

Conditions

1. Two components, two sets of lecturers.
2. Lectures 1-5 Prof. F. Hudecz
Lectures 6-9 Dr. Gy. Domány
Lectures 10-12 Dr. P. Buzder-Lantos
3. Examination: two parts determined by the lecturers and one mark.
 - option A: written test
 - option B: presentation based on literature
 - option C: oral examination
4. Participation at lectures > 70 %

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Some Approved Peptide Pharmaceuticals and their Methods of Manufacture

<u>First generation</u>	<u>Second generation</u>	<u>New generation</u>
Oxytocin (L)	Carbetocin (S)	Abarelix (GnRH) (L)
ACTH (1-24) & (1-39) (L,S)	Terlipressin (L,S)	Cetrorelix (GnRH) (L)
Vasopressin (L,S)	Felypressin (L,S)	Ganirelix (GnRH) (L)
Insulin (E,SS, R)	Buserelin (L,S)	Eptifibatide
Glucagon (E,S,R)	Deslorelin (L,S)	Bivalirudin (L)
Calcitonins (L,S,R)	Goserelin (L)	Copaxone (L)
TRH (L)	Histrelin (L)	Techtide P-289(S)
Gonadorelin (L,S)	Leuprolide (L,S)	Cubicin (F)
Somatostatin (L,S)	Nafarelin (S)	Fuzeon (antiHIV) (H)
GHRH (1-29) & (1-44) (S)	Tryptorelin (L,S)	Ziconotide (pain) (S)
CRF (Human & Ovine) (S)	Lecirelin (S)	Pramlintide (diabetes) (S)
Cyclosporin (F)	Lanreotide (S)	Exenatide (diabetes) (S)
Thymopentin (L)	Octreotide (L,S)	Icatibant (brady-rec)
Thymosin Alpha-1 (S)	Atosiban (L)	Romiplostim (hormon)
Secretins (Human & Porcine) (E,S)	Desmopressin (L,S)	Degarelix (GnRH)
Parathyroid Hormone (1-34) & (1-84)(S)	Lypressin (L)	Mifamurtide (rák, adj.)
Vasoactive Intestinal Polypeptide (S)	Ornipressin	Ecallantide (ödéma)
Brain Natriuretic Peptide (R)	Pitressin (L)	Liraglutide (diabetes)
Cholecystokinin (L)	ACE Inhibitors (Enalapril, Lisinopril) (L)	Tesamorelin
Tetragastrin (L)	HIV Protease Inhibitors (L)	Surfaxin
Pentagastrin (L)		Peginesatide
Eledoisin (L)		Carfilzomib
		Linaclotide (enz.inh)

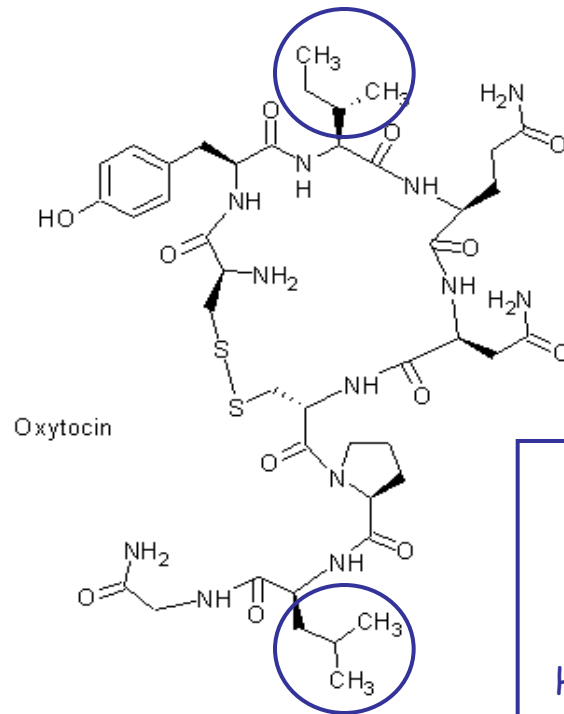
L = in solution; S = on solid phase; E = extraction; F = fermentation; H = hybrid synthesis;
R = recombinant; SS = semi-synthesis.

First generation: the first ones

Hormones: hypophysis



V. du Vigneaud
(Nobel prize 1955)



Oxytocin

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH₂

Oxytocin

Structure: 1953 V. du Vigneaud
Synthesis: 1954 V. du Vigneaud

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly-NH₂

Vasopressin

ACTH

(Adrenocorticotrophic hormone,
corticotropin) (1-39, 1-24)

H-Ser¹-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-
-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-
-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Leu-Ala-
-Glu-Ala-Phe-Pro-Leu-Glu-Phe³⁹-OH

Synthesis (1971):

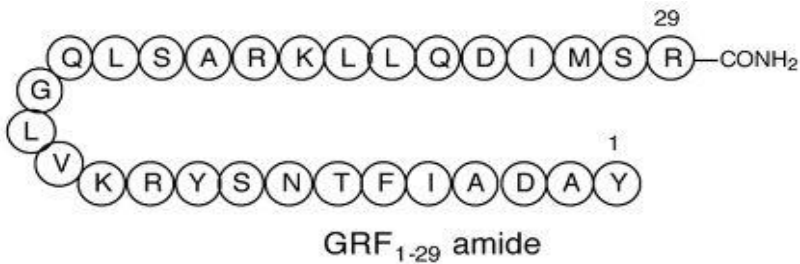
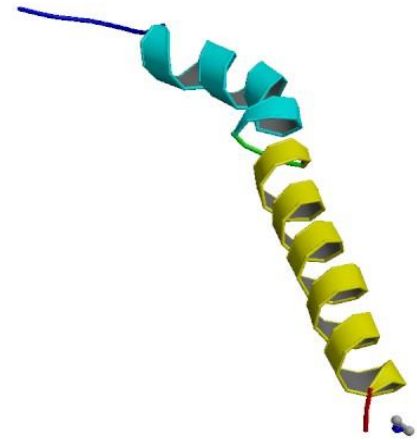
S. Bajusz, L. Kisfaludy, K. Medzihradszky

The first generation

Hormones: hypothalamus

CRH (Corticotropin-releasing hormone, corticotropin-releasing factor, CRF, corticoliberin)

H-Ser¹-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-
-Leu-Arg-Glu-Val-Leu-Glu-Met-Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-
-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala⁴¹

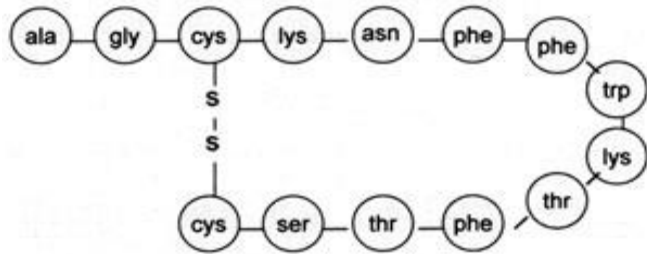


Sermorelin (GHRH 1-29, GRF 1-29)

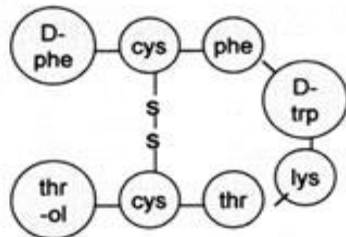
GHRH (growth hormone releasing hormone, 1-44)

