Approaches of Classical Medicinal Chemistry

Optimizing Drug Properties
Lead-to-Drug Design

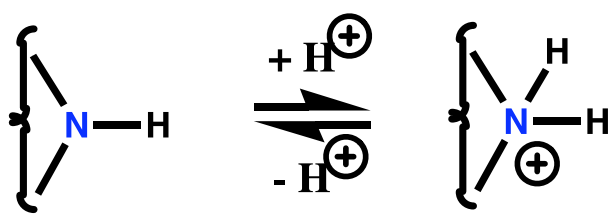
Optimizing Pharmacokinetics

Aims

- To improve pharmacokinetic properties of lead compound
- To optimize chemical and metabolic stability (stomach acids / digestive enzymes / metabolic enzymes)
- To optimize hydrophilic / hydrophobic balance (solubility in blood / solubility in GIT / solubility through cell membranes / access to CNS / excretion rate)

Pharmacokinetics – drug design

- Drugs must be polar - to be soluble in aqueous conditions- to interact with molecular targets
- Drugs must be 'fatty' - to cross cell membrane - to avoid rapid excretion
- Drugs must have both hydrophilic and lipophilic characteristics
- Many drugs are weak bases with pK_a's 6-8



Crosses membranes

Receptor interaction & water solubility

Solubility and membrane permeability

Vary alkyl substituents

Rationale:

- Varying the size of alkyl groups varies the hydrophilic / hydrophobic balance of the structure
- Larger alkyl groups increase hydrophobicity

Disadvantage:

- May interfere with target binding for steric reasons

Methods:

- Often feasible to remove alkyl groups from heteroatoms and replace with different alkyl groups
- Usually difficult to remove alkyl groups from the carbon skeleton - full synthesis often required

'Masking' or removing polar groups

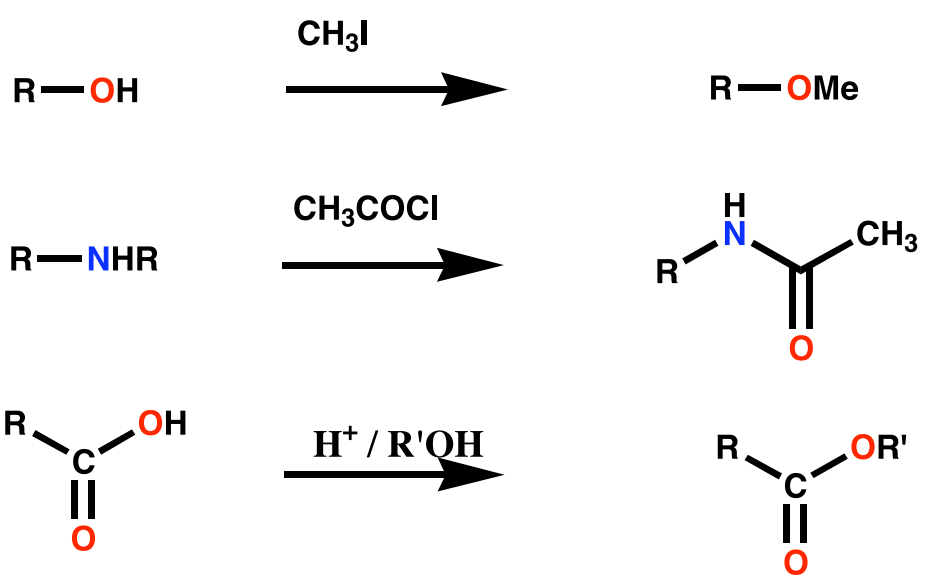
Rationale:

- Masking or removing polar groups decreases polarity and increases hydrophobic character

Disadvantages:

- Polar group may be involved in target binding
- Unnecessary polar groups are likely to have been removed already (simplification strategy)

