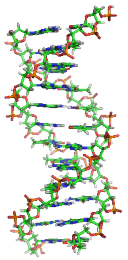
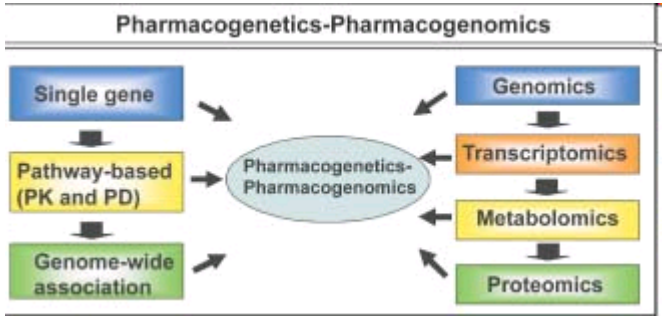
Pharmacogenetics

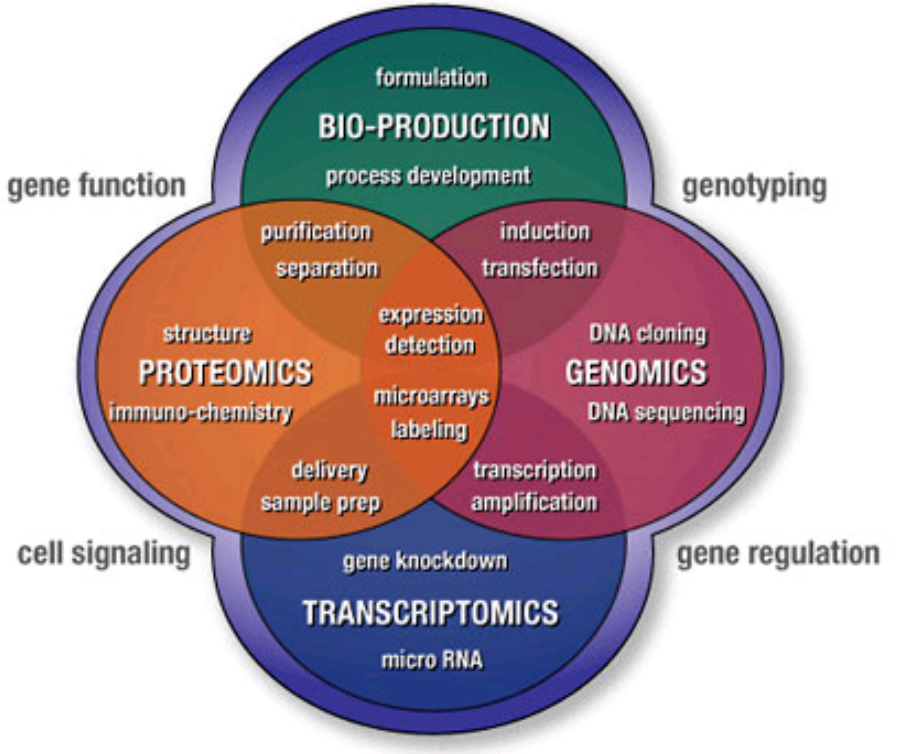


Pharmaceutical biotechnologies:

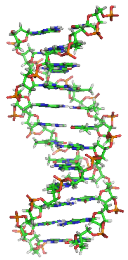
- Gene identification
- Target identification
- Personalized medicine development:

-omics: correlation with chemistry



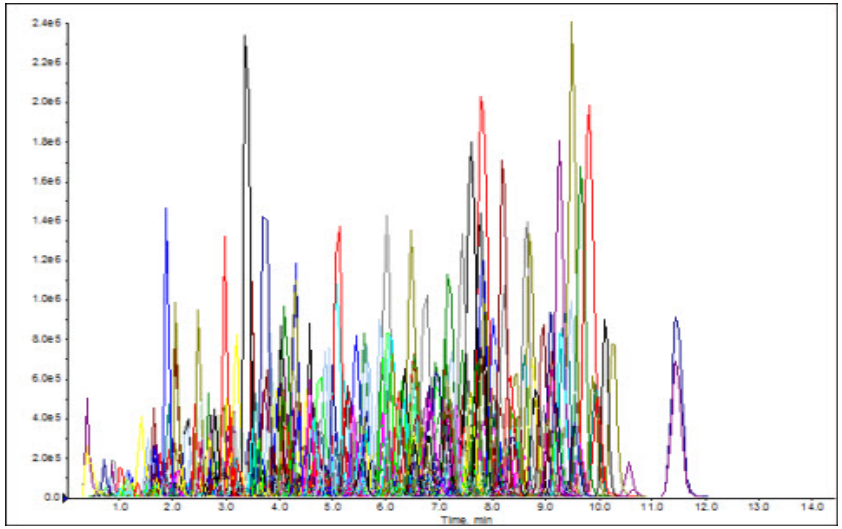


Pharmacogenetics - Peptides

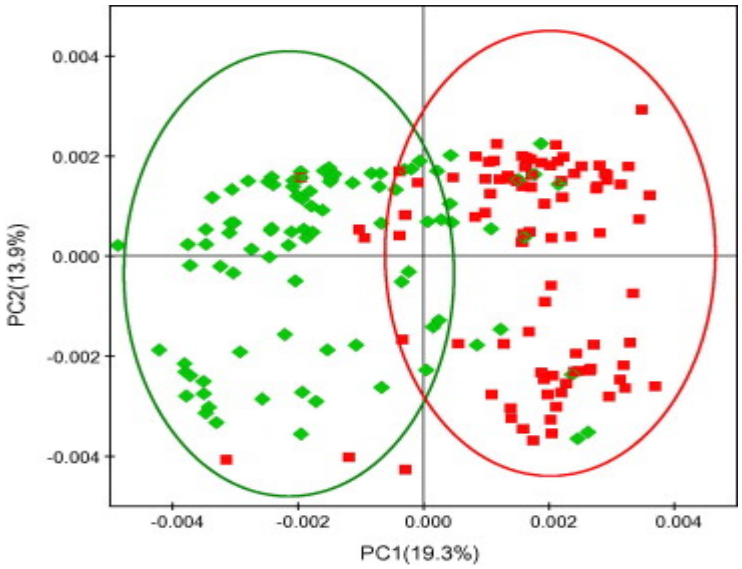


Pharmaceutical biotechnologies:

-omics: correlation with chemistry

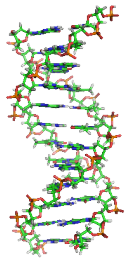


Chromatography



Principal Component Analysis

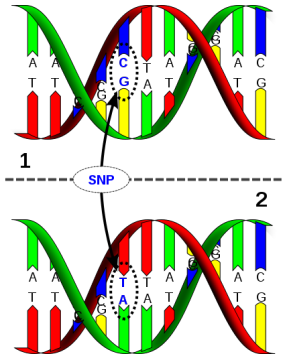
Pharmacogenetics - Peptides



-omics nomenclature:

ENVIROMICS environmental effects on pathology diagnosis

GENOMICS **S**ingle **N**ucleotide **P**olimorphism:
SNP → biomarkers, amplified via PCR



TRANSCRIPTOMICS

transcripted mRNA

PROTEOMICS

peptides, proteins

METABOLOMICS

small/macromolecules

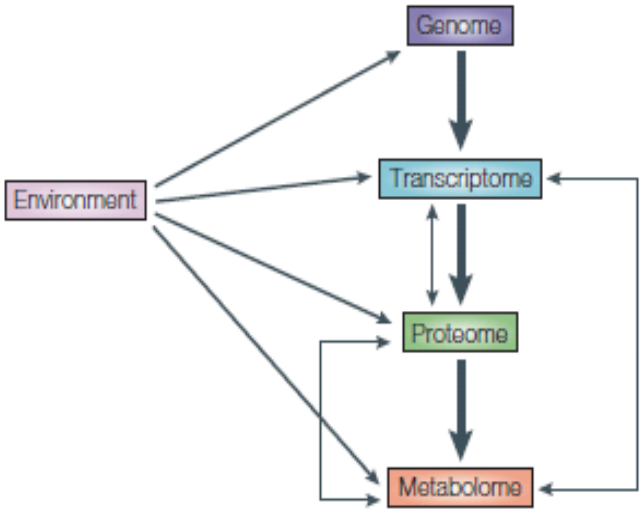


Figure 1 | **The biological organization of the '-omes'.** The classical view of biological organization is to consider the flow of information from the genome to the transcriptome, to the proteome and then the metabolome. However, each tier of organization depends on the other, so a perturbation in one network can affect another. Furthermore, the environment has a crucial impact on not only expression and concentrations of transcripts, proteins and metabolites, but also on the genome by selecting for adaptive changes in subpopulations of cells within a tumour. Metabolomics can potentially probe much more than classical metabolism and metabolic disorders — it can also be used to monitor changes in the genome or to measure the effects of downregulation or upregulation of a specific gene transcript.

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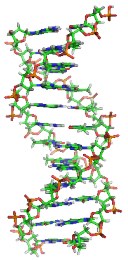
Pharmacogenetics - Peptides



Biomarkers:

natural products that can be traced to a particular biological origin. They are powerful tools that can be used to trace diseases, drugs and environmental contaminants in modern systems

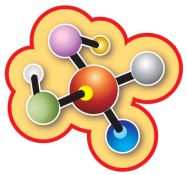


Table 2 | **Metabolic biomarkers of tumours**

Metabolite*	Metabolic function	Associated tumours/characteristics
Alanine	In conjunction with lactate, increases in tissues during hypoxia; made by transamination of pyruvate to prevent further increases in lactate[81]	Hepatoma and brain tumours, including astrocytomas, metastases, gliomas, meningiomas and dysembryoplastic neuroepithelial tumours
Saturated lipids	An important constituent of cell membranes, although membrane lipids are poorly resolved by NMR; lipid peaks detected by NMR are believed to either be present in cell-membrane microdomains or in cytoplasmic vesicles	Alterations in levels associated with proliferation, inflammation, malignancy, necrosis and apoptosis[20,29,33,39,82]
CCMs	Include choline, phosphocholine, phosphatidylcholine and glycerophosphocholine; these are key constituents of cell membranes	Levels change during apoptosis and necrosis; the tumour types that these changes have been found in include brain, sarcomas, prostate and hepatoma[31–34]
Glycine	An amino acid and an essential precursor for *de novo* purine formation	Decreased following disruption of the HIF-1 signalling pathway[36]
Lactate	An end product of glycolysis	Increases rapidly during hypoxia and ischaemia; poorly vascularized tumours have a low intracellular pH as a result of increased lactate production; increased rates of lactate production have been associated with a range of tumours and, in particular, certain types of neoplasms[83]
Myo-inositol	Involved in osmoregulation and volume regulation	Increased in colon adenocarcinoma, glioma, schwannomas, ovarian carcinoma, astrocytoma and endometrial cells[43,55]; decreased in breast tumours[84]
Nucleotides	Used to manufacture DNA and RNA; also key metabolic intermediates in fatty-acid and glycogen metabolism; changes in ATP concentration also indicate the energetic status of the tumour	Increased in glioma during apoptosis[41]; CDP-choline is also increased during apoptosis[19,85]
PUFAs	Constituents of cell membranes, especially mitochondrial	Increased in glioma cells undergoing apoptosis[20,21], and in dedifferentiated and pleomorphic liposarcomas[86]
Taurine	Important in osmoregulation and volume regulation; hypotaurine is also an antioxidant and might protect cells from free-radical damage	Increased in squamous-cell carcinoma[87], prostate cancer and liver metastasis[88]

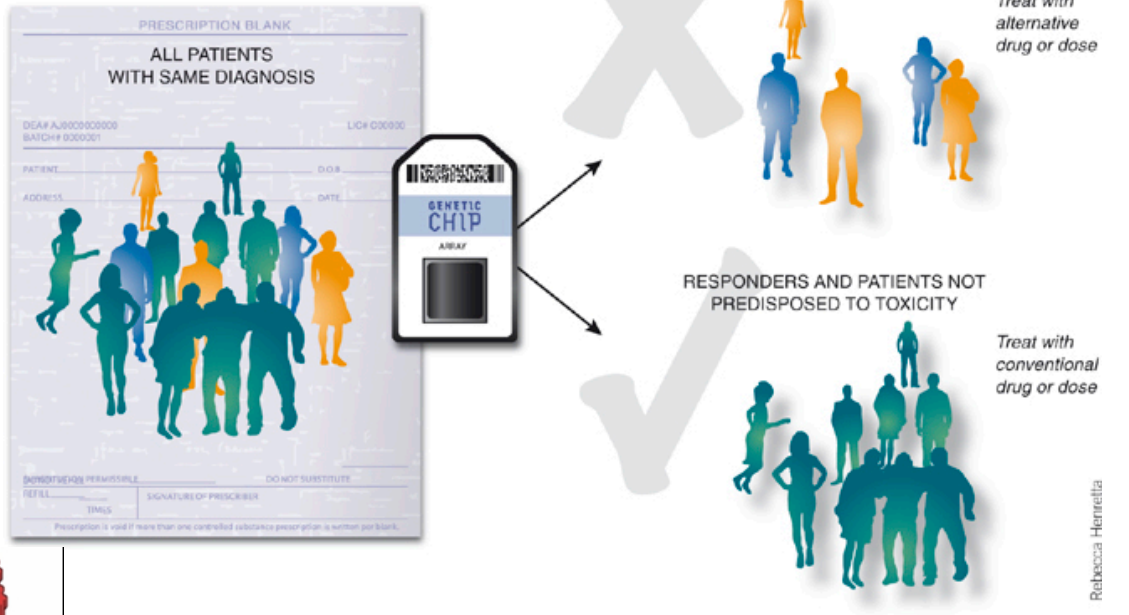
*Metabolites identified by NMR spectroscopy of tissue extracts or magnetic resonance spectroscopy *in vivo*. CCM, choline-containing metabolites; CDP-choline, cytidine diphosphocholine; HIF-1, hypoxia-inducible factor 1; NMR, nuclear magnetic resonance; PUFAs, polyunsaturated fatty acids.