HO-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys- Val-
Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile- -Met-
Ser-Arg²⁹-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly- -Ala-Arg-
Ala-Arg-Leu-NH₂

GhRH, gonadotropin-releasing hormone
Gonadorelin, Luteinizing-hormone-releasing hormone LHRH)

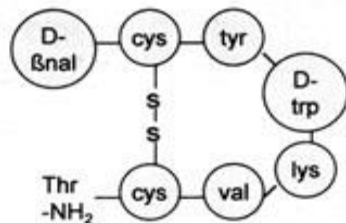


Human somatostatin



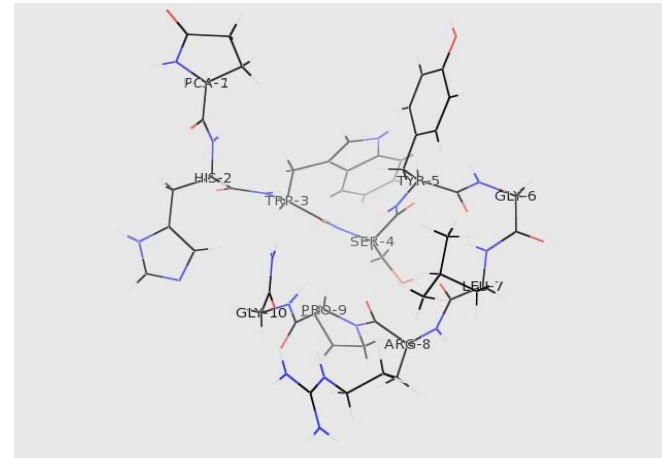
Octreotide acetate

Sandostatin (Peptidomimetic)



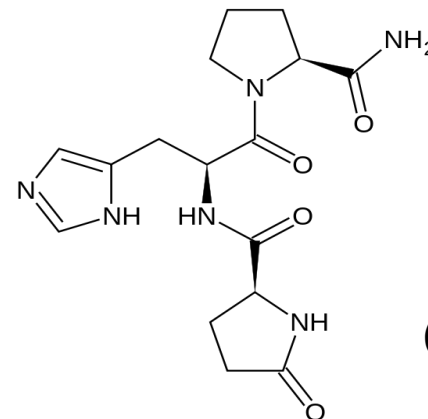
lanreotide

A **long-acting** derivative of somatostatin.



(pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂

TRH, thyrotropin-releasing hormone
(thyrotropin-releasing factor, TRF)

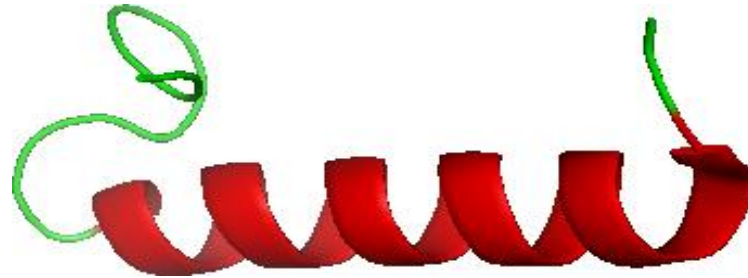


(pyro)Glu-His-Pro-NH₂

The first generation

Hormones

Calcitonin (32 amino acids) linear polypeptide produced in humans primarily by the parafollicular cells (also known as C-cells) of the thyroid, Calcitonin can be used therapeutically for the treatment of hypercalcemia or osteoporosis. Its structure comprises a single alpha helix



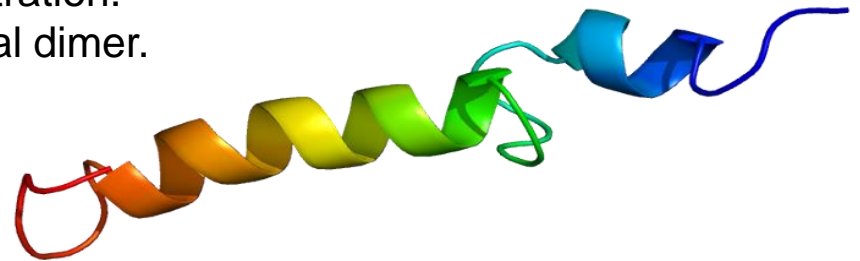
salmon: Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro

human: Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro.

Parathyroid hormone (PTH) is secreted by the chief cells of the parathyroid glands as a polypeptide with 84 amino acids. It acts to increase the concentration of Ca_2^+ in the blood, whereas calcitonin acts to decrease calcium concentration.

hPTH-(1-34) crystallizes as a slightly bent, long helical dimer.

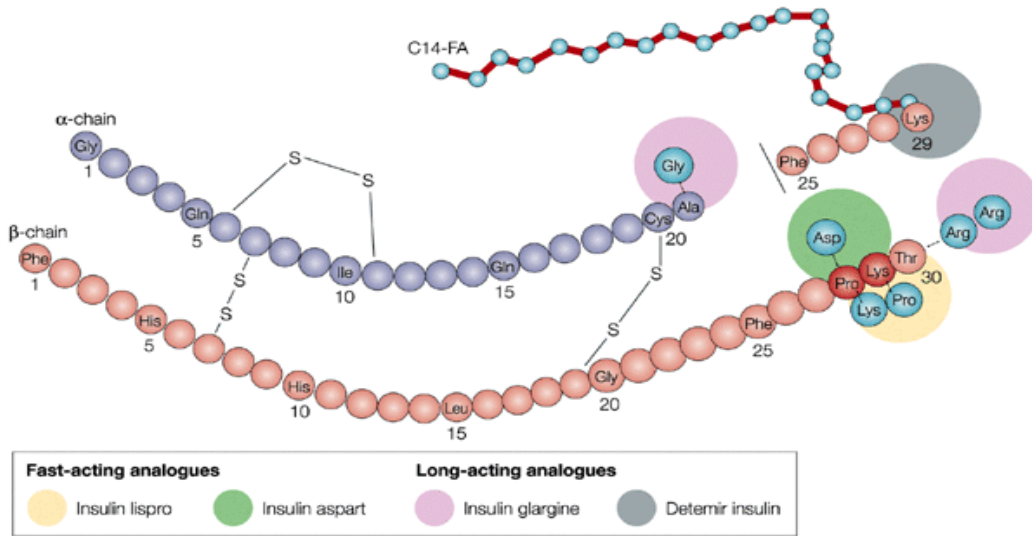
The extended helical conformation of hPTH-(1-34) is the likely bioactive conformation.



The first generation

Hormones: pancreas

Insulin



Nature Reviews | Drug Discovery

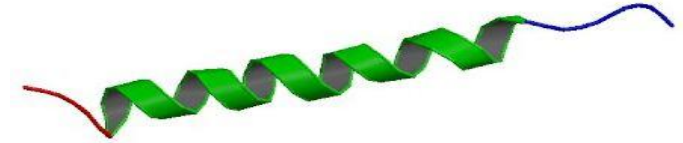
Isolation: 1922 , F. Banting (N.d. 1923, 32-year old)

Structure: 1953 , F. Sanger

Synthesis: 1969, H. Zahn, P.G. Katsoyannis

Conformation: 1965, D. Hodgkin

Glucagon (29 amino acids)



H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-
-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-
-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH

Secreted by the pancreas, raises blood glucose levels.
Its effect is opposite that of insulin, which lowers
blood glucose levels .

