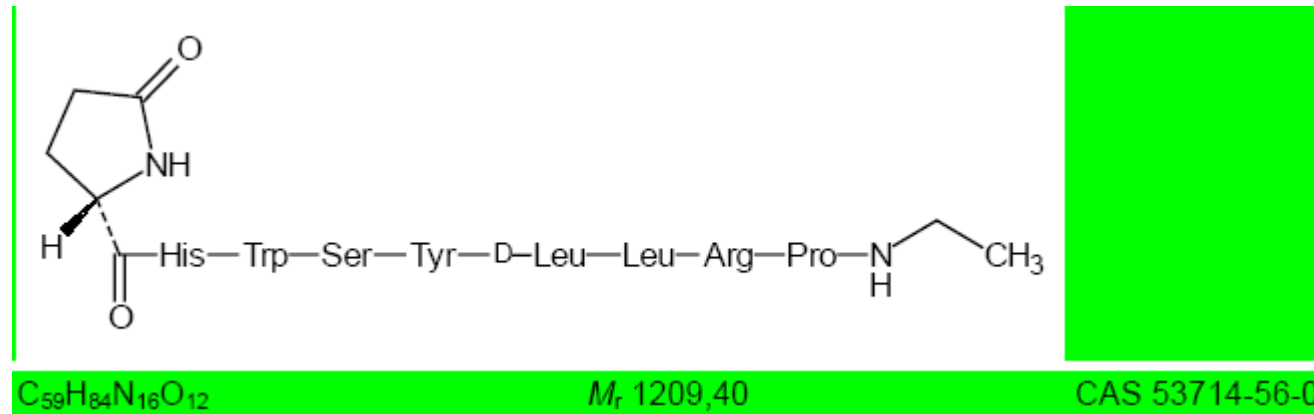
1.1 Liberins and statins („releasing“ & „inhibiting“)

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



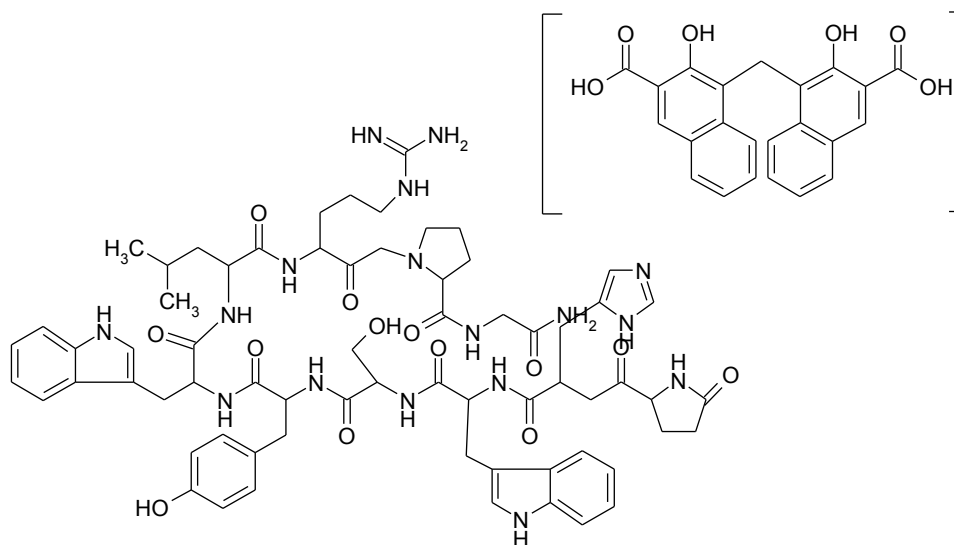
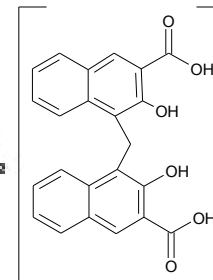
leuprorelin (syn. leuprolide) *Leuprorelinum EP*

Eligard ®

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

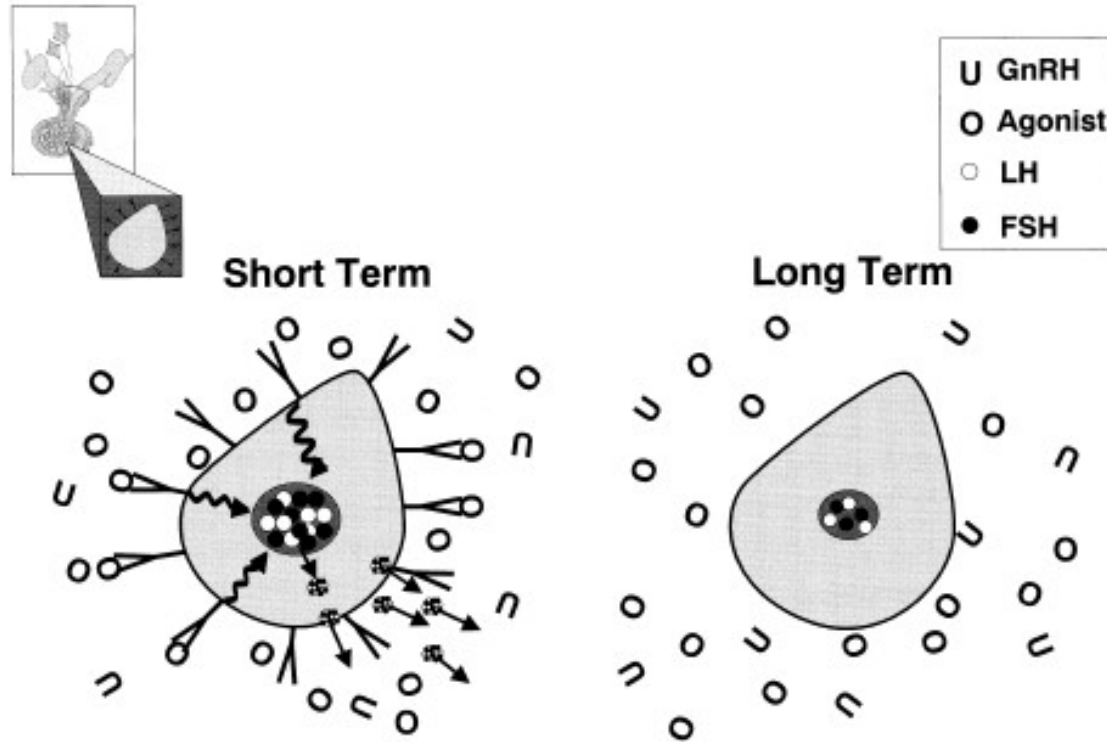
Gonadorelin and its analogues Agonists

Trelstar™ (triptorelin pamoate)



Short- and long term action of gonadorelin agonists

Effects of GnRH agonist.

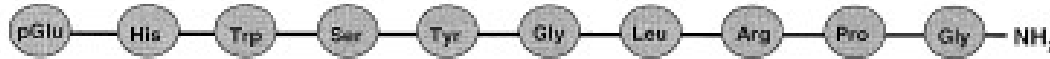


- long term action leads to receptors internalisation and stopping of the effect (due to decreasing LH and FSH levels and thus also levels of sexual hormones)

Gonadorelin analogues Gonadorelin antagonists

The GnRH antagonists.

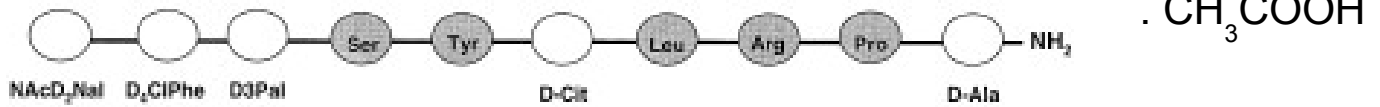
GnRH



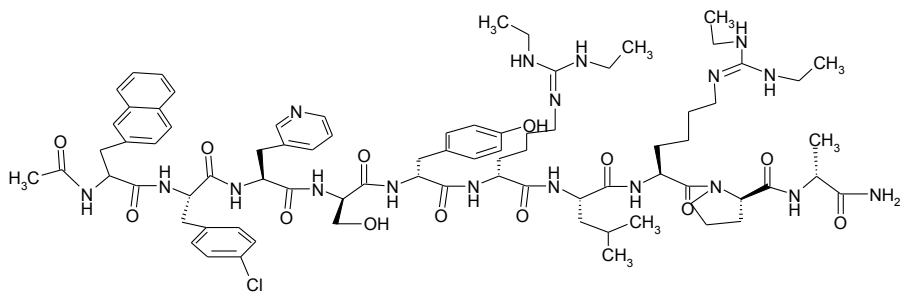
Antagon™ (ganirelix acetate)



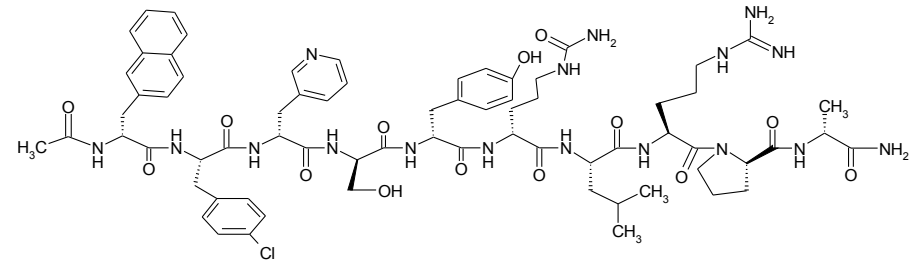
Cetrotide® (cetrorelix acetate)



Amino Acid Number	1	2	3	4	5	6	7	8	9	10
	NAcD ₂ Nal	D ₂ CIPhe	D ₃ 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)

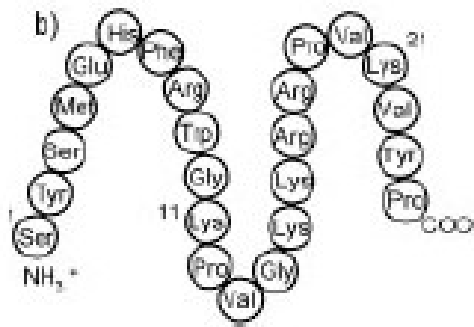
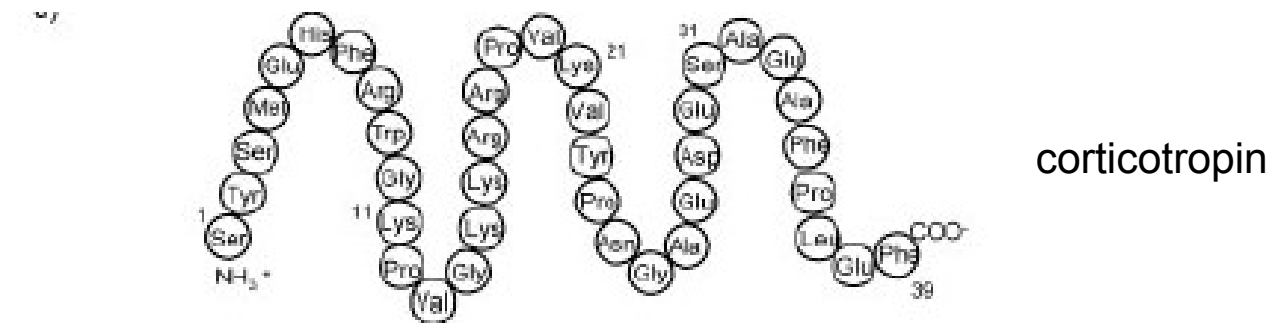
- replacement of Gly in position 6 with a more bulky amino acid leads to stability increase
- the sequence of the first three amino acids is needed for receptor binding and is kept in agonists
- antagonists have Trp in position 3 replaced with a non-physiologic amino acid, they bind to GnRH and avoid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