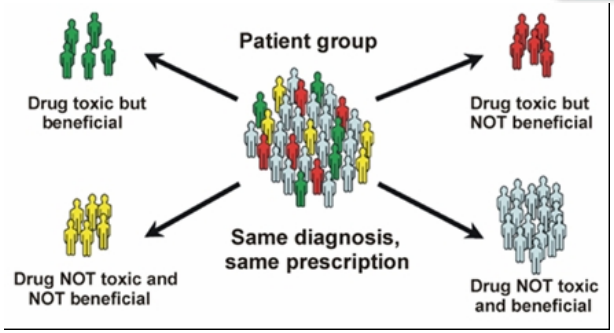
Pharmacogenetics - Peptides

Personalized medicine:

Pharmaceuticals

Diagnostics



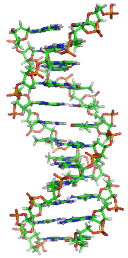


Clinical Pharmacology & Therapeutics **81**, 311-315 (March 2007)

The Art and Science of Personalized MedicineEditorial

M Piquette-Miller and D M Grant

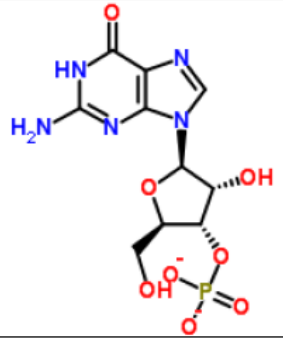
Pharmacogenetics - Peptides



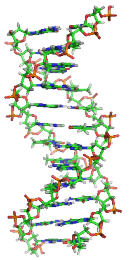
Nucleic acids:

Gene: DNA segment involved in polypeptides production

Clone: identical rDNA molecules originated by bacterial replication

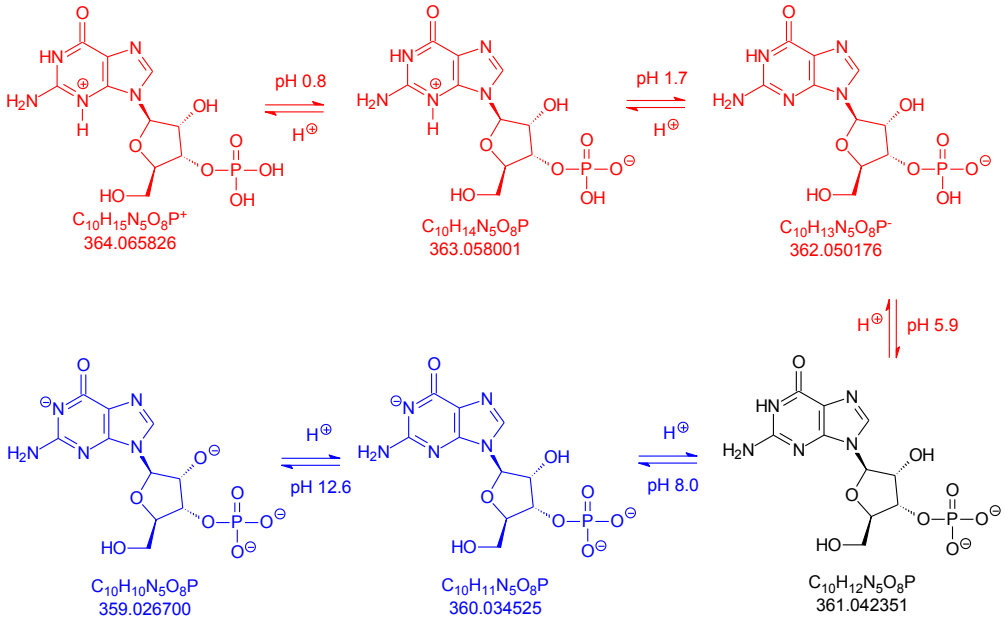
Name	**GUANOSINE-3'-PHOSPHATE**
Structure	
Systematic name	[(2R,3S,4R,5R)-5-(2-amino-6-oxo-1H-purin-9-yl)-4-hydroxy-2-(hydroxymethyl)tetrahydrofuran-3-yl] phosphate
Formula	$C_{10}H_{12}N_5O_8P$
MW	361.2047
Monoisotopic mass	361.042348897
Mp	--
H bond acceptors	13
H bond donors	7
Acid pK*a*	0.78; 5.90 (PO_4) 10.16 (amide) 12.6 (4'-OH)
Basic pk*a*	2.60 (N3)
ACD Log D pH 5.5	-5.09
ACD Log D pH 7.4	-6.03
Solubility	water

Pharmacogenetics - Peptides



Nucleic acids:

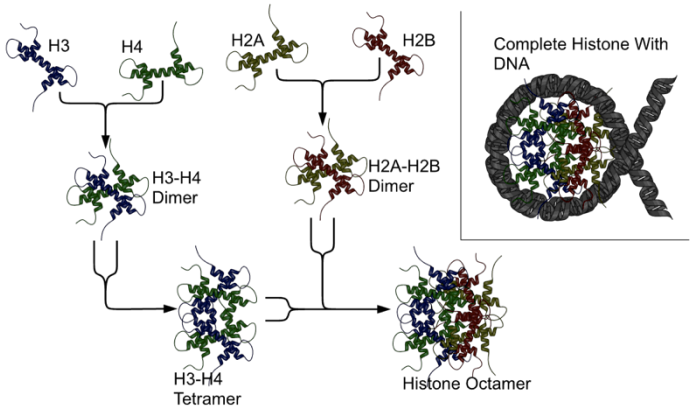
Guanosin-3’-phosphate species at different pH values



pH 7

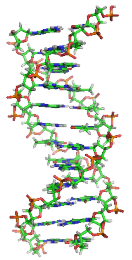
Histones:

basic proteins



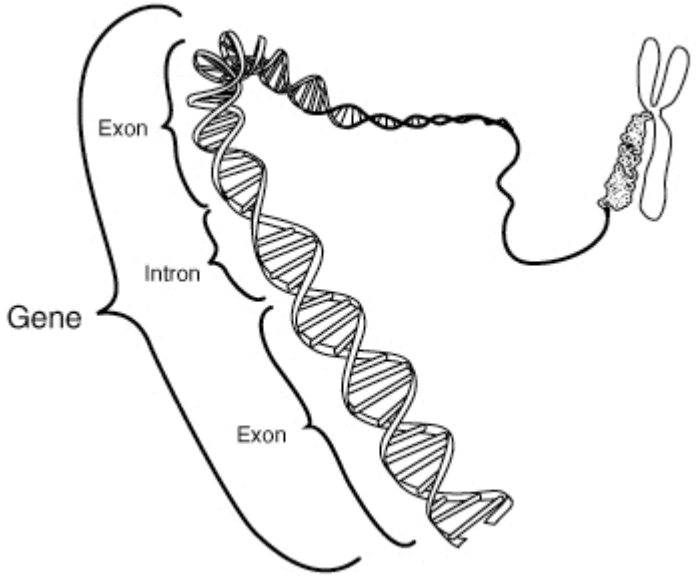
Example: *histone H2A-Bbd type 1 [Homo sapiens]*
MPRRRRRRGSSGAGGRGRTCSRTV
RAELSFSVSQVERSLREGHYAQRLSR
TAPVYLAAVIEYLTAKVPEL
AGNEAQNSGERNITPLLLDMVVHN
DRLLSTLFNTTTISQVAPGED

Pharmacogenetics - Peptides



Chromosomes and genes:

Human cells: 23 pairs (mother/father) chromosomes



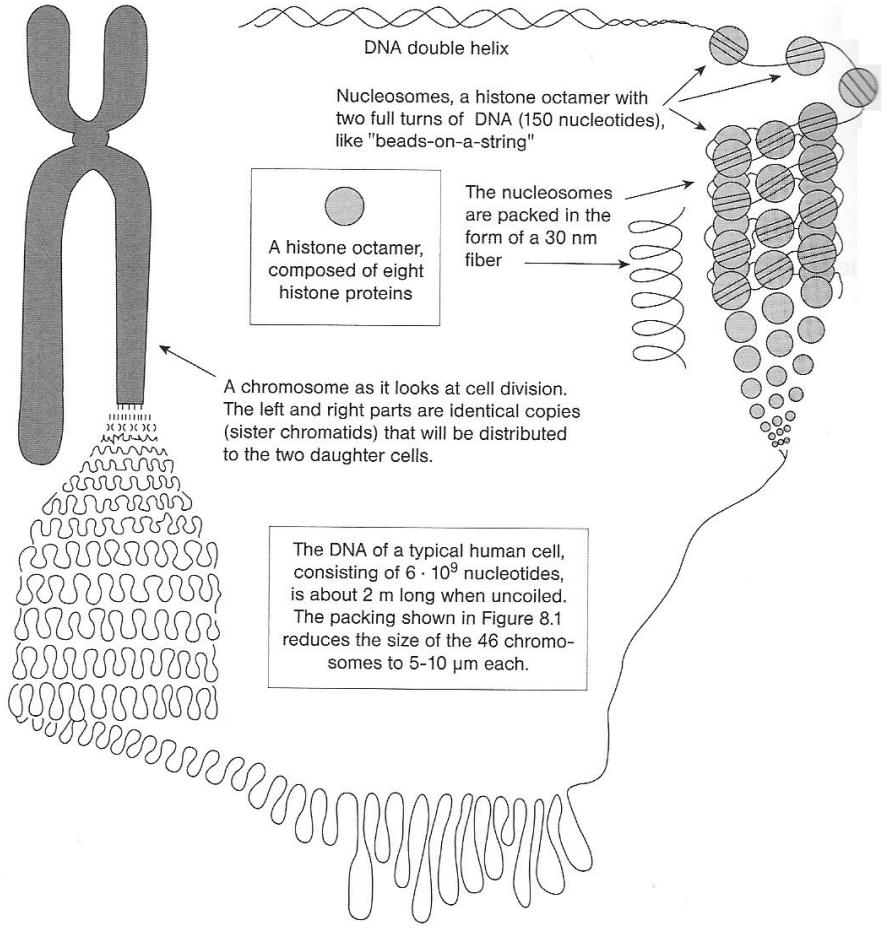
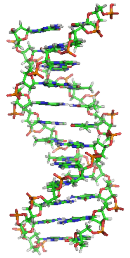
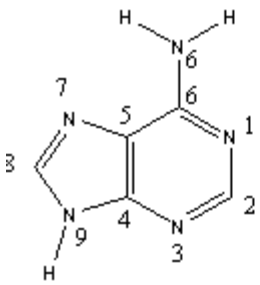


Figure 8.1 The construction of a human chromosome, down to the DNA double helix.

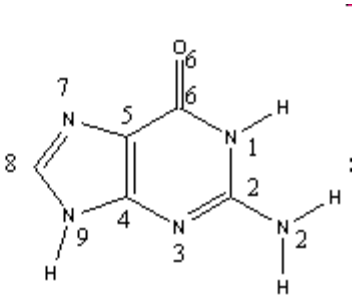
Pharmacogenetics - Peptides



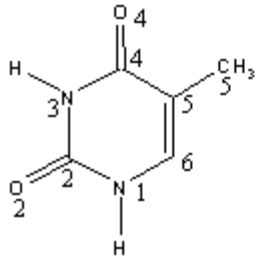
DNA nomenclature:



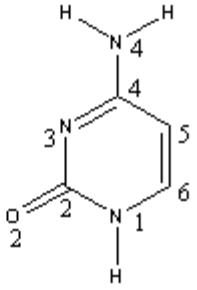
Adenine (Ade)



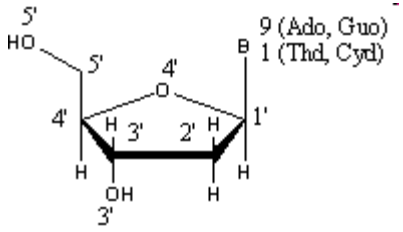
Guanine (Gua)



Thymine (Thy)



Cytosine (Cyt)



http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html

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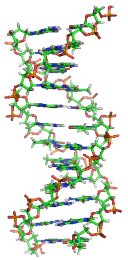
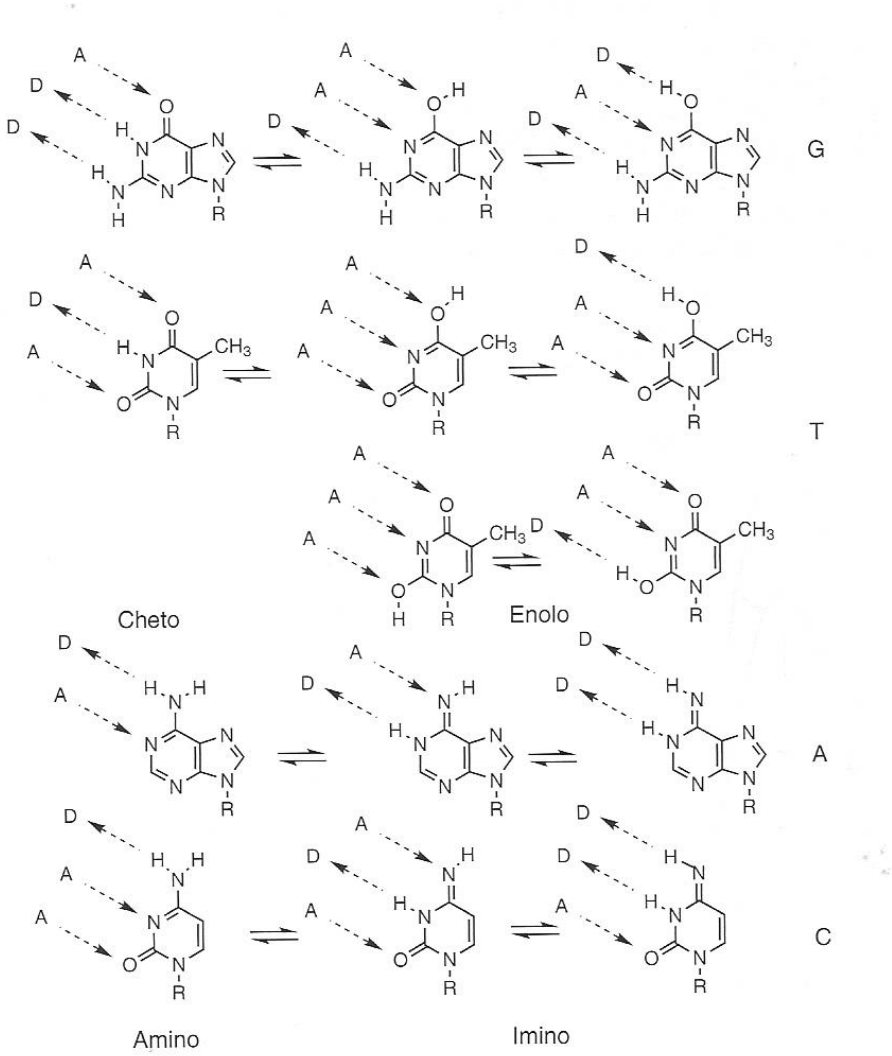


DNA

H bonds

tautomery



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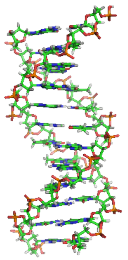
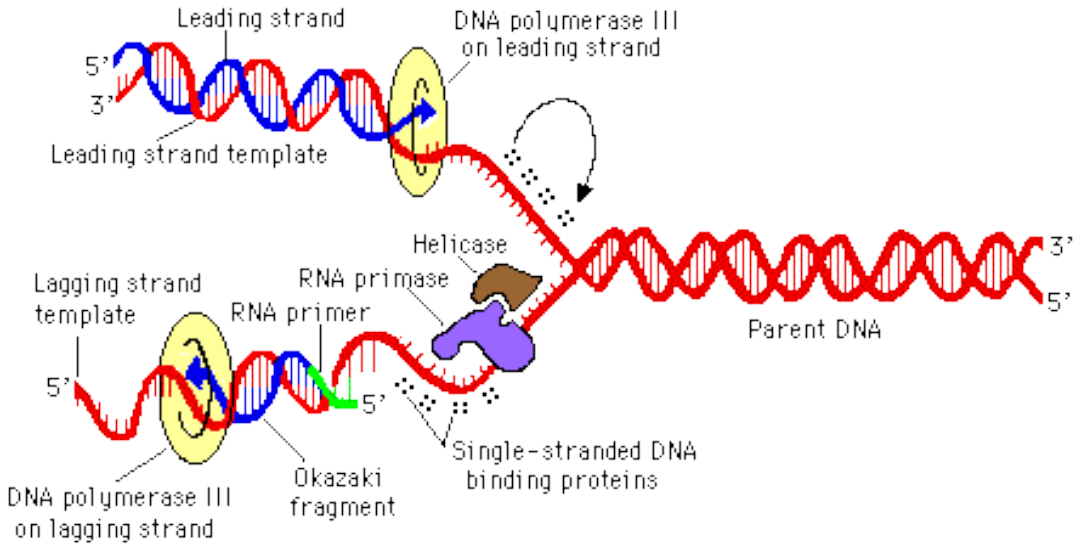


