Therapeutic peptides

Classification of therapeutic peptides

1. Hormones

1.1 Liberins a 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

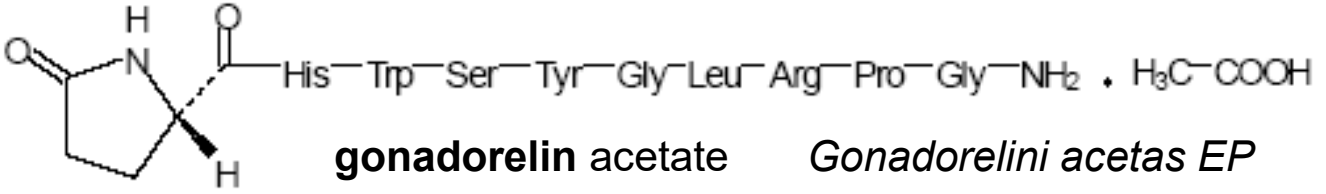
One-letter	Three-letter	
A	Ala	alanine
B	Asx	asparaginic acid or asparagine
C	Cys	cysteine
D	Asp	asparaginic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Leu	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
Q	Gln	glutamine
R	Arg	arginine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

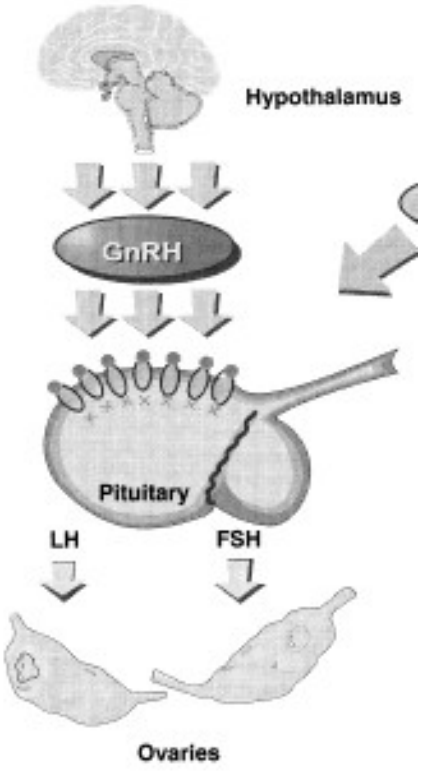
1.1 Liberins and statins („releasing“ & „inhibiting“)

Gonadorelin (GnRH = LHRH) and its analogues

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

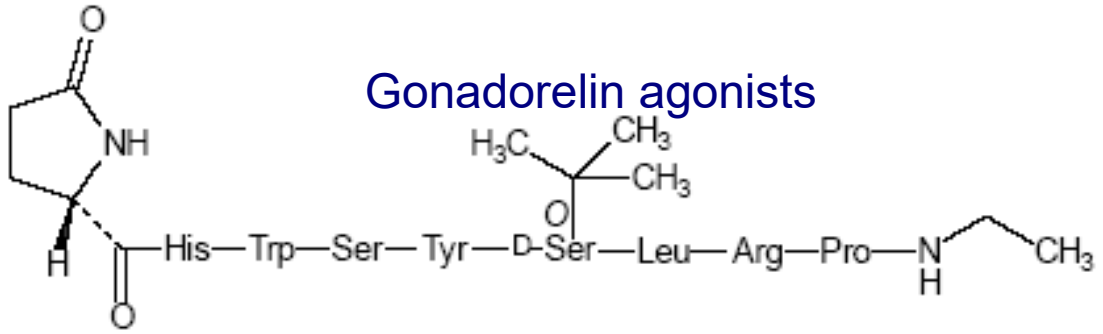


gonadorelin acetate *Gonadorelini acetas EP*



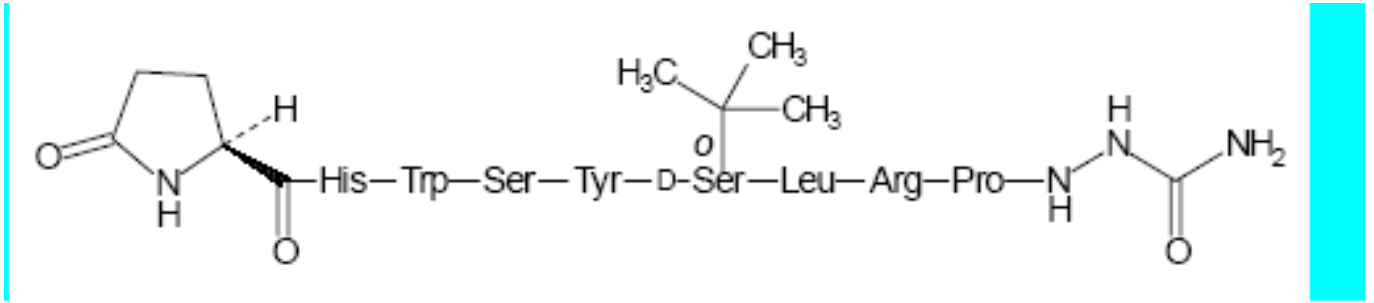
$C_{57}H_{79}N_{17}O_{15}$ M_r 1242,35

Gonadorelin agonists



$C_{60}H_{86}N_{16}O_{13}$ M_r 1239,44 CAS 57982-77-1

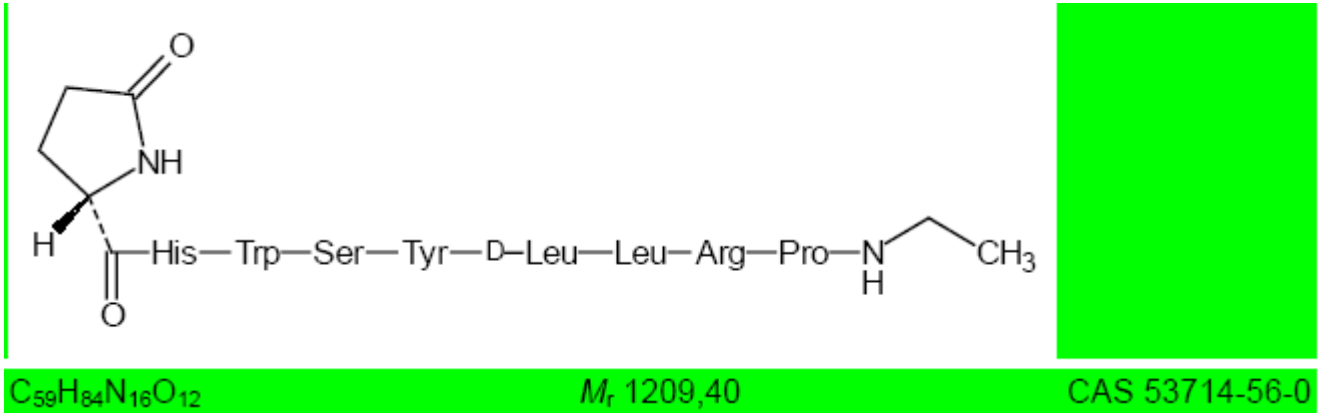
buserelin *Buserelinum EP*



$C_{59}H_{84}N_{18}O_{14}$ M_r 1269,42 CAS 65807-02-5

goserelin *Goserelinum EP*

Gonadorelin and its analogues
Agonists

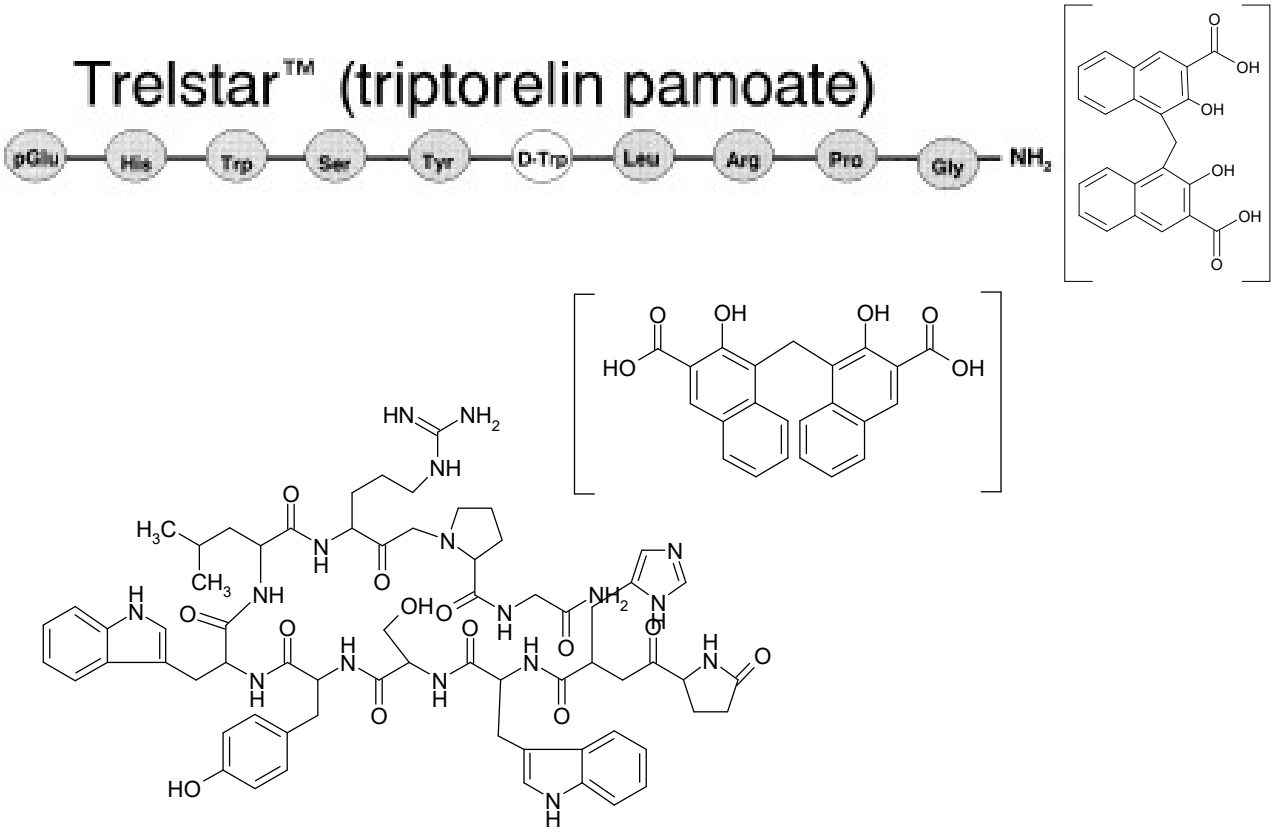


$C_{59}H_{84}N_{16}O_{12}$ M_r 1209,40 CAS 53714-56-0

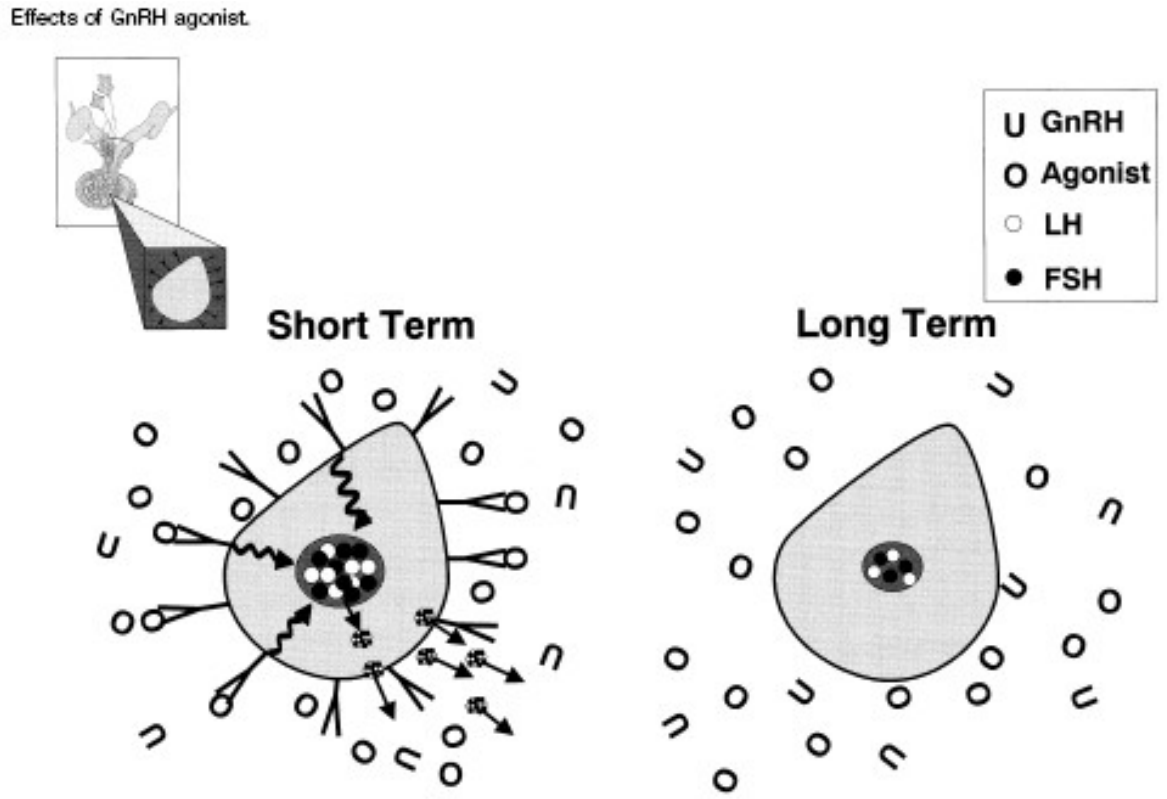
leuprorelin (syn. leuprolide) *Leuprorelinum EP*
Eligard ®

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

Gonadorelin and its analogues
Agonists



Short- and long term action of gonadorelin agonists

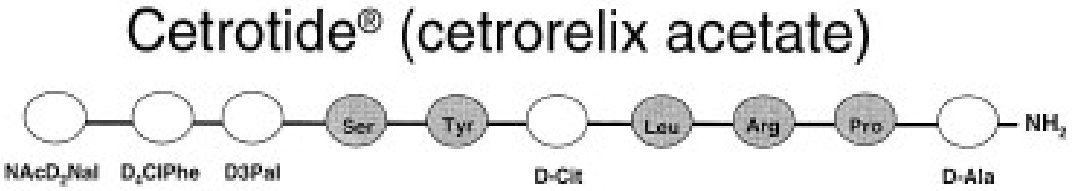


•long term action leads to receptors internalisation and stopping of the effect (due to decreasing LH and FSH levels and thus also levels of sexual hormones)

Gonadorelin analogues
Gonadorelin antagonists

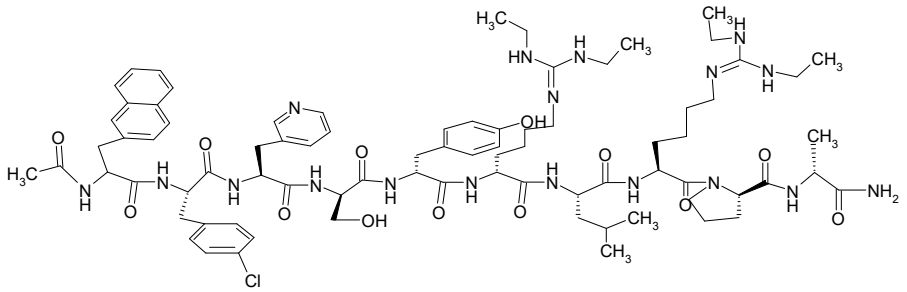
The GnRH antagonists.