Vasoactive intestinal peptide (VIP) (28 amino acids)

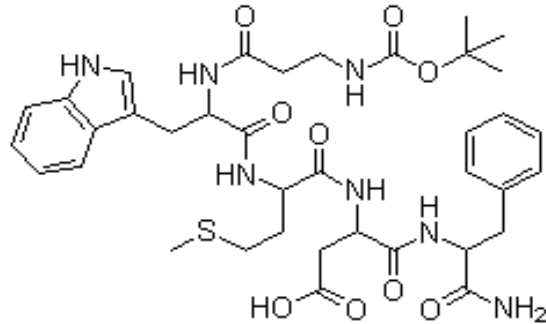


Produced by tissues of vertebrates (gut, pancreas, suprachiasmatic nuclei of the hypothalamus in the brain). The highest levels are normally found in the nervous system and gut. It is a neuromodulator/neurotransmitter. Regulates muscle activity, epithelial cell secretion, and blood flow in the gastrointestinal tract

The first generation

Hormones: digestion

Gastrin is a linear peptide stimulating secretion of gastric acid (HCl) by the parietal cells of the stomach. It is released by G cells in the antrum of the stomach, duodenum, and the pancreas into the bloodstream. Gastrin is found primarily in three forms: 1-34 (big gastrin), 1-17 (little), 1-14 (mini)



N-t-Boc-β-Ala-Trp-Met-Asp-Phe-NH₂

Pentagastrin (Peptavlon)

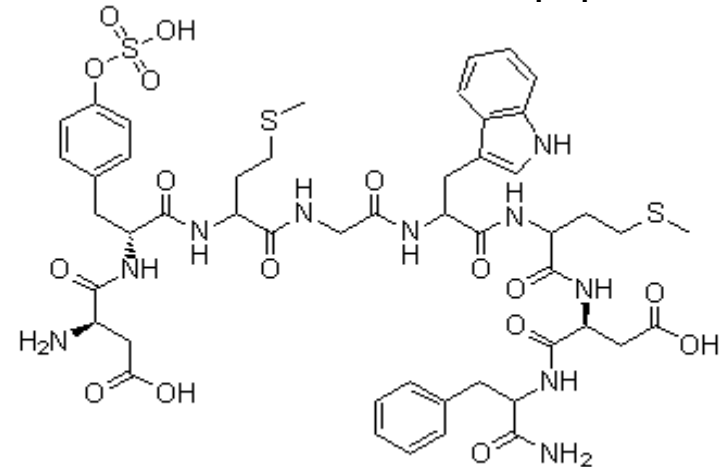
5 amino acids of the C-terminus end of gastrin



Secretin (27 amino acids)

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-
-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-
-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-CONH₂

Cholecystokinin (CCK-8) C-terminal octapeptide



Asp-Tyr(SO₃H)-Met-Gly-Trp-Met-Asp-Phe-NH₂

From Greek *chole*, "bile"; *cysto*, "sac"; *kinin*, "move"; hence, *move the bile-sac (gallbladder)*. Responsible for stimulating the digestion of fat and protein.

CCK is composed of varying numbers of amino acids depending on post-translational modification of the CCK gene product: e.g., CCK58, CCK33, and CCK8. CCK58.

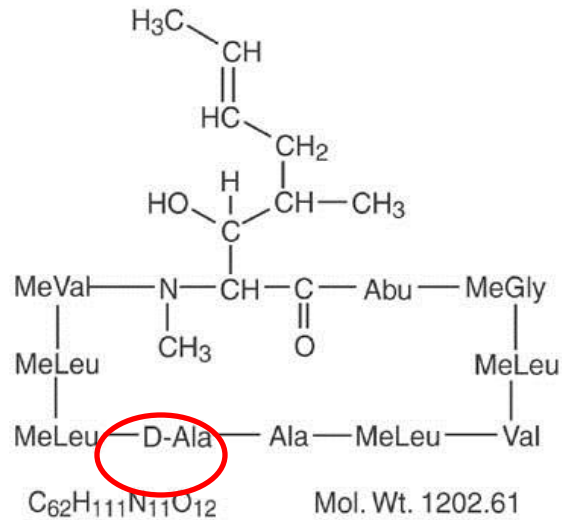
Kinevac (Sincalide for Injection)

The first generation

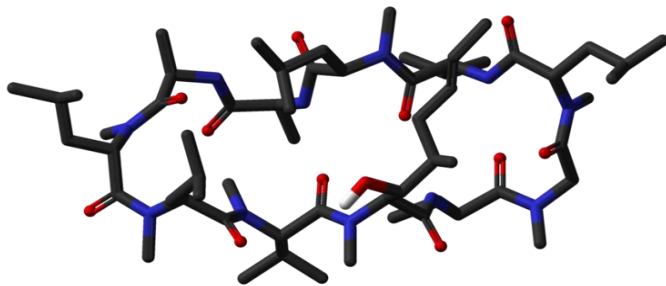
Peptides acting on the immune system

Cyclosporin (cyclosporin A)

immunosuppressant, fungi, natural product
11 amino acids, cyclic, D-amino acids

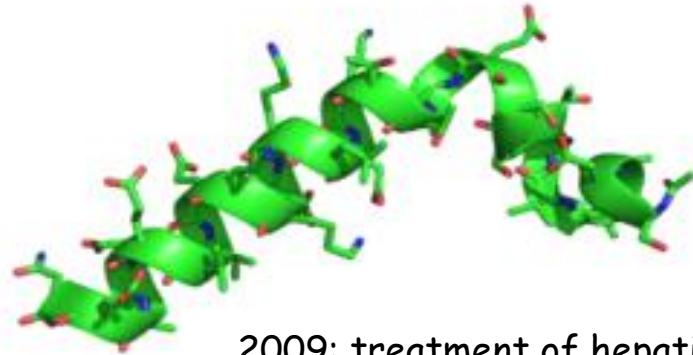


Isolation: 1971 from *Tolypocladium inflatum*
Medical use: 1983



Thymosin $\alpha 1$

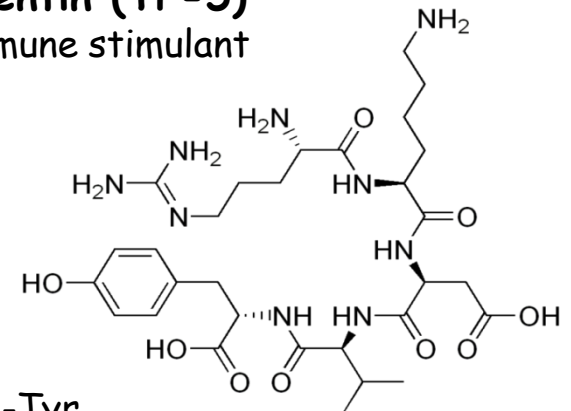
T-cell immune stimulant,
28 amino acids fragment



2009: treatment of hepatitis B/C

Thymopentin (TP-5)