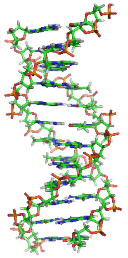
DNA synthesis

Enzymatic synthesis

PCR: polymerase chain reaction: amplification of DNA fragments



Pharmacogenetics - Peptides



From DNA synthesis

to protein production

Endonuclease

Ligase

Inverse transcriptase

DNA polymerase

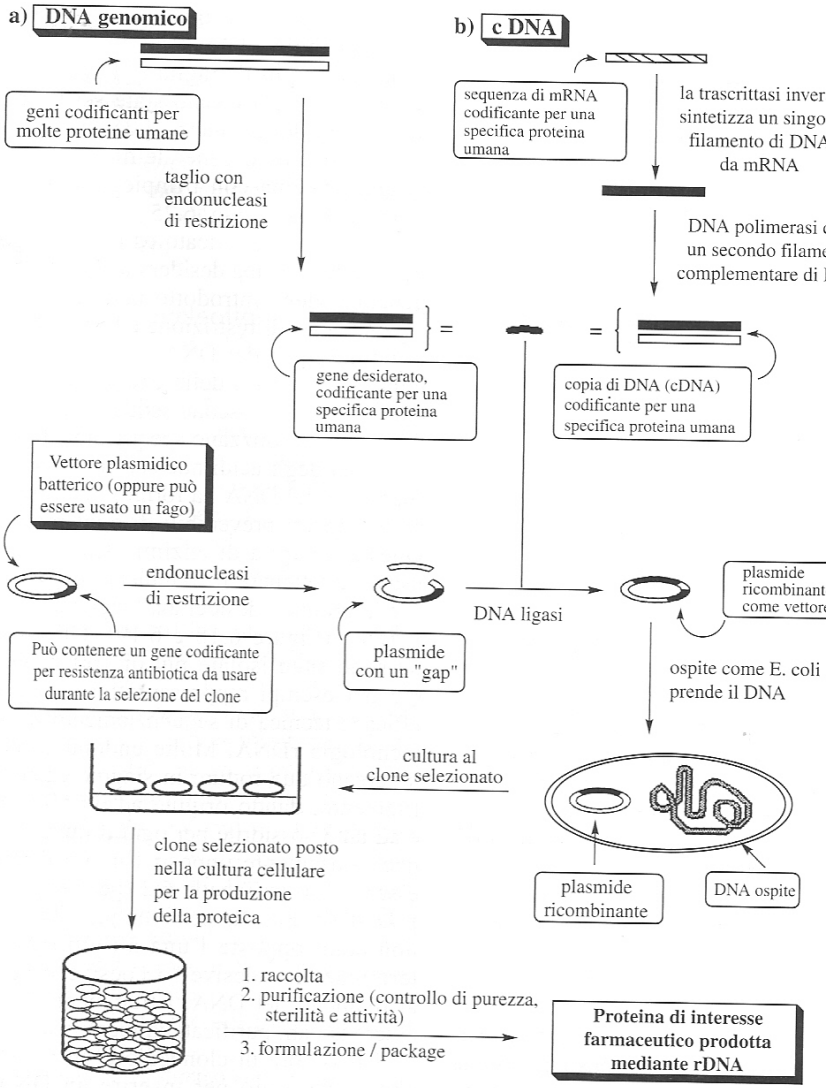
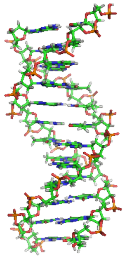


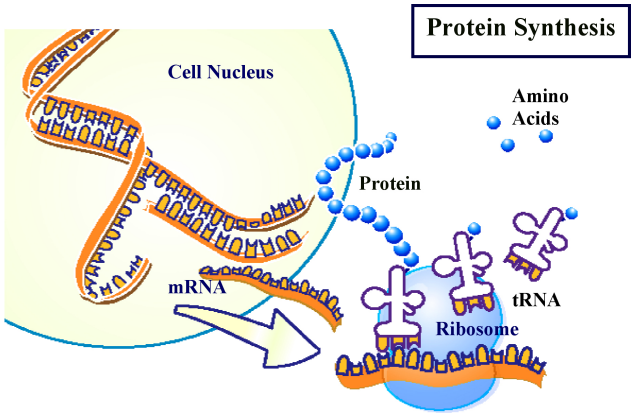
Fig. 6.25. Riassunto di una produzione tipica mediante una proteina rDNA da (a) DNA genomico o (

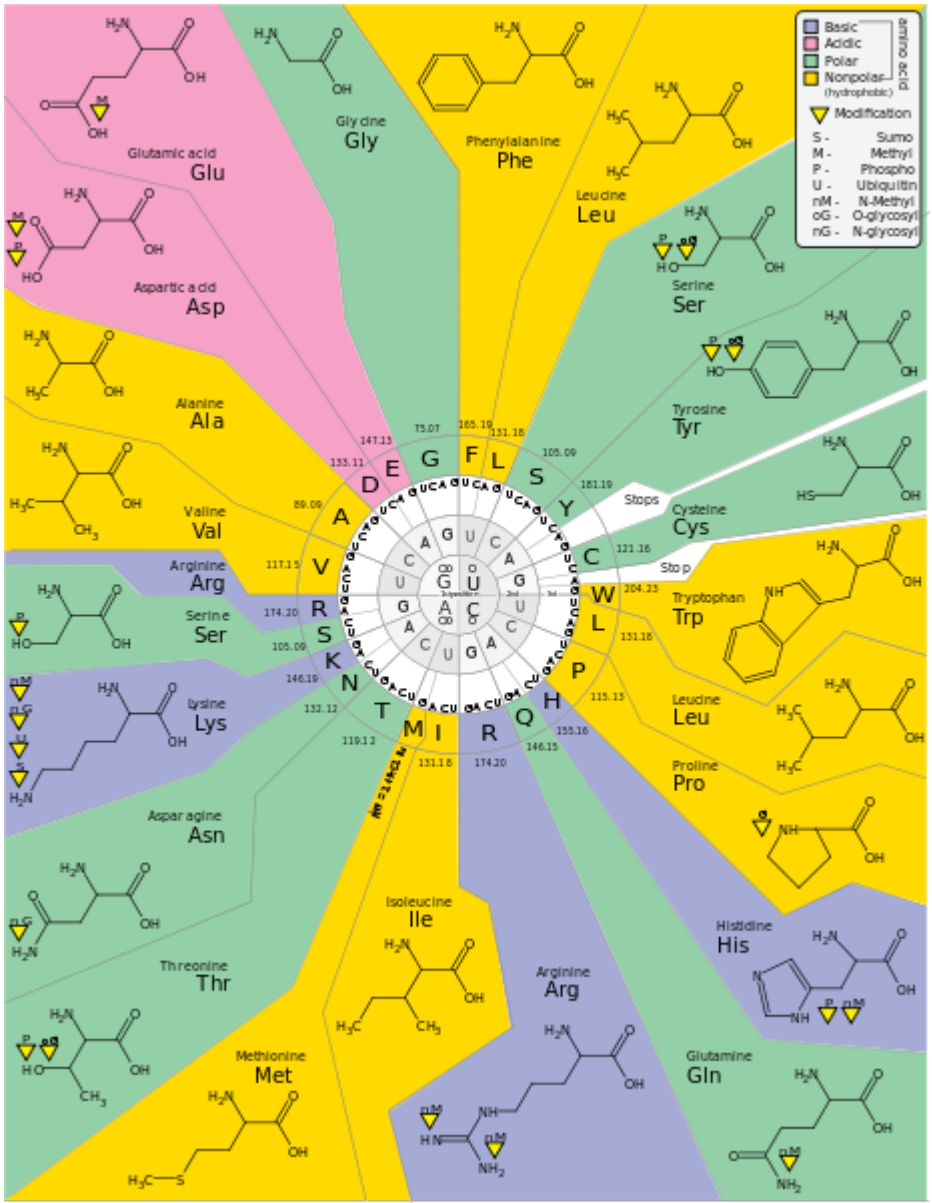
Pharmacogenetics - Peptides



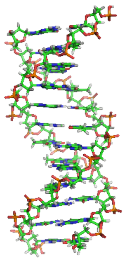
From DNA synthesis

to protein production





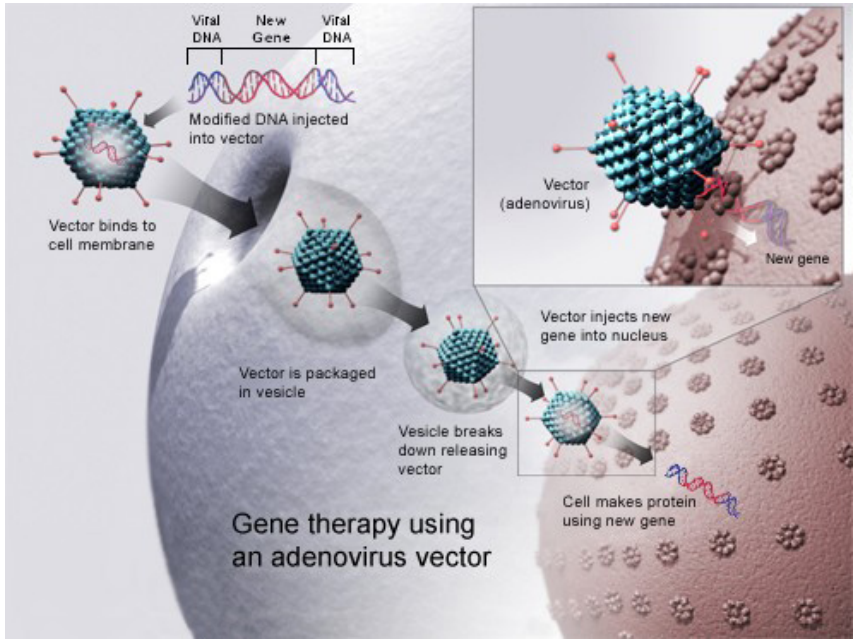
Pharmacogenetics - Peptides



Gene therapy:

In vivo promotion of therapeutic proteins synthesis by nucleic acid introduction:

- Viral vectors (AV, retrovirus, HS,…)
- Non-viral vectors (plasmides) introduced by:
    - Physical methods (electroporation, Gene gun, magnetoporation,…)
    - Chemical methods (cationic lipids (cytofectins), calcium phosphate precipitation, artificial chromosomes [yeasts],…)

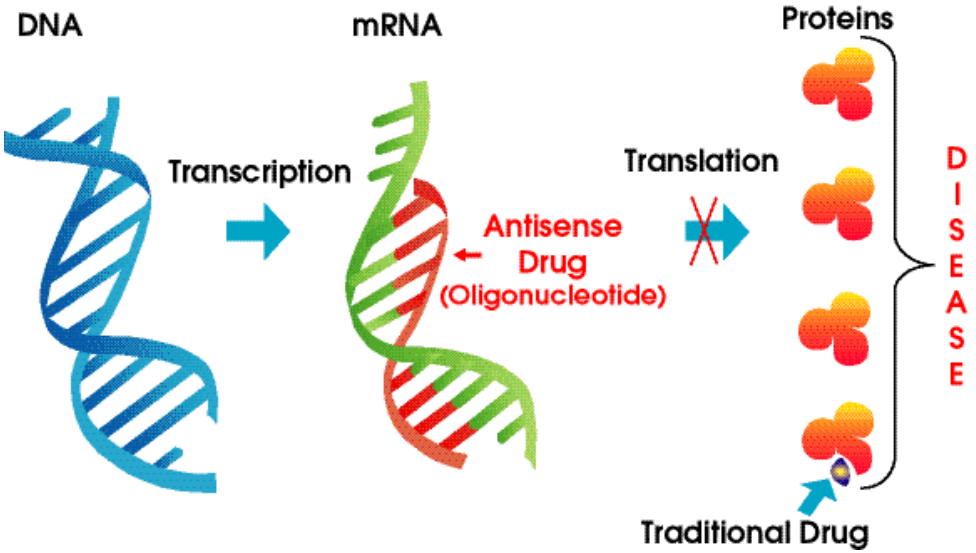


Pharmacogenetics - Peptides

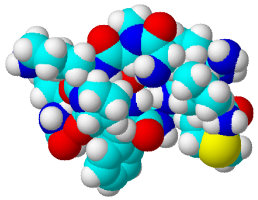
Antisense therapy:

DNA-based drugs for genetic disorders or (viral) infections and cancer:

- oligonucleotides
- bioisosteric replacement (S/O)



Peptides



Peptide/protein drugs:

2-15 aa: oligopeptides

15-50 aa: polypeptides

> 50 aa: proteins

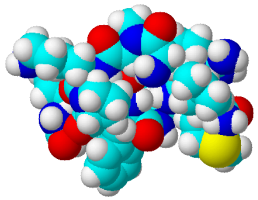
L-aminoacids

Enzymes: carboxypeptidases; dipeptidylcarboxypeptidases; aminopeptidases; amidases

Inactive on D-aminoacids.

Stability to hydrolysis: insulin oral absorption 0.05%. Nasal: 30%. Subcutaneous: 80%.

Peptides, WADA S2



Peptide/protein drugs:

S2. PEPTIDE HORMONES, GROWTH FACTORS AND RELATED SUBSTANCES

The following substances, and other substances with similar chemical structure or similar biological effect(s), are prohibited:

1. **Erythropoiesis-Stimulating Agents** [e.g. **erythropoietin (EPO), darbepoetin (dEPO), hypoxia-inducible factor (HIF) stabilizers, methoxy polyethylene glycol-epoetin beta (CERA), peginesatide (Hematide)];**

2. **Chorionic Gonadotrophin (CG) and Luteinizing Hormone (LH)** and their releasing factors, in males;

3. **Corticotrophins** and their releasing factors;

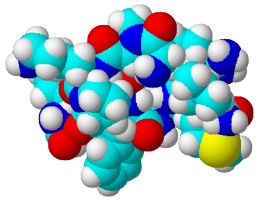
4. **Growth Hormone (GH)** and its releasing factors and **Insulin-like Growth Factor-1 (IGF-1).**

In addition, the following growth factors are prohibited

Fibroblast Growth Factors (FGFs), Hepatocyte Growth Factor (HGF), Mechano Growth Factors (MGFs), Platelet-Derived Growth Factor (PDGF), Vascular-Endothelial Growth Factor (VEGF) as well as any other growth factor affecting muscle, tendon or ligament protein synthesis/degradation, vascularisation, energy utilization, regenerative capacity or fibre type switching;

and other substances with similar chemical structure or similar biological effect(s).