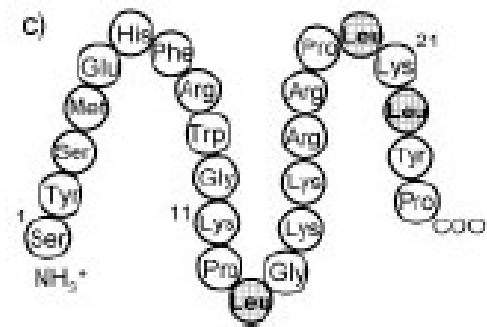


tetracosactide

syn. cosyntropin [USAN]

Tetracosactidum EP

Synacten®

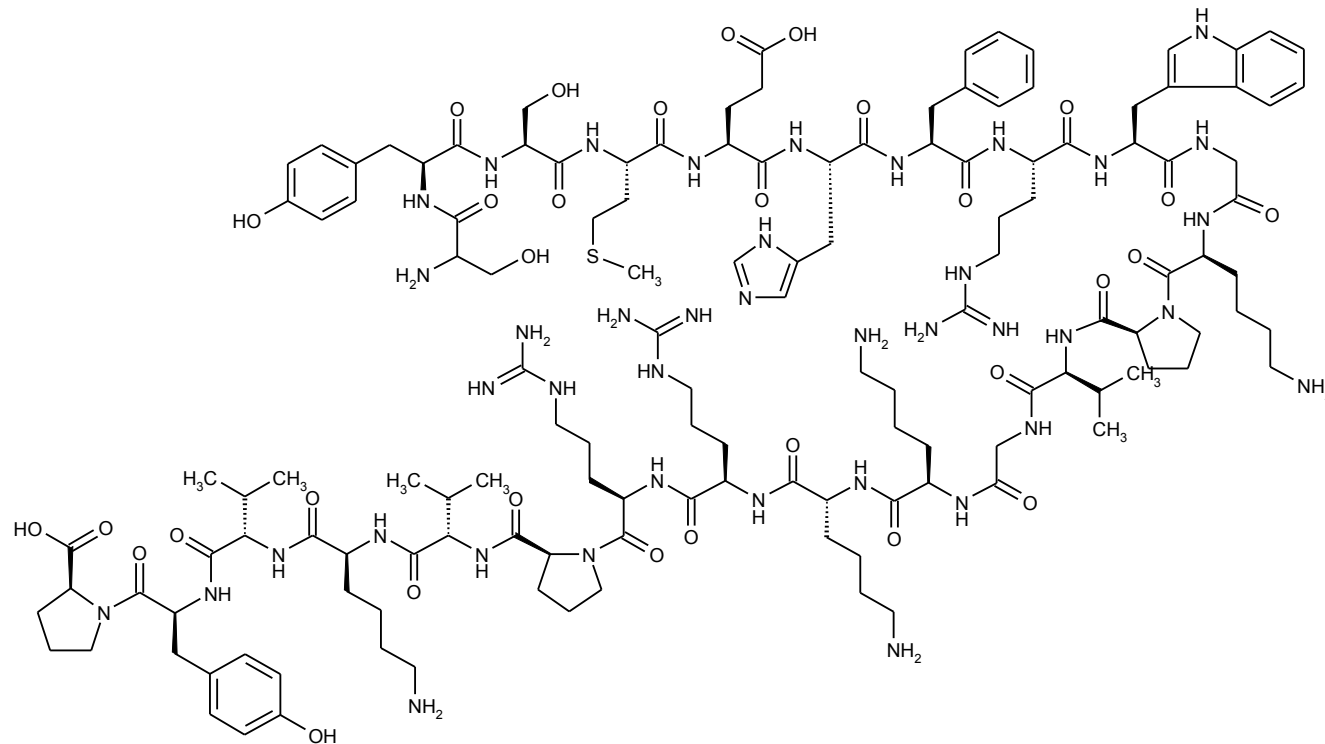


SynVL

• compound used as a standard for determination of tetracosactide 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

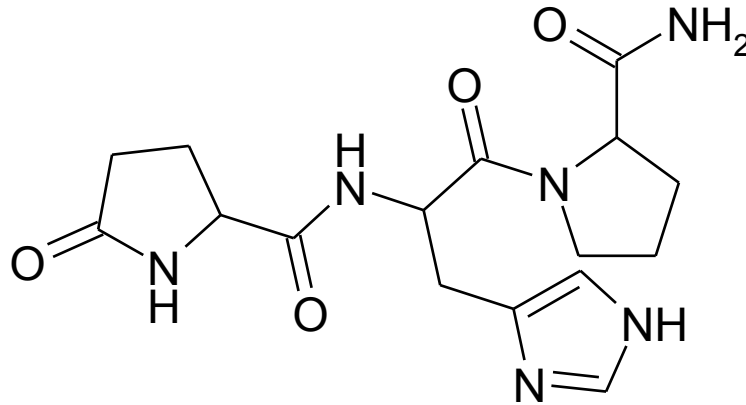


tetracosactide

- 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.



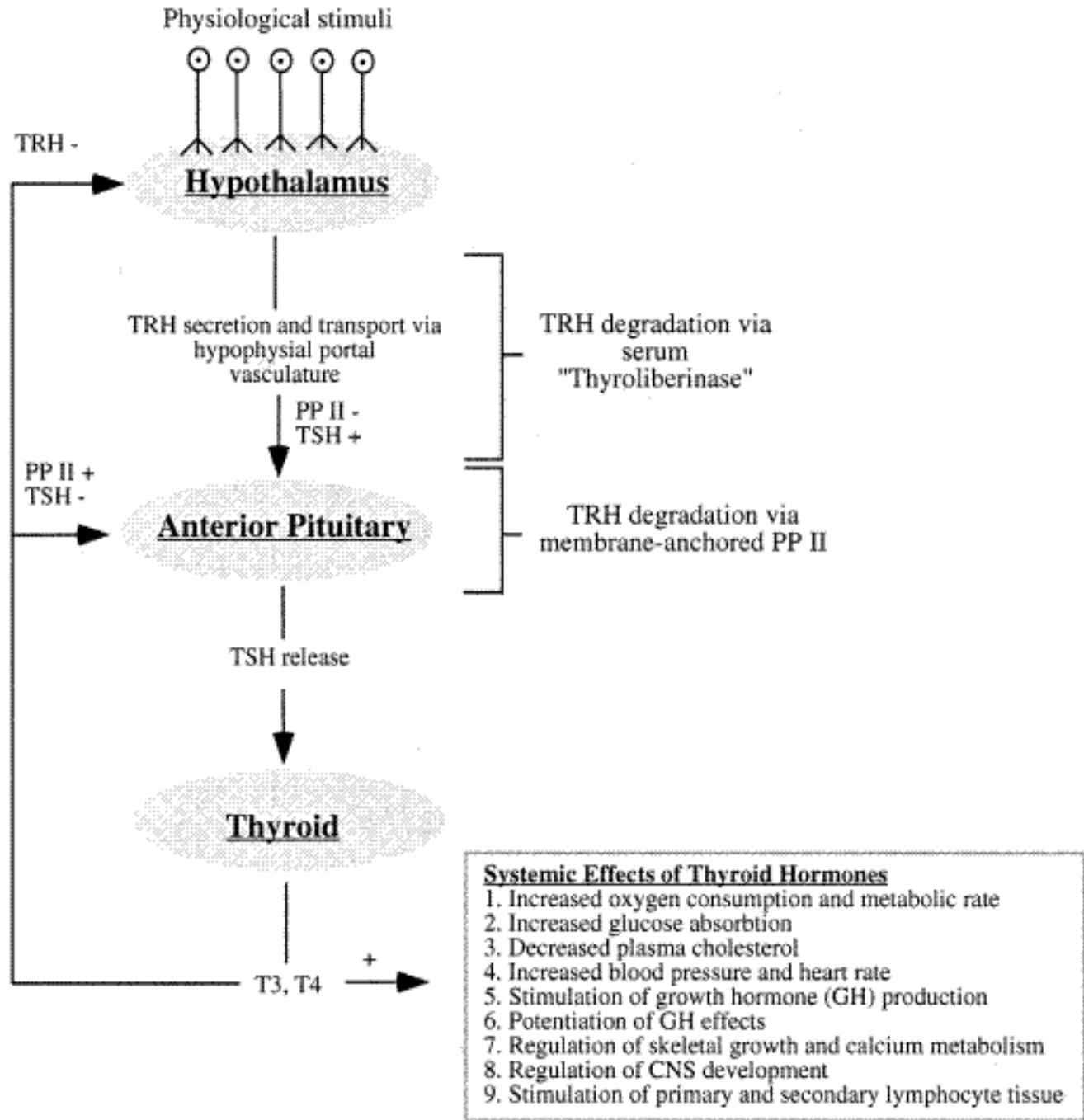
protirelin

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.)