. CH_3COOH

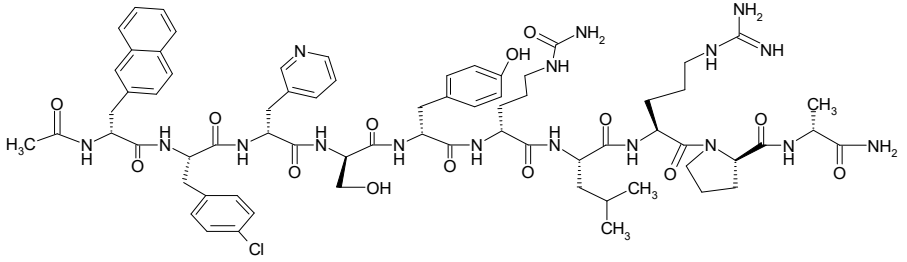


. CH_3COOH

Amino Acid Number	1	2	3	4	5	6	7	8	9	10



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 an non-physiologic amino acid, they bind to GnRH and avoid its action on receptors

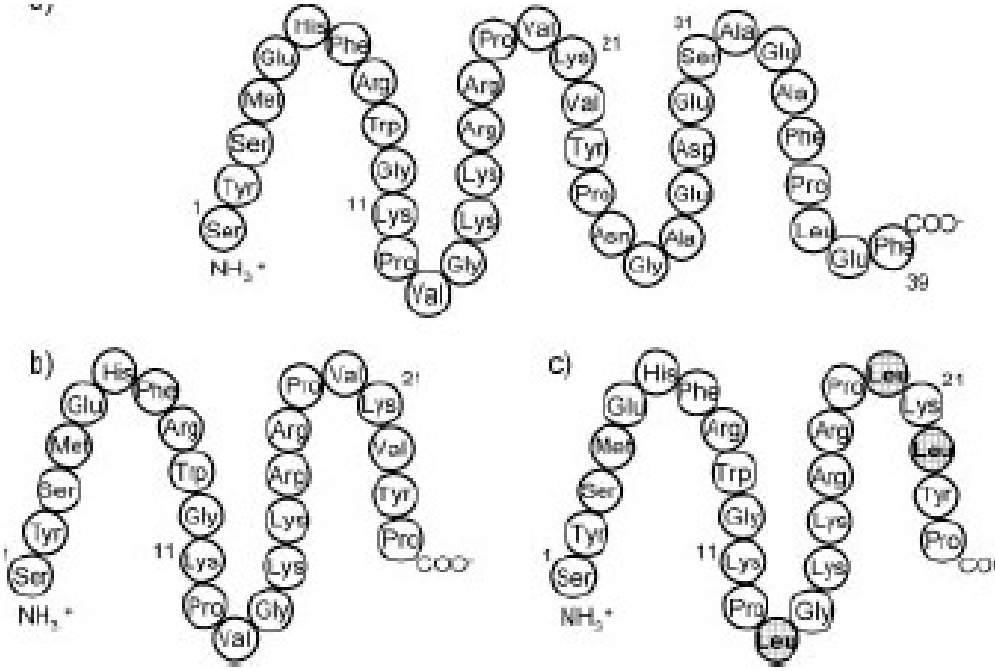
Corticotropin and its analogues

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



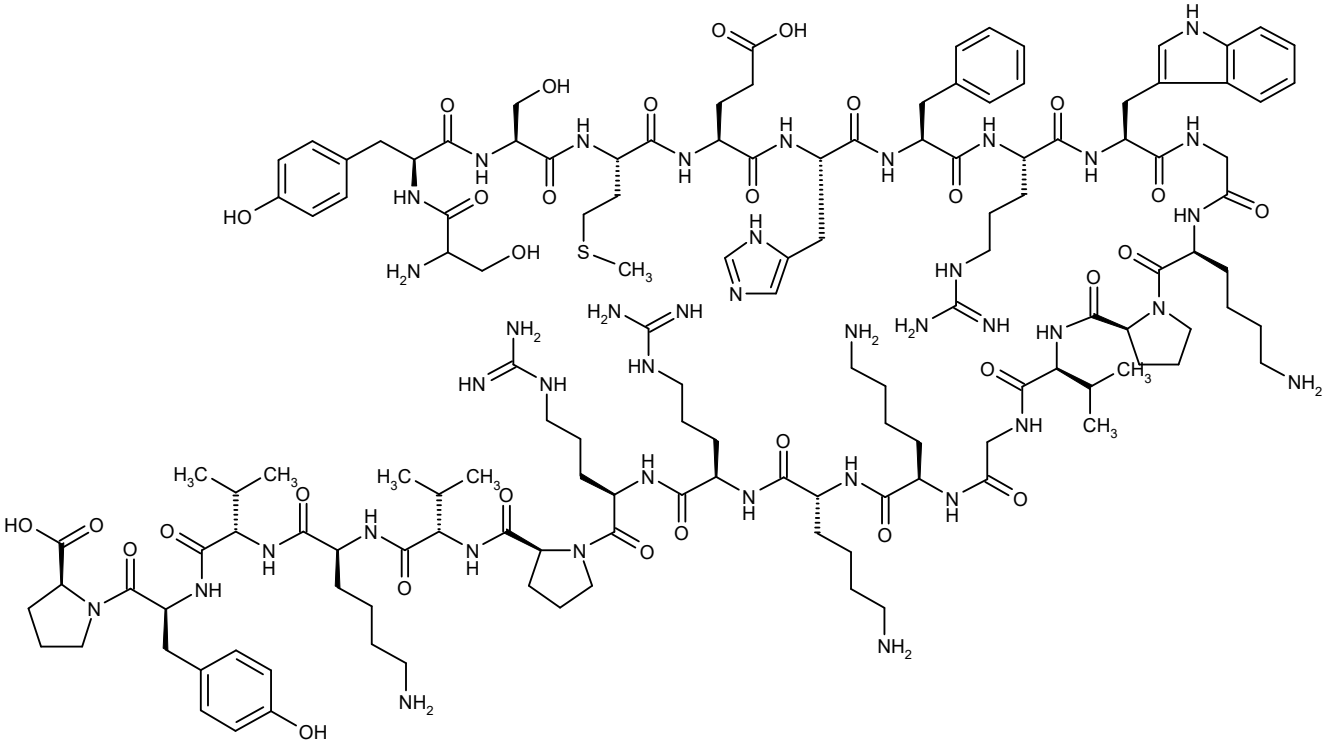
corticotropin

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



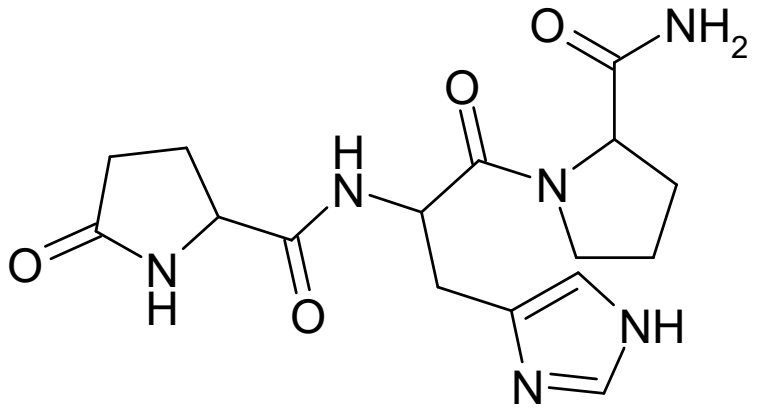
tetracosactide

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

Protirelin – synthetic thyreotropin-releasing hormone (TRH)

•a hormone sythetized in paraventricular nucleus of hypothalamus, stimulating release of thyreotropin and prolactin from the anterior pituitary gland

•also neurotransmitter in CNS, takes part in food intake regulation, control of energy metabolism etc.



protirelin

5-oxoprolyl-histidyl-prolinamide

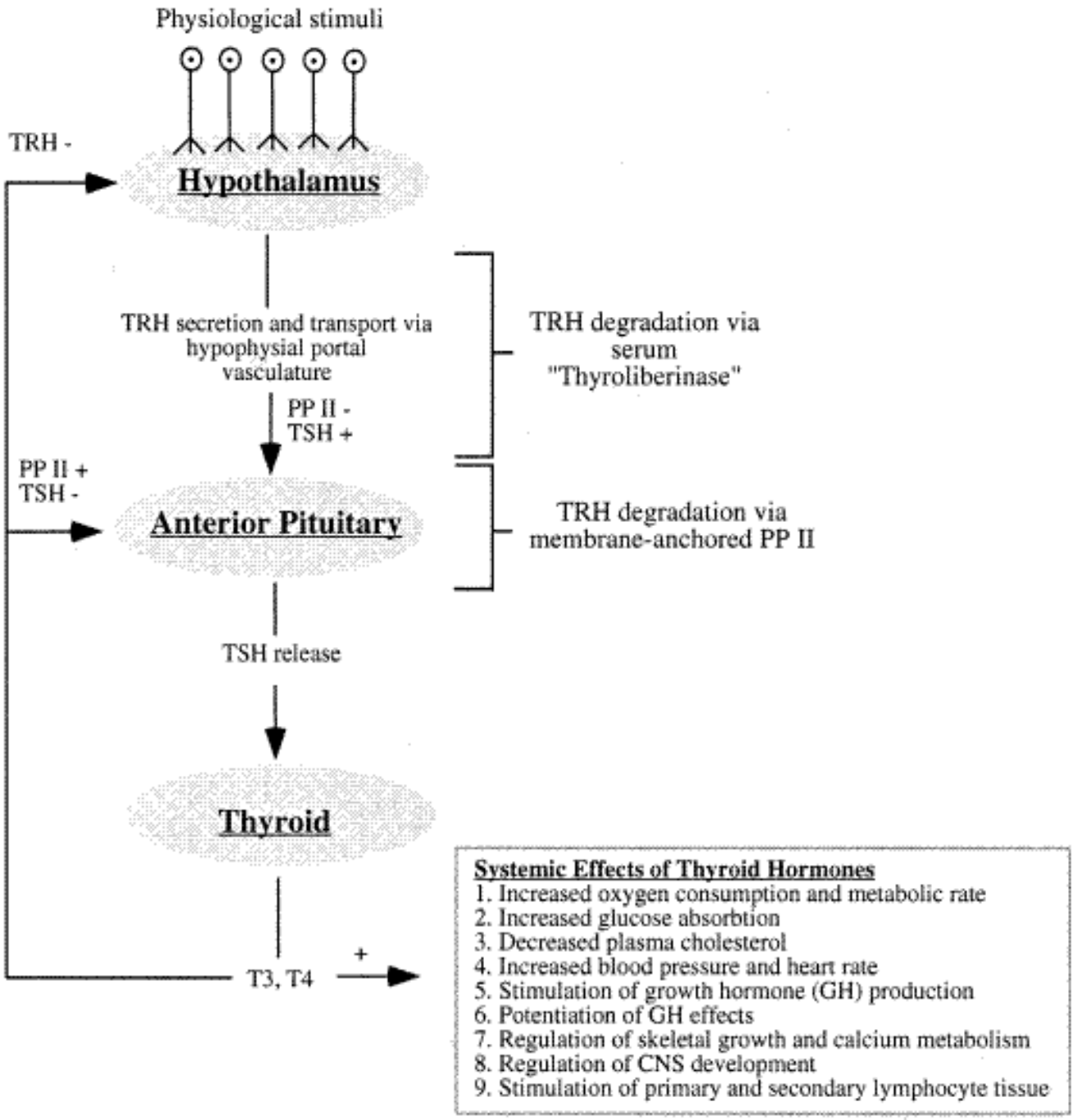
Protirelinum EP

•structure elucidated 1969, used approx. 1976 – 1991, then abandoned

•administered *p.o.*

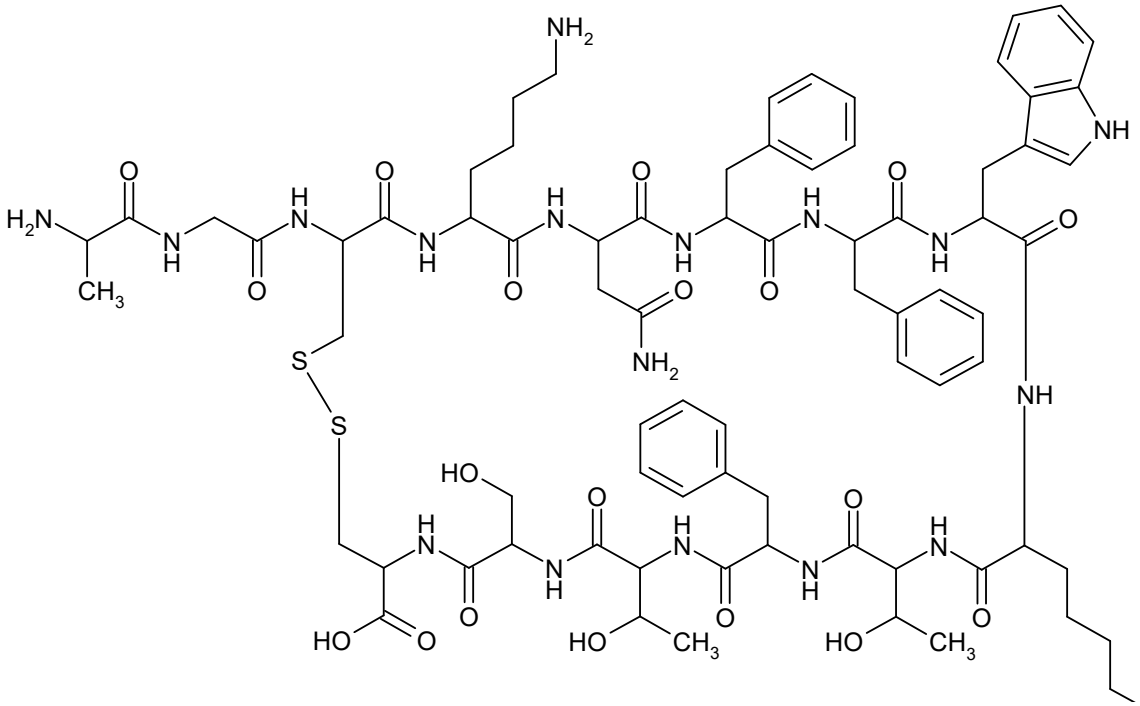
•used as cognitive functions enhacer for treatment of post-traumatic conditions in injuries of brain and spinal cord and of neurodegeneration diseases (Alzheimer, Parkinson, motoric neuronal disease etc.)

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“)



H—Ala—Gly—Cys—Lys—Asn—Phe—Phe—Trp—Lys—Thr—Phe—Thr—Ser—Cys—OH

$C_{76}H_{104}N_{18}O_{19}S_2$ M_r 1637,89 CAS 38916-34-6

somatostatin

Somatostatinum EP

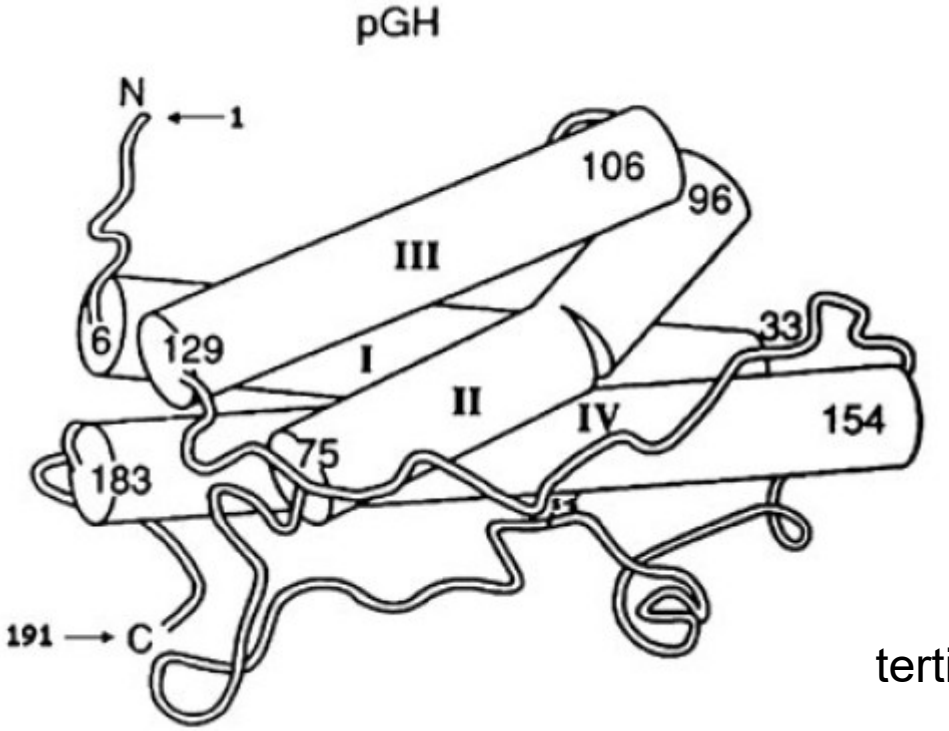
Somatostatin Eumedica® inf.

•prepared by synthesis
•treatment of acromegaly

1.2 Soma(to)tropin

= growth hormone (GH)

- peptide consisted of 191 AA secreted from anterior pituitary gland
- stimilates 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