Peptides, WADA S2

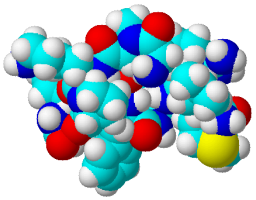


Peptide/protein drugs:

ORMONI:	***ADRENOCORTICOTROPI***	**COSYNTROPIN**
	DI RILASCIO DELLE GONADOTROPINE	**LEUPROLIDE, ABARELIX**
	GONADOTROPINE	**LUTROPINE**
	DELLA CRESCITA/SOMATOSTATINE	**PEGVISOMANT, PENTETREOTIDE**
	IPOFISARI	**OXYTOCIN, VASOPRESSIN**
	PLACENTARI	**CORYONIC GONADOTROPIN**
	PANCREATICI	**INSULIN*, PRAMLINTIDE**
	PARATIROIDEI	**TERIPARATIDE, CALCITONIN**
	FATTORI DI CRESCITA EMATOPOIETICI	**EPO, EPOETIN**

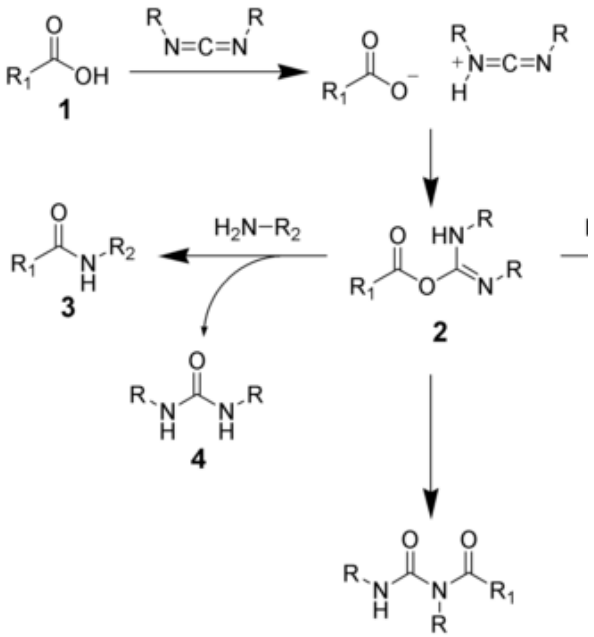
***WADA S4**

Peptides



Peptide synthesis in solution:

- Risk of racemization
- Activation of carboxyl groups: DCC, dehydrating agent



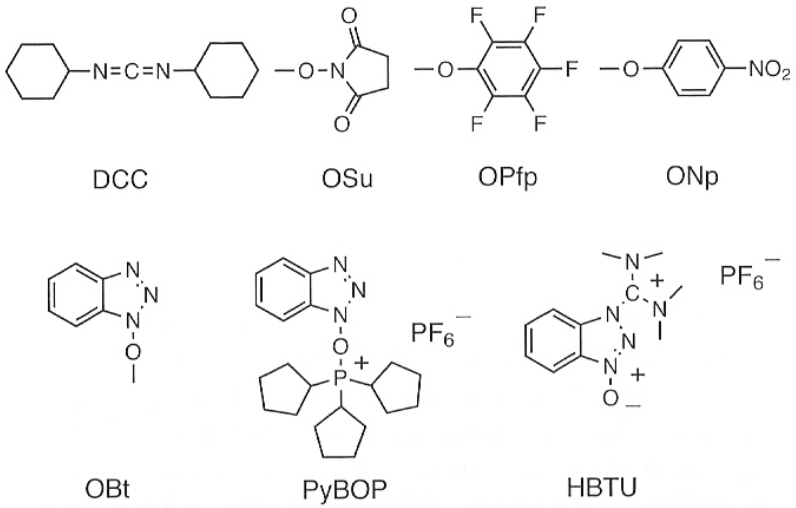
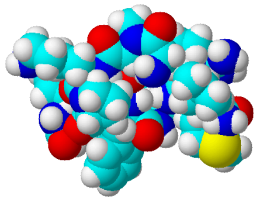


Fig. 7.6. Struttura chimica di alcuni reagenti usati nella reazione di formazione del legame peptidico

Peptides



Peptide synthesis in solution:

- protection/deprotection of aminogroups

Cbz: stable in bases, removed by HBr, HF or CH_3COOH or Pd/H_2

Boc: removed by TFA. Used in catalytic hydrogenation with Pd

Fmoc: stable in acids, removed by piperidine.

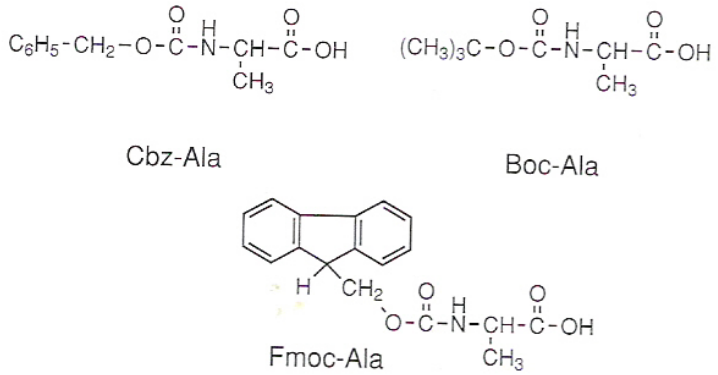


Fig. 7.5. Tre gruppi protettori di tipo uretanico - Cbz, Boc e Fmoc, legati all'alanina.

- protection/deprotection of carboxyl groups

Me/Et esther: removed by alkaline hydrolysis.

Benzyl esther: removed by HF or Pd/H_2

t-Bu esther: removed by TFA, HF. Stable with Pd/H_2.

- protection/deprotection of lateral chains

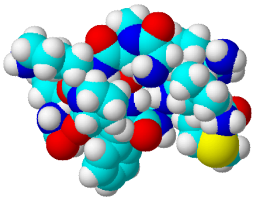
Benzyl, t-Bu, cyclohexyl esther: COOH

Benzyl ether: OH (Ser; Thr)

Tosyl (p-Me-phenylsulphonyl): guanidine (Arg)

2-chlorocarbobenzoxy: Lys

Peptides



Peptide solid phase synthesis:

-Merrifield reaction

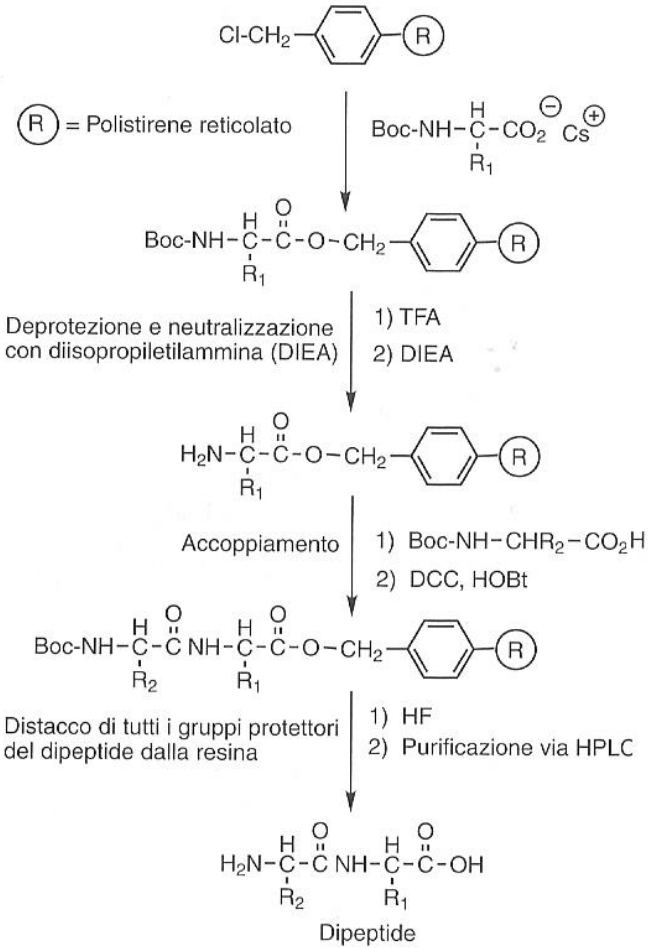
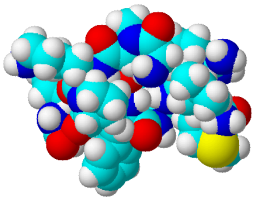


Fig. 7.7. Schema della sintesi chimica su fase solida basata sulla metodica di Merrifield.

Peptides



Peptide/protein drugs chemical instability:

hydrolysis, oxidation (S of cys and met, aromatic: tyr, trp and his), racemization, beta-elimination, disulfide exchange

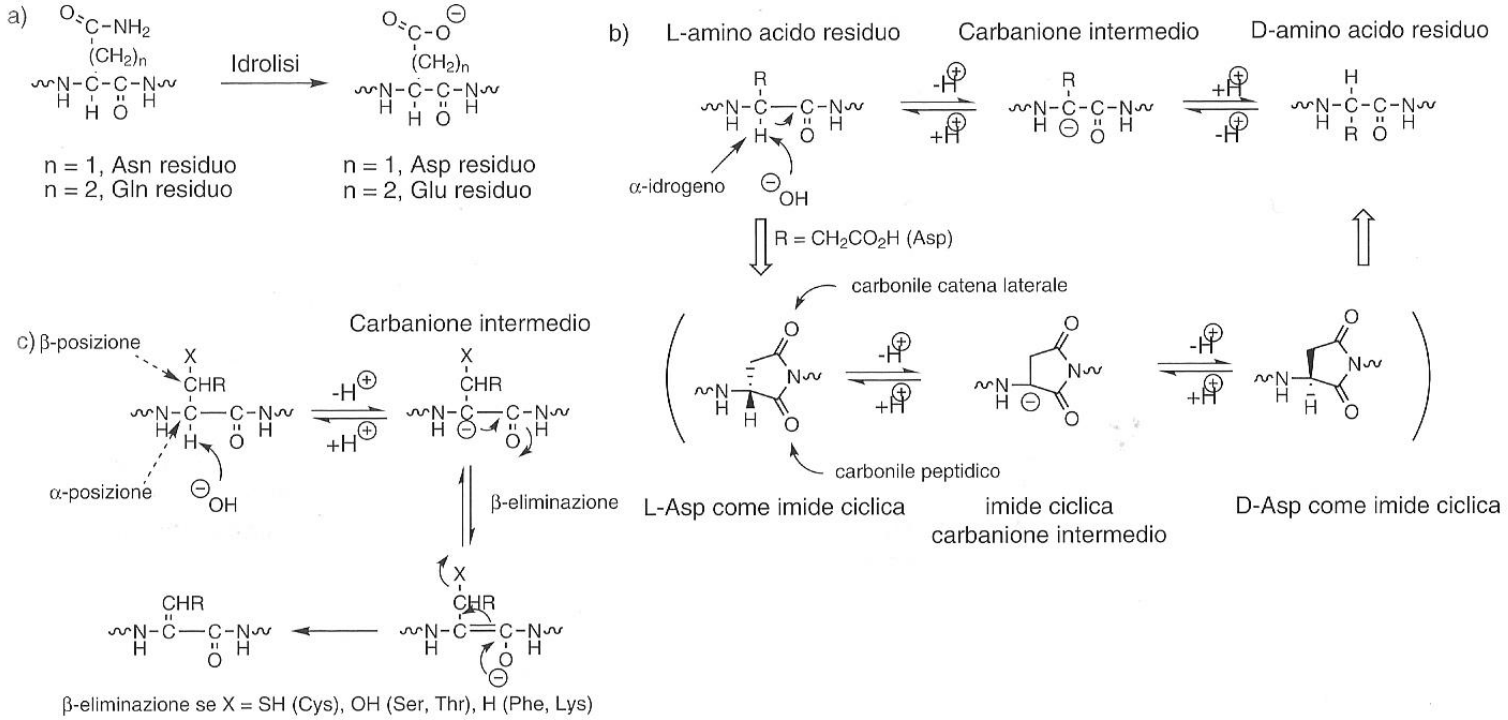
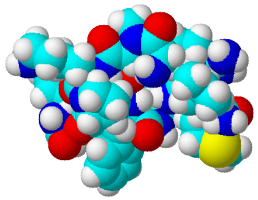


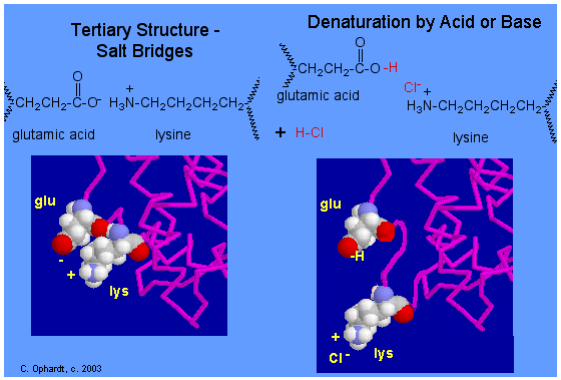
Fig. 6.26. Instabilità chimica di biofarmaceutici proteici. (a) Idrolisi. (b) Racemizzazione catalizzata da basi. (c) β-eliminazione.

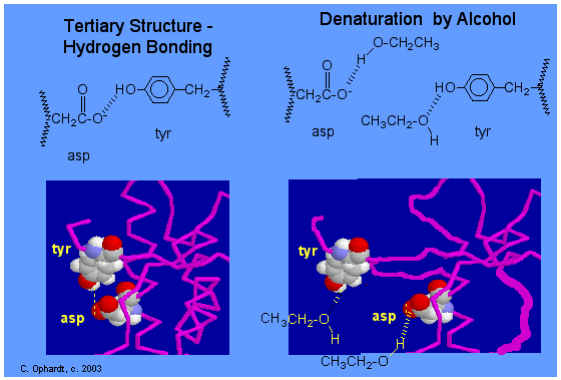
Peptides

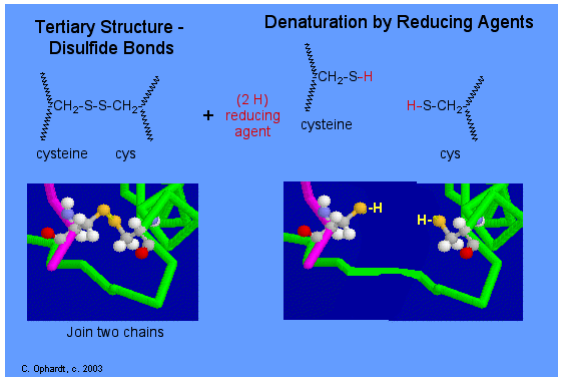


Peptide/protein drugs chemical instability:

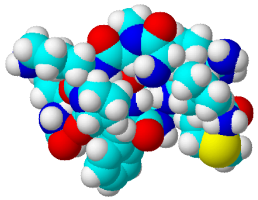
- acid/basic denaturation: by salt bridge disruption / formation
- organic solvent (R-OH) denaturation: by H bonds disruption (secondary and tertiary structure)
- reducing agent denaturation: by disulfide bond disruption
- heavy metals denaturation (Zn^{+2}, Hg^{+2}, Pb^{+2}, Ag^{+1} Tl^{+1}, Cd^{+2}) : by salt bridge formation (disinfection) and





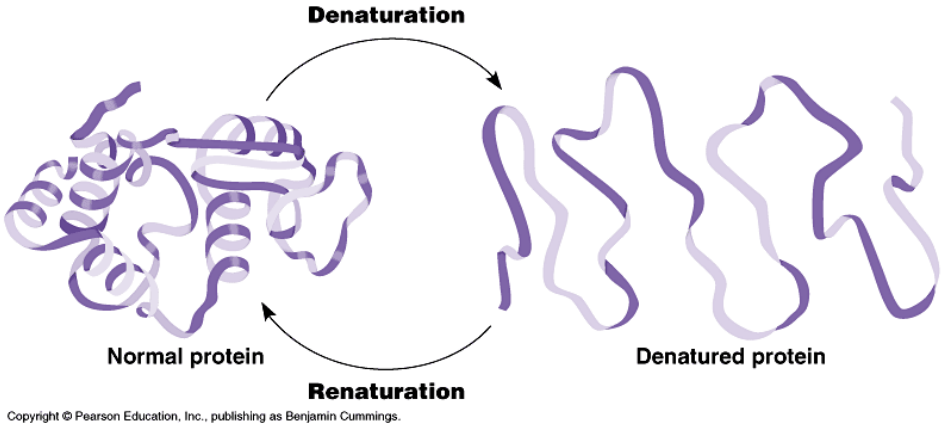


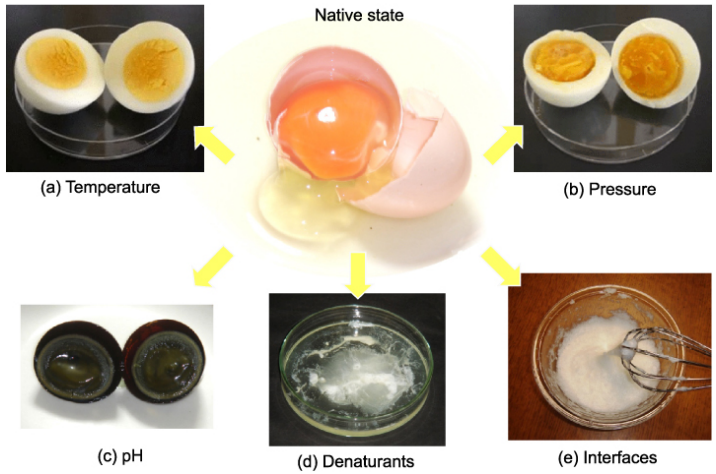
Peptides



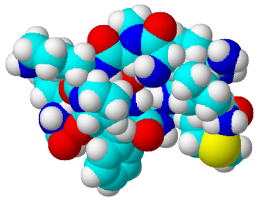
Peptide/protein drugs physical instability:

consequence of polymeric nature: denaturation (temperature, pH, organic solvents); surface adsorption; non-covalent aggregation (soluble of with precipitation)





Peptides



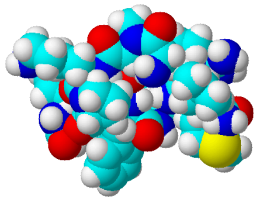
Peptide/protein drugs metabolic (ADME) instability:

- low **a**dsorption (membrane crossing)
- gastric pH (acid)
- gastro-intestinal peptidases (enzymatic)
- metabolic hydrolysis, oxidation, racemization, beta-elimination, disulfide reduction
- short half-life (but low toxicity of degradation products)

Novel release modes:

- conjugation with co-solvents (glycol)
- liposomes and microspheres
- viral and erythrocytes carriers
- transdermal, nasal spray, buccal and ocular, rectal systems
- prodrugs, co-administration of peptidase inhibitors, etc.