T-cell immune stimulant



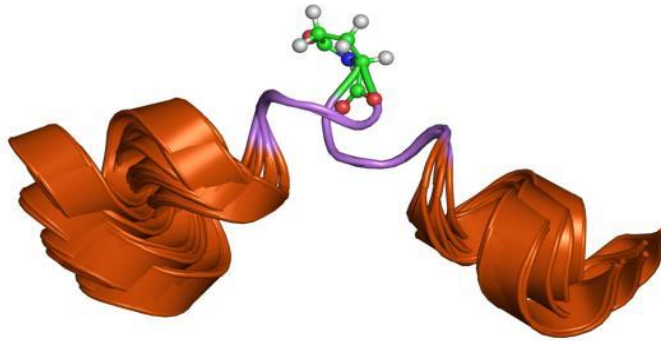
Arg-Lys-Asp-Val-Tyr

The first generation

Antihypertensive drugs

Eledoisin

11 amino acids, octopus (*Eledone*) origin

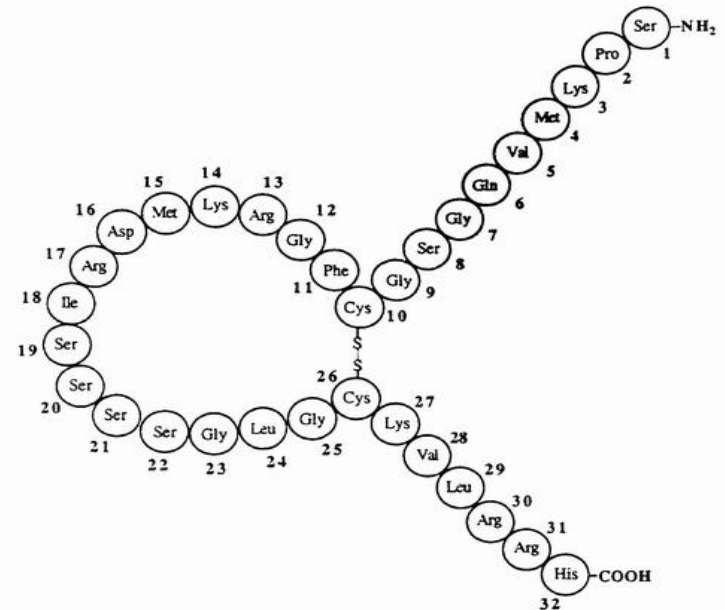


Belonging to the tachykinin family of neuropeptides. it has vasodilator, hypotensive, and extravascular smooth muscle stimulant properties, The amino acid sequence:

pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH₂.

Brain natriuretic peptide (BNP)

32 amino acids, cyclic



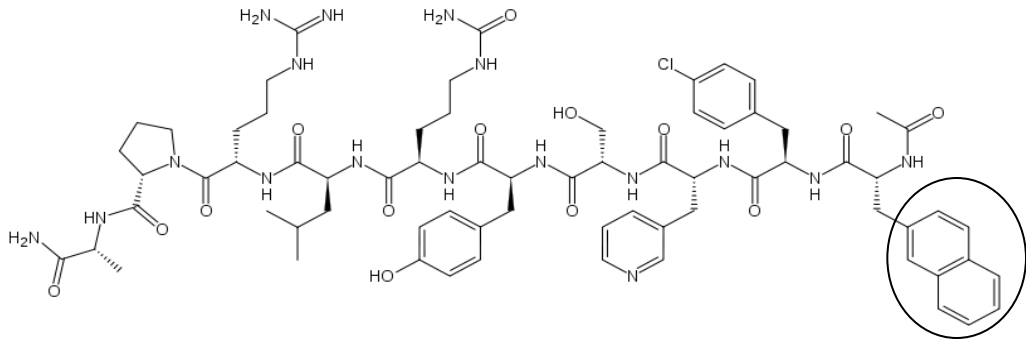
Secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells. The physiologic actions include decrease in systemic vascular resistance and central venous pressure as well as an increase in natriuresis.

The net effect: a decrease in blood volume, which lowers systemic blood pressure .

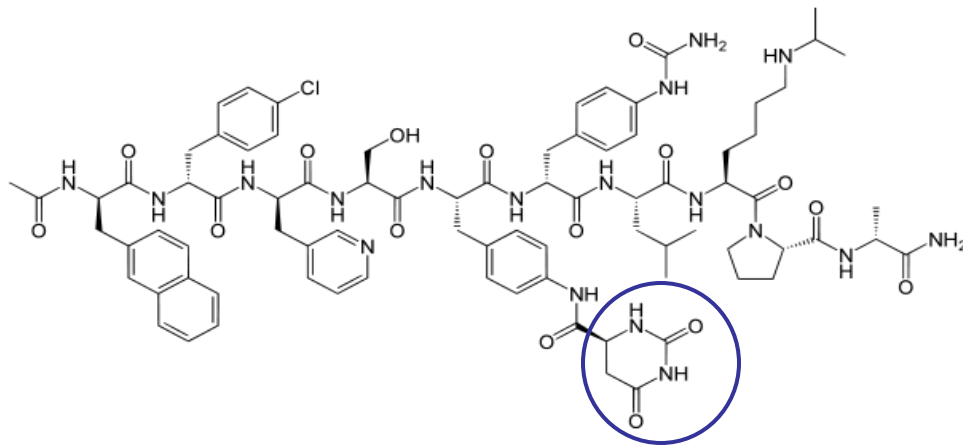
New generation

New generation

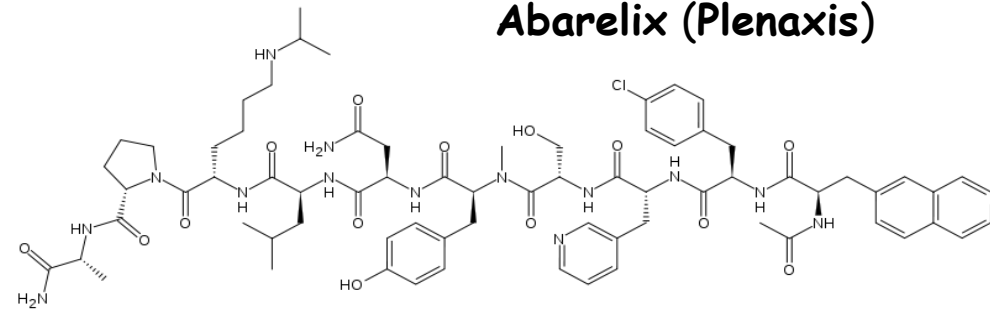
Hormones: GnRH antagonists - peptidomimetics



Cetrorelix
(synthetic)

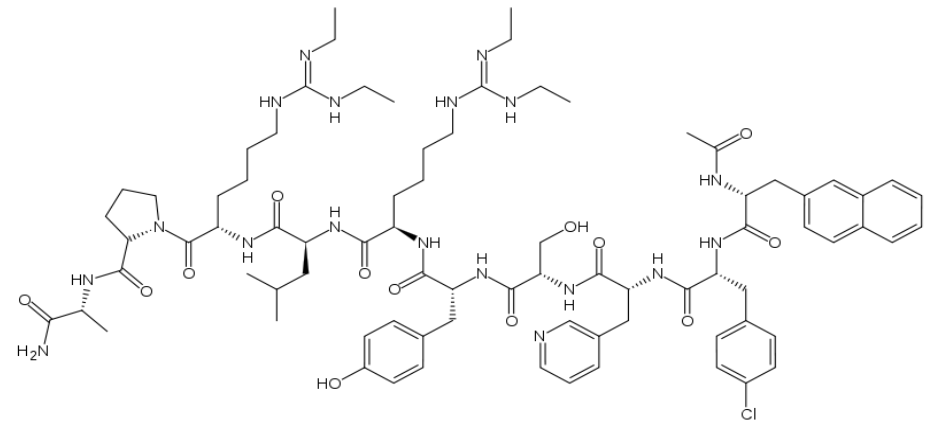


Degarelix



Abarelix (Plenaxis)

In oncology to reduce the amount of testosterone in patients with advanced symptomatic prostate cancer.