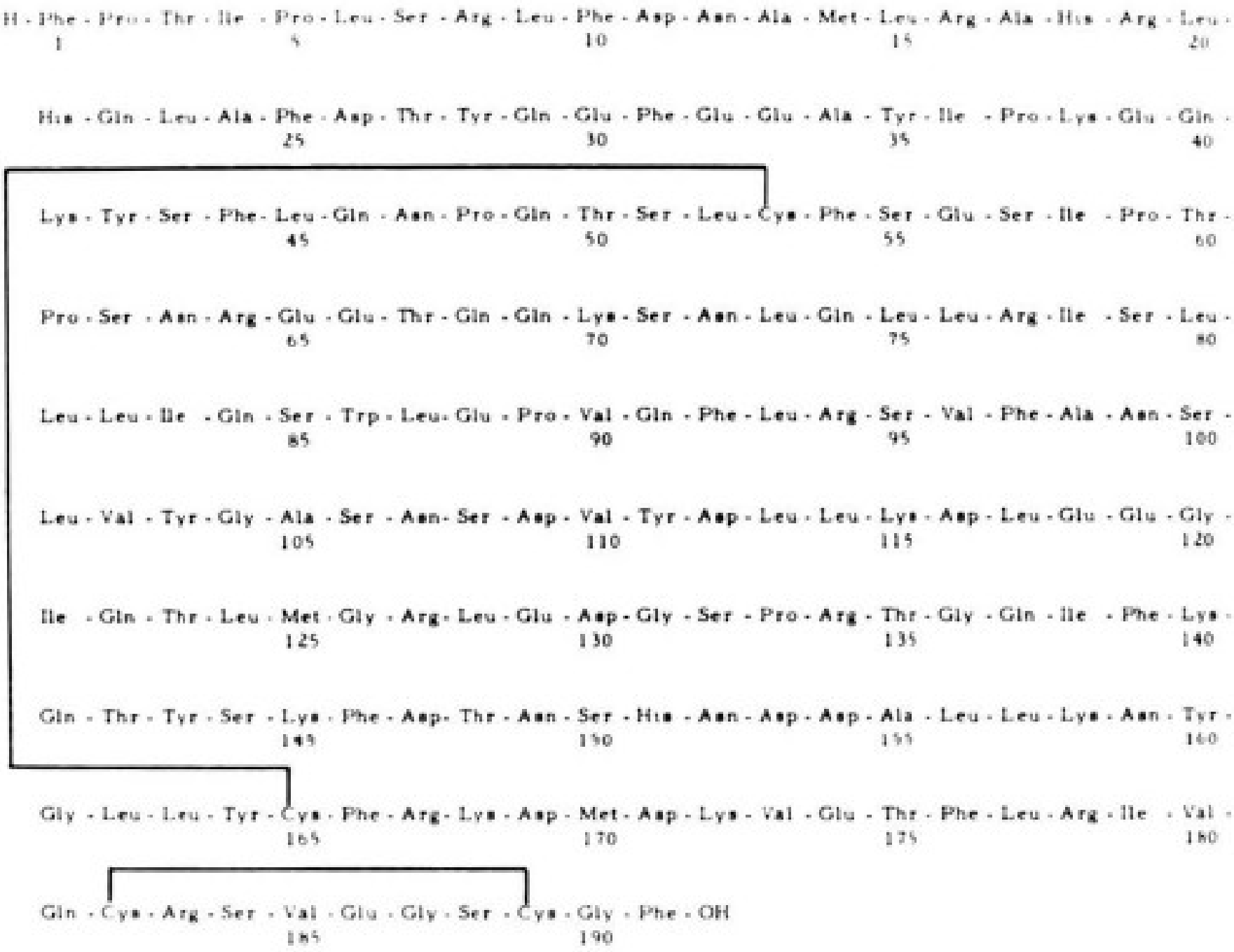
Somatropinum EP

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

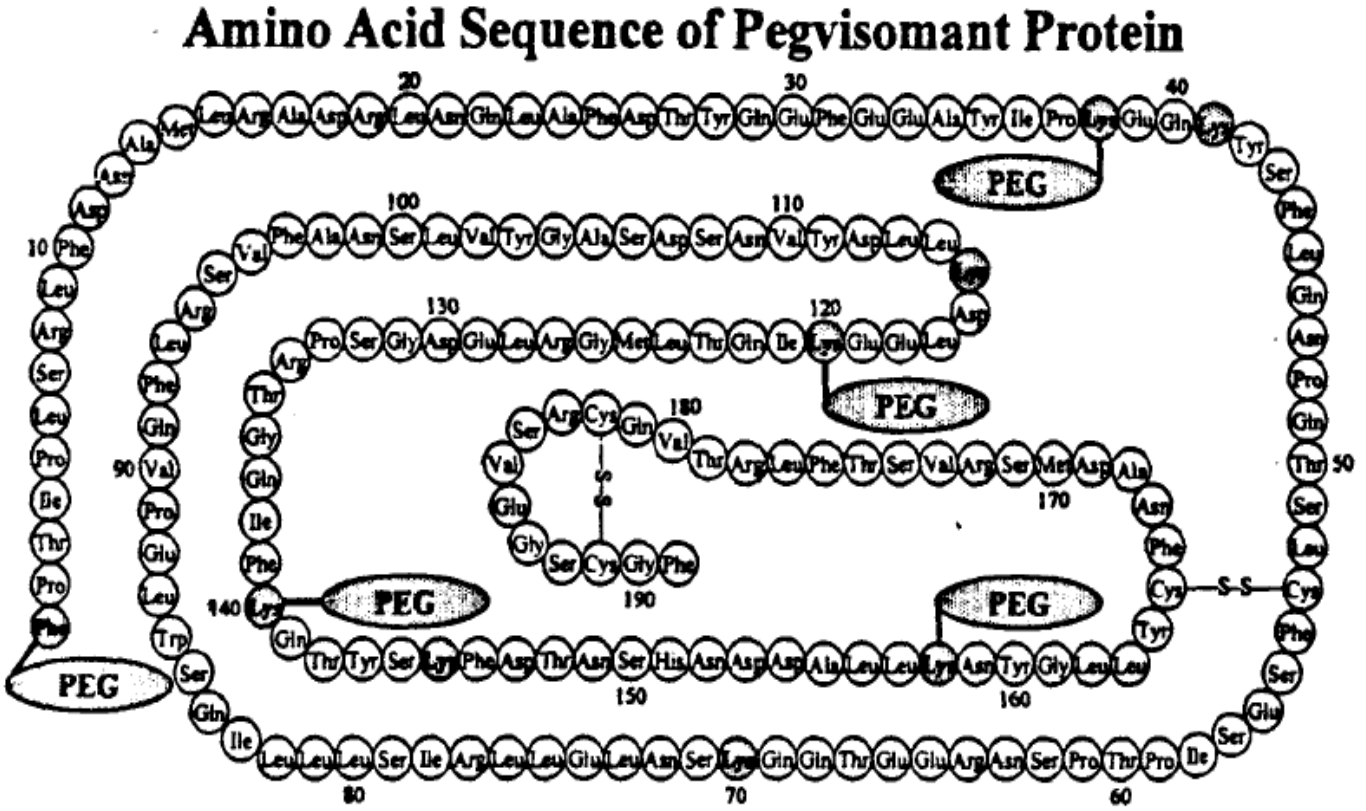
Genotropin ® , Humatrope ® , Nutropinaq ® , Omnitrope ® ...

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

Primary structure of human somatropin



Śomatropin (GH) analogues



* *Stippled residues indicate PEG attachment sites (Phe_1, Lys_{38}, Lys_{41}, Lys_{70}, Lys_{115}, Lys_{120}, Lys_{140}, Lys_{145}, Lys_{158})*

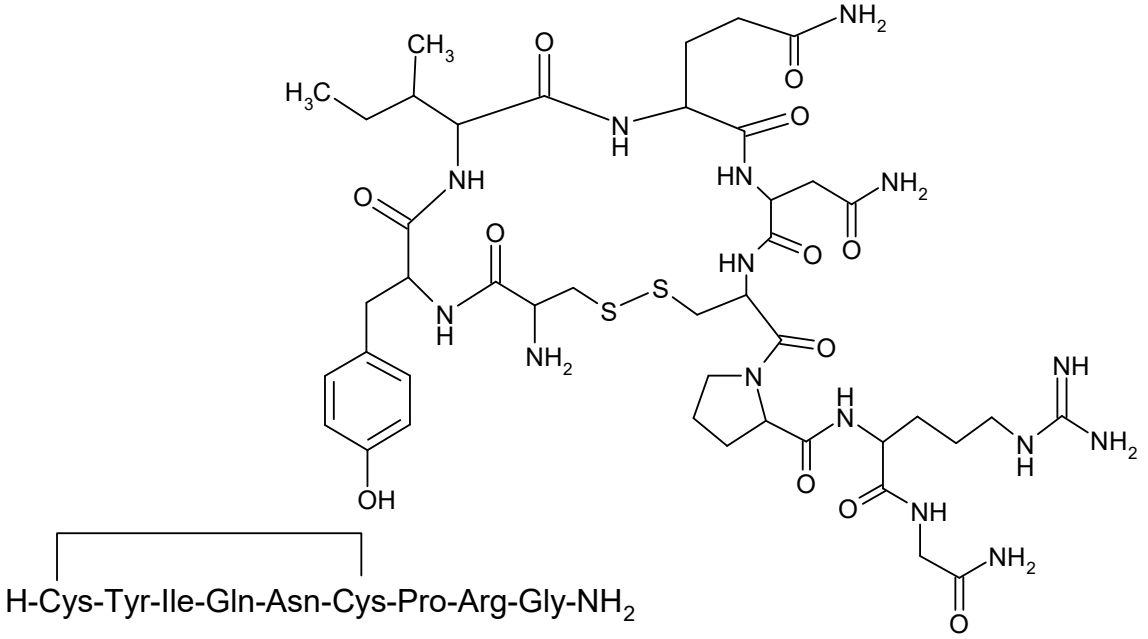
pegvisomant

- 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_1 and various 8 Lys residues
- prepared by the recombinant technology followed by a controlled reaction with oxiran (polyadition) which results to covalent binding of 4 – 5 polyoxoethylene chains of $M_r \sim 500$
- pegylation lowers antigenicity and prolongs the biologic half-time
- using: treatment of acromegaly

1.3 Oxytocin, vasopressins and their analogues

Vasotocin

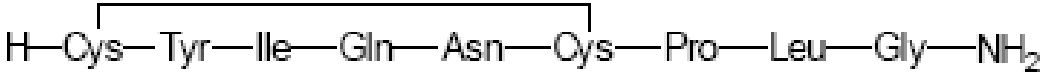
= fylogenetic precursor of oxytocine and vasopressins in organisms lower than mammals



H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly-NH_2

Oxytocin

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



H—Cys—Tyr—Ile—Gln—Asn—Cys—Pro—Leu—Gly—NH_2

$C_{43}H_{66}N_{12}O_{12}S_2$ 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)

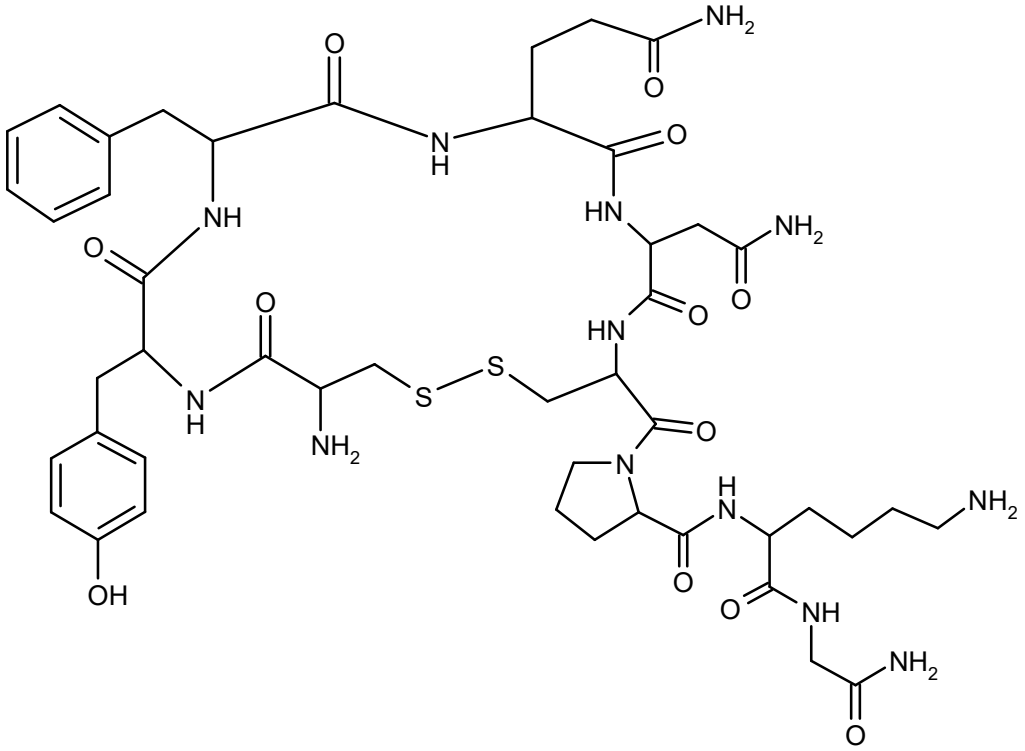
=antidiuretic hormone(s) (ADH)

•octapeptides released from the neurohypophysis of all vertebrates (precursor synthetized in hypothalamus)

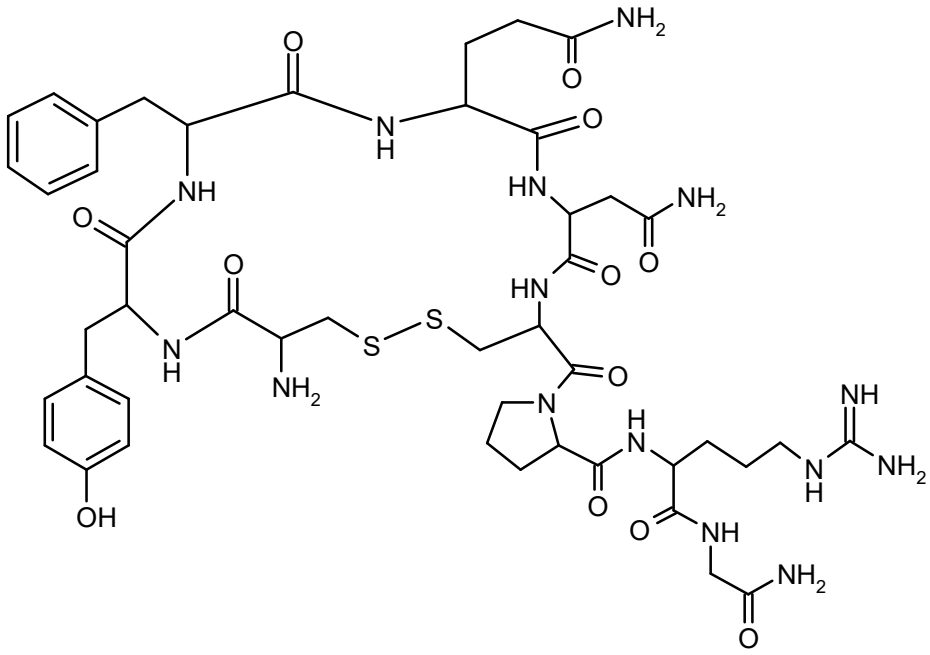
•control body water content (regulation of kidneys, lungs etc.)

•potential neurotransmitters

•semi-synthetic derivatives used predominantly



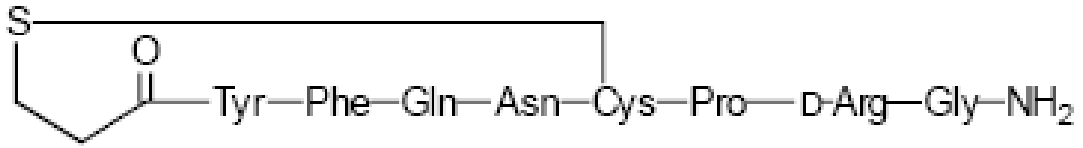
lysine-vasopressin
lypressin
•*Suidae* family only



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*, ...)

Vaspressin analogues

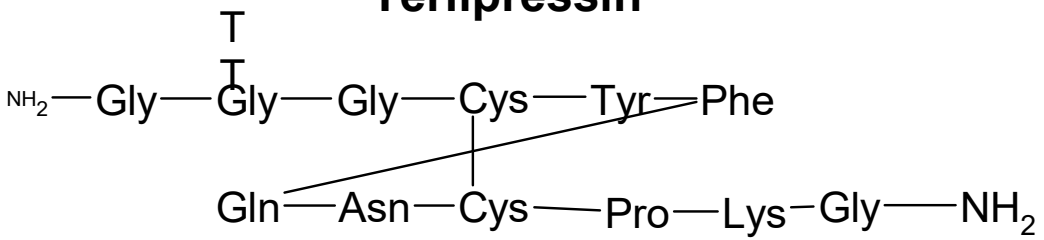
Felypressin

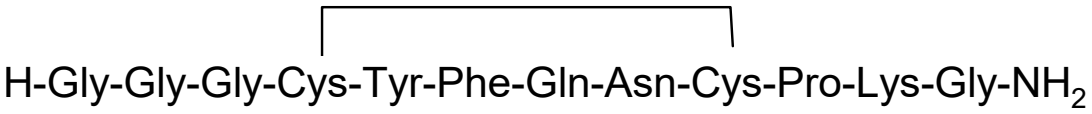
$C_{46}H_{65}N_{13}O_{11}S_2$ M_r 1040,22 CAS 56-59-7

Felypressinum EP

•vasoconstrictor with reduced antidiuretic activity

Terlipressin





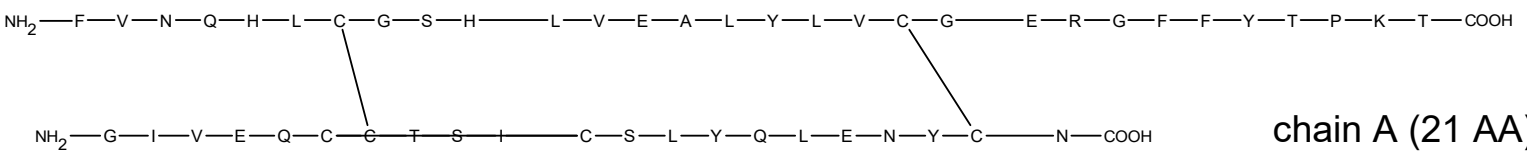
•vasoconstrictor, treatment of variceal bleeding, circulation and septic shock
Glypressin ® inj., Remestyp ® inj.