Peptides



Hypothalamic hormones:

Superagonists of gonadotropin realising hormone: Gly-6 substitution with D-Leu and elimination of terminal gly:

LEUPROLIDE

$C_{59}H_{84}N_{16}O_{12}$ MW 1209.40

L02AE02

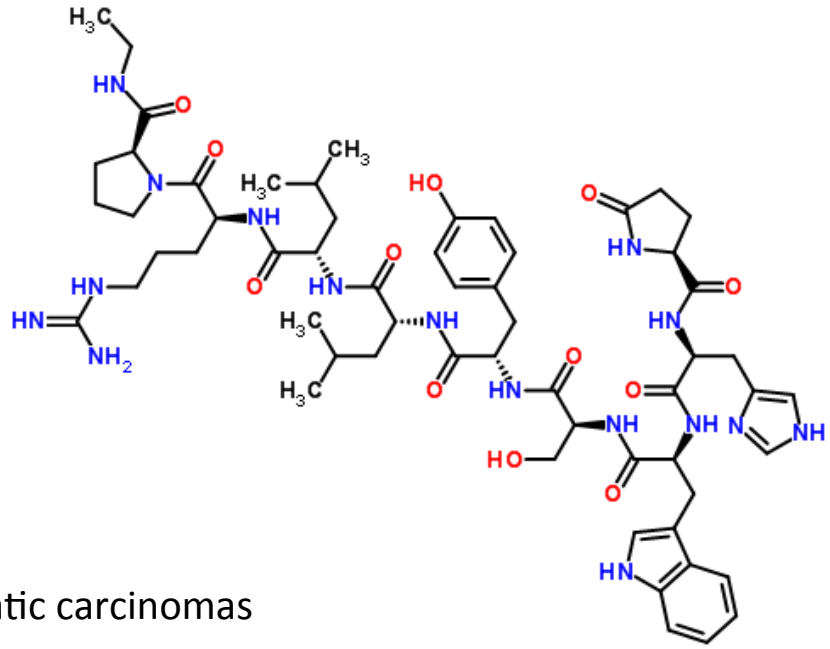
L ANTINEOPLASTIC AND

IMMUNOMODULATING AGENTS

L02 ENDOCRINE THERAPY

L02A HORMONES AND RELATED AGENTS

L02AE Gonadotropin releasing hormone analogues

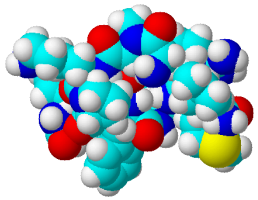


Used in the treatment of mammal and prostatic carcinomas

Analogues:

GOSERELIN, NAFARELIN, TRIPTORELIN

Peptides



Hypothalamic hormones:

Antagonists of gonadotropin realising hormone: LH peaks control. Ac-D-NaphtylAla in 1; p-Cl D-Phe in 2; 3-pyridyl-D-Ala in 3; i-Pr-Lys in 8; D-Asn in 6:

ABARELIX

$C_{72}H_{95}ClN_{14}O_{14}$ MW 1414.68

L02BX01

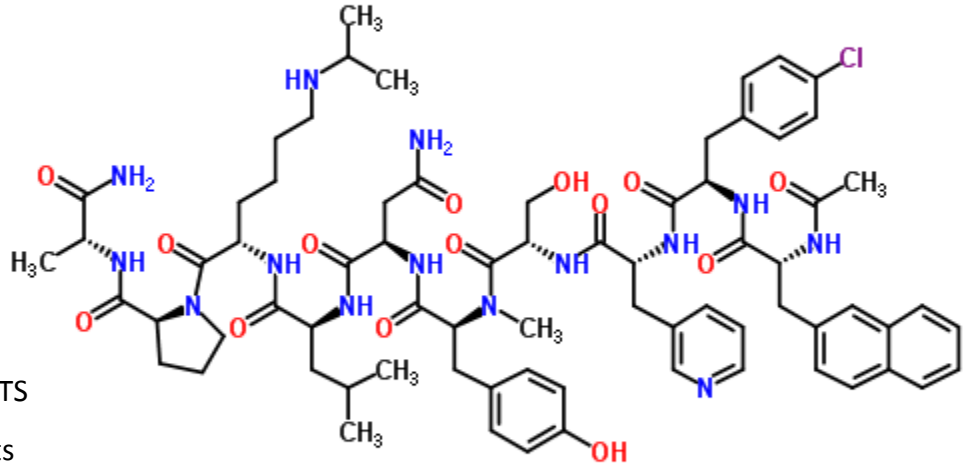
L ANTINEOPLASTIC AND

IMMUNOMODULATING AGENTS

L02 ENDOCRINE THERAPY

L02B HORMONE ANTAGONISTS AND RELATED AGENTS

L02BX Other hormone antagonists and related agents

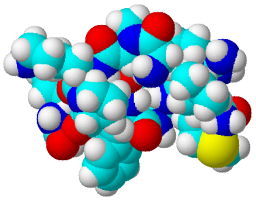


Used in the ART (assisted reproduction technology)

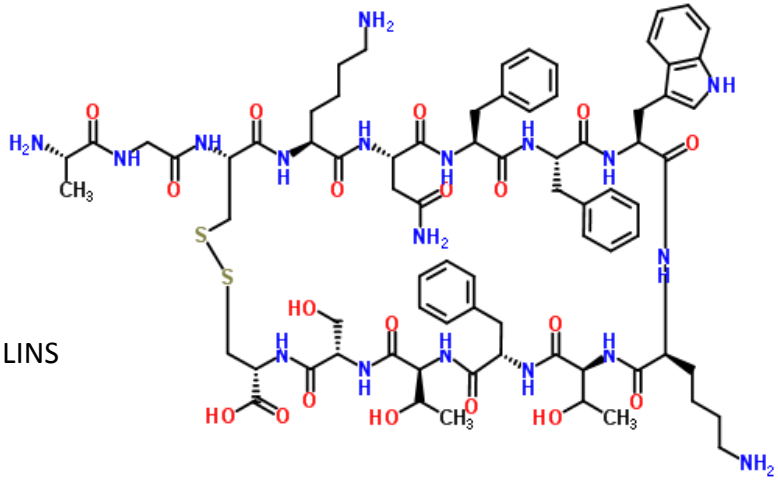
Analogues:

GANIRELIX, CETRORELIX

Peptides



Other hypothalamic hormones:



SOMATOSTATIN, 14 aa

$C_{76}H_{104}N_{18}O_{19}S_2$ MW 1636.72

H01CB01

H SYSTEMIC HORMONAL PREPARATIONS, EXCL. SEX HORMONES AND INSULINS

H01 PITUITARY AND HYPOTHALAMIC HORMONES AND ANALOGUES

H01C HYPOTHALAMIC HORMONES

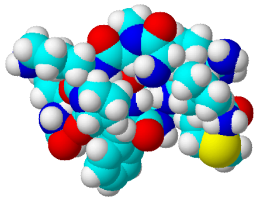
H01CB Somatostatin and analogues

Used in the treatment of acromegaly

Synthetic analogues:

OCTREOTIDE, ^{111}In-PENTETREOTIDE (tumor diagnosis)

Peptides



Hypophysis hormones:

h GROWTH HORMONE, 191 aa

Direct effect and induction of IGF-1 (insulin-like growth factor)

The 22 kDa protein is secreted by the pituitary gland. rhGH: recombinant.

Induces lipolysis and lean body mass growth. Effects on bone growth (especially in children). It has a short $t_{½}$ and is secreted in pulsatile manner. Stimulates protein turnover.

Synthetic analogues:

SOMATOTROPIN, PEGVISOMANT

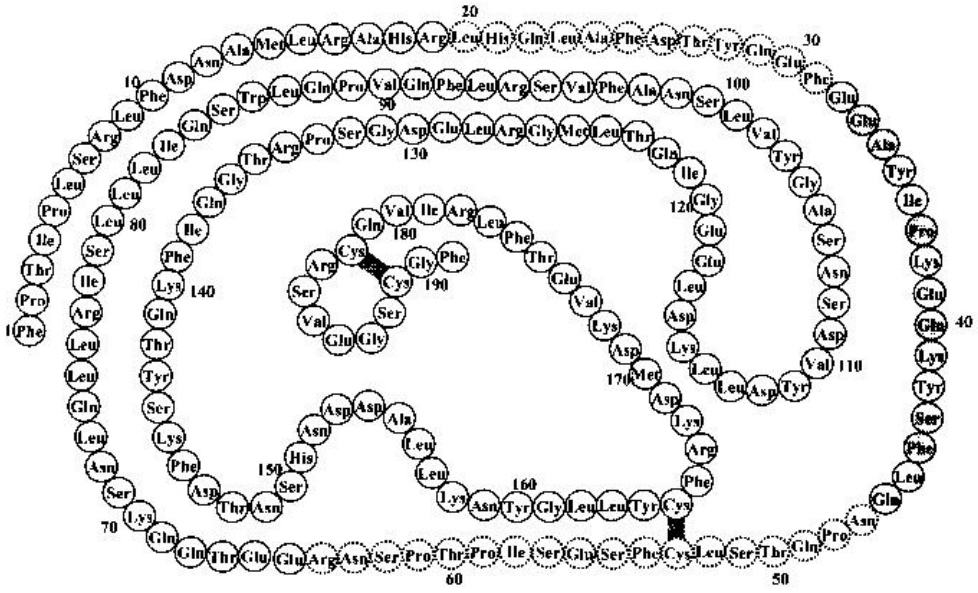
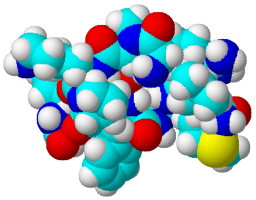


Fig. 2 The 20 kDa variant of hGH is characterized by deletion of residues 32–46 (*gray circles*) from the 22 kDa molecule. Resulting diagnostic peptides for LC–MS identification (compare Fig. 3) are signified by dotted circles

Peptides



Hypophysis hormones:

h GROWTH HORMONE, 191 aa

Chronic effect: acromegaly.

Analytical procedures: immunochemistry (RIA, ELISA). MS

Marker approach: changes in hGH-dependent parameters (IGF-1 level; IGFBP 3; IGF-1/IGPF 3 ternary complex)

Isoform approach: 20 kDa fragment and various smaller fragments, circulating in monomeric, homodimeric and heterodimeric forms. rhGH: 22 kDa isoform only.

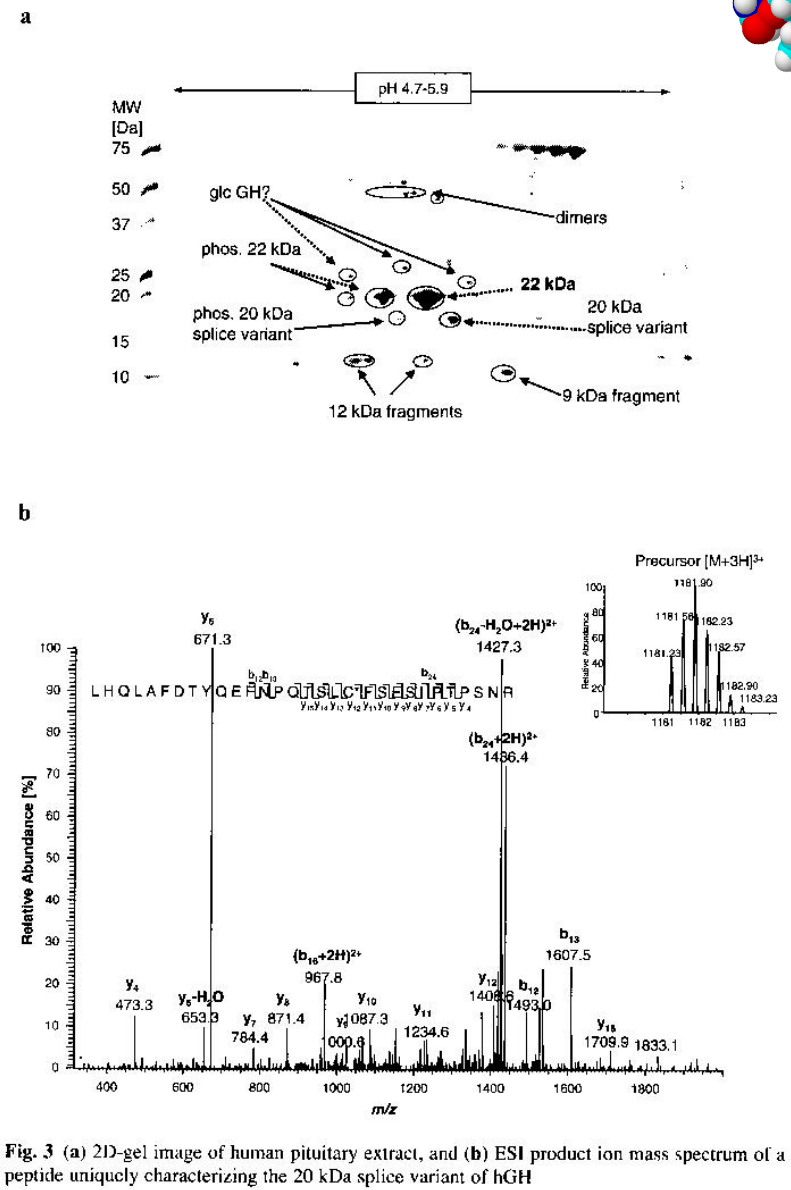
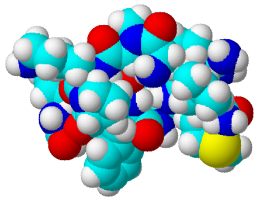


Fig. 3 (**a**) 2D-gel image of human pituitary extract, and (**b**) ESI product ion mass spectrum of a peptide uniquely characterizing the 20 kDa splice variant of hGH

Peptides



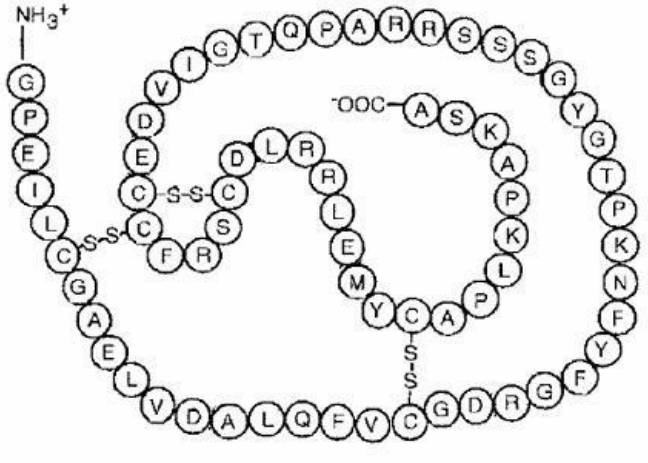
Hypophysis hormones:

IGF-1 (insulin-like growth factor) 70 aa.

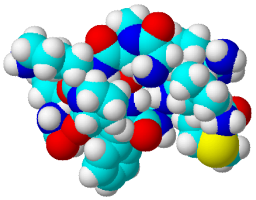
Up to 800 ng/mL level in plasma

IGF-1/IGF binding protein 3 complex has to be disrupted by the use of strong acids and ethanol or SDS.