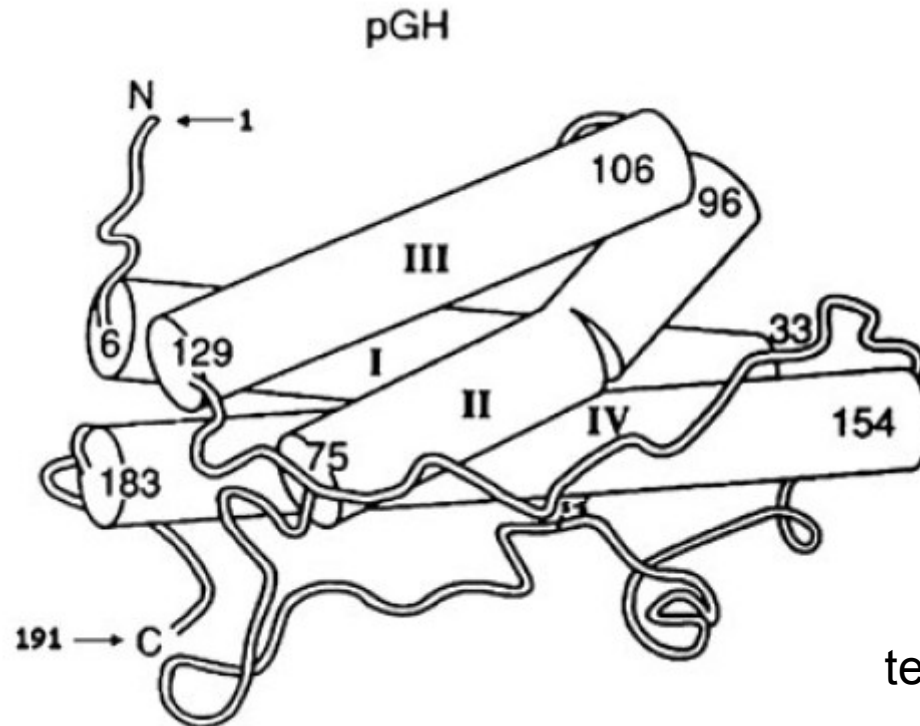
Metabolism of TRH and its regulation



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



tertiary structure of porcine GH

somatropin

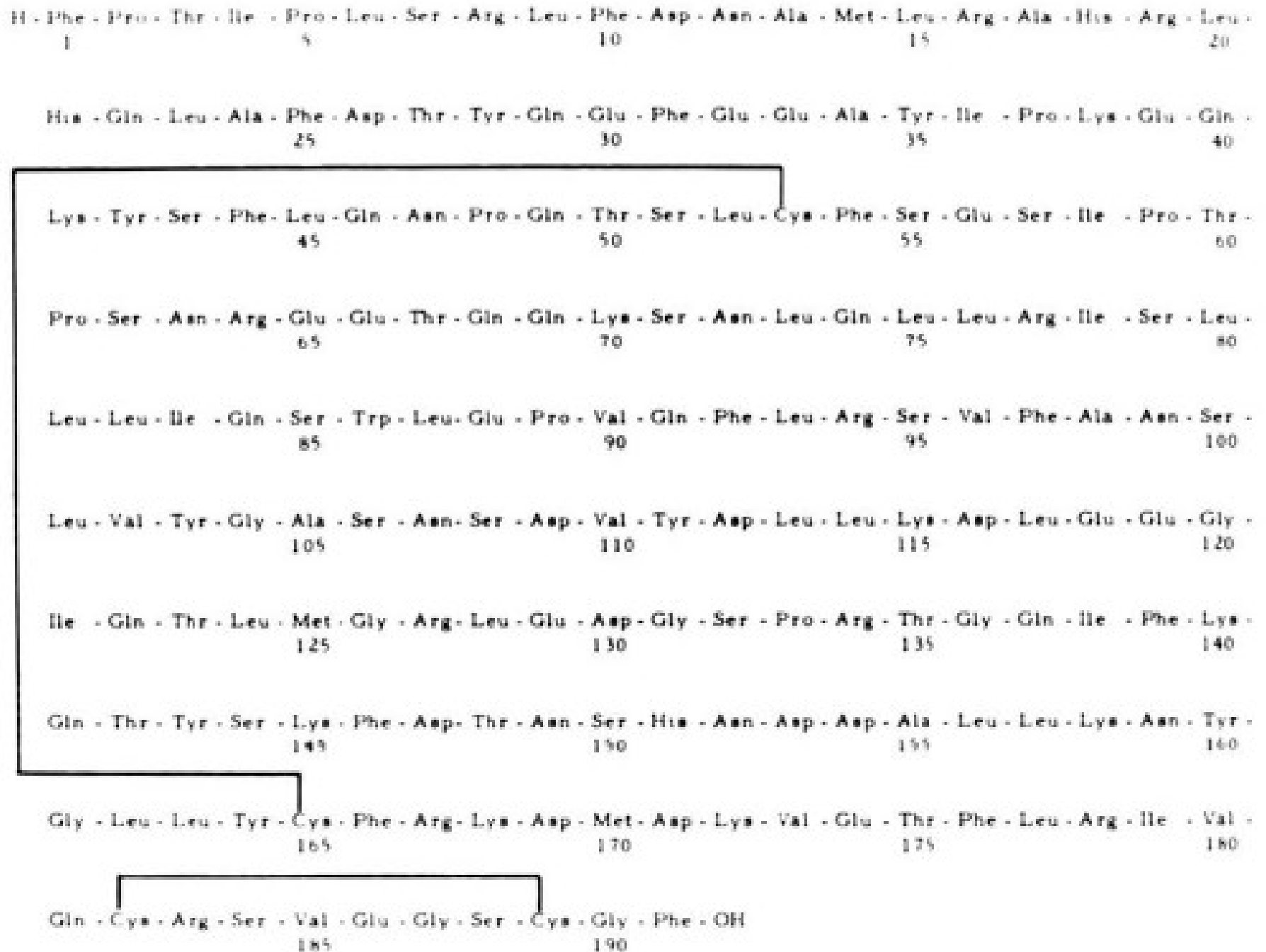
Somatropinum EP

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

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

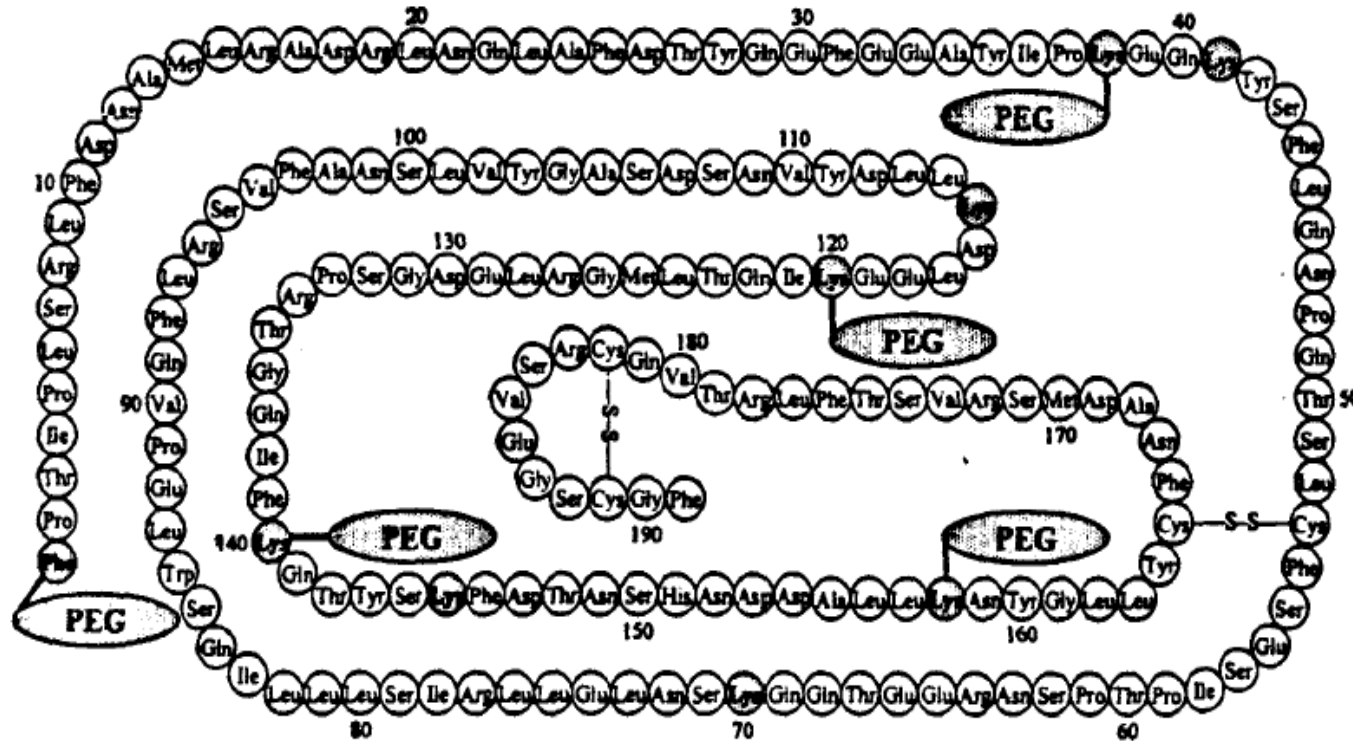
```
1 MATGSRTSLL LAFGLLCLPW LQEGSAFPTI PLSRLFDNAM LRAHRLHQLA FDTYQEFEEA YIPKEQKYSF LQNPQTSLCF SESIPTPSNR EETQQKSNLE 100
101 LLRISLLLIQ SWLEPVQFLR SVFANSLVYG ASDSNVYDLL KDLEEGIQLT MGRLEDGSPR TGQIFKQTYS KFDNTSHNDD ALLKNYGLLY CFRKDMDKVE 200
201 TFLRIVQCRS VEGSCGF
```

Primary structure of human somatotropin



Somatropin (GH) analogues

Amino Acid Sequence of Pegvisomant Protein



* Stippled residues indicate PEG attachment sites (Phe₁, Lys₃₈, Lys₄₁, Lys₇₀, Lys₁₁₅, Lys₁₂₀, Lys₁₄₀, Lys₁₄₅, Lys₁₅₈)

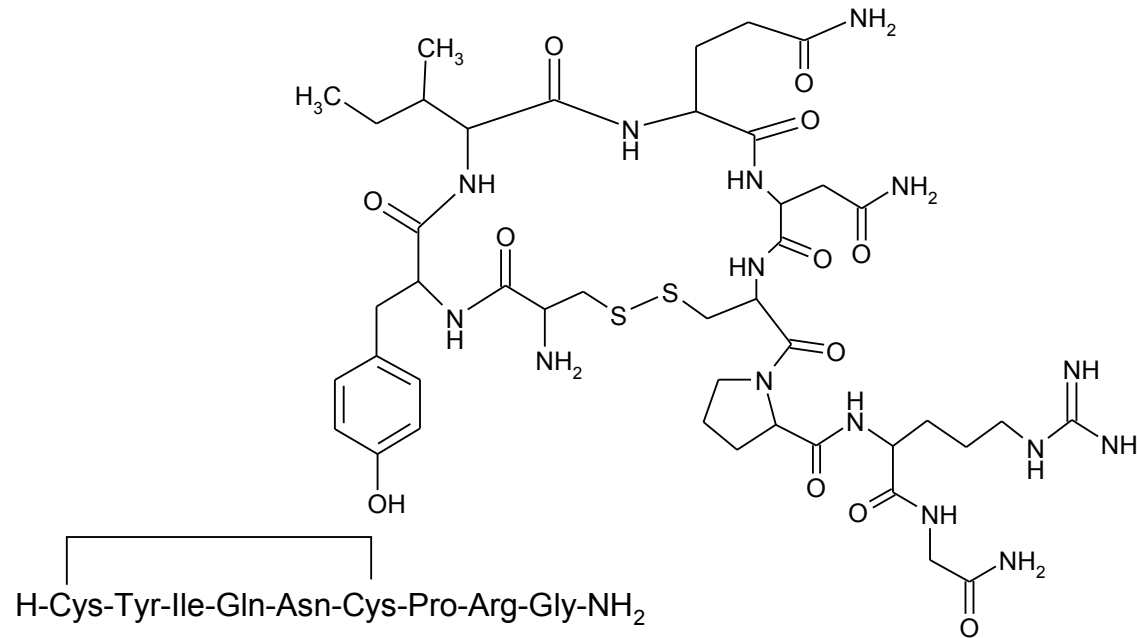
pegvisomant

- 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 Phe₁ 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_r ~ 500
- pegylation lowers antigenicity and prolongs the biologic half-time
- using: treatment of acromegaly

1.3 Oxytocin, vasopressins and their analogues

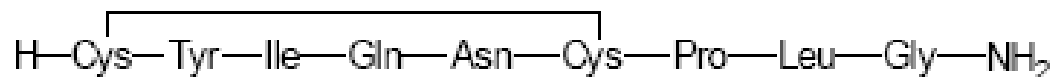
Vasotocin

= 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



C₄₃H₆₆N₁₂O₁₂S₂

M_r 1007,19

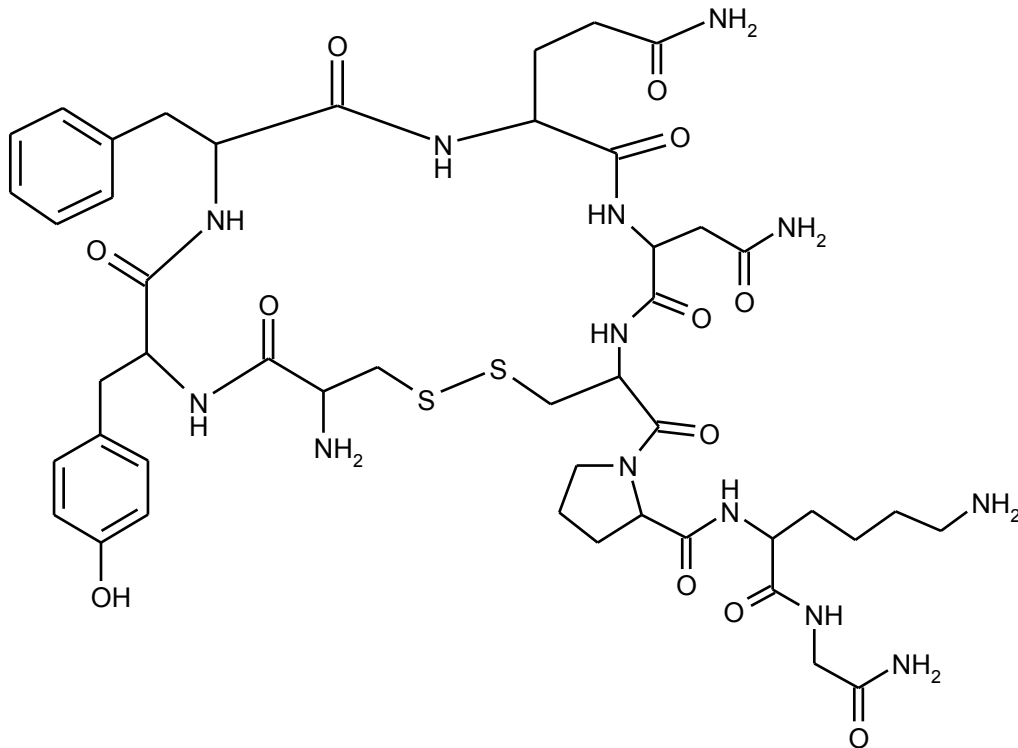
CAS 50-56-6

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

Vasopressin(s)