1.4 Insulines, glucagon and GLP-1 analogues

Insuline

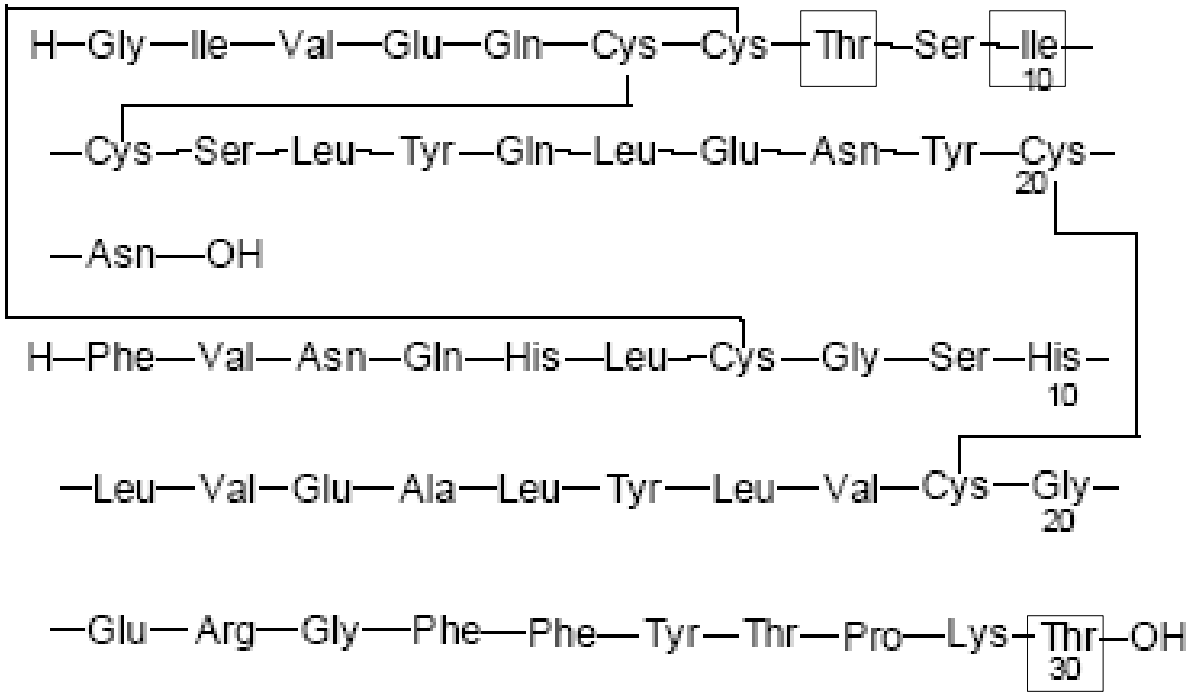
- Secreted mostly by β-cells of Langerhans islets of pancreas
- Enables utilisation of glucose by cells of body
- First isolated by Banting and Best from dog´s pancreas in 1921

Human insuline



chain B (30 AA)

chain A (21 AA)



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

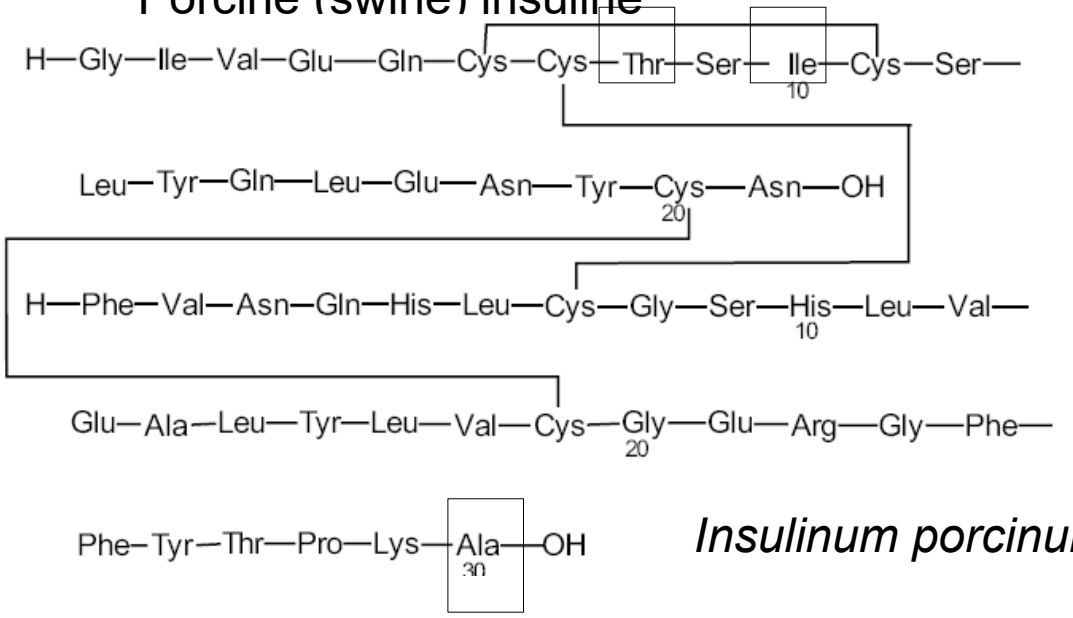
•syn. humuline

Bovine (cow´s) insuline

Insulinum bovinum PhEur

- isolation from beef pancreases

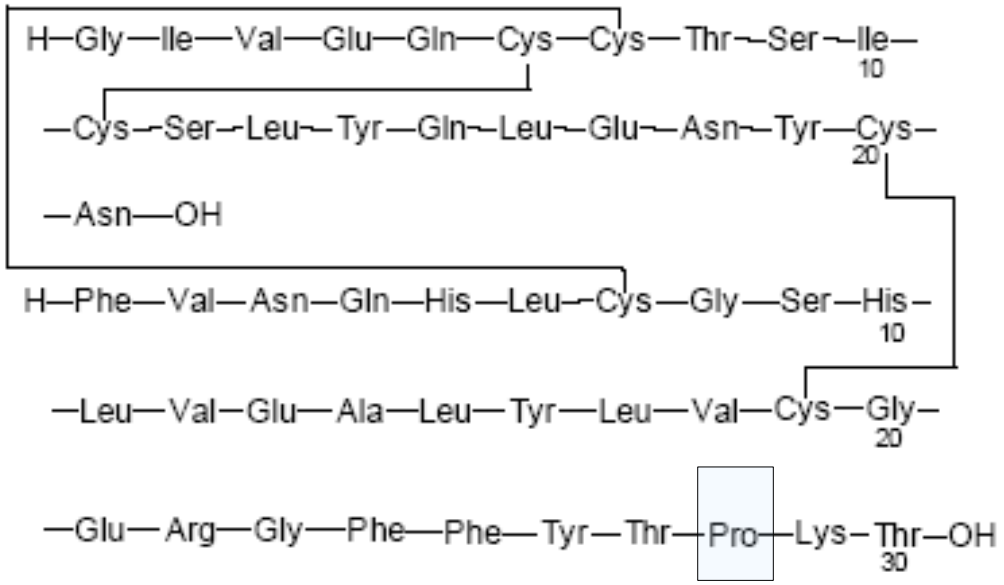
Porcine (swine) insuline



Insulinum porcinum PhEur

Insuline analogues

human

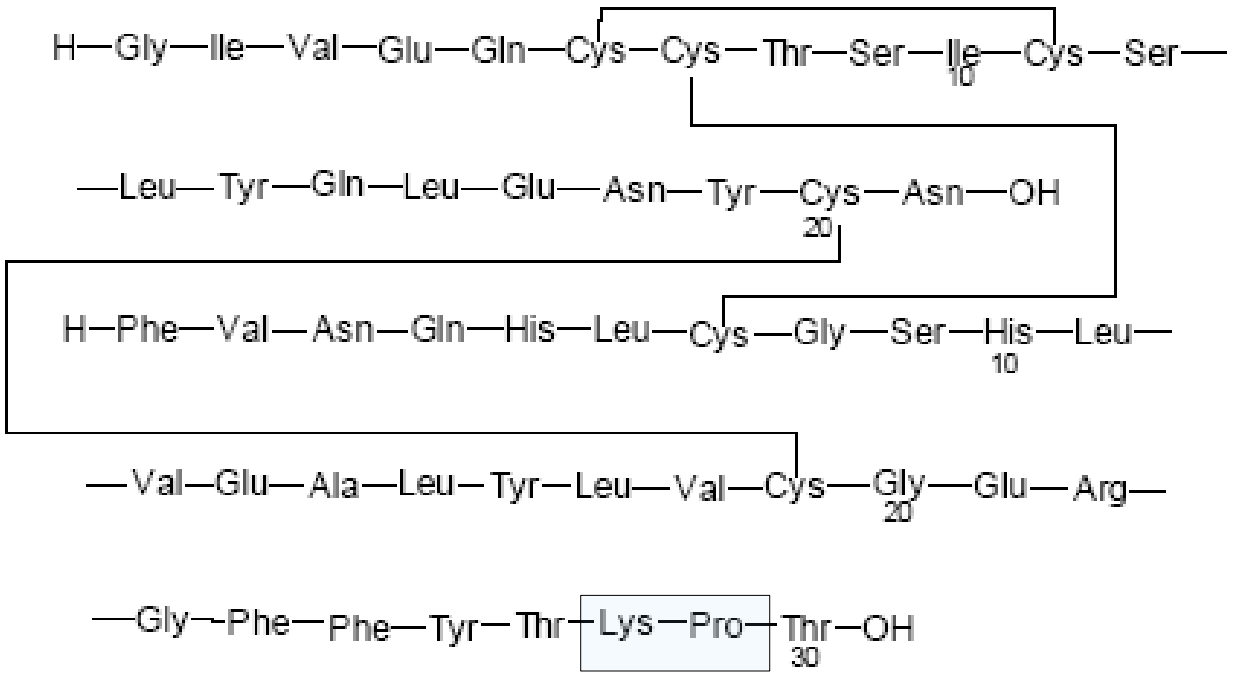


$C_{257}H_{383}N_{65}O_{77}S_6$ M_r 5807,60 CAS 11061-68-0

aspart
Insulinum aspartum
PhEur
Novorapid ®

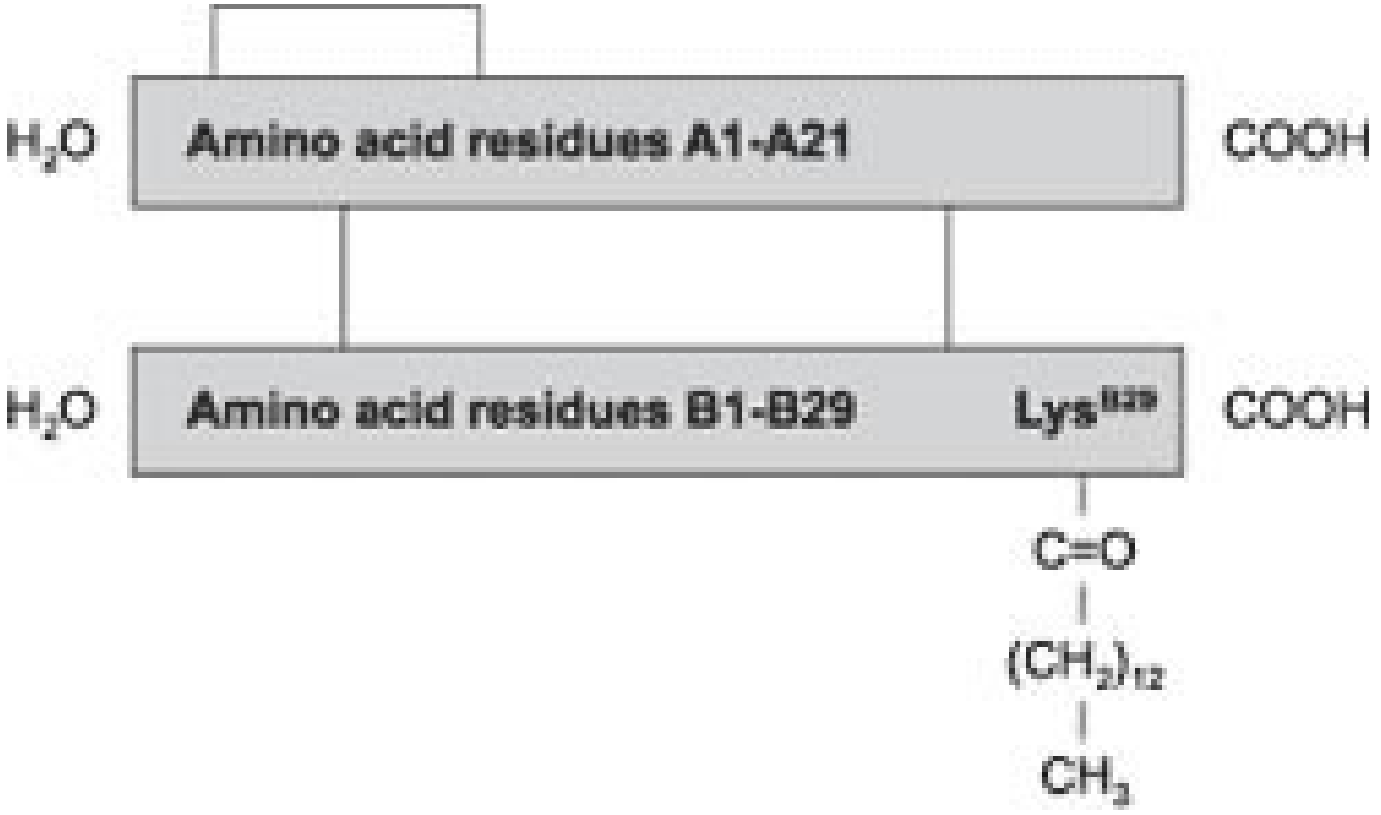
$C_{256}H_{381}N_{65}O_{79}S_6$ M_r 5825,58 CAS 116094-23-6

•recombinant technology



$C_{257}H_{383}N_{65}O_{77}S_6$ M_r 5807,61 CAS 133107-64-9

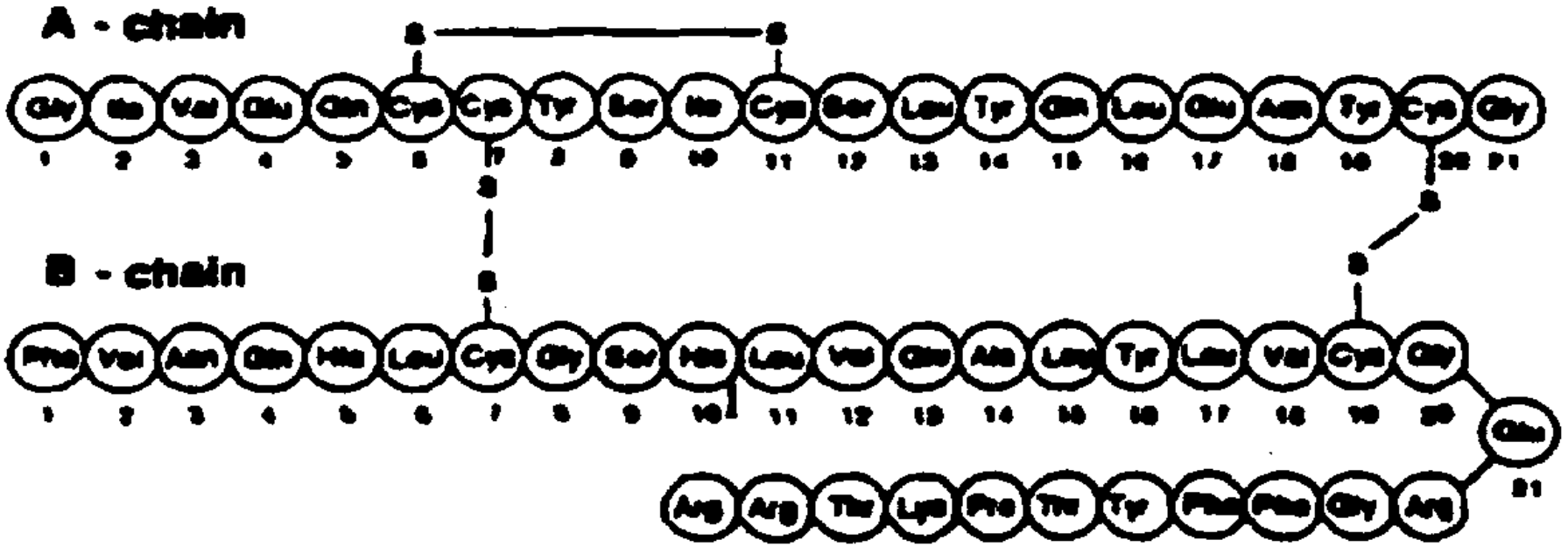
insulin-lispro
Insulinum lisprum PhEur
•recombinant
Humalog ®, Liprolog ®



insulin-detemir

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

Levemir ®

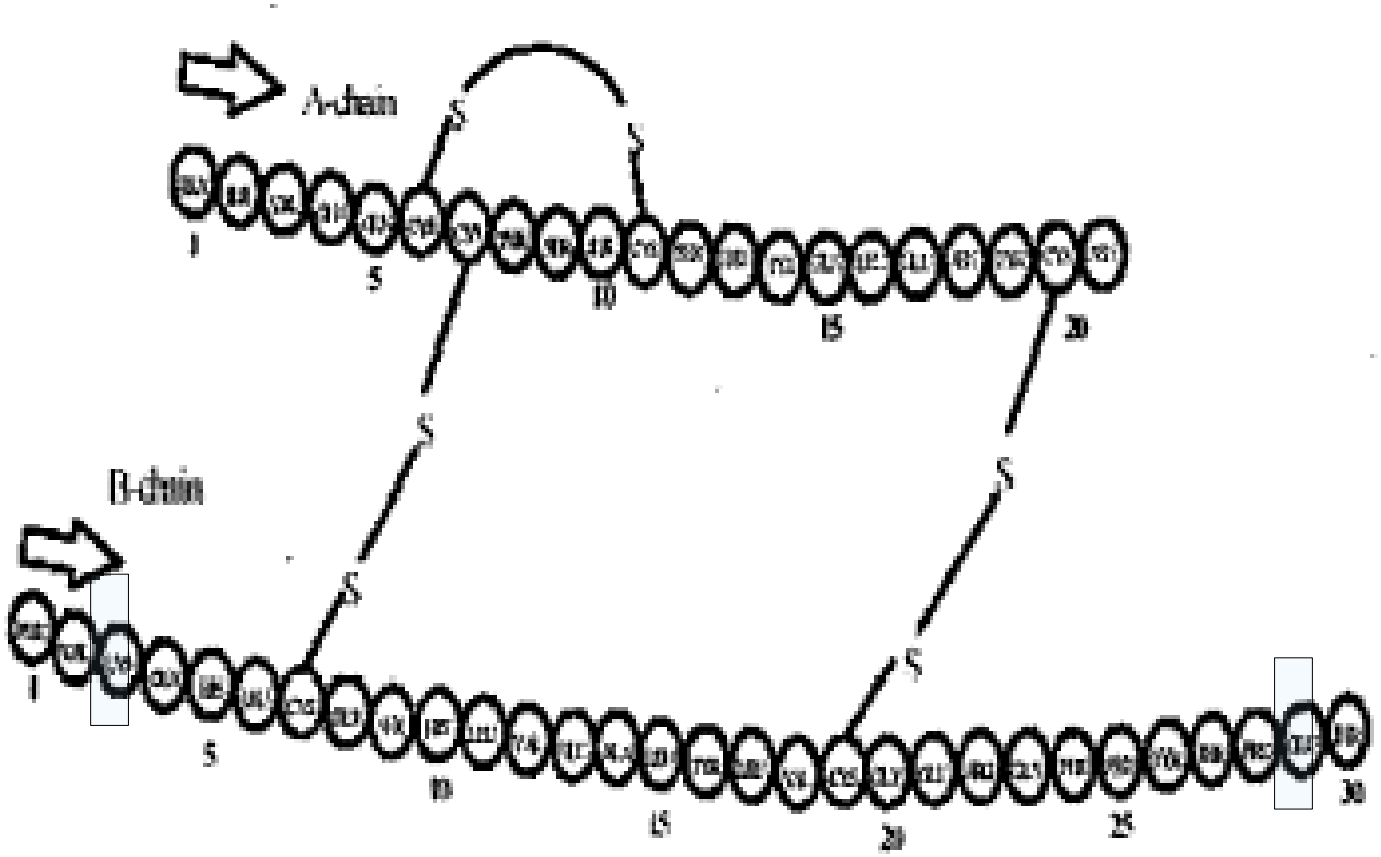


insulin-glargin

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 $T_{½}$, typically administered 1x daily s.c. before sleeping