Ganirelix

In assisted reproduction to control ovulation

New generation

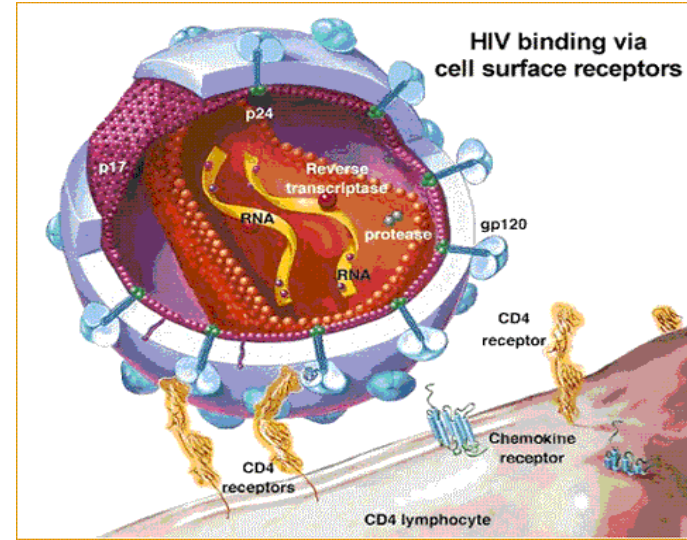
Antibacterial drugs: inhibitors of interactions

Enfuvirtide

36 amino acids,
HIV fusion inhibitor,
antiviral
(binding to gp41 protein)

Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-
-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu--
Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-
-Trp-Phe-NH₂

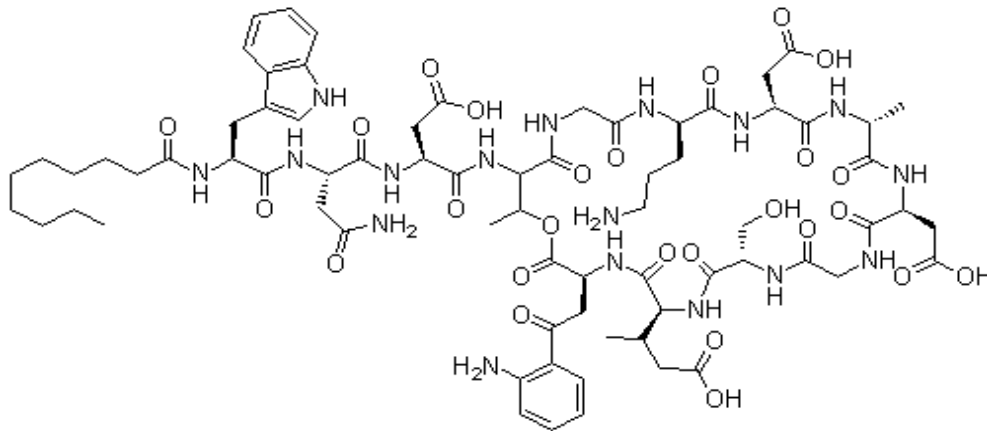
<http://www.usermeds.com>



Daptomycin (Cubicin)

Lipopeptide antibiotics (Gram positive)

13 amino acids,
D-amino acid, non-natural amino acid



N-decanoyl-L-Trp-L-Asn-L-Asp-L-Thr-Gly-L-Orn-L-Asp-D-Ala-L-Asp-Gly-D-Ser-*threo* -3-methyl-L-Glu-3-anthraniloyl-L-Ala[egr]1-lactone

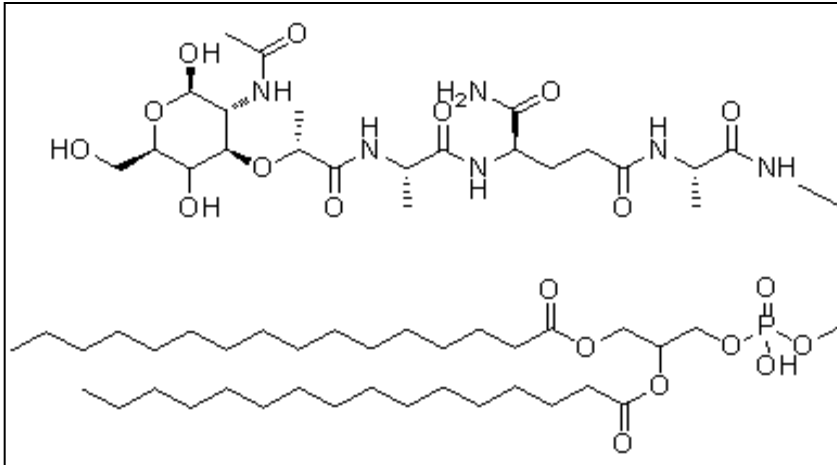
New generation

Glatiramer acetate (Copolymer 1, Copaxone)

immunomodulator, multiple sclerosis

random copolymer polymer

$\text{poly}(\text{Glu}_{1,4-1,8}-\text{Ala}_{4,1-5,8}-\text{Lys}_{3,2-4,2}-\text{Tyr}_{1,0})$



Mifamurtide (Mepact)

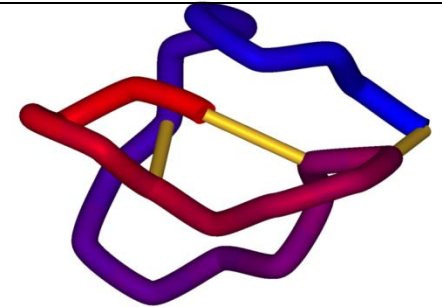
muramyl tripeptide
phosphatidylethanolamine
osteosarcoma

<http://www.medkoo.com/Anticancer-trials/Mifamurtide.htm>



Ziconotide

ω -conotoxin peptide, „Conus magus“
Ca channel blocking
(non-opioid, non-NSAID), pain killer



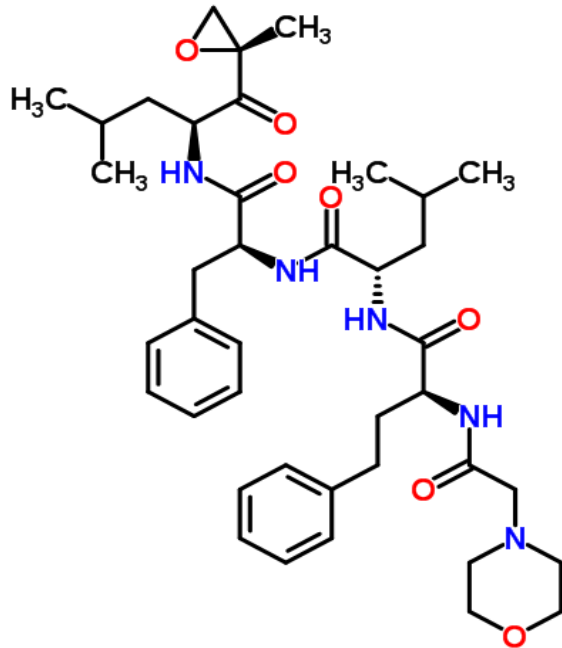
H-Cys-Lys-Gly-Lys-Gly-Ala-Lys-Cys-Ser-Arg-Leu-Met-Tyr-Asp-Cys-Cys-Thr-Gly-Ser-Cys-Arg-Ser-Gly-Lys-Cys-NH₂