By MS it is possible to discriminate between the natural IGF-1 and synthetic, more bioavailable analogues as the long-R^3-IGF-1 variant.



Peptides



Hypophysis hormones:

IGF-1 (insulin-like growth factor) 70 aa.

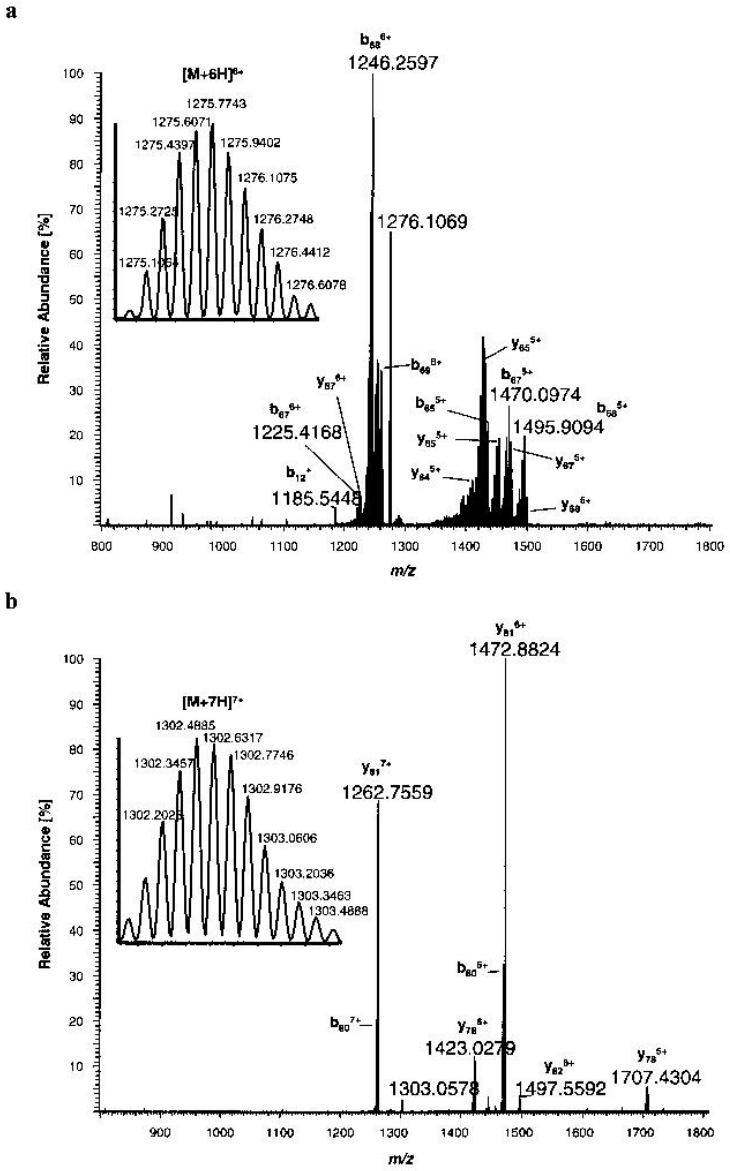
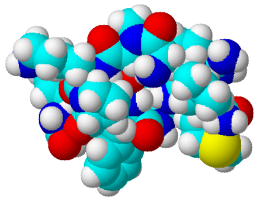


Fig. 1 ESI product ion mass spectra of multiply charged precursor ions of (a) $[M+6]^{6-} = 1{,}276.8$ of human IGF-1, and (b) $[M+7]^{7-} = 1{,}302.5$ of long-R^3-IGF-1

Peptides

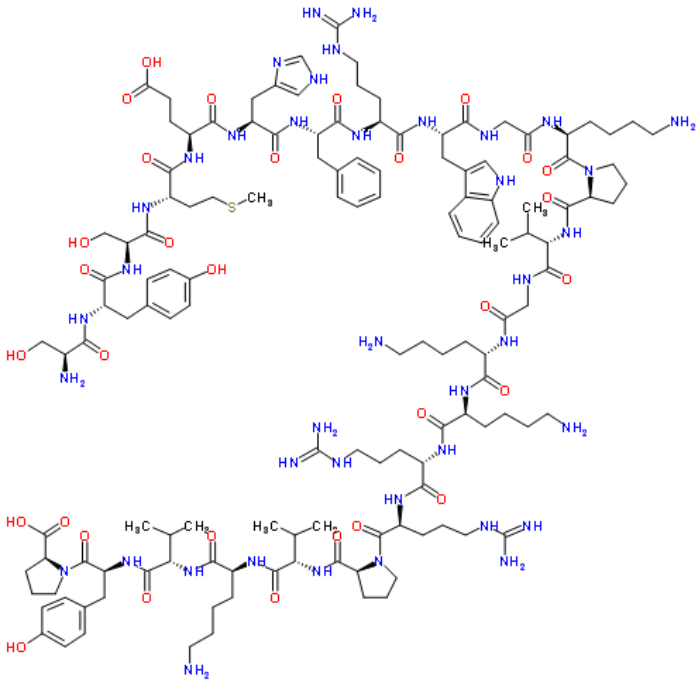


Hypophysis hormones:

Gonadotropins: FSH, LH

COSYNTROPIN, a1-24-Acth (entire human ACTH: 39 aa)

$C_{136}H_{210}N_{40}O_{31}S$ MW 2931.58



H01AA02

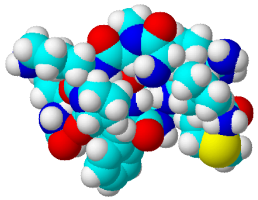
H SYSTEMIC HORMONAL PREPARATIONS, EXCL. SEX HORMONES AND INSULIN

H01 PITUITARY AND HYPOTHALAMIC HORMONES AND ANALOGUES

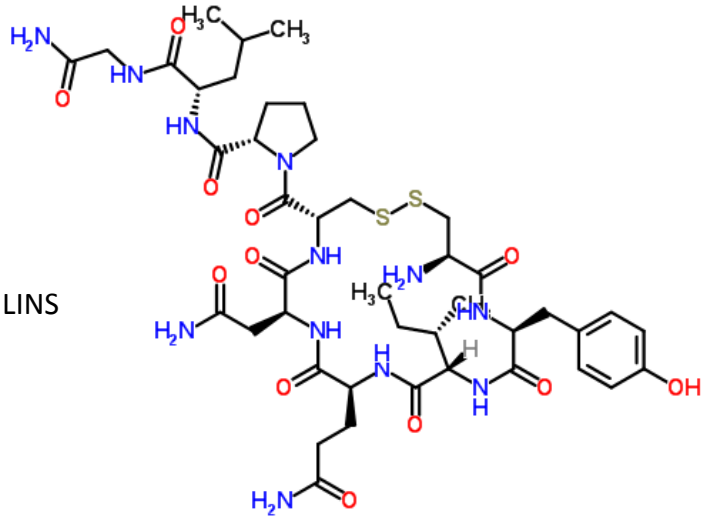
H01A ANTERIOR PITUITARY LOBE HORMONES AND ANALOGUES

H01AA ACTH

Peptides



Other hypophysis hormones:



OXYTOCIN, nonapeptide

$C_{43}H_{66}N_{12}O_{12}S_2$ MW 1007.19

H01BB02

H SYSTEMIC HORMONAL PREPARATIONS, EXCL. SEX HORMONES AND INSULINS

H01 PITUITARY AND HYPOTHALAMIC HORMONES AND ANALOGUES

H01B POSTERIOR PITUITARY LOBE HORMONES

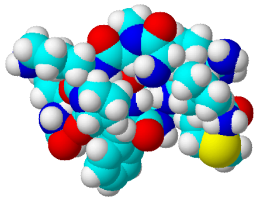
H01BB Oxytocin and analogues

VASOPRESSIN, [Phe-3, Arg-8]-oxytocin

H01BA01

Antidiuretic effect

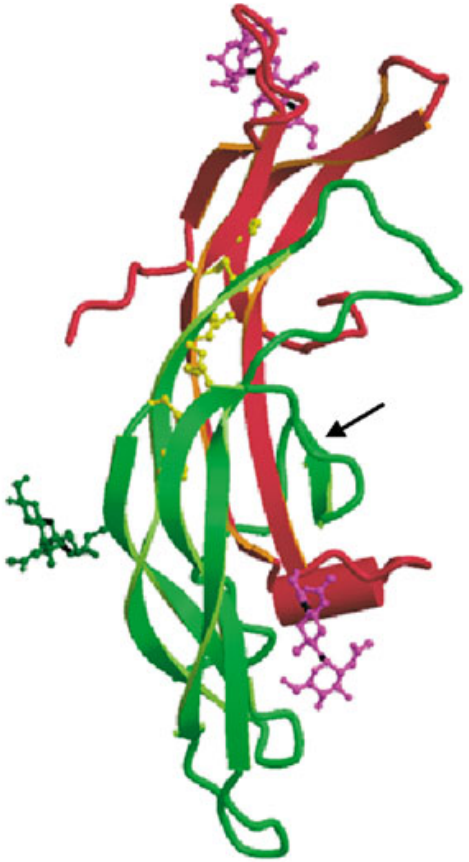
Peptides



Placenta hormones:

h CHORIONIC GONADOTROPIN, α and β subunits. Stimulates testosterone production in males and ovulation in females. Glycoprotein.

244 aa MW 36000



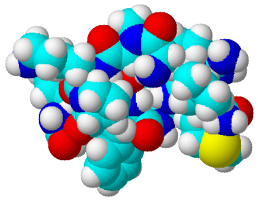
G03GA01 G GENITO URINARY SYSTEM AND SEX HORMONES

G03 SEX HORMONES AND MODULATORS OF THE GENITAL SYSTEM

G03G GONADOTROPINS AND OTHER OVULATION STIMULANTS

G03GA Gonadotropins

Peptides



Erythropoiesis-stimulating agents:

ERYTHROPOIETIN, glycoprotein

165 aminoacids, MW 30.4 kDa

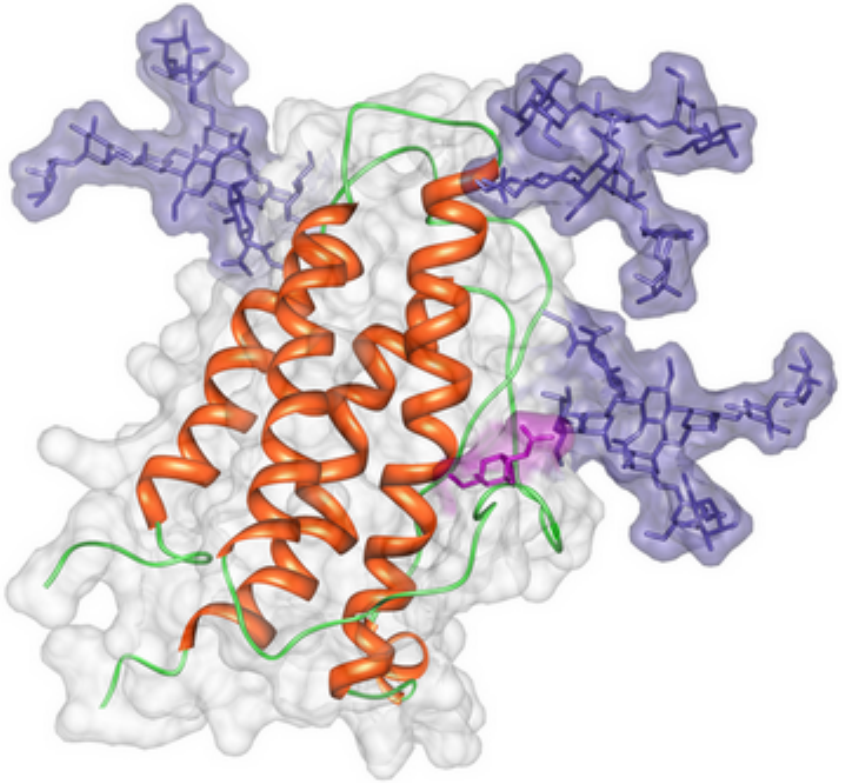
B03XA01

B BLOOD AND BLOOD FORMING ORGANS

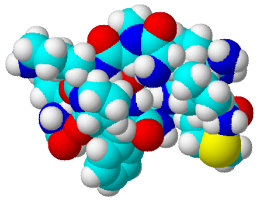
B03 ANTIANEMIC PREPARATIONS

B03X OTHER ANTIANEMIC PREPARATIONS

B03XA Other antianemic preparations



Peptides

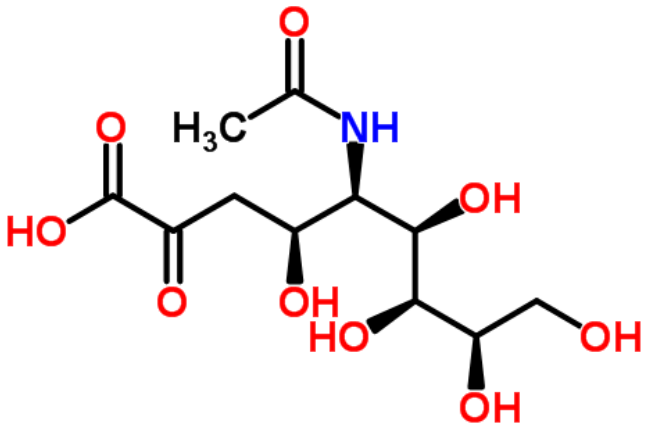


Erythropoiesis-stimulating agents:

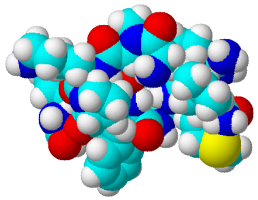
ERYTHROPOIETIN, 40% carbohydrates. N-glycosylation sites: 24, 38, 83 Asn. O-glycosylation site: 126 Ser. Two disulfide bonds: 7 to 161, 29 to 33 Cys. ***Sialic acid***:

Four helical bundles:

- **A)** residues 8-26
- **B)** residues 56-83
- **C)** residues 90-112
- **D)** residues 137-161

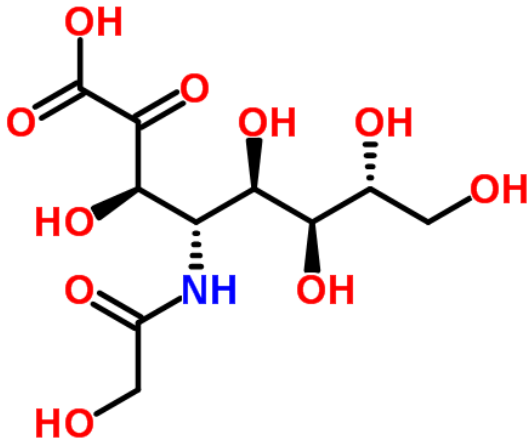


Peptides



Erythropoiesis-stimulating agents:

EPOETIN alfa, first rhEPO, epo- :identical primary sequence. Greek letters: different degree of glycosylation. Chinese-hamster-ovary cell lines used for transfection: production of ***N-glycolylneuraminic acid***, which humans cannot synthetize:

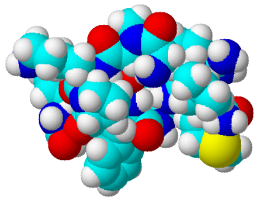


EPOETIN beta, EPOETIN omega: : higher and higher amount of basic isoforms.

EPOETIN delta (Dynepo): produced in human fibrosarcoma cell lines: no NGNA.