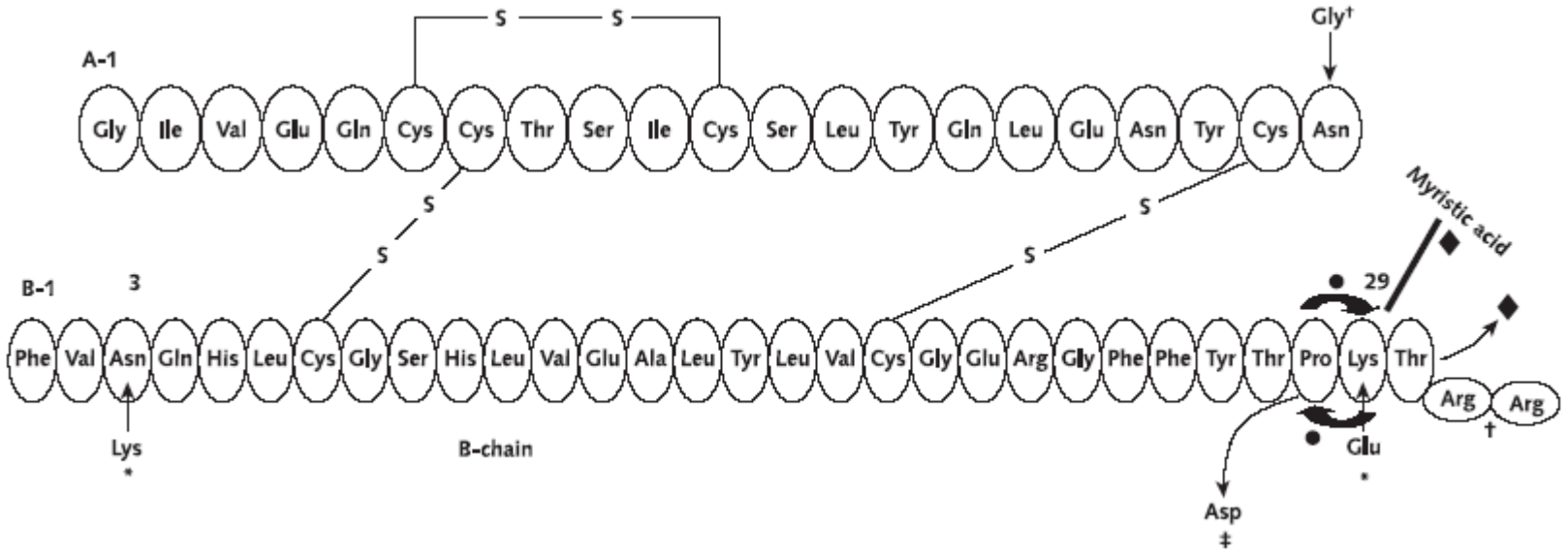


Chemical name: 3βLys-29βGlu-human insulin
CAS registry number: 207748-29-6
Molecular formula/molecular weight: $C_{258}H_{384}O_{78}N_{64}S_6$/5823

insulin-glulisin

Apidra ®

Summary of the used insuline analogues

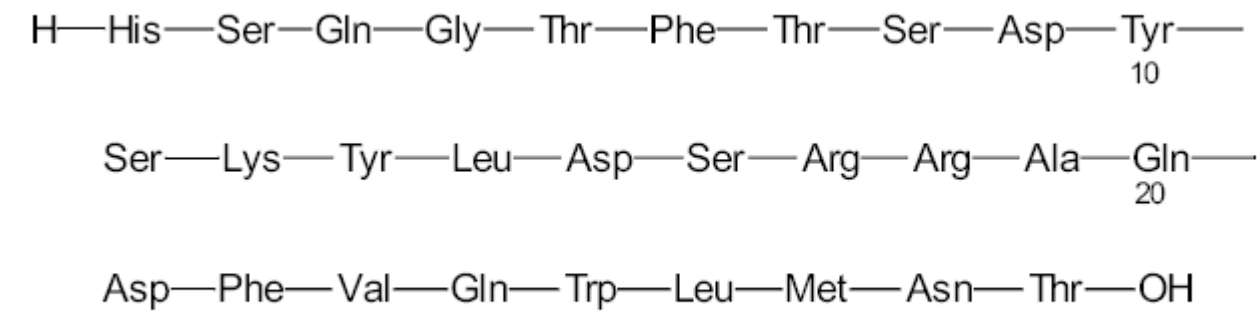


● = Insulin lispro differs from human insulin by the substitution of proline with lysine at position 28 and the substitution of lysine with proline at position 29 of the insulin β chain.
‡ = Insulin aspart is designed with the single replacement of the amino acid proline by aspartic acid at position 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 muscules similarly to cholinergics



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

•potetiates all steps of insulin biosynthesis; has positive impact to function and surviving of β-cells

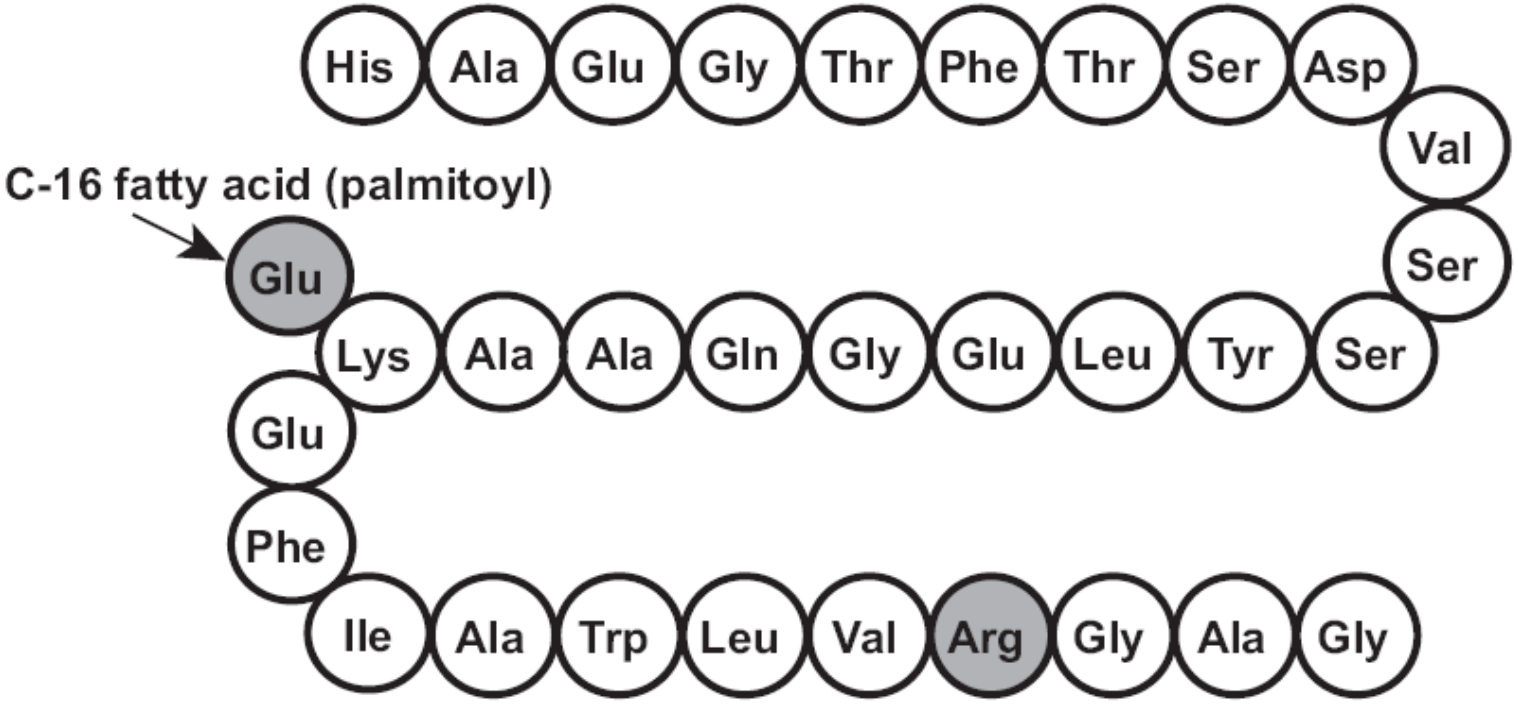
•decreases redundant glucose production in liver, slows down stomach emptying leading to postprandial hypoglycaemia, its central effect leads to appetite decrease ($\Rightarrow$ body weight loss), probably also positive effects to cardiovascular system

•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)-Lys^{26}, Arg^{34}-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

Calcitonin

•released from thyroidal C-cells (= parafolicular cells – Baber 1876), in lower vertebrates from ultimobranchial bodies, originated from 5th branchial fissure

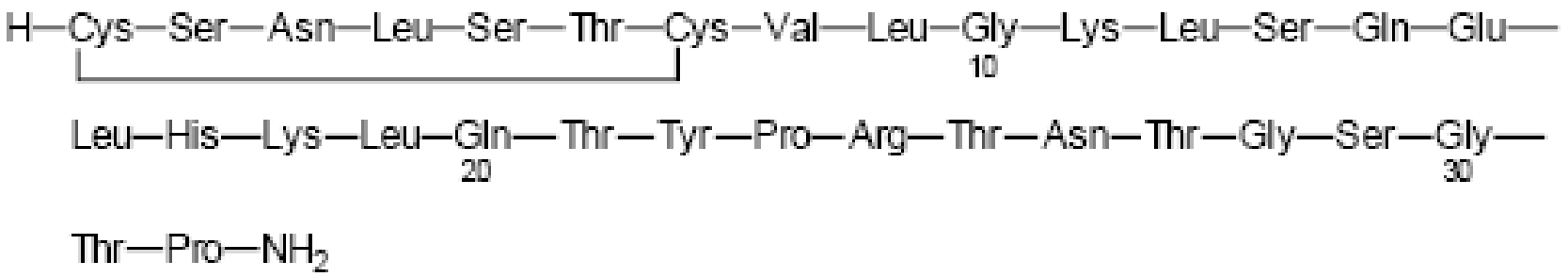
•peptide from 32 amino acid residues (salmon's – *Onchorhyncus kisutch;* human has 139 AA)

•receptors on osteoclasts (also in kidneys and brain)

•↓ excretion of Ca^{2+} from the bone (⇒ ↓ calcaemia)

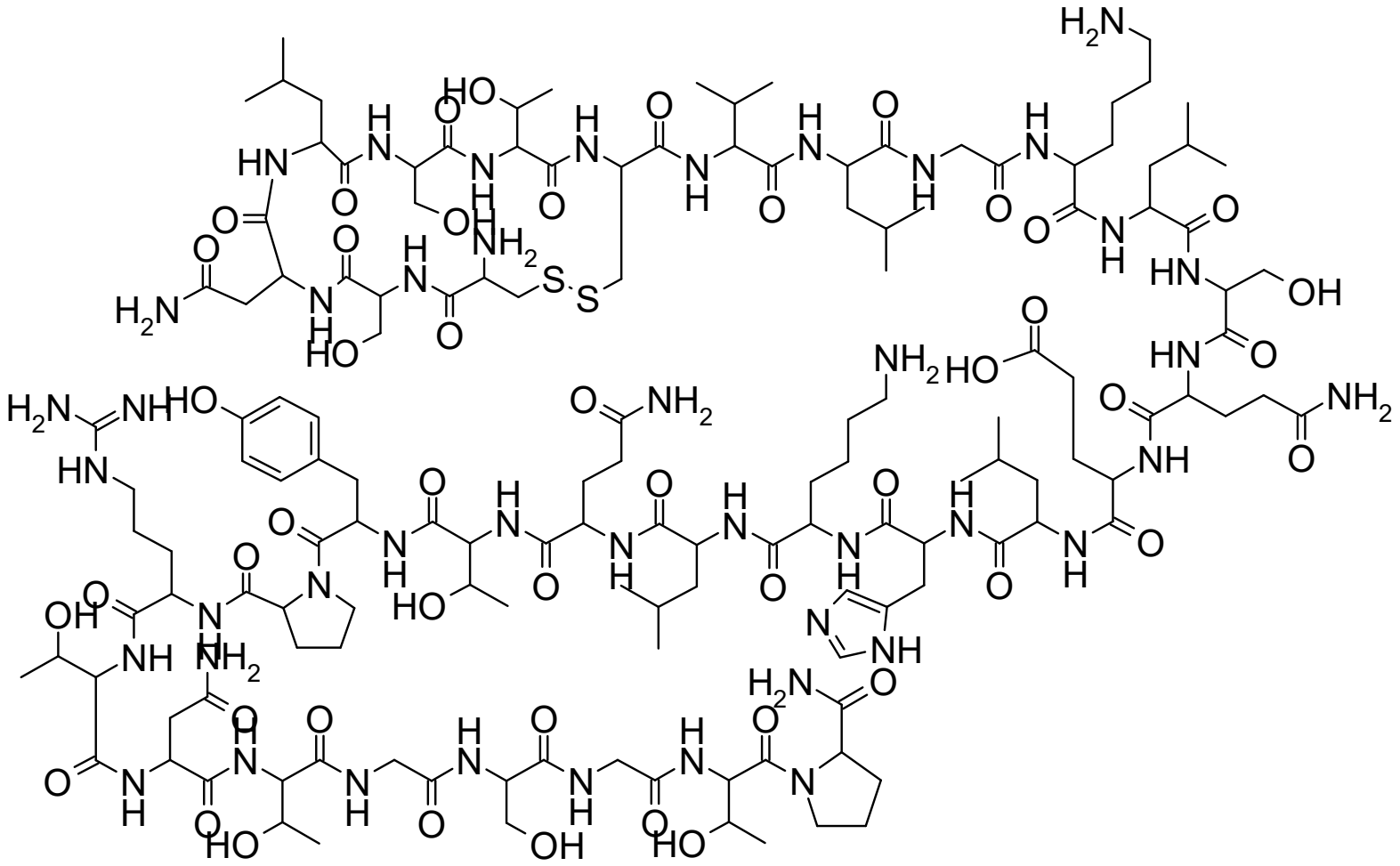
•↓ osteoclasts formation

•used together with Ca^{2+} for treatment of osteoporosis



$C_{145}H_{240}N_{44}O_{48}S_2$ M_r 3431,88 CAS 47931-85-1

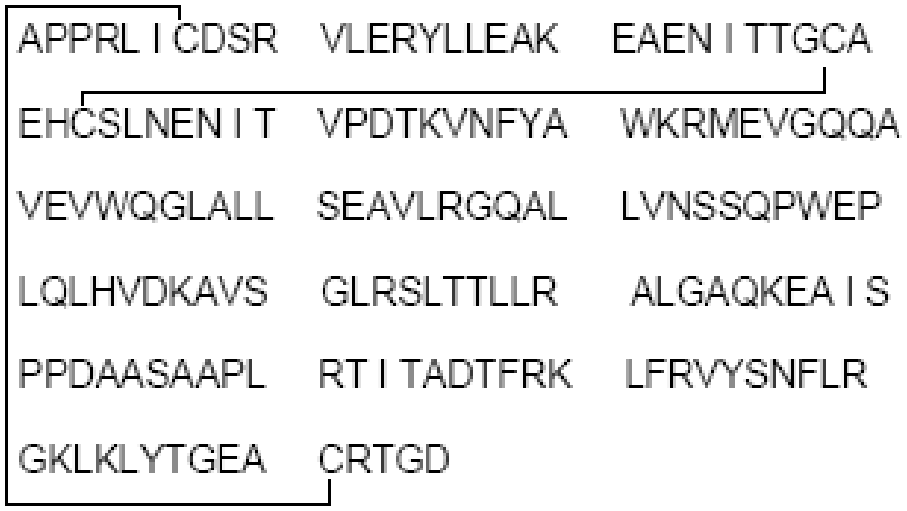
Calcitonin



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

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

2. Blood factors of erythropoetine type



erythropoietin (EPO)
= glycosylated protein from 165 AA M_r about 30 600 CAS 113427-24-0
Erythropoietini solutio concentrata EP
= a solution containing a group of closely related glycoproteins, which are not to distinguish from the natural human erythopoietin (human urine erythropoietin, huEPO), 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* mostly in rodent cell lines by a method based on the recombinant DNA
- technology
- **INN names: epoetin + greek letter spelt in full** (eg. epoetin beta)
- various epoetins differ in glycosylation, complex branched oligomeric sugar chains are attached
- treatment of anaemia in chronic kidney failure, missused for doping

Epoetin Alfa
(Genetical Recombination)

エポエチン　アルファ（遺伝子組換え）

Protein moiety

APPRLICDSR VLERYLLEAK EAENITTGCA EHCSLNENIT VPDTKVNFYA

WKRMEVGQQA VEVWQGLALL SEAVLRGQAL LVNSSQPWEP LQLHVDKAVS

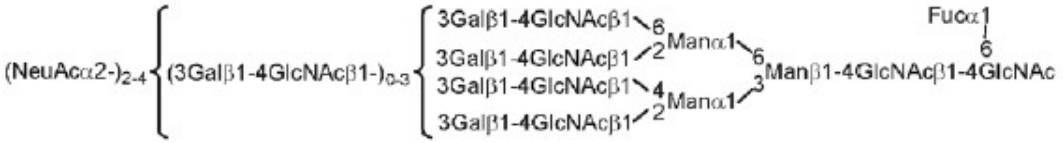
GLRSLTTLLR ALGAQKEAIS PPDAASAAPL RTITADTFRK LFRVYSNFLR

GKLKLYTGEA CRTGD

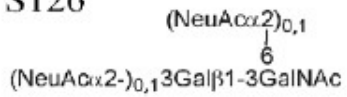
N24, N38, N83 and S126 : glycosylation

Carbohydrate moiety (structure of major glycans)