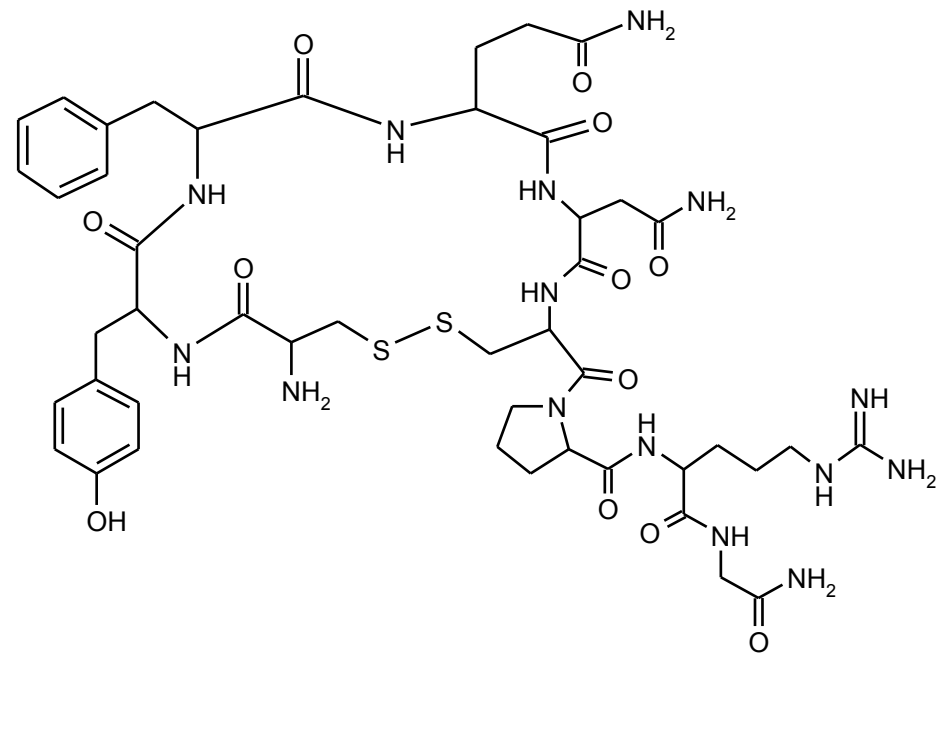
=antidiuretic hormone(s) (ADH)

- 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

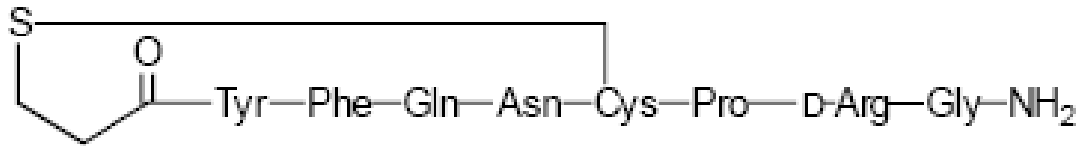


arginine-vasopressin
argipressin

•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

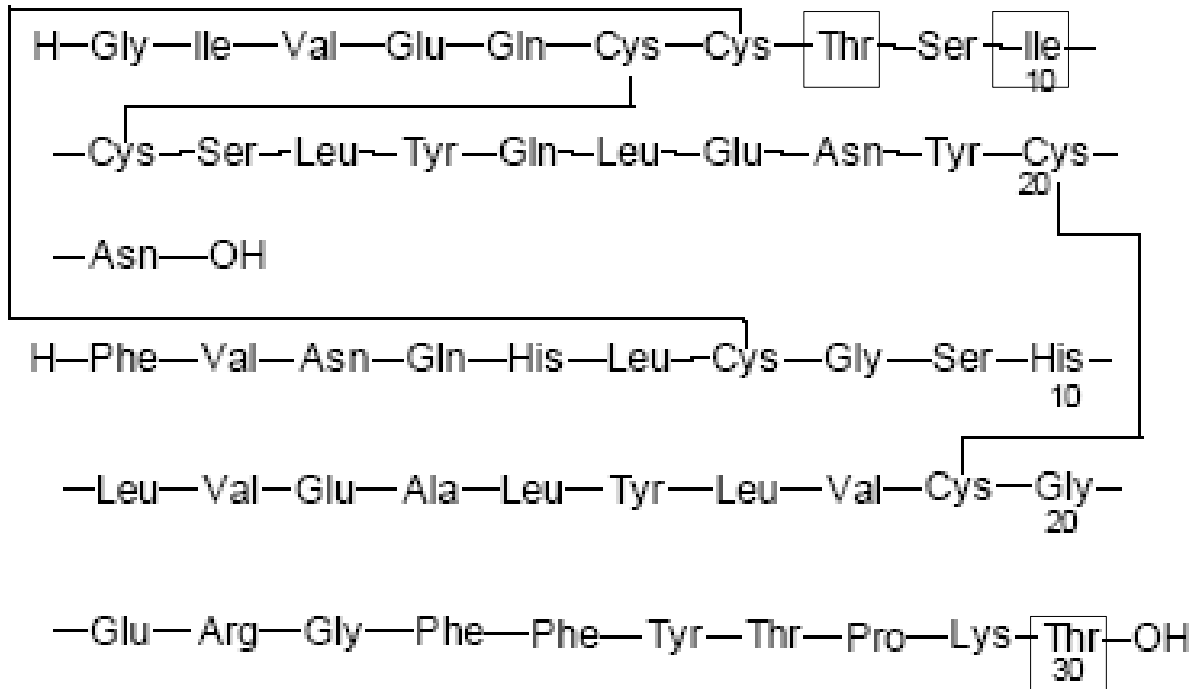
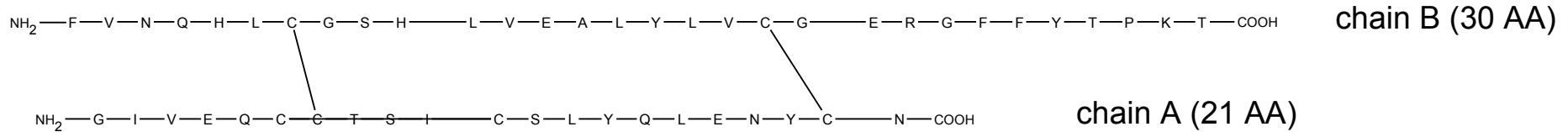
- cyclic pseudononapeptide
- prepared by synthesis
- antidiuretic (*enuresis nocturna*, ...)

1.4 Insulines, glucagon and GLP-1 analogues

Insuline

- Secreted mostly by β -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



C₂₅₇H₃₈₃N₆₅O₇₇S₆

M_r 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

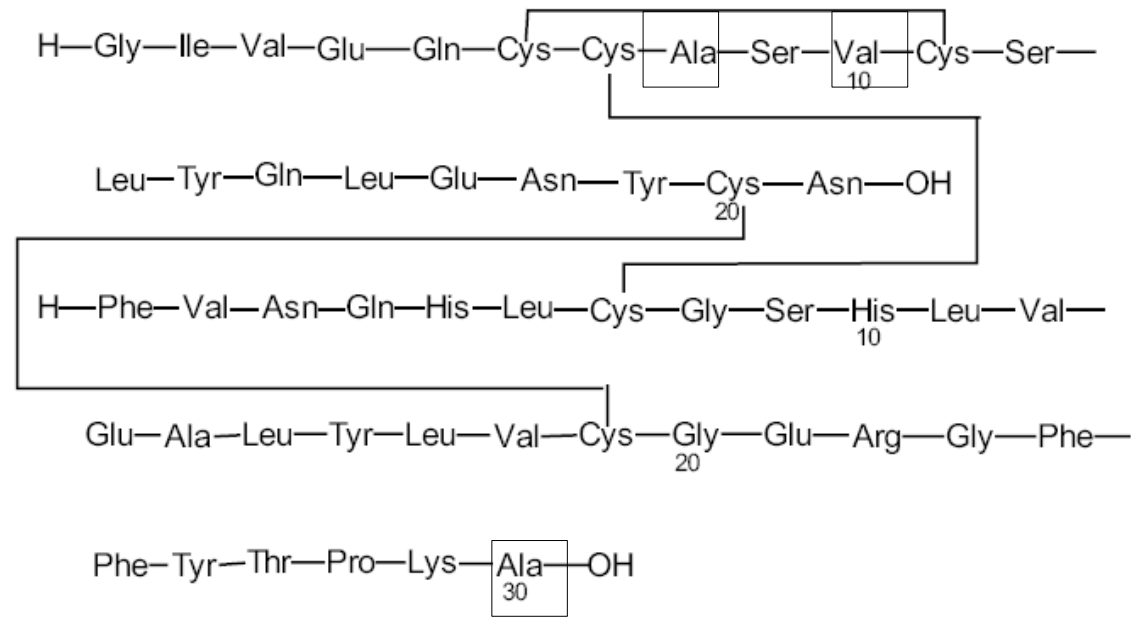
1-24 signal sequence; 25-54 chain B; 57-87 peptide C; 90-110 chain A