New generation

Enzyme inhibitors

Carfilzomib

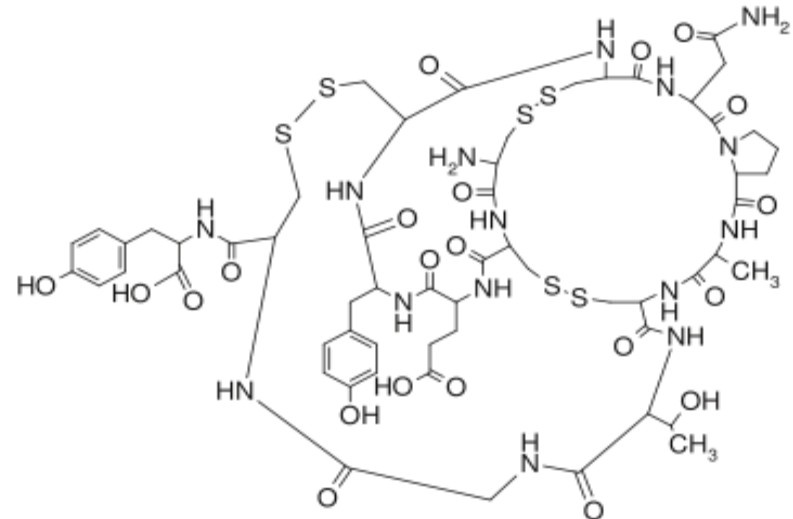
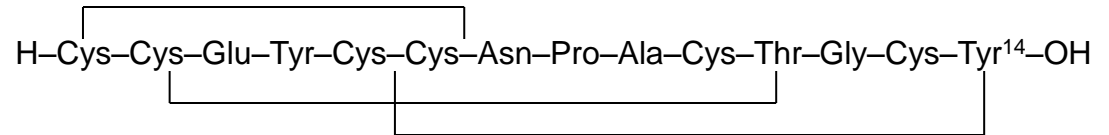
tetrapeptide, multiple myeloma
proteasome inhibitor, chymotripsine-like enzyme
FDA: 20 July, 2012



N-((2S)-2-[(4-Morpholinylacetyl)amino]-4-phenylbutanoyl)-
L-Leu-N-((2S)-4-methyl-1-[(2R)-2-methyl-2-oxiranyl]-1-oxo-
2-pentanyl)-L-Phe-amide

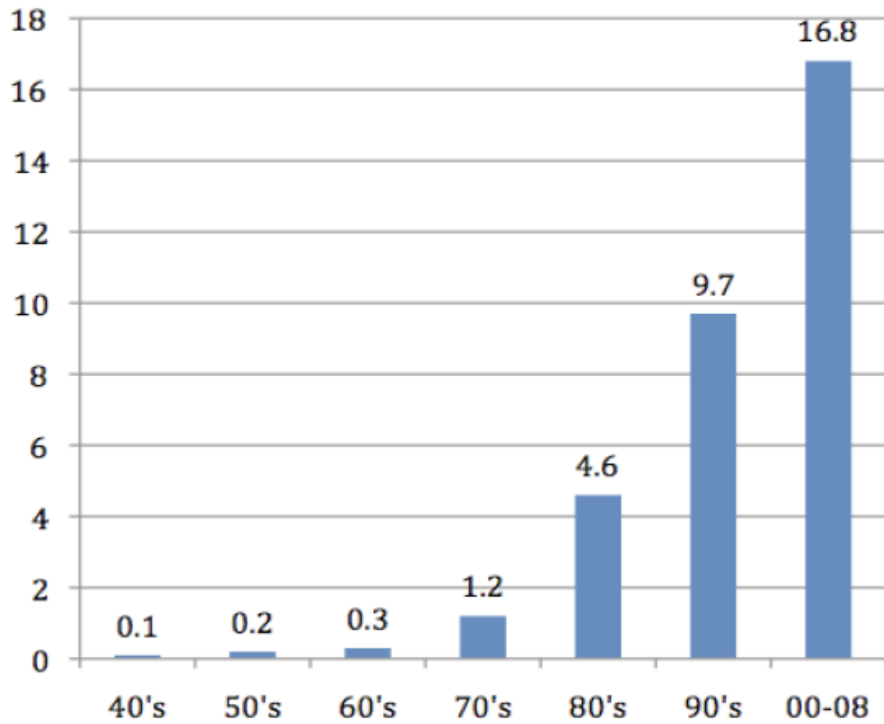
Linacotide (Linzess)

14 amino acids, 3 cycles
guanylate cyclase 2C inhibitor
irritable bowel syndrome
FDA: 30 August, 2012



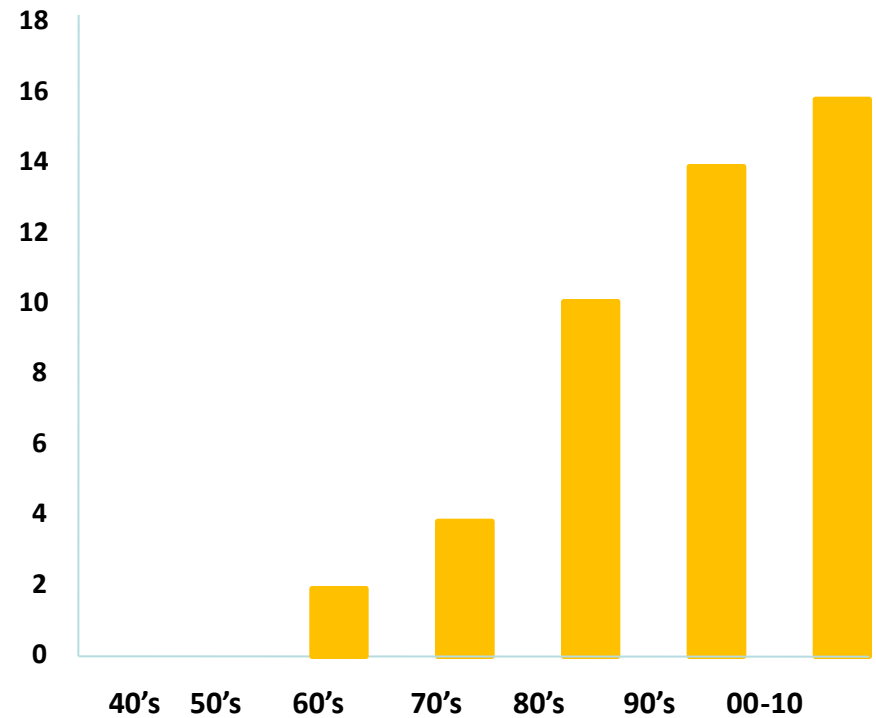
Peptide *

Year of clinical studies (1940 -)