N24, N38 and N83



S126



$C_{809}H_{1301}N_{229}O_{240}S_5$: 18235.70 (Protein moiety)
[*113427-24-0*]

Epoetin Beta
(Genetical Recombination)

エポエチン　ベータ（遺伝子組換え）

Protein moiety

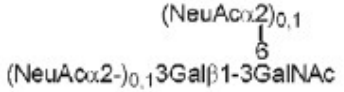
APPRLICDSR VLERYLLEAK EAENITTGCA EHCSLNENIT VPDTKVNFYA
WKRMEVGQQA VEVWQGLALL SEAVLRGQAL LVNSSQPWEP LQLHVDKAVS
GLRSLTTLLR ALGAQKEAIS PPDAASAAPL RTITADTFRK LFRVYSNFLR
GKLKLYTGEA CRTGD

N24, N38, N83 and S126: glycosylation

Carbohydrate moiety (structure of major glycans)

N24, N38 and N83

S126

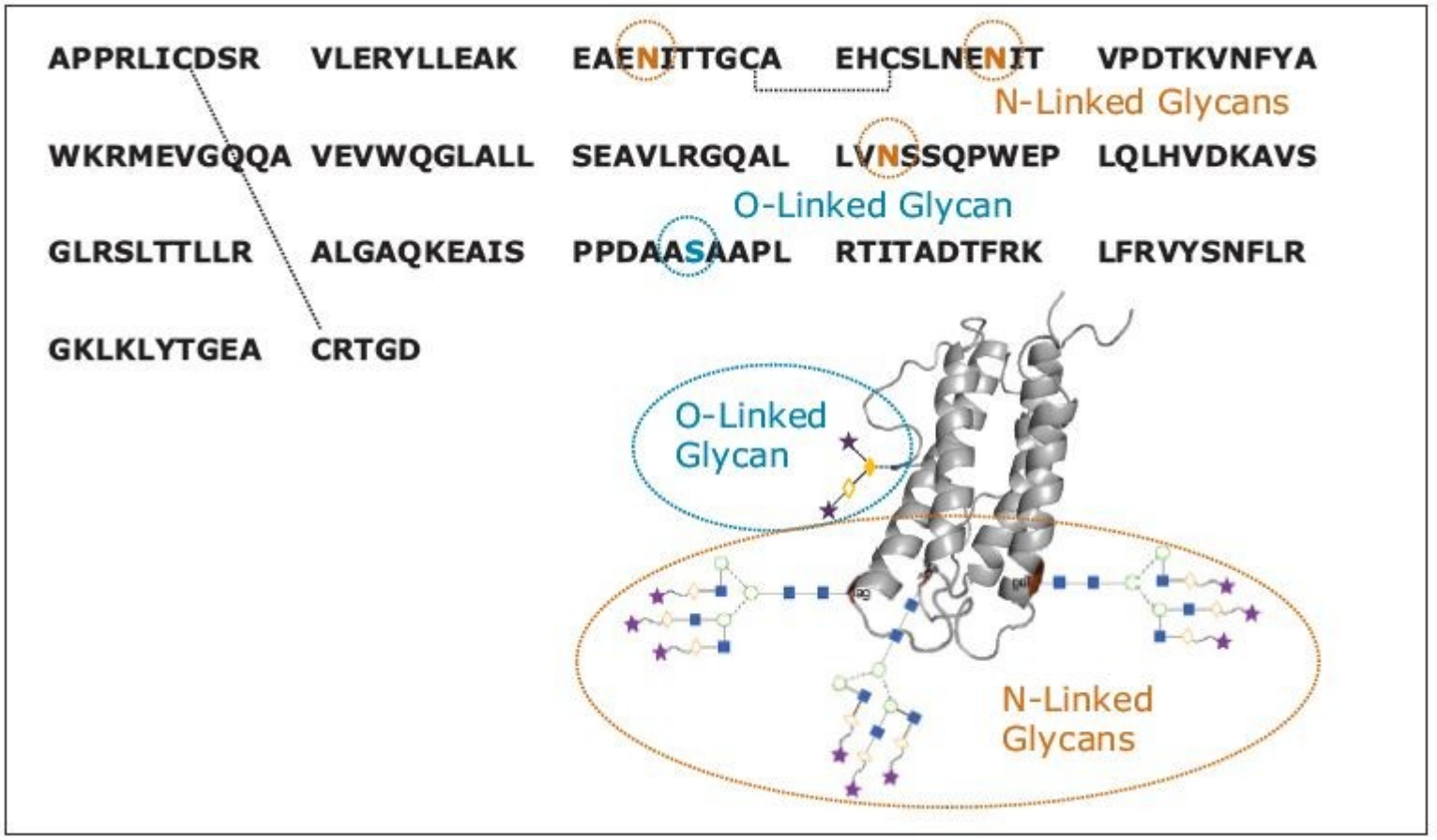


$C_{809}H_{1301}N_{229}O_{240}S_5$: 18235.70 (Protein moiety)
[*122312-54-3*]

Overview of epoetins

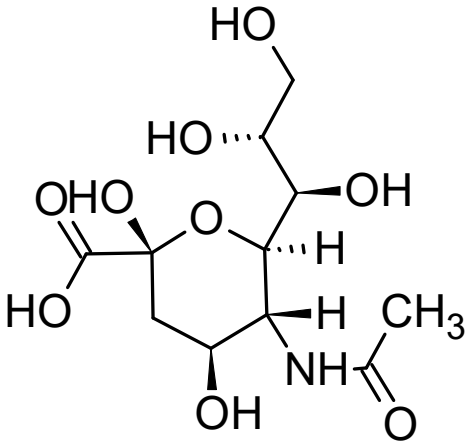
INN name: epoetin	Year of discovery/approval	Production organism / tissue	M_r CAS	Glycosylation pattern	Originator product/biosimilar	Brand names ®, generic codes
alfa	2000	Chinese hamster ovary	113427-24-0	similar to uhEPO	orig/biosim	**Eprex,** Binocrit, Abseamed
beta	1997	Chinese hamster ovary	122312-54-3		orig	Neorecormon
gama	1990	C127 murine cells transfected with huEPO cRNA	28 000-31 000 130455-76-4		orig	TYB-5220
delta	2002 - 2009	human fibrosarcoma cell line HT-1080	261356-80-3	less *O*-acetyls in *O*-glycan chains; similar to uhEPO	orig	Dynepo
epsilon	1995		154725-65-2		orig	
zeta	2007	Chinese hamster ovary	32 000-40 000 604802-70-2		biosim. of EPO alfa	Silapo, Retacrit
theta	2009	Chinese hamster ovary	762263-14-9	sugars represent 40 % of total M_r	orig	Biopoin, Eporatio
kappa	2010	Chinese hamster ovary	11096-26-7		biosim. of EPO alfa	Epoetin alfa BS injection ®

Epoetins´ glycosylation

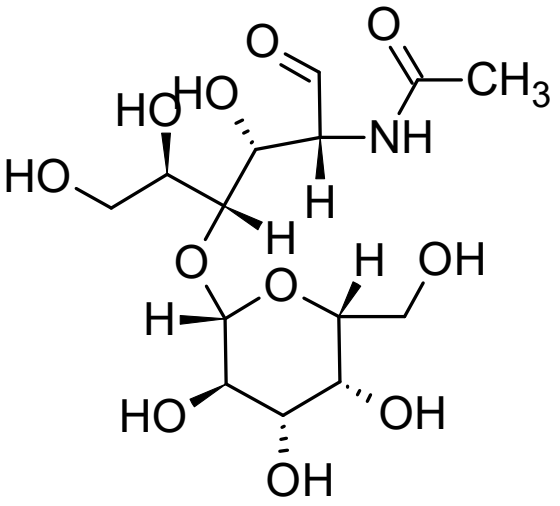


Sites of *N*-glycosylation: Asn24, Asn38, Asn83 (= N24, N38, N83)
Site of *O*-glycosylation*:* Ser126 (= S126)

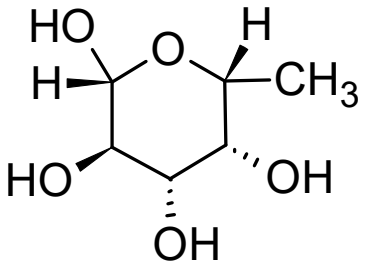
Epoetins´ glycosylation: some more specific occuring sugars



N-acetylneuraminic acid (= sialic acid)



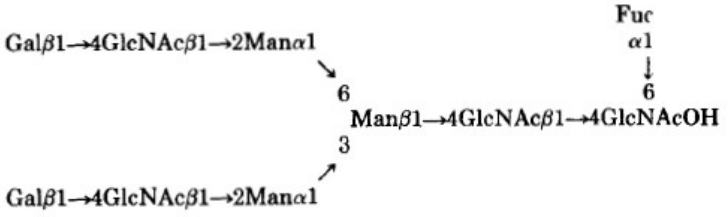
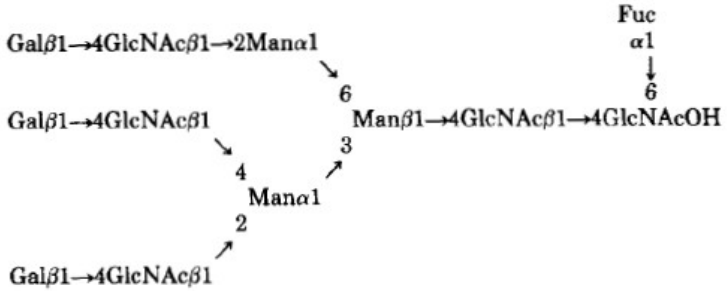
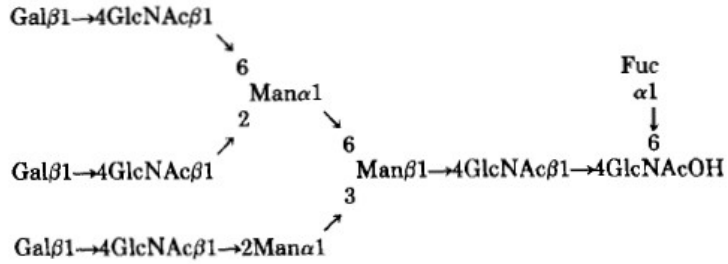
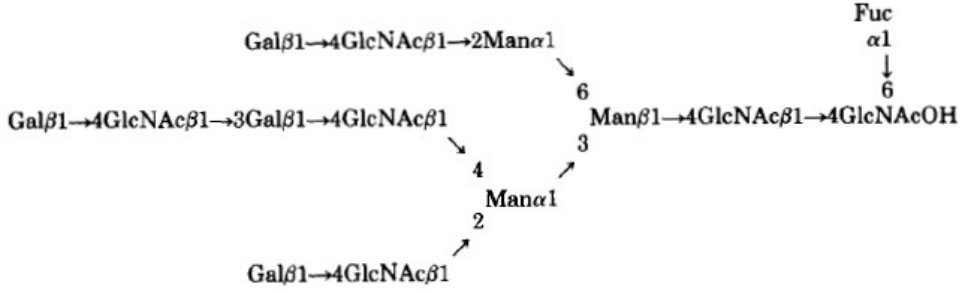
N-acetyllactosamine



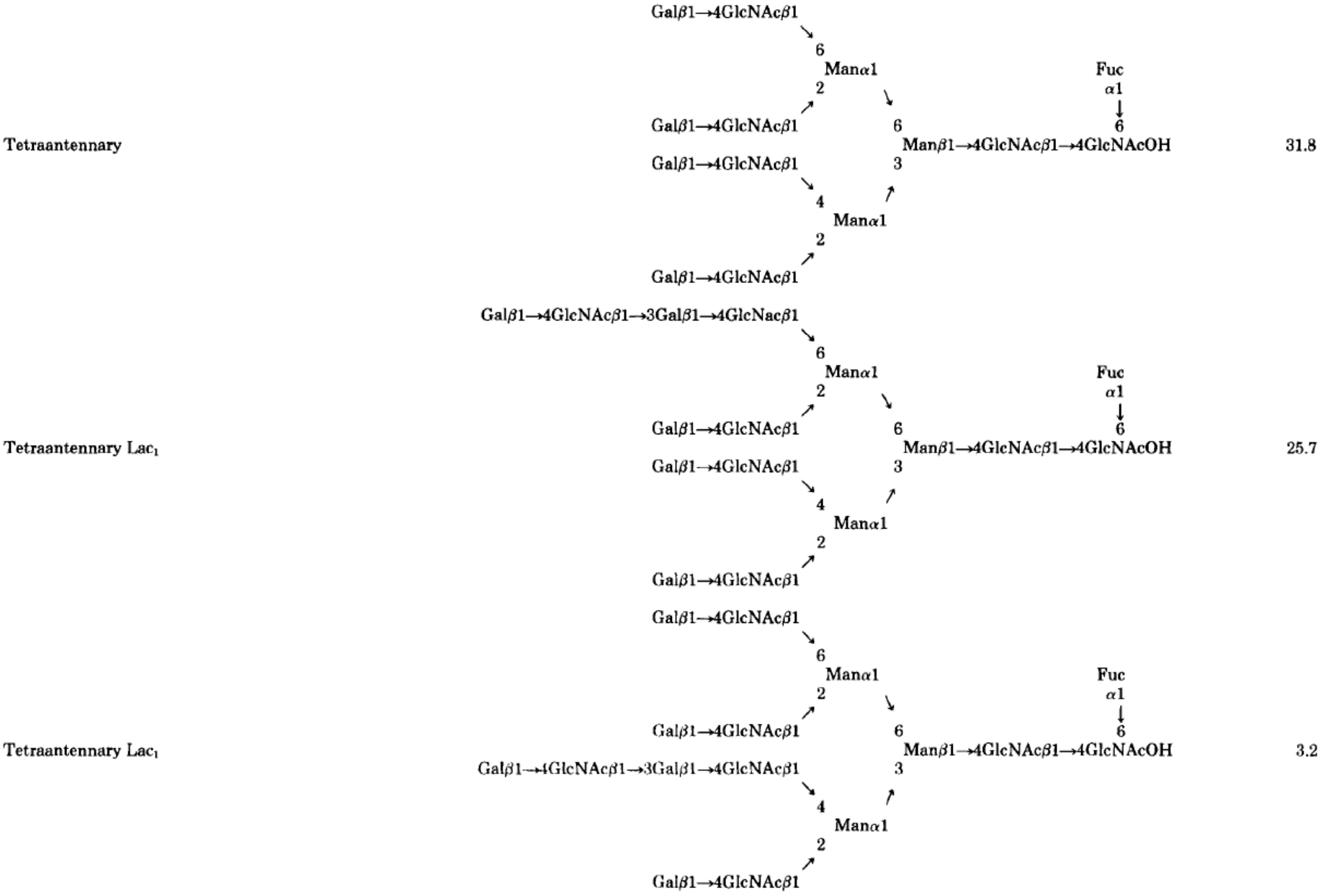
L-fucose

Epoetins´ glycosylation: secondary structure of *N*-attached oligosaccharide chains

Structures of asialo N-linked saccharides obtained from recombinant erythropoietin

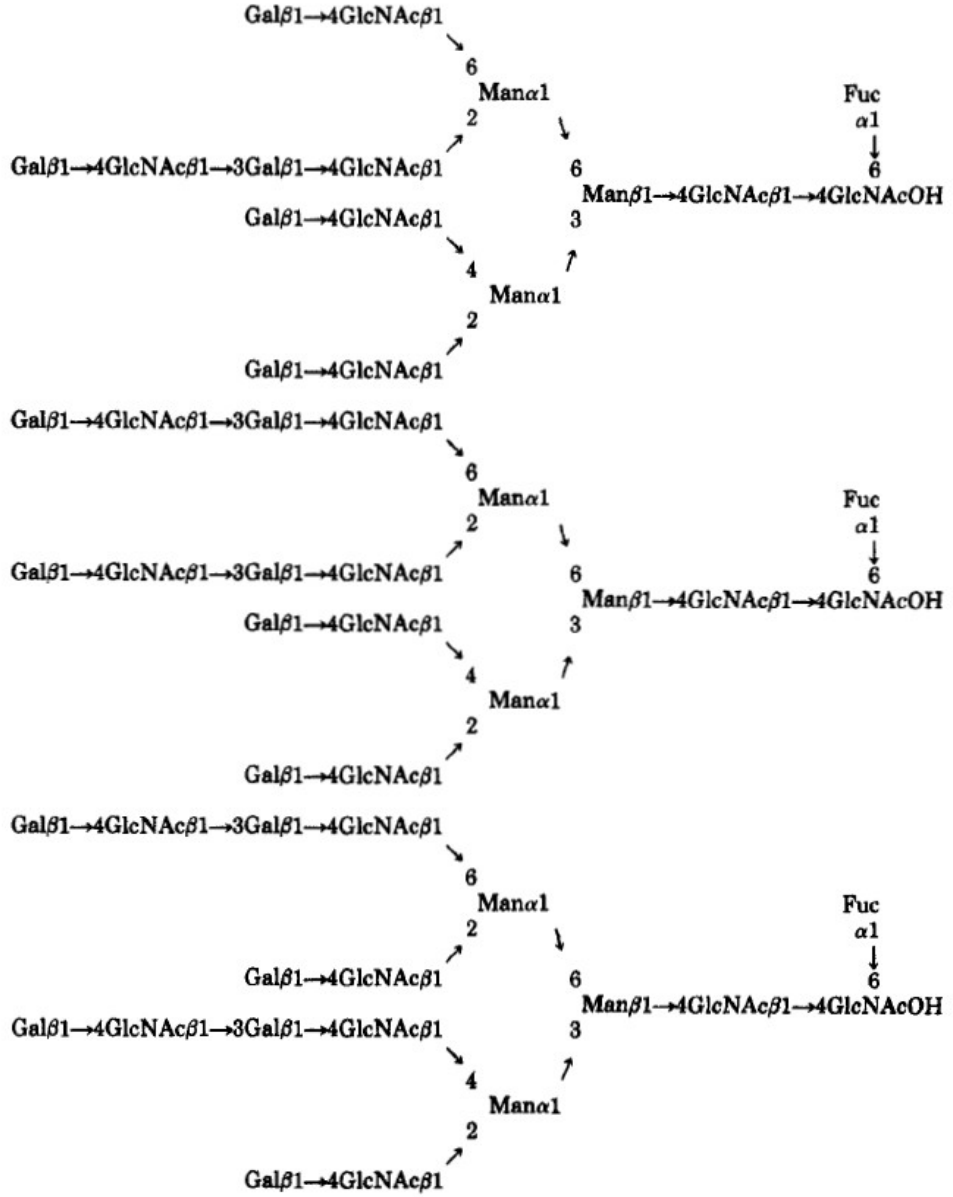
Saccharide	Structure[a]	Relative amount
		%
Biantennary		1.4
Triantennary		3.5
Triantennary		6.5
Triantennary Lac_1		3.5