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

Insulinum humanum PhEur

- syn. humuline

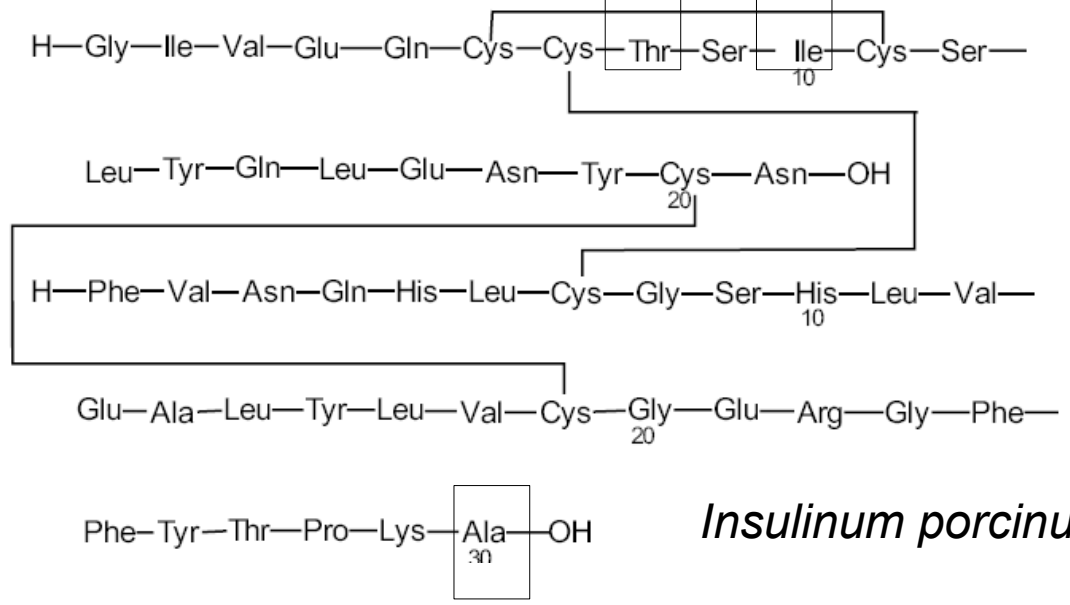
Bovine (cow's) insuline



Insulinum bovinum PhEur

- isolation from beef pancreases

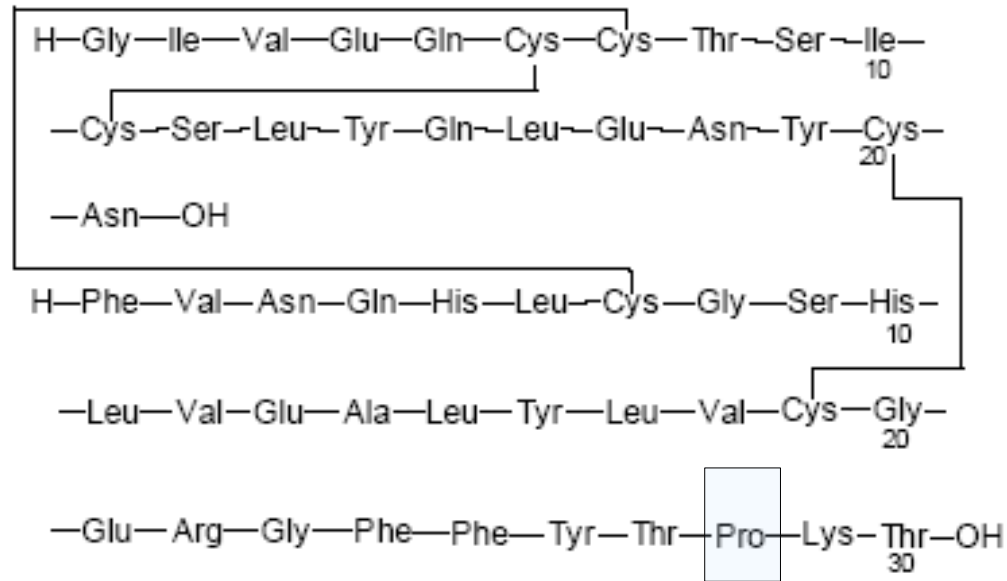
Porcine (swine) insuline



Insulinum porcinum PhEur

Insuline analogues

human

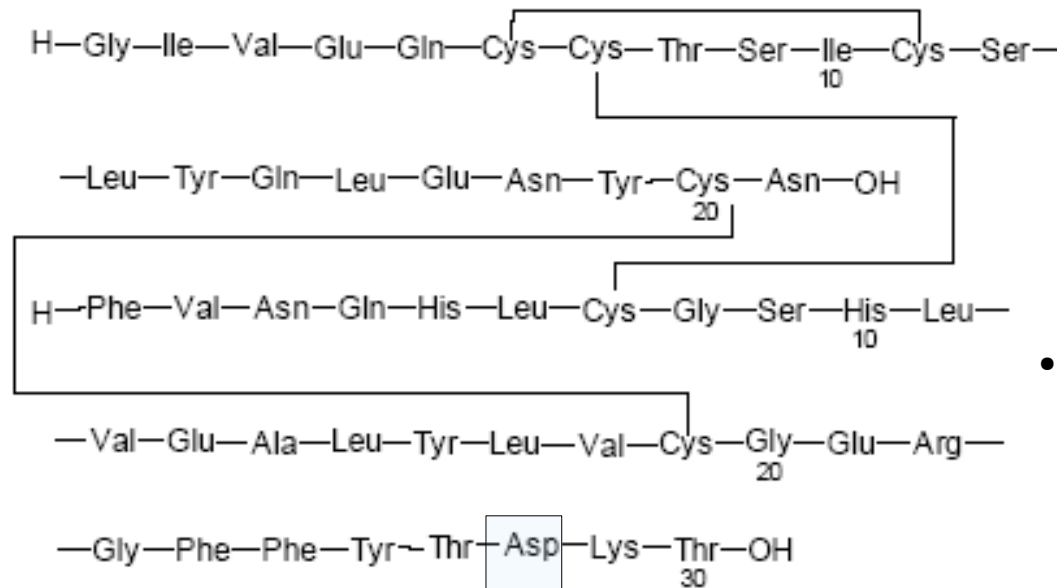


$C_{257}H_{383}N_{65}O_{77}S_6$

M_r 5807,60

CAS 11061-68-0

aspart
Insulinum aspartum
PhEur
 Novorapid®

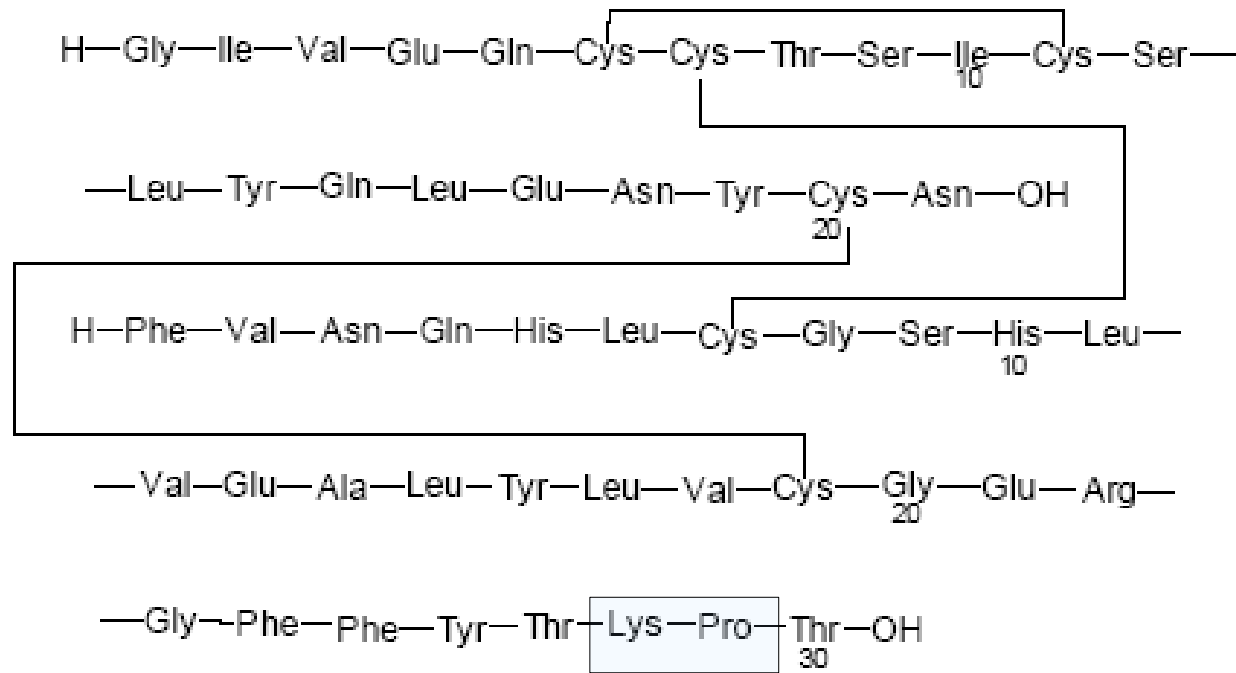


•recombinant technology

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

M_r 5825,58

CAS 116094-23-6



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

M_r 5807,61

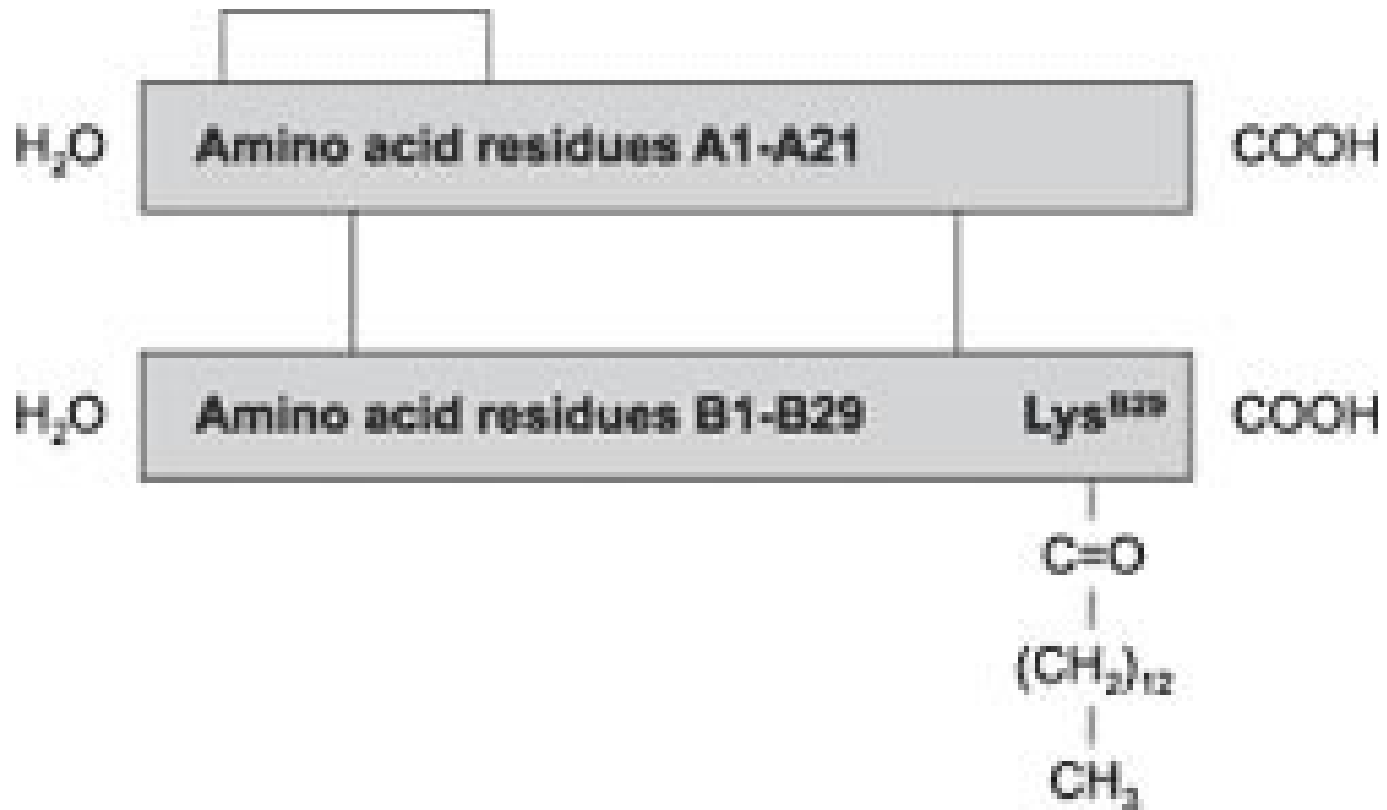
CAS 133107-64-9

insulin-lispro

Insulinum lisprum PhEur

•recombinant

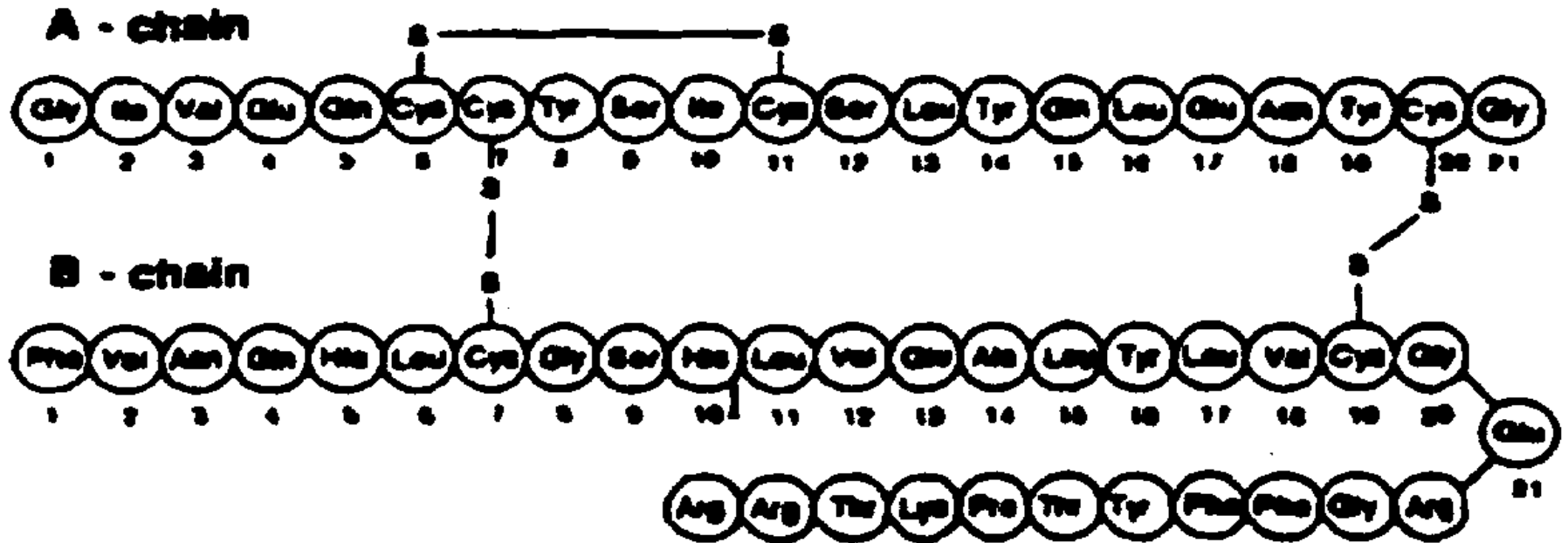
Humalog ®, Liprolog ®



insulin-detemir

- chain B has only 29 AA, tetradecanoyl (myristoyl) attached to Lys^{B29}
- recombinant-semi synthetic