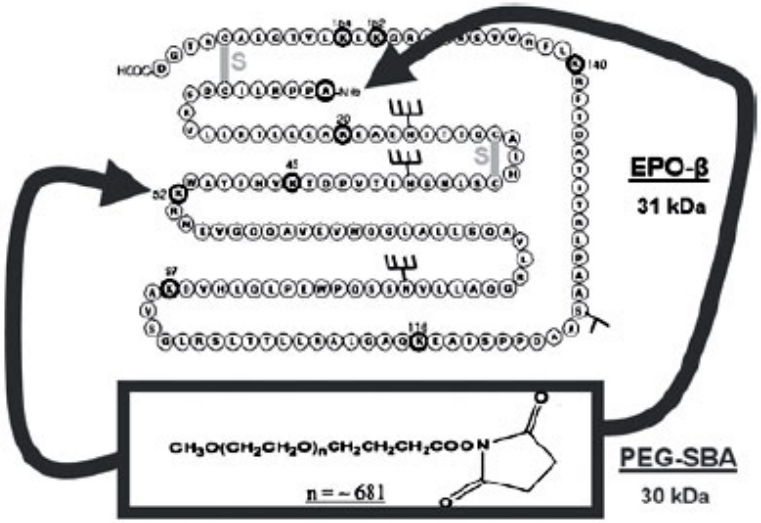
DARBEPOETIN alfa (Nespo): 5 different aminoacids and additional glycosilation sites.

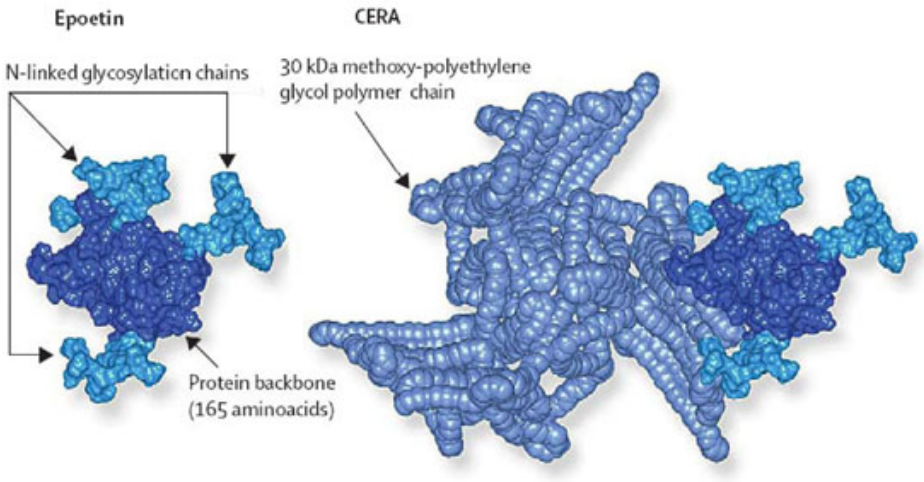
Peptides



Erythropoiesis-stimulating agents:

CERA (Continuos Erythropoietin Receptor Activator): PEGylated form of EPOETIN beta on terminal Ala or on ε—amino group of Lys52 and Lys45. 60 kDa.





(Peptides)

Plasma substitutes

Cristalloids: electrolytic aqueous solutions, without proteins

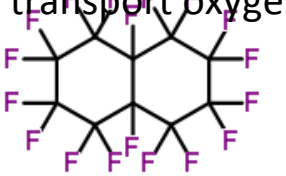
Proteic colloids: plasma protein fraction PPF (from human blood)

Non-proteic colloids: **DEXTRANS, HEStarch (WADA S5)**

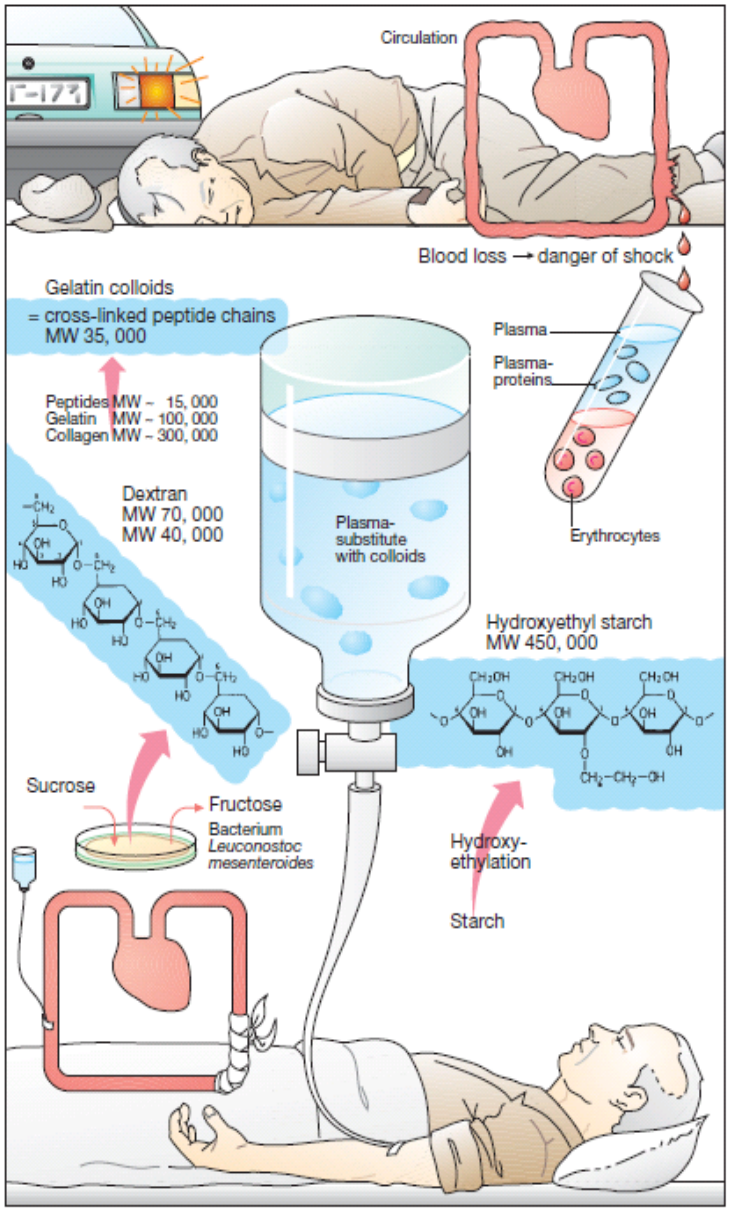
Artificial blood:

Perfluorocarbons: capable to transport oxygen

PERFLUORODECALINE

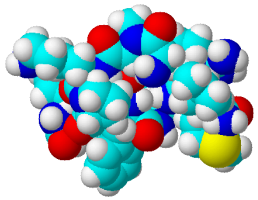


Genetically-modified Hemoglobins



A. Plasma substitutes

Peptides, WADA S4



Peptides / related compounds:

S4. HORMONE AND METABOLIC MODULATORS

The following are prohibited:

1. **Aromatase inhibitors** including, but not limited to: **aminoglutethimide, anastrozole, androsta-1,4,6-triene-3,17-dione (androstatrienedione), 4-androstene-3,6,17 trione (6-oxo), exemestane, formestane, letrozole, testolactone.**

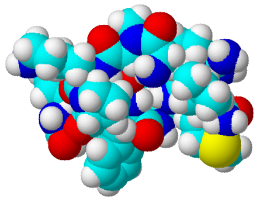
2. **Selective estrogen receptor modulators (SERMs)** including, but not limited to: **raloxifene, tamoxifen, toremifene.**

3. **Other anti-estrogenic substances** including, but not limited to: **clomiphene, cyclofenil, fulvestrant.**

4. **Agents modifying myostatin function(s)** including, but not limited, to: **myostatin inhibitors.**

5. **Metabolic modulators:**
   a) Insulins
   b) Peroxisome Proliferator Activated Receptor δ (PPARδ) agonists (e.g. GW 1516), PPARδ-AMP-activated protein kinase (AMPK) axis agonists (e.g. AICAR)

Peptides, WADA S4.5

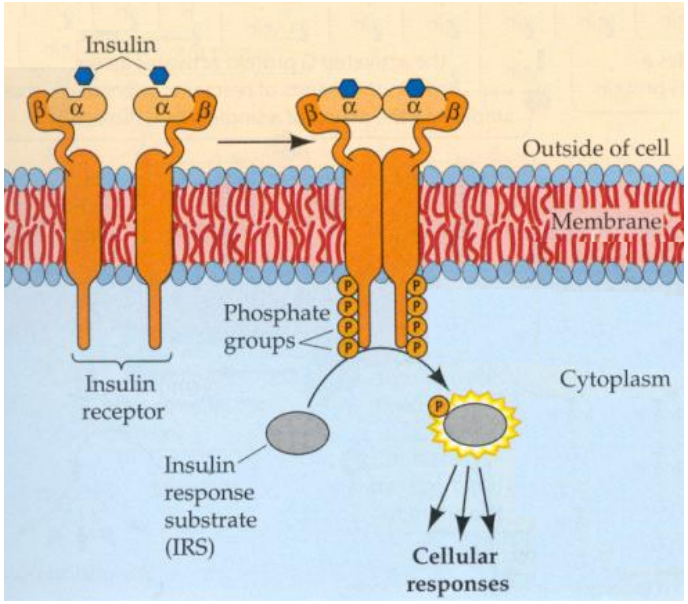


Pancreas hormones:

Insulin (β-cells)

Induces glycogen formation, protein biosynthesis and inhibits protein breakdown.

Insulin bonding results in intramolecular autophosphorylation of tyr residues on β subunits: tyr-kinase activation.



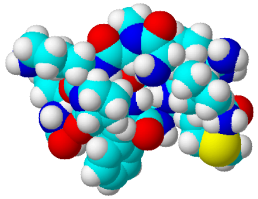
***Insulin receptor*:** glycoproteic receptor with 2 α extracellular subunits and 2 β transmembrane subunits.

Glucagon (α-cells): stimulates glycogenolysis and gluconeogenesis

Somatostatin (δ-cells): inhibits insulin and glucagon secretion

Others: Amylin; Glucagon-like peptide 1 (GLP-1); Adiponectine: food assumption/ obesity

Peptides, WADA S4.5



Pancreatic hormones:

Insulin:

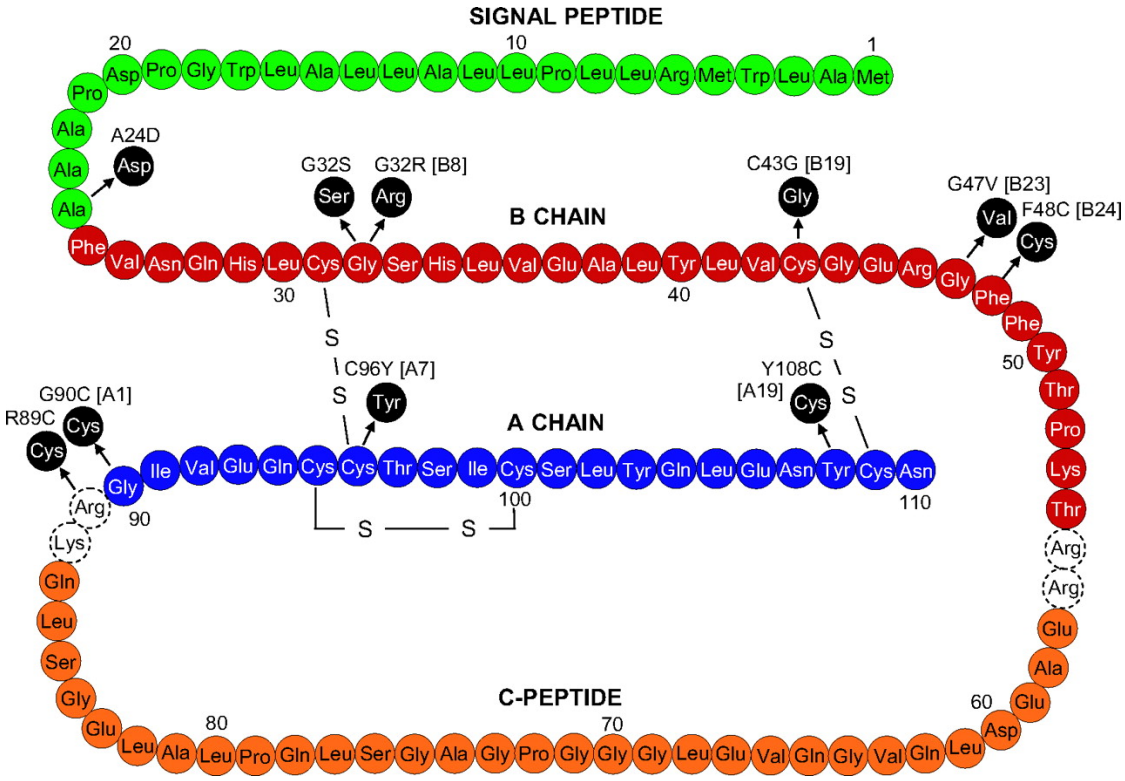
51 aa peptide. A-chain (21) / B-chain (30). 3 S-S bridges (1 intra-chain). Formed from proinsulin *via* PC2 and PC3 calcium-dependent endopeptidases: 4 basic aa and C-peptide removed.

Bovine/Porcine Insulin: ‘50s

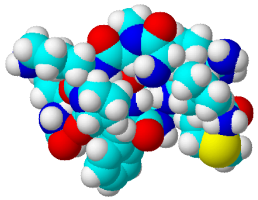
Total synthesis: 1963

Recombinant DNA: 1978

Main metabolites: DesB30, DesB24-30, DesB25-30



Peptides, WADA S4.5



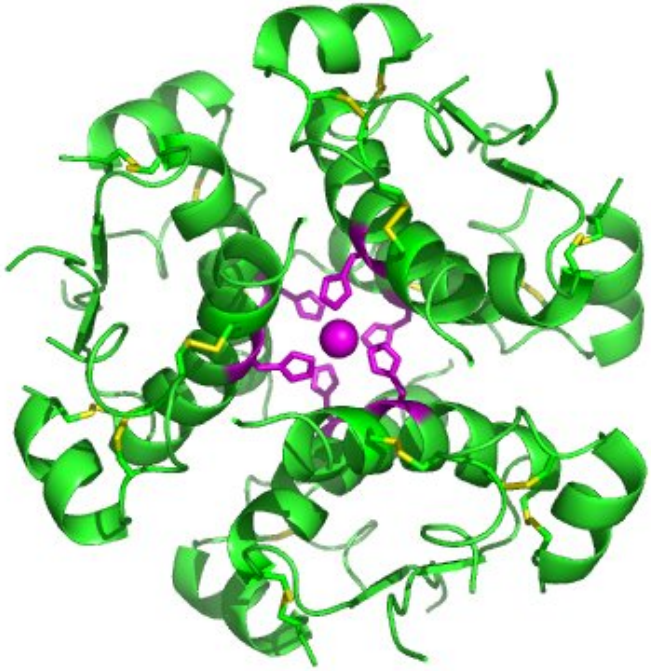
Pancreatic hormones:

Insulin structure in solution:

A-chain: 2 α-helices. B-chain: 1 α-helix / 1 β-turn (hydrophobic residues in the internal folding): hydrosolubility and stability.

Interaction with receptor: monomeric form (stable at concentration < 0.1 µM).

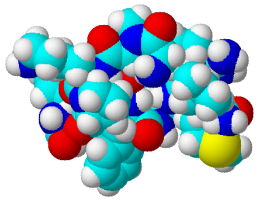
It forms hexameric aggregates with Zn^{2+} ions, at higher concentration and neutral pH value.



Hyperglycemia: glucose > 126-240 mg/dL (7-13.5 mM)

Hypoglycemia: glucose < 72 mg/dL (4 mM)

Peptides, WADA S4.5



Metabolic modulators:

INSULIN

Produced with rDNA (*Escherichia coli*).