Epoetins´ glycosylation: secondary structure of *N*-attached oligosaccharide chains continued

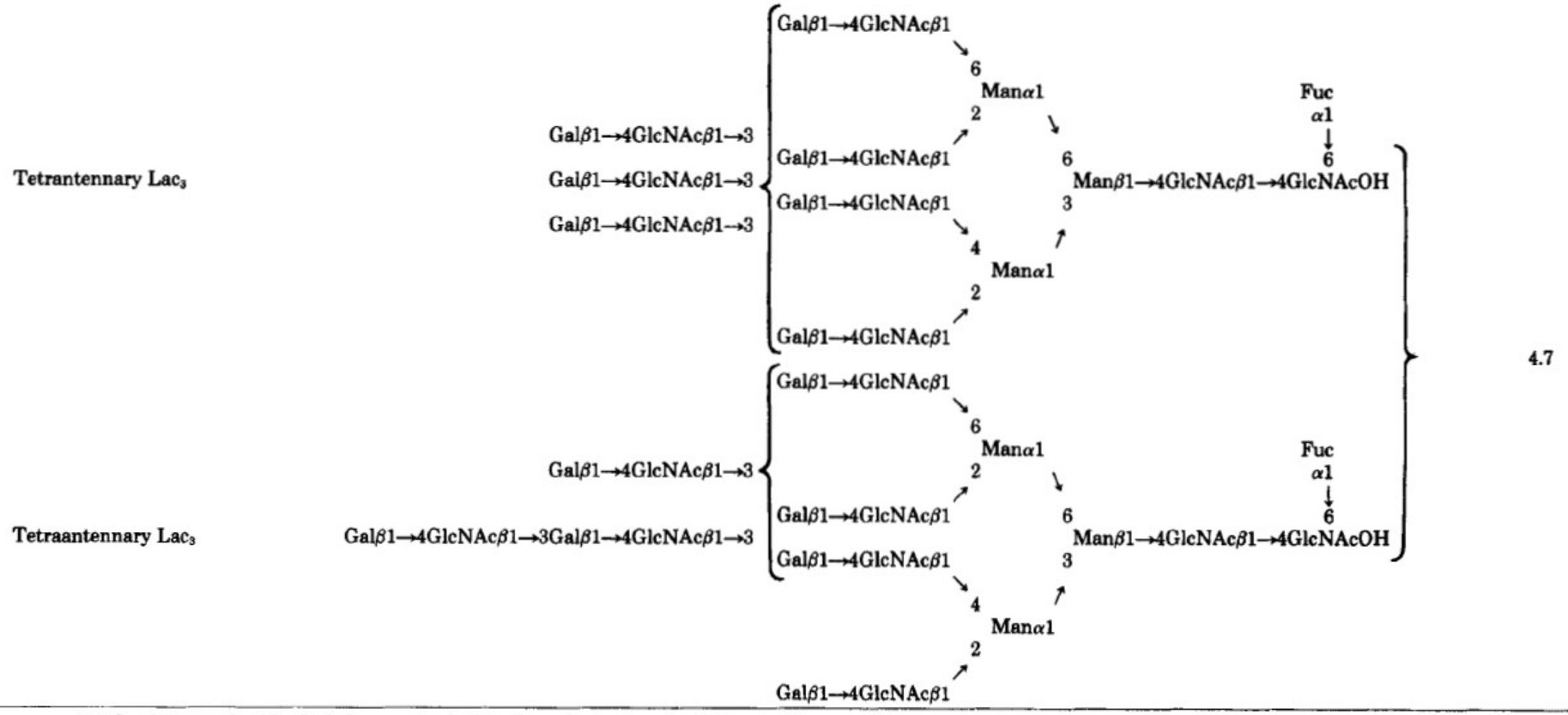


Epoetins´ glycosylation: secondary structure of *N*-attachedoligosaccharide chains continued

TABLE III–*Continued*

Saccharide	Structure[a]	Relative amount
		%
Tetraantennary Lac_1		3.2
Tetraantennary Lac_2		13.2
Tetraantennary Lac_2		3.3

Epoetins´ glycosylation: secondary structure of *N*-attached oligosaccharide chains continued



[a] 15% of the saccharides lack fucose attached to the reducing terminal *N*-acetylglucosamine.

Differences in individual epotins' glycosylation pattern: CZE in accordance with the European Pharmacopoea continued

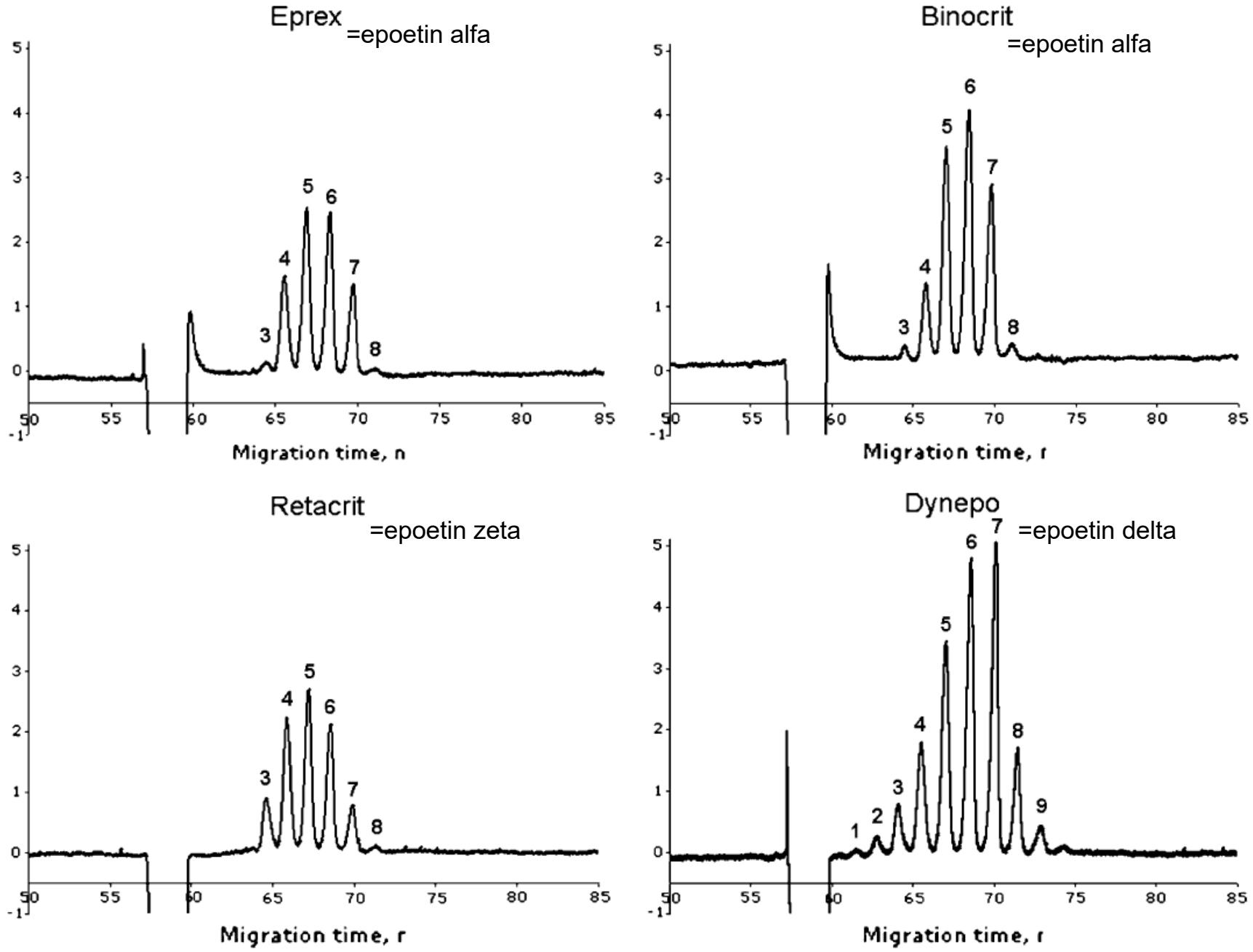
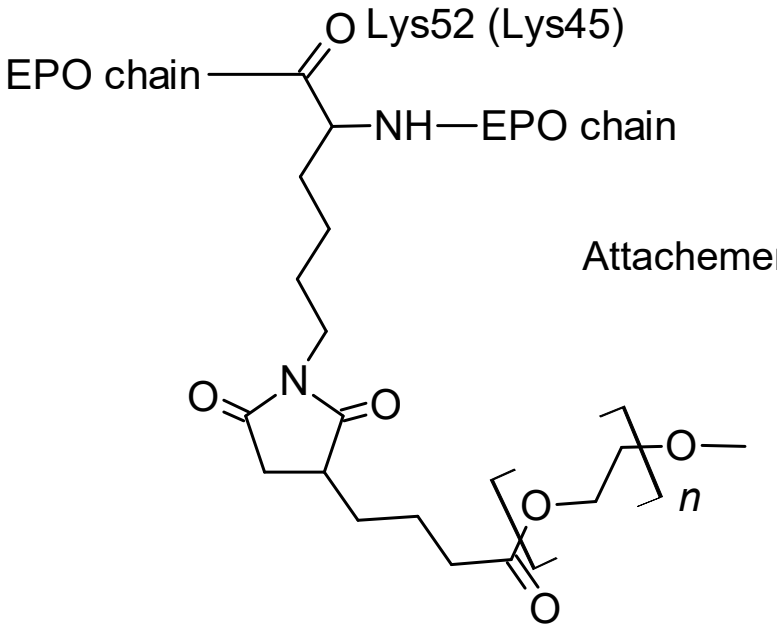


Fig. 3 CE-UV analysis of the four EPO products.

Brinks V. et al., Pharm. Res. 28, 386-393 (2011)

Epoetin conjugates
Methoxy-polythylenglycol-epoetin beta

Total M_r cca 60 000



Attachement of poly(oxoethylene) chain to EPO by a „monamide“ linker

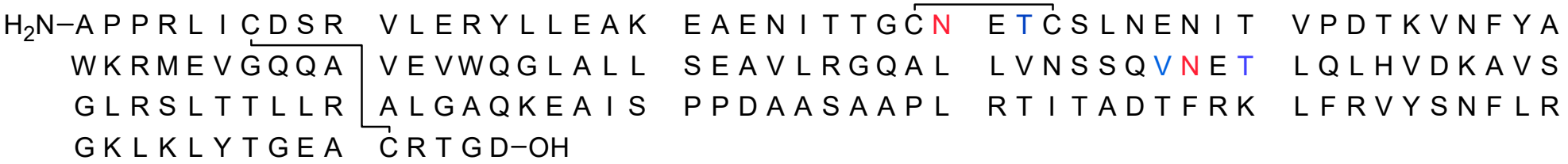
Plasmatic $T_{1/2}$ cca 139 h ⇒ „continuous erythropoietin receptor activator“, CERA
Mircera ® (*s.c.* or *i.v.*) for treatment of anemia in chronic renal disease

Epoetin analogues with altered protein sequence

Darbepoetin alfa

- sequence of EPO alfa changed: Asn30, Thr32, Val87, Asn88 and Thr90 ⇒ 2 new sites of *N*-glycosylation ⇒ 5 sites of *N*-glycosylation in total; 2 new oligosaccharide chains attached
- total M_r 30 000 – 37 000
- recombinant
- indicated for treatment of anemia caused by a chemotherapy of non-myeloid cancers or by chronic renal failure

Aranesp ® (originator); Nespo ® (biosimilar – approved in EU 2001 - 2008)



Primary structure of darbepoetin alfa aglycone. New asparagine residues, to which new cabohydrate chains are attached, are in red, other changed amino acid residues in blue.

Colony stimulating factors (CSFs)

= proteins supporting survival and expansion of pluripotent stem cells and stimulate them to differentiation into various types of leukocytes

GM-CSF: Granulocyte macrophage colony stimulating factor

```
          10         20         30         40         50
MWLQSLLLLG TVACSISAPA RSPSPSTQPW EHVNAIQEAR RLLNLSRDTA
          60         70         80         90        100
AEMNETVEVI SEMFDLQEPT CLQTRLELYK QGLRGSLTKL KGPLTMMASH
         110        120        130        140
YKQHCPPTPE TSCATQIITF ESFKENLKDF LLVIPFDCWE PVQE
```

signaling peptide
GM-CSF

Sites of glycosylation: *O*-: Ser22, Ser24, Ser26, Thr27; *N*-: Asn44, Asn54

https://www.uniprot.org/uniprot/P04141

Colony stimulating factors

APARSPSPST QPWEHVNAIQ EARRLLNLSR

DTAAEMNETV EVISEMFDLQ EPTCLQTRLE

LYKQGLRGSL TKLKGPLTMM ASHYKQHCPP

TPETSCATQI ITFESFKENL KDFLLVIPFD

CWEPVQE

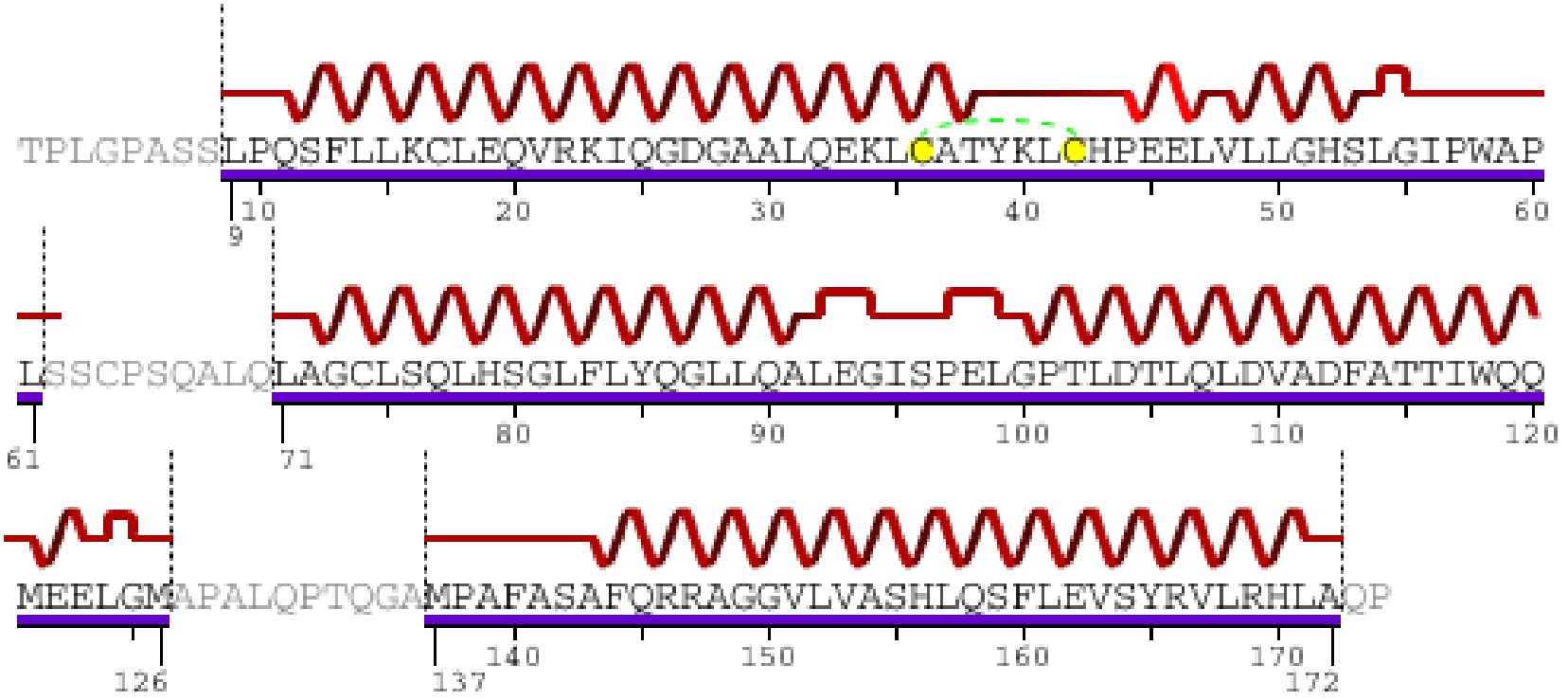
$C_{639}H_{1007}N_{171}O_{196}S_8$ M_r 14 477,49 CAS 99283-10-0

molgramostim
= a factor stimulating granulocytes and macropfages coloies 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