Levemir ®

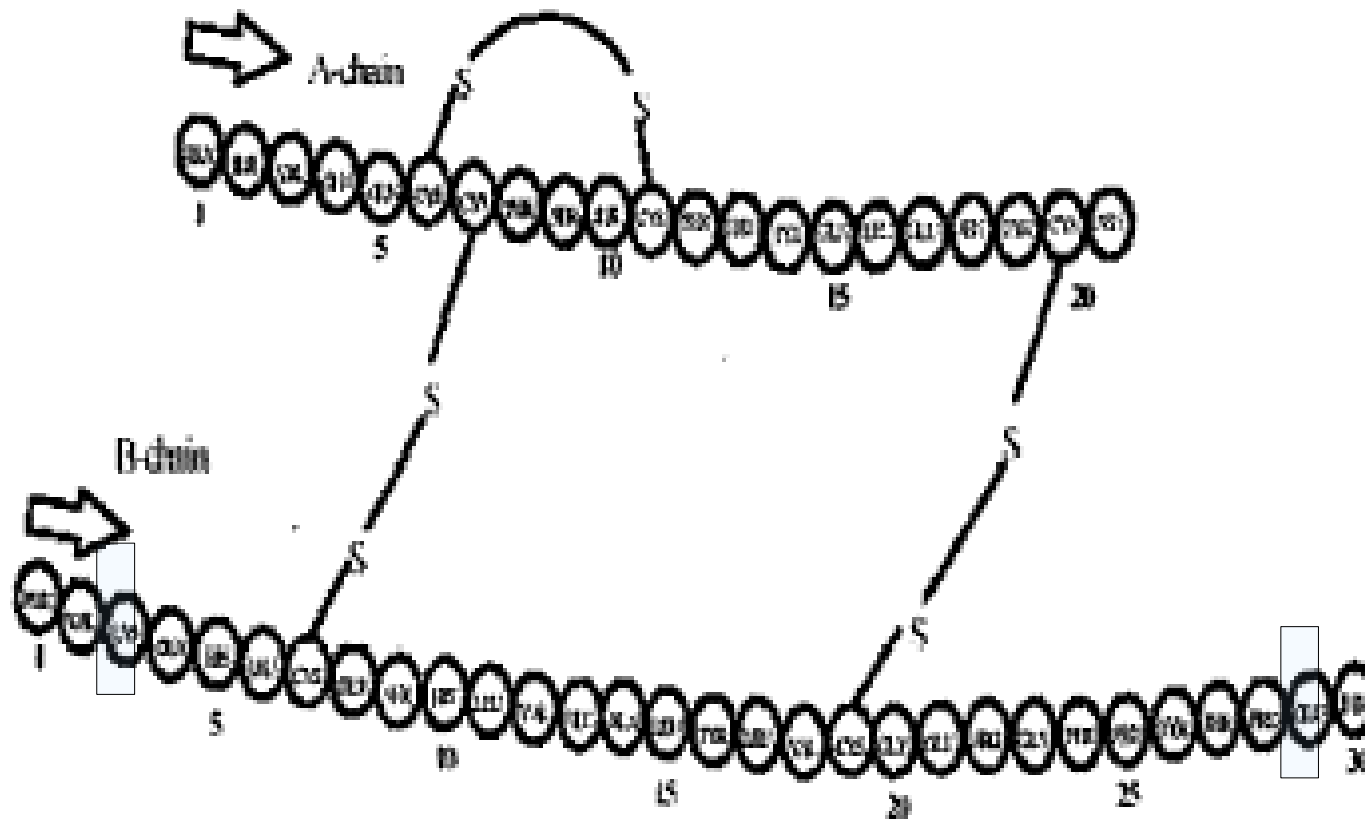


insulin-glargin

Gly^{21A}-L-Arg^{30B}-L-Arg^{31B}-insulin

Lantus[®], Optisulin[®]

- insulin of 1st choice in diabetes of 2nd 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

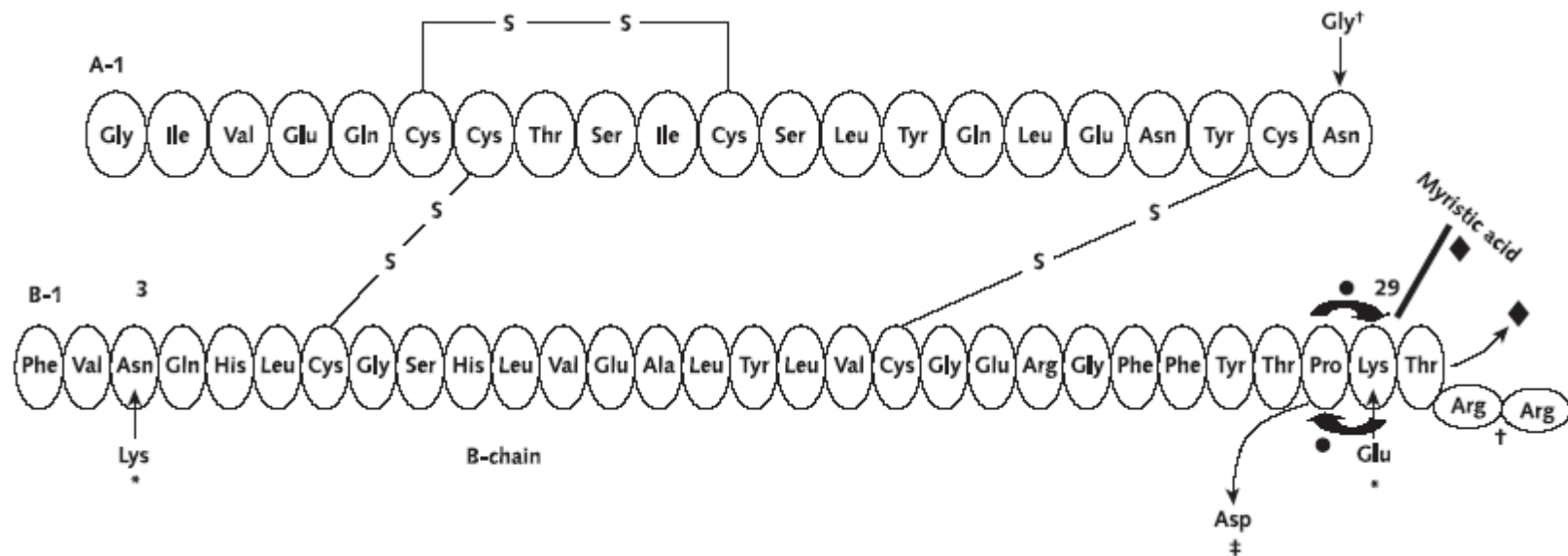
CAS registry number: 207748-29-6

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 β chain.

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

* = Insulin glulisine is designed with the substitution of the amino acid lysine with asparagine at position 3 of the human insulin β 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 β chain.

◆ = Insulin detemir is designed to bind albumin in plasma after absorption. Threonine is omitted from position 30 of the insulin β 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

$C_{153}H_{225}N_{43}O_{49}S$

M_r 3482,78

CAS 16941-32-5

Glucagonum PhEur

- isolated from porcine or bovine pancreases

Glucagonum humanum PhEur

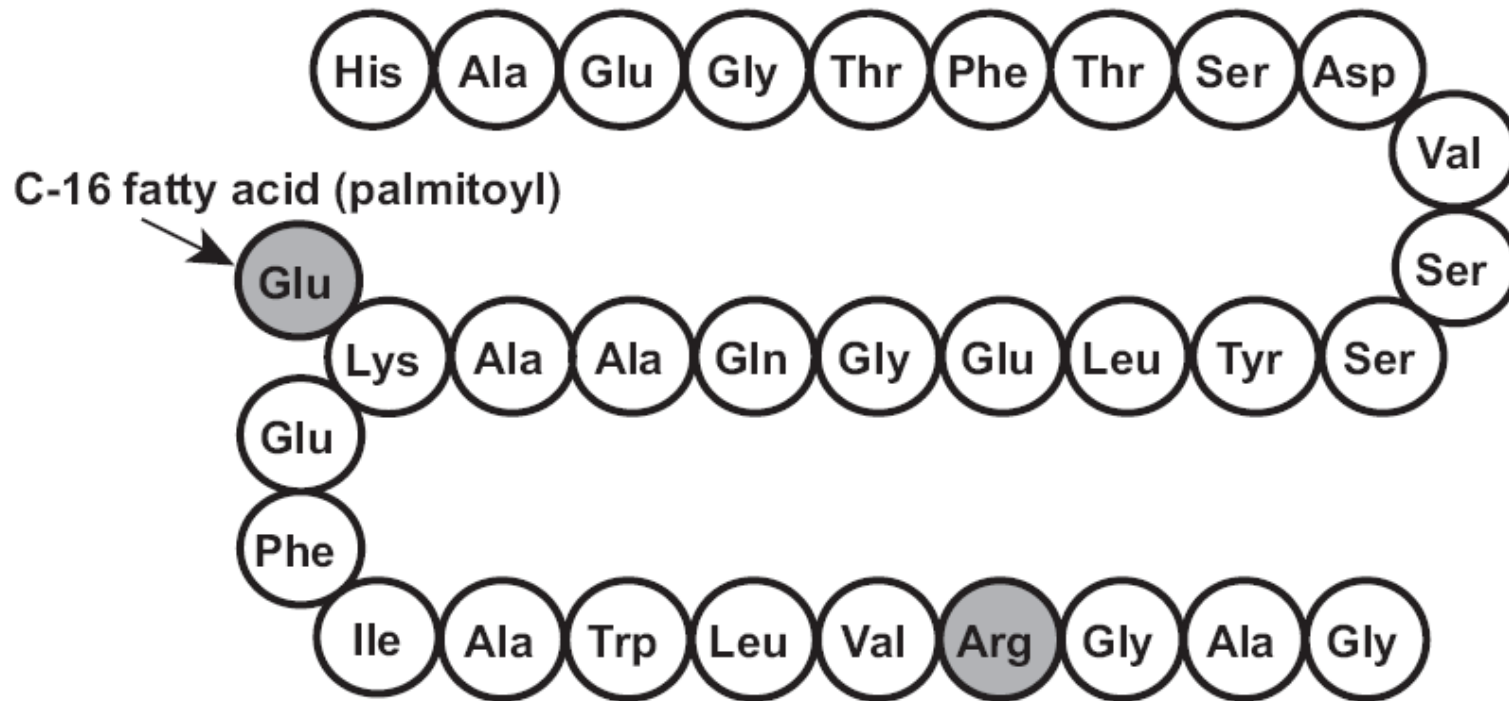
- produced by recombinant technology; AA sequence is identical
- usage: treatment of serious hypoglycaemia, X-ray GIT diagnostic etc.