*Peptide Therapeutics Foundations 2010

Number of peptide drugs on the market (1970 -) **



**appr. 50 approved peptide (API) until 2002

SOME EXAMPLES OF PEPTIDES IN LATE STAGE CLINICAL DEVELOPMENT

<u>Product</u>	<u>Indication</u>	<u>Length</u>	<u>Status</u>
Degludec	Type 1 and type 2 diabetes	--	NDA Pending
Teduglutide	Short bowel syndrome (GLP-2 analog)	33	NDA Pending
Lixisenatide (ZP10) (GLP-1 Agonist)	Type 2 diabetes	44	Phase III
Stimuvax (BLP-25 lipopeptide)	Non-small cell lung carcinoma (therapeutic vaccine)	25	Phase III
MX-226 (Omigantan)	Topical antimicrobial for catheter-related infections	12	Phase III
Pasireotide	Cushing's disease	6	Phase III
Albiglutide	Type 2 diabetes	--	Phase III
E75	Breast cancer (therapeutic vaccine)	--	Phase II/III
Pexiganan	Diabetic foot infections	22	Phase II/III
Cilengitide	Glioblastoma	5	Phase III
KAI-4169	Secondary hyperparathyroidism	--	Phase II
TRV120027	Acute heart failure	--	Phase II
MIM-D3	Dry eye	--	Phase II

RECENTLY APPROVED PEPTIDES

API	Date	Manufacturing Method	Indication
Teriparatide	11/26/02	Recombinant	Osteoporosis [PTH (1-34)]
Fuzeon (T20)	3/13/03	Hybrid synthesis	AIDS
Cubicin	9/12/03	Fermentation	Bacterial skin infections
Abarelix	11/25/03	Solution-phase synthesis	Prostate cancer
Human Secretin	4/9/04	Solid-phase synthesis	Diagnostic for pancreatic function
Prialt (Ziconotide)	12/29/04	Solid-phase synthesis	Chronic pain
Symlin (Pramlintide)	3/16/05	Solid-phase synthesis	Types 1 and 2 diabetes
Byetta (Exenatide)	4/29/05	Solid-phase synthesis	Type 2 diabetes
Preos/Preotact	4/26/06	Recombinant	Osteoporosis [PTH (1-84)]
Romiplostim	8/22/08	Recombinant	Chronic idiopathic thrombocytopenia
Degarelix	12/24/08	Solution-phase synthesis	Prostate cancer
Mefamurtide	3/6/09	Solution-phase synthesis	Osteosarcoma
Ecallantide	11/27/09	Recombinant	Hereditary angioedema
Liraglutide	2/25/10	Recombinant	Type 2 diabetes
Tesamorelin	11/12/10	Solid-phase synthesis	HIV lipodystrophy
Surfaxin	3/16/12	Solid-phase synthesis	Respiratory distress syndrome
Peginesatide	3/27/12	Solid-phase synthesis	Anemia
Carfilzomib	7/20/12	Solution-phase synthesis	Multiple myeloma
Linaclotide	8/30/12	Solid-phase synthesis	Irritable bowel syndrome

Main fields of applications

AIDS

Allergies

Analgesia

Arthritis

Birth Control

Cardiovascular Diseases

CNS Disorders

Cystic Fibrosis

Diabetes

Epilepsy

Gastrointestinal Disorders

Growth Deficiencies

Gynecological Disorders

Hypertension

IBD/IBS

Immune Deficiencies

Infections (anti-viral, anti-microbial)