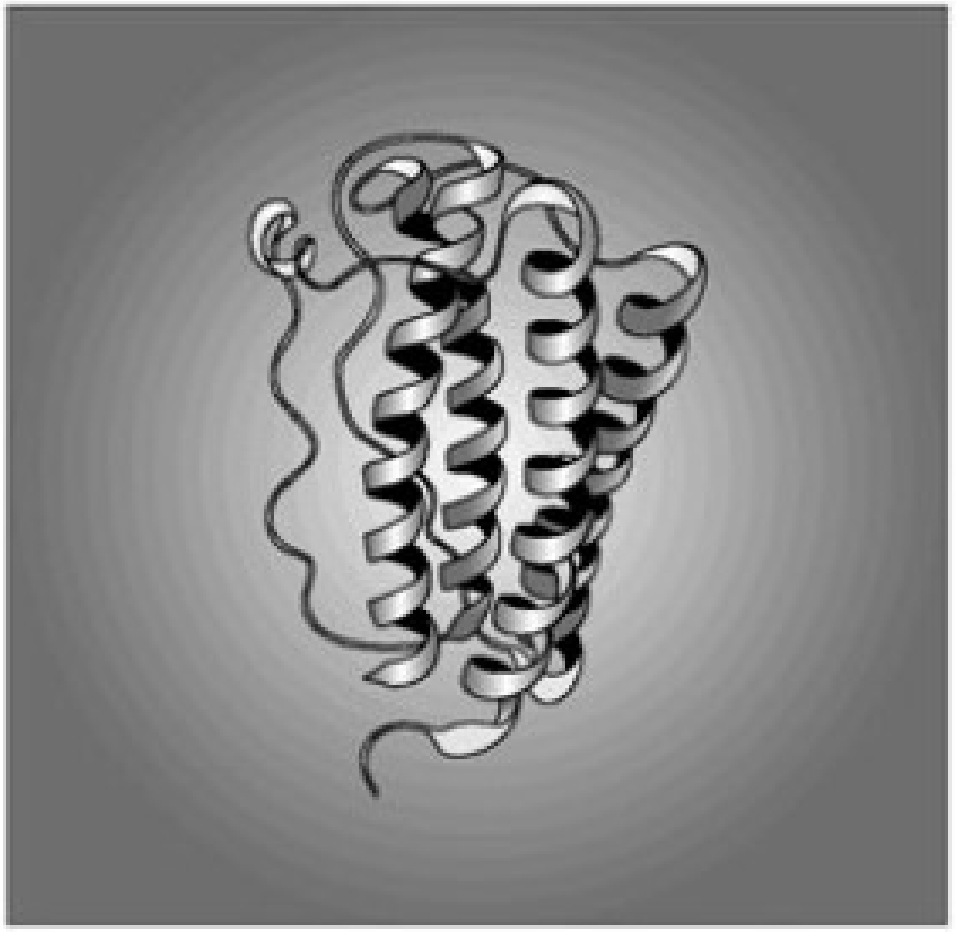
Sequence of filgrastim precursor



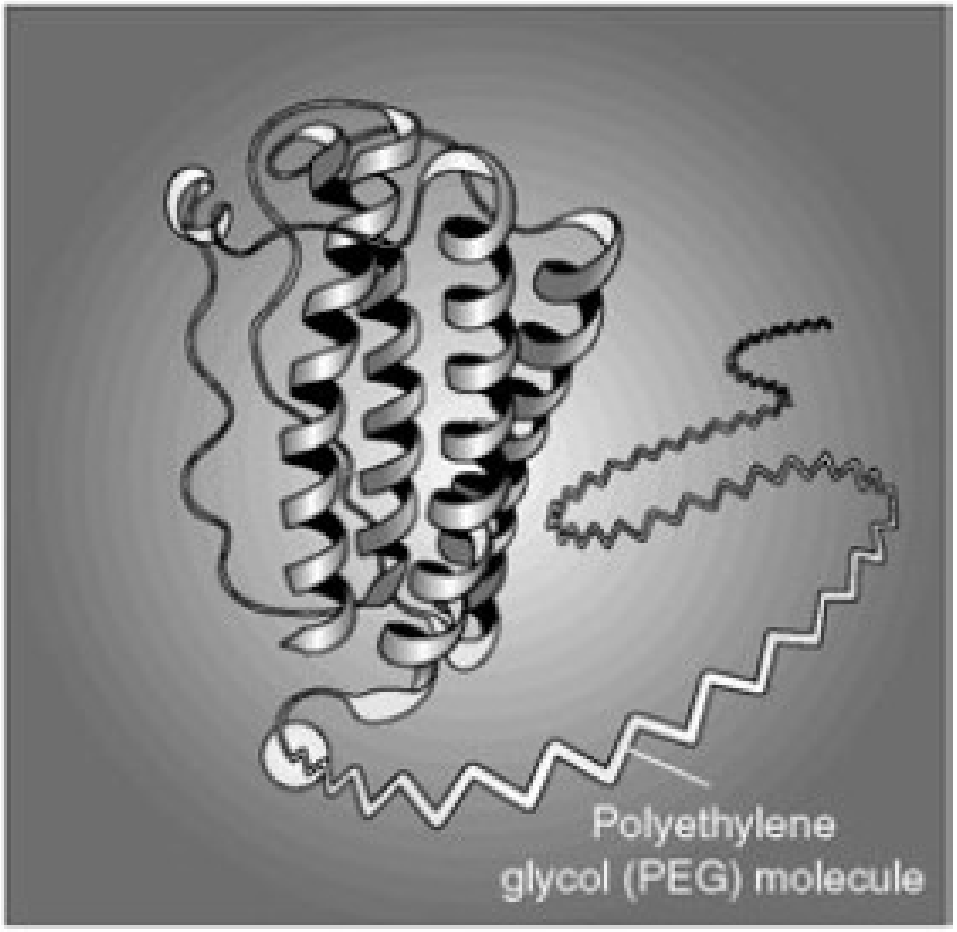
•treatment of neutropenia in cancer chemotheapy and in AIDS

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

Human stem cell factor (SCF)

1 MEGICRNRVT NNVKDVTKLV ANLPKDYMIT LKYVPGMDVL PSHCWISEMV
51 VQLSDSLTDL LDKFSNISEG LSNYSIIDKL VNIVDDLVEC VKENSSKDLK
101 KSFKSPEPRL FTPEEFFRIF NRSIDAFKDF VVASETSDCV VSSTLSPEKD
151 SRVSVTKPFM LPPVAA

- ligand for the receptor-type protein-tyrosine kinase KIT ($\Rightarrow$ synonym KITLG).
- plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration and function, and in melanogenesis
- KITLG/SCF binding can activate several signaling pathways
- 166 AA
- two differentially glycosylated forms, LMW-SCF and HMW-SCF
- peripheral blood progenitor cell mobilization

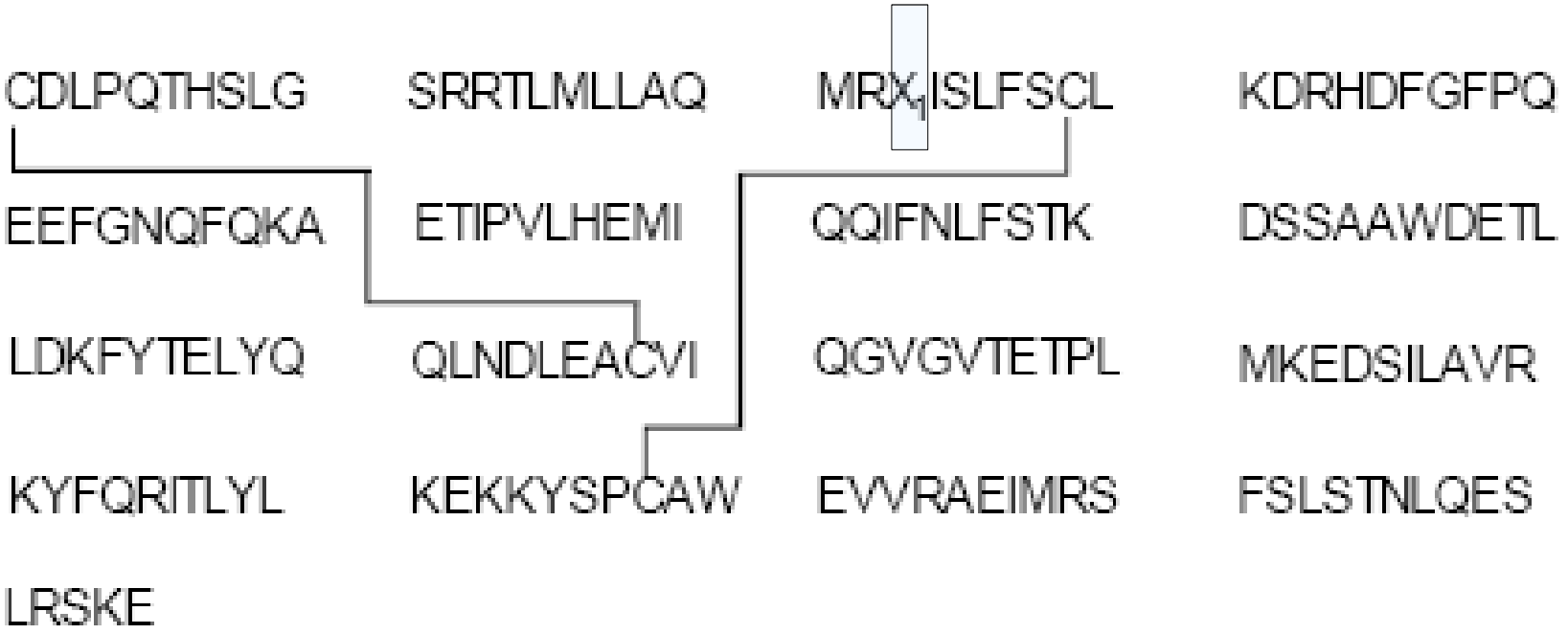
http://www.uniprot.org/uniprot/P21583

A recombinant form of SCF: **ancestim** (Stemgen ®)

- dimer
- non-glycosylated
- indicated for the setting of autologous peripheral blood progenitor cell transplantation in patients at risk of poor peripheral blood progenitor cell mobilisation combined with filgrastim
- temporarily approved e.g. in Canada and New Zealand, currently withdrawn
- replaced in therapy with a small molecule – **plerixafor** Mozobil ®

http://www.medsafe.govt.nz/regulatory/ProductDetail.asp?ID=8093

4. Non-specific antibodies - interferons



interferon α_2

Interferoni alfa-2 solutio concentrata EP

X1 = Lys α_{2a}

X1 = Argα_{2b}

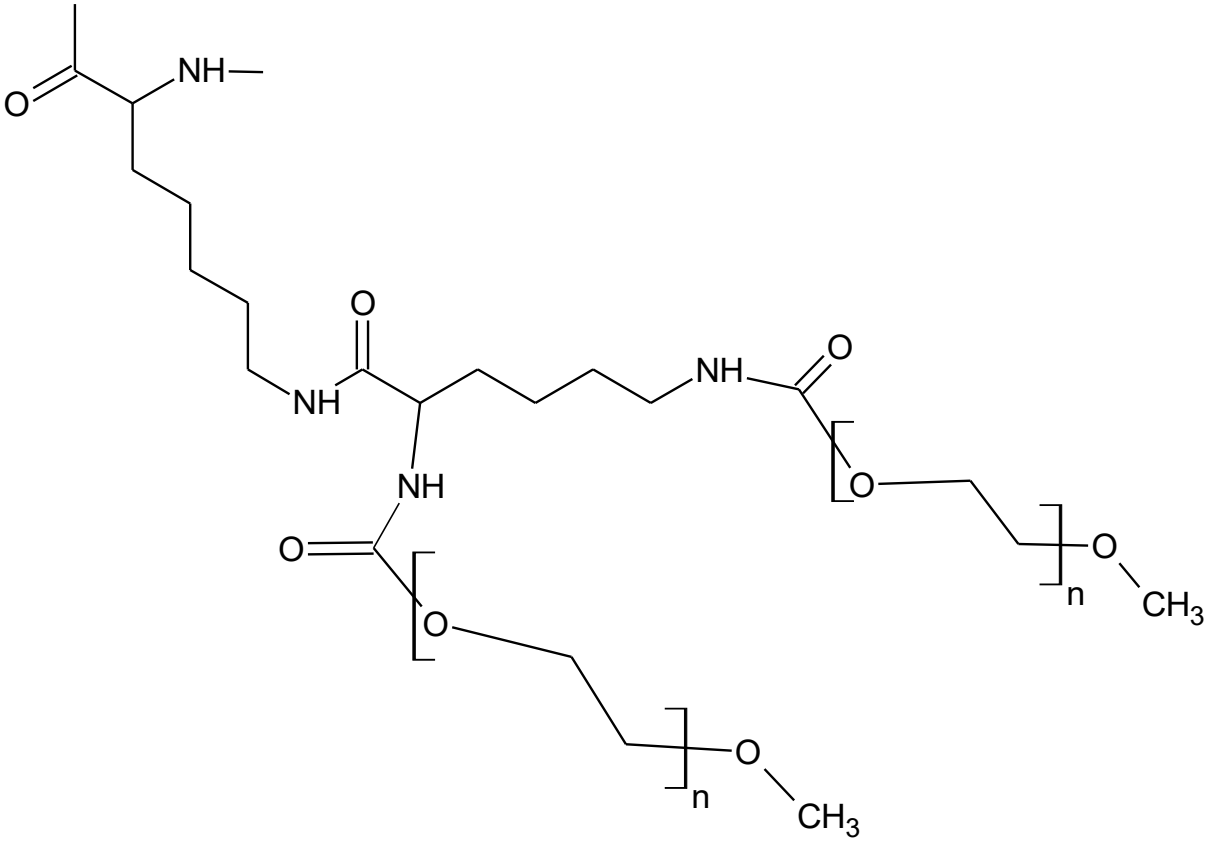
- antiviral activity during viral RNA and protein syntheses
- antiproliferation activity
- produced by a recombinant technology on bacteria

Pegylated interferons α

- **peginterferon α_{2a}** (Pegasys ®) - on some Lys residues attached N^2, N^6-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 His_{34}
  - urethane moiety is labile, free interferon α_{2b} is released into the circulation and directly interacts with receptors
  - treatment of hepatitic C

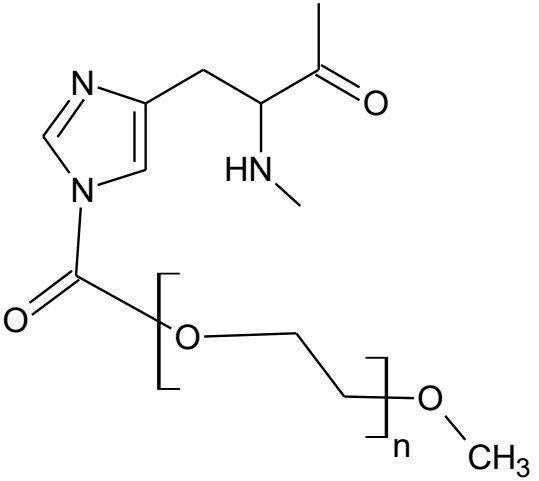
Pegylated interferons α

Differences in their substitutions



Lys

α_{2a}



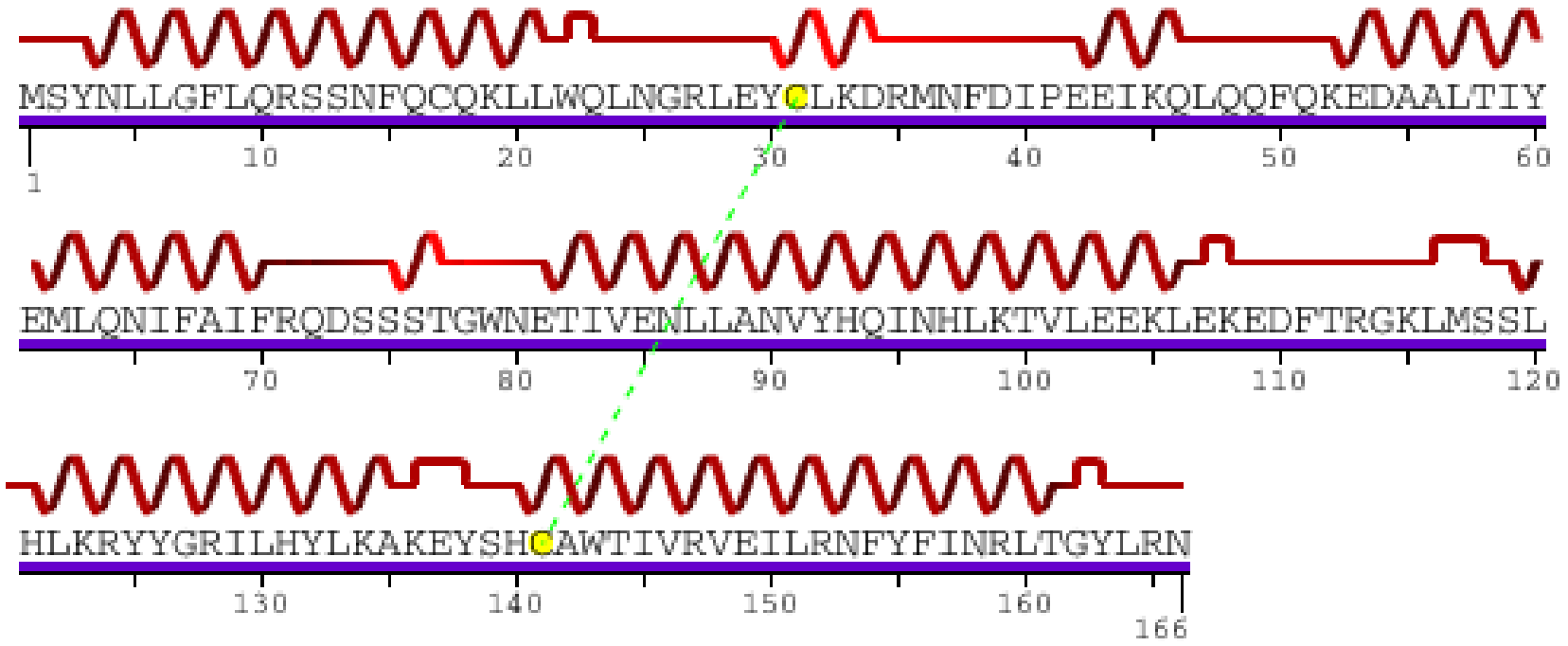
His^{34}

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 painfull than β_{1b}
- β_{1b} (Extavia ®)
  - Cys_{17} 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