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 β -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

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

GLP-1 analogues



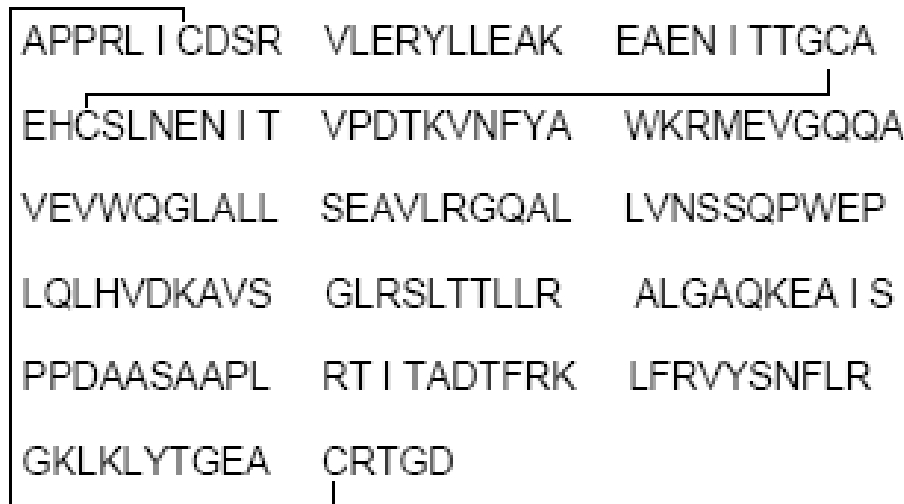
liraglutide

Victoza® inj. sol.

γ -L-glutamoyl(N- α -hexadecanoyl)-Lys²⁶, Arg³⁴-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 α and β cells

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 EVISEMFDLQ EPTCLQTRLE
LYKQGLRGSL TKLKGPLTMM ASHYKQHCPP
TPETSCATQI ITFESFKENL KDFLLVIPFD
CWEPVQE

$C_{639}H_{1007}N_{171}O_{196}S_3$

M_r 14 477,49

CAS 99283-10-0

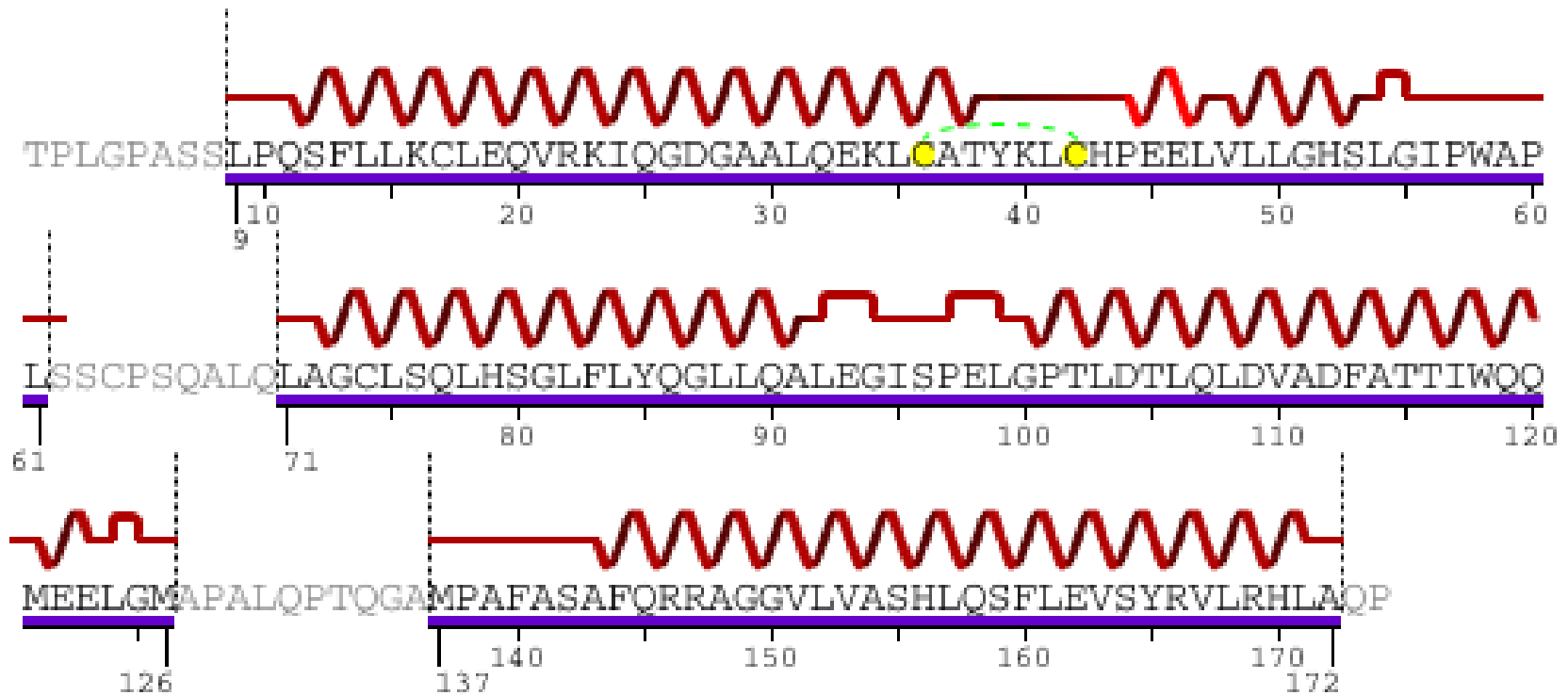
molgramostim

= 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

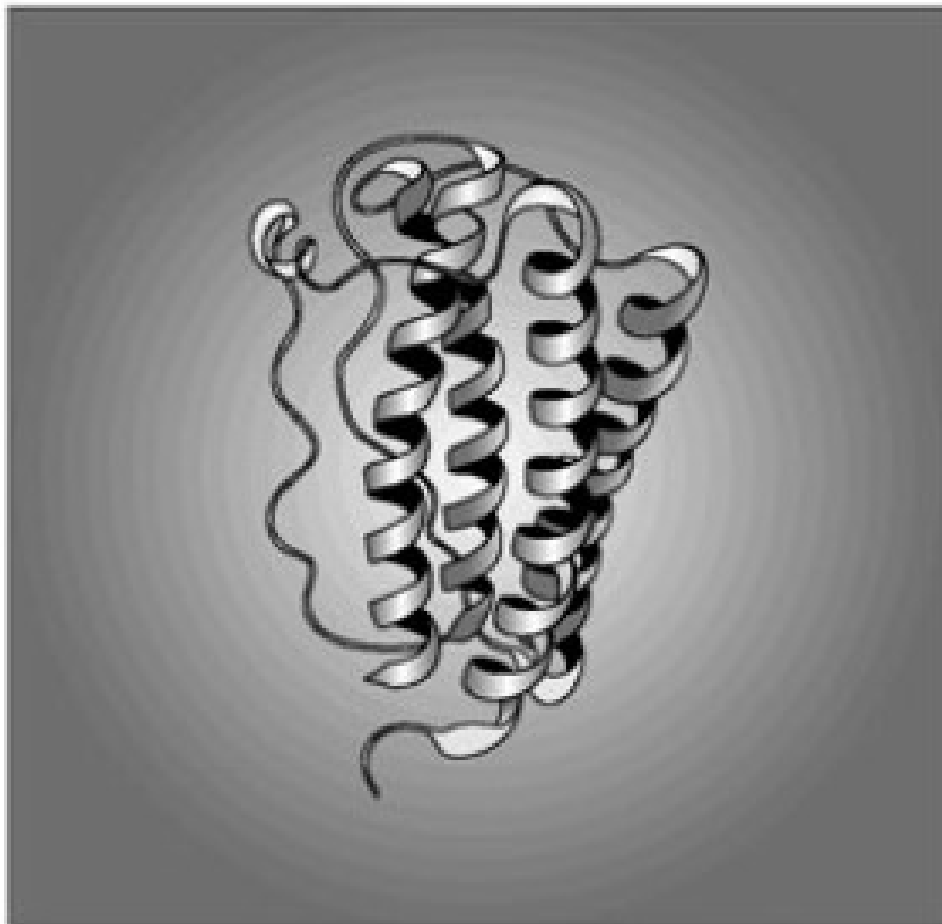
Filgrastim = human granulocytes colony-stimulating factor (G-CSF); glycosylated, 174 AA
Sequence of filgrastim precursor



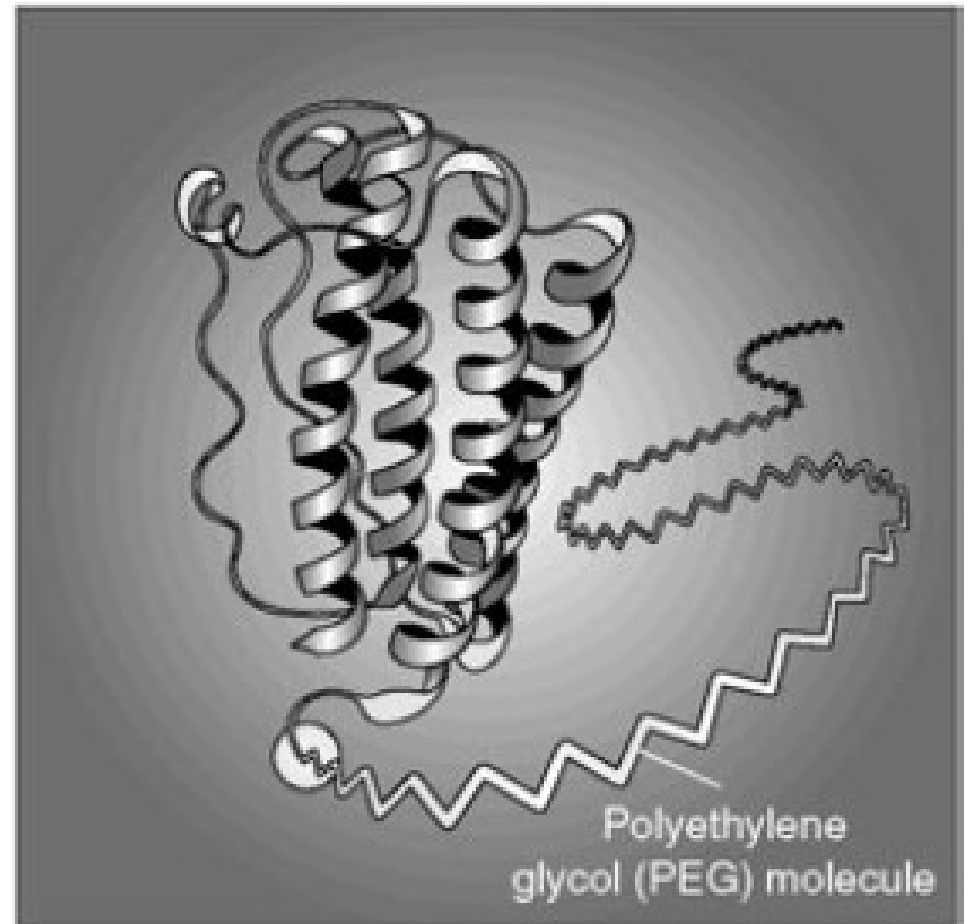
- treatment of neutropenia in cancer chemotheapy and in AIDS