Insulin analogues:

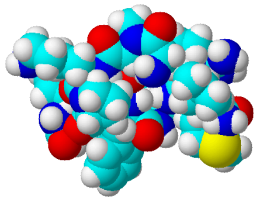
LISPRO: the penultimate lysine and proline residues on the C-terminal end of the B-chain are reversed: *rapid action.*

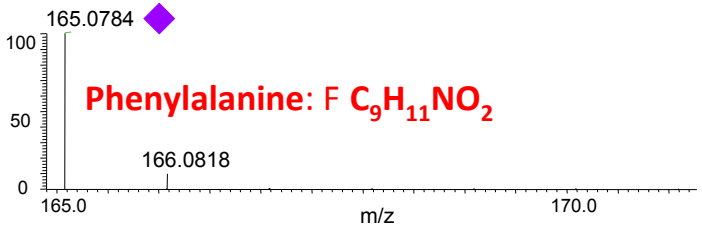
GLARGINE: A21 Asn replaced by Gly. 2 Arg added to B-chain. *22 h action*.

Name	**HUMAN INSULIN**
Structure	 GIVEQCCTSICSLYQLENYCN (A chain) FVNQHLCGSHLVEALYLVCGERGFFYTPKT (B chain)
Systematic name	--
Formula	$C_{257}H_{383}N_{65}O_{77}S_6$
MW	5808
Monoisotopic mass	5803.671
Mp	229°C
H bond acceptors	142
H bond donors	28
Acid p*Ka*	10: 6 COOH (2 term + 4 Glu), 4 Ph-OH
Basic p*ka*	6: 3 NH_2 (2 term + 1 Lys), 1 Arg, 2 His
ACD Log D pH 5.5	--
ACD Log D pH 7.4	1.76
Solubility	Water, Dilute acids (20 mg/mL in 0.01M HCl), Aqueous alkali
LD50	40 mg/Kg rat s.c.
Therapeutic cat	antidiabetic
ATC **A10AB01** **A10AC01** **A10AD01** **A10AE01** **A10AF01**	**A10AB01** A ALIMENTARY TRACT AND METABOLISM A10 DRUGS USED IN DIABETES A10A INSULINS AND ANALOGUES A10AB Insulins and analogues for injection, fast-acting
Receptors	insulin receptor
Notes	endogenous hormone

Nomi commerciali (IT)	
ACTRAPHANE, HUMULIN	A, RR, iniettabile

Peptides, WADA S4.5





Phenylalanine: F $C_9H_{11}NO_2$

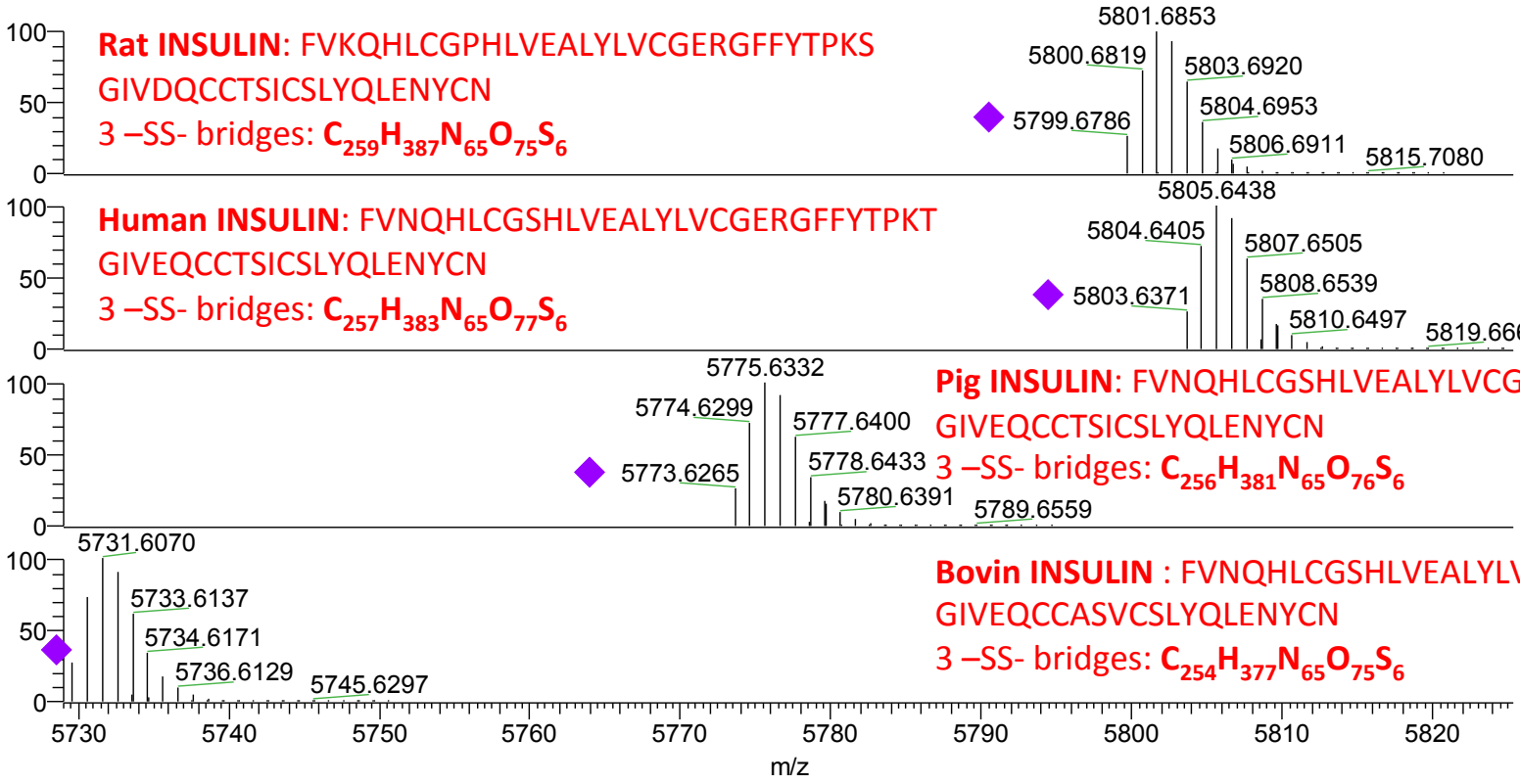
monoisotopic masses ◆

Rat INSULIN: FVKQHLCGPHLVEALYLVCGERGFFYTPKS
GIVDQCCTSICSLYQLENYCN
3 –SS- bridges: $C_{259}H_{387}N_{65}O_{75}S_6$

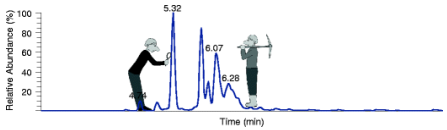
Human INSULIN: FVNQHLCGSHLVEALYLVCGERGFFYTPKT
GIVEQCCTSICSLYQLENYCN
3 –SS- bridges: $C_{257}H_{383}N_{65}O_{77}S_6$

Pig INSULIN: FVNQHLCGSHLVEALYLVCGERGFFYTPKA
GIVEQCCTSICSLYQLENYCN
3 –SS- bridges: $C_{256}H_{381}N_{65}O_{76}S_6$

Bovin INSULIN : FVNQHLCGSHLVEALYLVCGERGFFYTPKA
GIVEQCCASVCSLYQLENYCN
3 –SS- bridges: $C_{254}H_{377}N_{65}O_{75}S_6$



Metabolic modulators, not in WADA S4.5



Oral hypoglycemics:

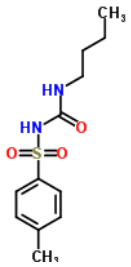
Sulfonylureas:

SAR: (huge) aliphatic chain on urea N. pK_A value 5.

Mechanism: action on ATP-dependent K^+-channels with sulfonylurea receptors (SUR).

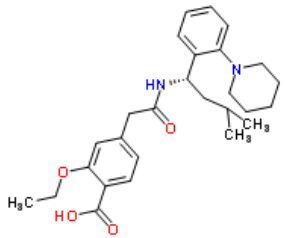
GLYBURIDE / GLIBENCLAMIDE

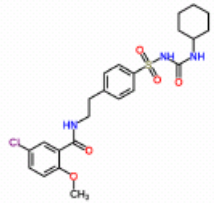
TOLBUTAMIDE



Meglitinides:

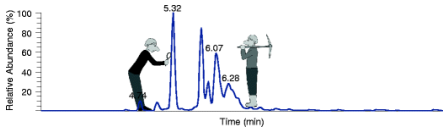
REPAGLINIDE



Name	**GLYBURIDE**
Structure	
Systematic name	5-chloro-N-(2-{4-[(cyclohexylcarbamoyl)sulfamoyl]phenyl}ethyl)-2-methoxybenzamide
Formula	$C_{23}H_{28}ClN_3O_5S$
MW	494.004
Monoisotopic mass	493.143819418
Mp	171-174°C
H bond acceptors	8
H bond donors	3
Acid p*Ka*	4.32 (sulfonylamide), 13.72 (carboxamide)
Basic p*ka*	--
ACD Log D pH 5.5	2.58
ACD Log D pH 7.4	1.79
Solubility	DMSO, ethanol, basic water
LD50	3250 mg/Kg mouse p.o.
Therapeutic cat	antidiabetic
ATC	**A10BB01** A ALIMENTARY TRACT AND METABOLISM A10 DRUGS USED IN DIABETES A10B BLOOD GLUCOSE LOWERING DRUGS, EXCL. INSULINS A10BB Sulfonamides, urea derivatives
Receptors	SUR on ATP-dependent K-channels

Nomi commerciali (IT)	
DAONIL, EUGLUCON, GLIBEN, GLIBORAL	A, RR, compresse

Metabolic modulators, WADA S4.5



PPARδ agonists:

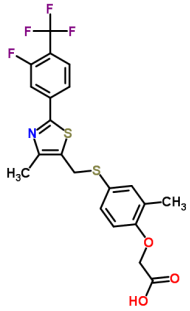
Methylthiazoles:

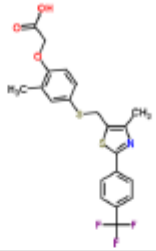
Peroxisome proliferator-activated receptor is a nuclear receptor.

Endurobol is carcinogenic in rats: stop to GSK research.

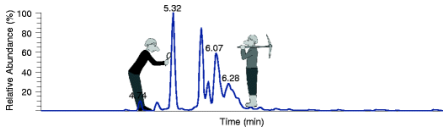
It increases fatty acid metabolism in skeletal muscle and protect against diet-induced obesity and type II diabetes.

GW0742:



Name	ENDUROBOL (GW501516)
Structure	
Systematic name	{2-Methyl-4-[({4-methyl-2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-5-yl}methyl)sulfanyl]phenoxy}acetic acid
Formula	$C_{21}H_{18}F_3NO_3S_2$
MW	453.4977
Monoisotopic mass	453.0680
Mp	134-136°C
H bond acceptors	4
H bond donors	1
Acid pK*a*	3.5 (carboxyl)
Basic pk*a*	2.1 (thiazol)
ACD Log D pH 5.5	3.98
ACD Log D pH 7.4	2.67
Solubility	Soluble in water (slightly), DMSO (50 mg/ml), DMF (25 mg/ml), 100% ethanol (12 mg/ml), and methanol (50 mg/ml).
LD50	Oral, rat: 980 mg/kg
Therapeutic cat	"ergogenic performance enhancing"
ATC	Investigational new drug
Receptors	PPARδ agonist

Metabolic modulators, WADA S4.5

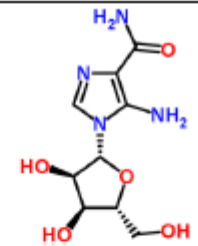


PPARδ AMPK axis agonists:

Aminoimidazoles:

It is used in the treatment of ischemic heart diseases.

It stimulates glucose uptake and it is used in combination with GW01516

Name	**ACADESINE (AICAR)**
Structure	
Systematic name	5-Amino-1-(β-D-ribofuranosyl)-1H-imidazole-4-carboxamide
Formula	$C_9H_{14}CN_4O_5$
MW	258.2313
Monoisotopic mass	258.0964
Mp	214-215°C
H bond acceptors	9
H bond donors	7
Acid pK*a*	--
Basic pk*a*	4.8 (imidazol)
ACD Log D pH 5.5	-2.93
ACD Log D pH 7.4	-2.93
Solubility	Soluble to 75 mM in water and to 75 mM in DMSO
LD50	980 mg/Kg, oral, rat.
Therapeutic cat	AMP-activated protein kinase activator
ATC	**C01EB13** C CARDIOVASCULAR SYSTEM C01 CARDIAC THERAPY C01E OTHER CARDIAC PREPARATIONS C01EB Other cardiac preparations
Receptors	AMP-activated protein kinase agonist

Metabolic modulators, WADA S4.4

Myostatin inhibitors

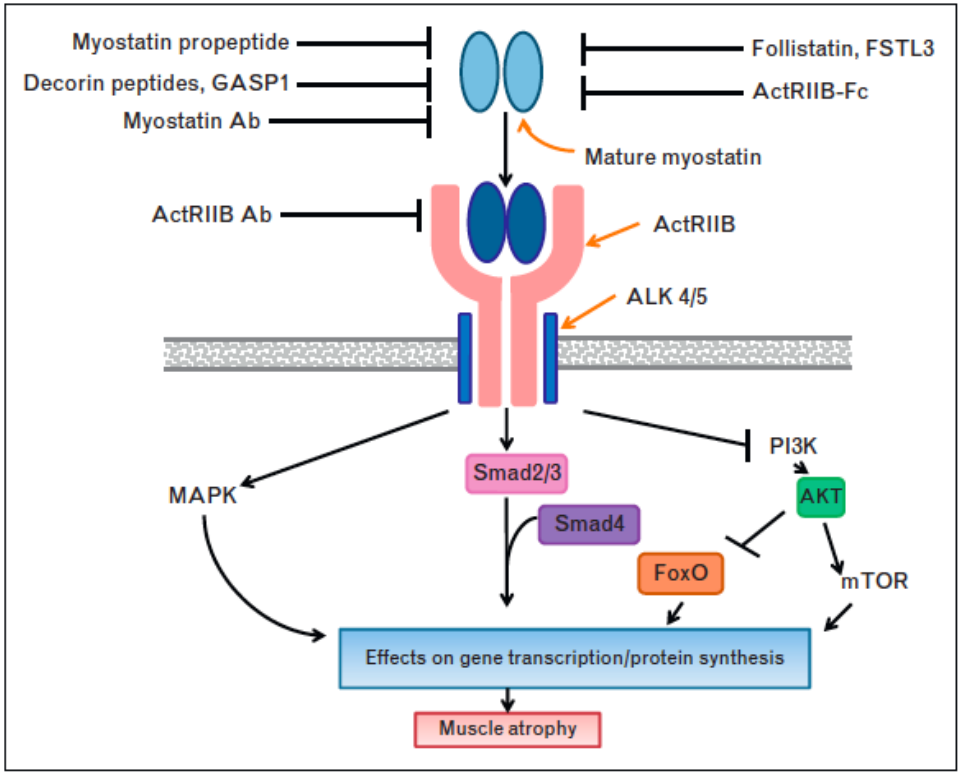


FIGURE 1. Summary of therapeutic invention points in the myostatin signaling pathway. Myostatin binds to its receptor complex ActRIIB/Alk 4 or 5 on skeletal muscle resulting in activation of the Smad 2/3, mitogen-activated protein kinase and inhibition of the PI3K intracellular signaling pathways that together result in gene transcriptional changes and effects on protein synthesis that ultimately give rise to muscle atrophy. Myostatin pathway inhibitors act extracellularly by either binding myostatin directly (Fstl3, Follistatin, myostatin antibody, GASP1, myostatin propeptide, decorin peptides, ActRIIB-Fc) or by binding its receptor complex (ActRIIB antibody) in order to block myostatin engaging its receptor complex and activating downstream signaling. Some of the inhibitors are naturally occurring (myostatin propeptide, Gasp1, follistatin, Fstl3) whereas others are engineered (myostatin antibody, ActRIIB antibody, ActRIIB-Fc). —I represent inhibitory activities. → represent activating activities. Ab = antibody.