Inflammation

Lung Surfactant

Obesity

Oncology

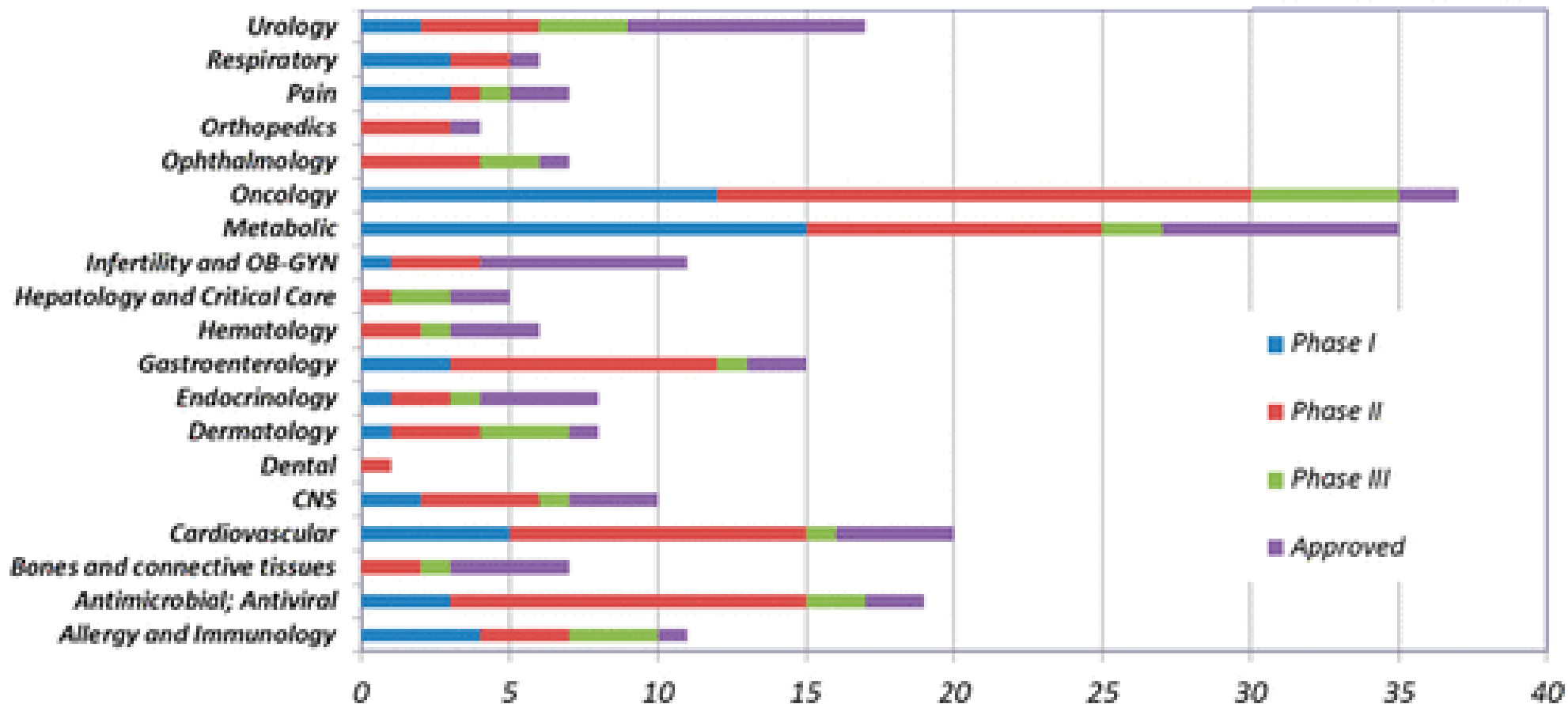
Ophthalmology

Osteoporosis

Urology

Vaccines

Number of Peptides Approved or in Active Development



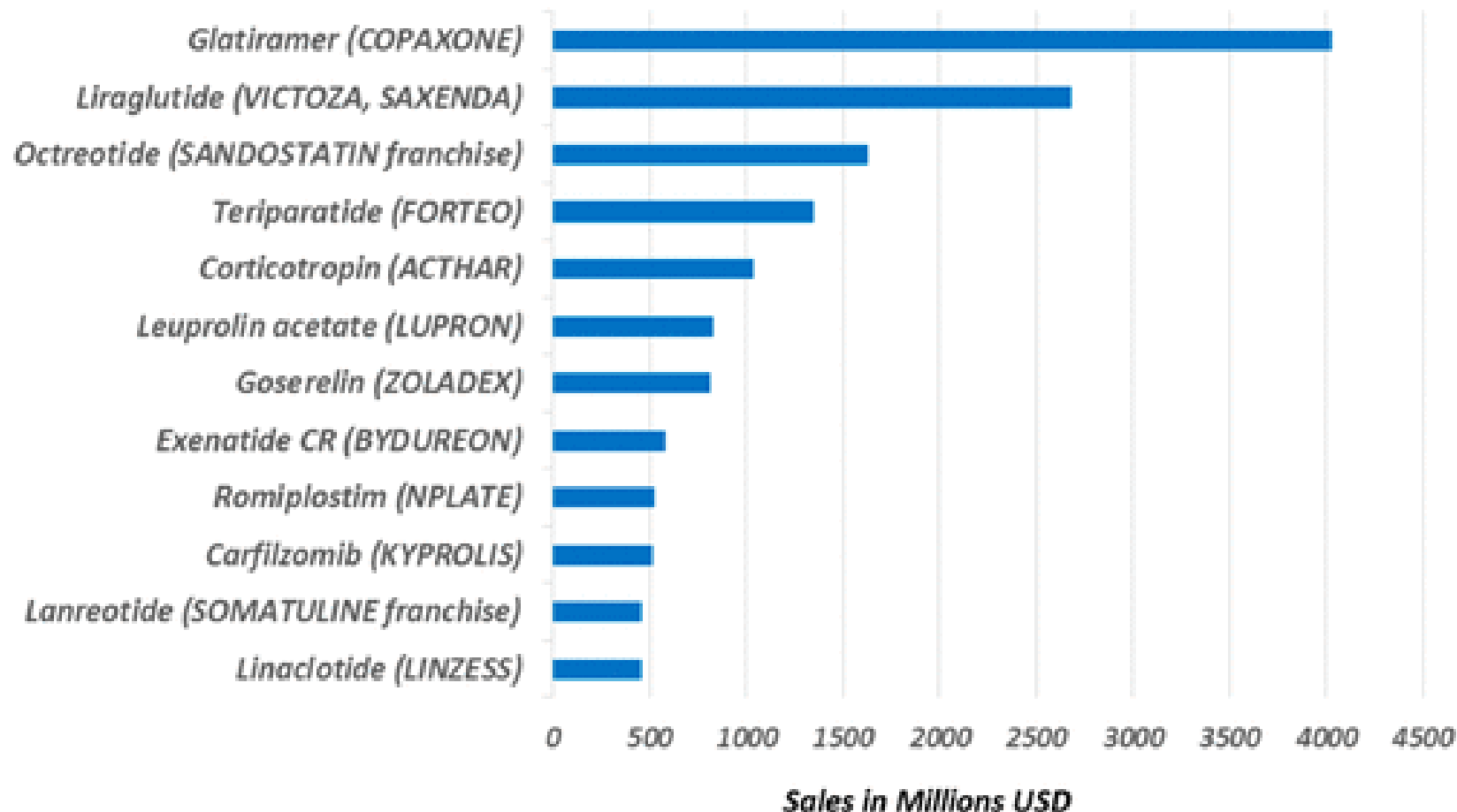
[Henninot A](#), [Collins JC](#), [Nuss JM](#). The Current State of Peptide Drug Discovery: Back to the Future? *J Med Chem.* 2018; 61:1382-1414.

Non-insulin Peptides Approved in the Years 2000-2016, Including Region of Launch

generic name	year of approval	therapeutic area	country of approval	generic name	year of approval	therapeutic area	country of approval
atosiban	2000	obstetrics	EU	mifamurtide	2009	oncology	EU
taltirelin	2000	CNS	JP	liraglutide	2009	metabolic disease	EU
aviptadil	2000	urology	EU	tesamorelin	2010	antiinfective	US
carbetocin	2001	obstetrics	EU	lucinactant	2012	pulmonary	US
nesiritide	2001	cardiovascular	US	peginesatide	2012	hematology	US
teriparatide	2002	osteoporosis	US	pasireotide	2012	endocrinology	EU
enfuvirtide	2003	antiinfective	US	carfilzomib	2012	oncology	US
abarelix	2003	oncology	US	linaclotide	2012	gastroenterology	US
ziconotide	2004	pain	US	teduglutide	2012	gastroenterology	EU
pramlintide	2005	metabolic disease	US	lixisenatide	2013	metabolic disease	EU
exenatide	2005	metabolic disease	US	albiglutide	2014	metabolic disease	EU
icatibant	2008	hematology	EU	oritavancin	2014	antiinfective	US
romiplostim	2008	hematology	US	dulaglutide	2014	metabolic disease	US
degarelix	2008	oncology	US	afamelanotide	2014	dermatology	

[Henninot A](#), [Collins JC](#), [Nuss JM](#). The Current State of Peptide Drug Discovery: Back to the Future? [J Med Chem.](#) 2018; 61:1382-1414.

Top Selling Non-Insulin Peptide Sales 2015



[Henninot A](#), [Collins JC](#), [Nuss JM](#). The Current State of Peptide Drug Discovery: Back to the Future? [J Med Chem](#). 2018; 61:1382-1414.

Targets

```
graph TD; A[Targets] --> B[Extracellular]; A --> C[Intracellular];
```

Extracellular

- receptors
(e.g. *G*-protein-coupled receptors)
- enzymes
- protein-protein interactions

Intracellular

- (10% of the compounds under development)
- Cell penetration
 - Reductive cytosol (Cys-Cys)

Sources



Isolation

Peptide antibiotics
Protein fragments
Peptide conjugates
Bispecific peptides
Disulfide-rich peptides" (DRP)
8-40 aminosav

Synthesis

Advantages - Disadvantages

Specificity
Potency
Low toxicity

Low stability
Short half-life
Enzymatic decomposition
Lack of oral application

Suggested readings

[Henninot A](#), [Collins JC](#), [Nuss JM](#).

The Current State of Peptide Drug Discovery: Back to the Future?

[J Med Chem](#). 2018; 61:1382-1414. doi: 10.1021/acs.jmedchem.7b00318.

[Eder J](#), [Herrling PL](#). Trends in Modern Drug Discovery.

[Handb Exp Pharmacol](#). 2016;232:3-22. doi: 10.1007/164_2015_20.

[Gaspar R](#), [Aksu B](#), [Cuine A](#), [Danhof M](#), [Takac MJ](#), [Linden HH](#), [Link A](#),
[Muchitsch EM](#), [Wilson CG](#), [Ohrngren P](#), [Dencker L](#).

Towards a European strategy for medicines research (2014-2020):
The EUFEPS position paper on Horizon 2020.

[Eur J Pharm Sci](#). 2012; 47:979-87. doi: 10.1016/j.ejps.2012.09.020.

[Parthasarathy A](#), [Anandamma SK](#), [Kalesh KA](#).

The Medicinal Chemistry of Therapeutic Peptides:

Recent Developments in Synthesis and Design Optimizations.

[Curr Med Chem](#). 2017; doi: 10.2174/0929867324666171012103559.

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