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

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

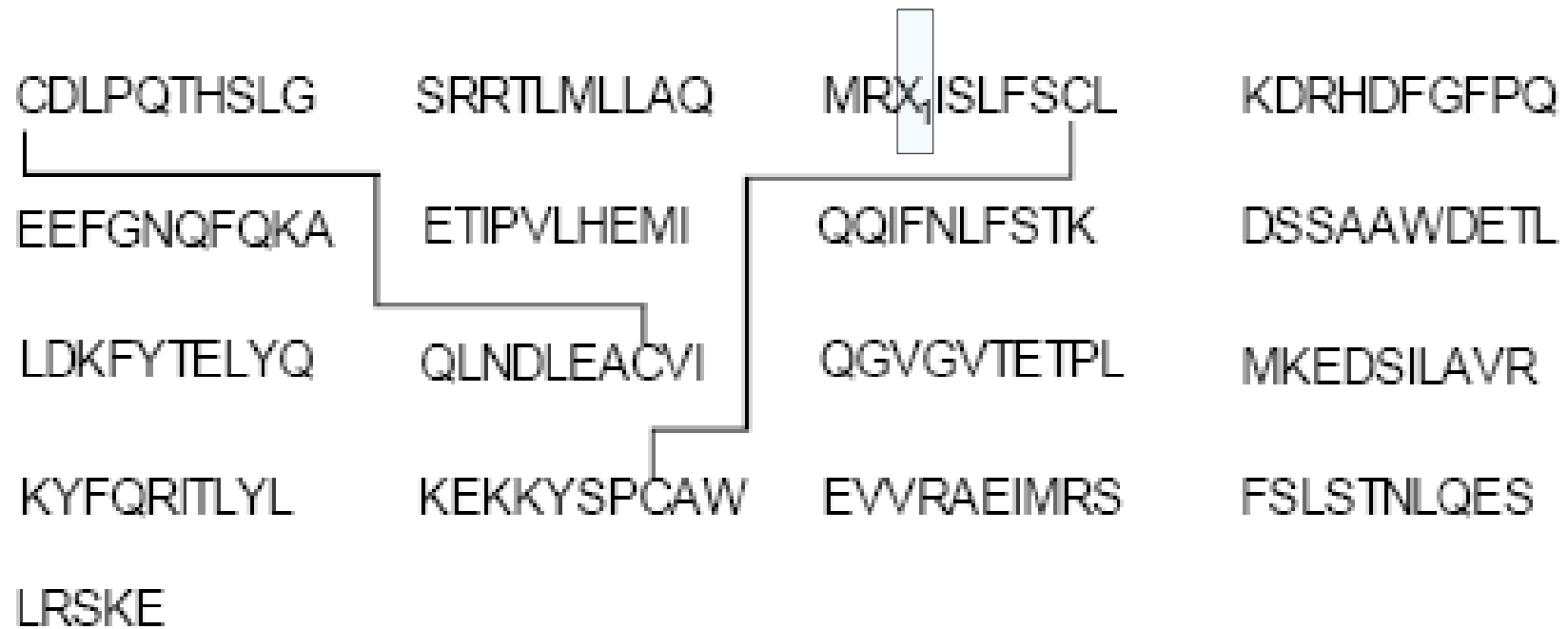


Filgrastim



Pegfilgrastim

4. Non-specific antibodies - interferons



interferon α_2

Interferoni alfa-2 solutio concentrata EP

X1 = Lys α_{2a}

X1 = Arg α_{2b}

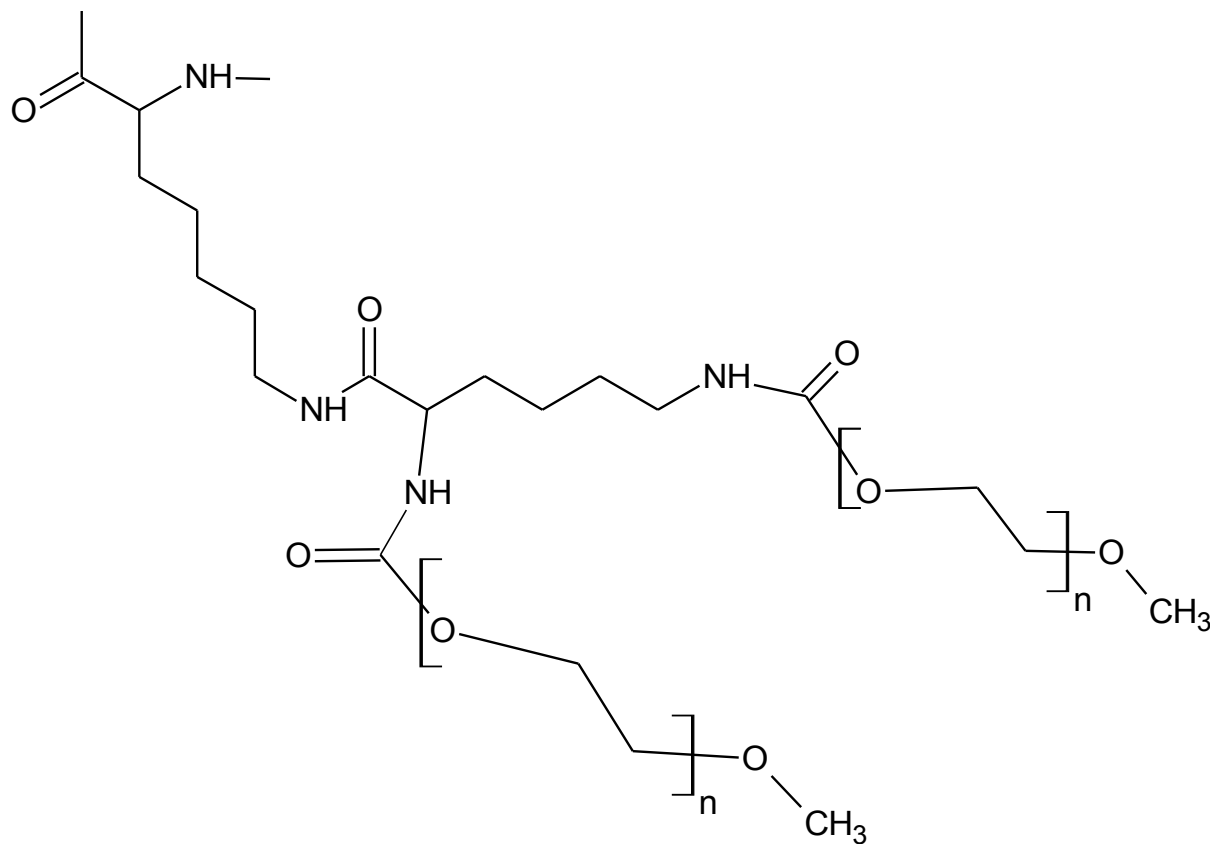
- antiviral activity during viral RNA and protein syntheses
- antiproliferation activity
- produced by a recombinant technology on bacteria

Pegylated interferons α

- **peginterferon α_{2a}** (Pegasys®) - on some Lys residues attached N², N⁶-dicarboxy-Lys esterified with PEG-monomethylether of M_r about 20 000
 - substitution is stable, free interferon is not released
 - peginterferon α_{2a} interacts directly with receptors on surface of the infected cell
 - lowered activity (only 7 % of free interferon α_{2a}) is counterbalanced by much longer half-time
 - treatment of hepatitis B and C combined with ribavirin
- **peginterferon α_{2b}** (Pegintron®) - only one PEG chain of M_r about 12 000 attached via urethane linker to a His, most frequently to His₃₄
 - urethane moiety is labile, free interferon α_{2b} is released into the circulation and directly interacts with receptors
 - treatment of hepatitis C

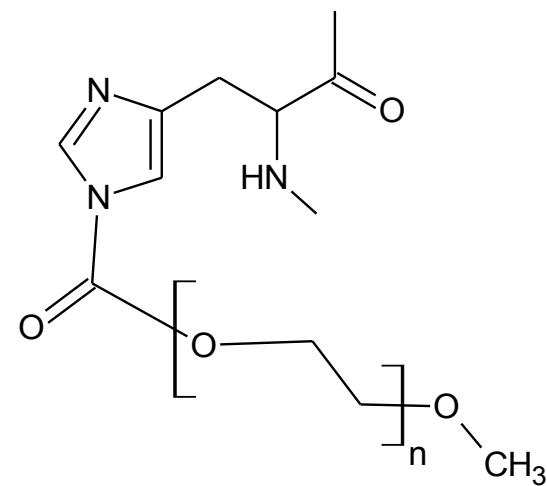
Pegylated interferons α

Differences in their substitutions



Lys

α_{2a}



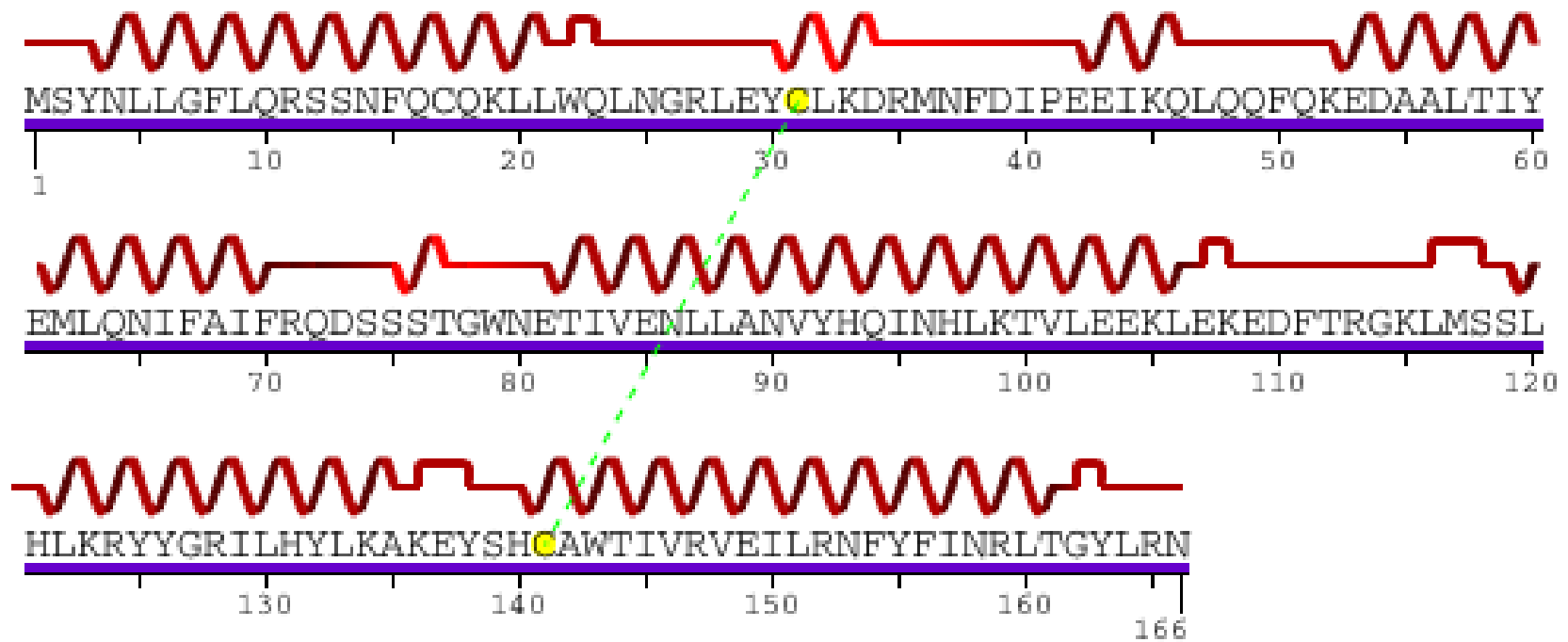
His³⁴

α_{2b}

interferon β

= 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 β

- β_{1a} (Avonex[®] , Betaferon[®] , Rebif[®])
 - 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 β_{1b}
- β_{1b} (Extavia[®])
 - Cys₁₇ changed to Ser
 - recombinant technology on *E. coli*
 - s.c. application every other day

interferon γ_{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