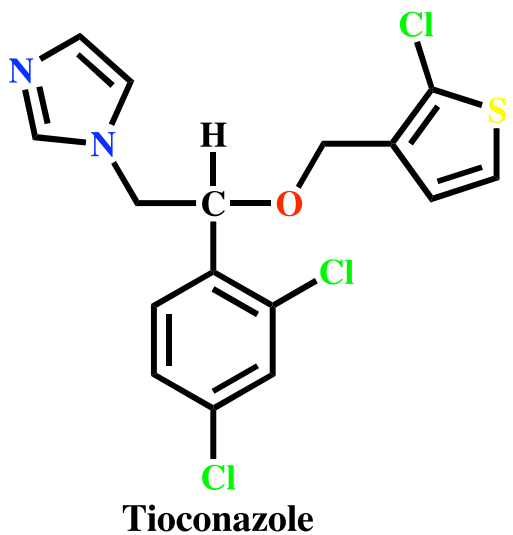
Methods:



Adding polar groups

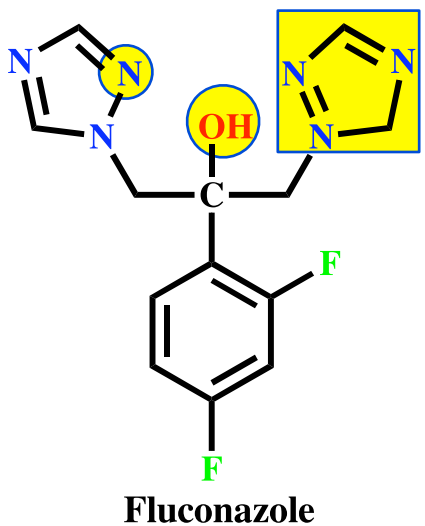
Rationale:

- Adding polar groups increases polarity and decreases hydrophobic character
- Useful for targeting drugs vs. gut infections
- Useful for reducing CNS side effects



Tioconazole

Antifungal agent with poor solubility - skin infections only



Fluconazole

Systemic antifungal agent improved blood solubility

Disadvantage:

- May introduce unwanted side effects

Vary pK_a

Rationale:

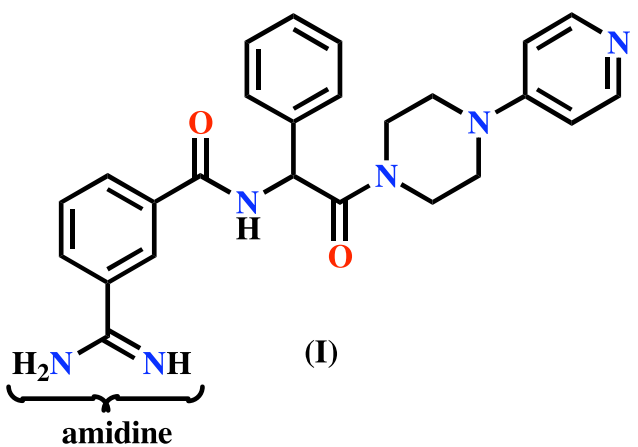
- Varying pK_a alters percentage of drug which is ionized
- Alter pK_a to obtain required ratio of ionized to unionized drug

Method:

- Vary alkyl substituents on amine nitrogens
- Vary aryl substituents to influence aromatic amines or aromatic carboxylic acids

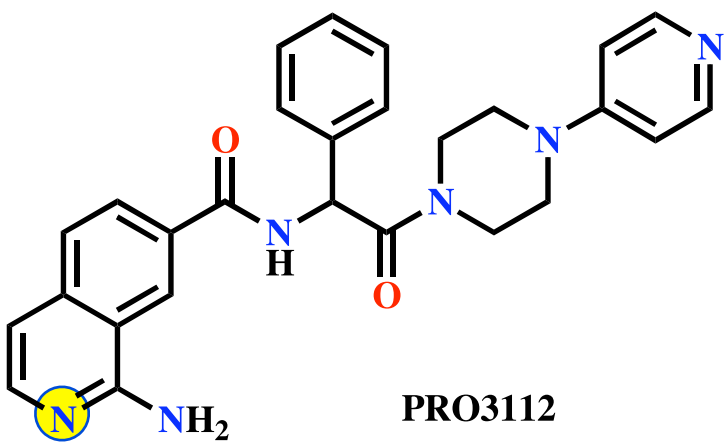
Disadvantage:

- May affect binding interactions



(I)

Antithrombotic
but too basic



PRO3112

Decreased basicity
N locked into heterocycle

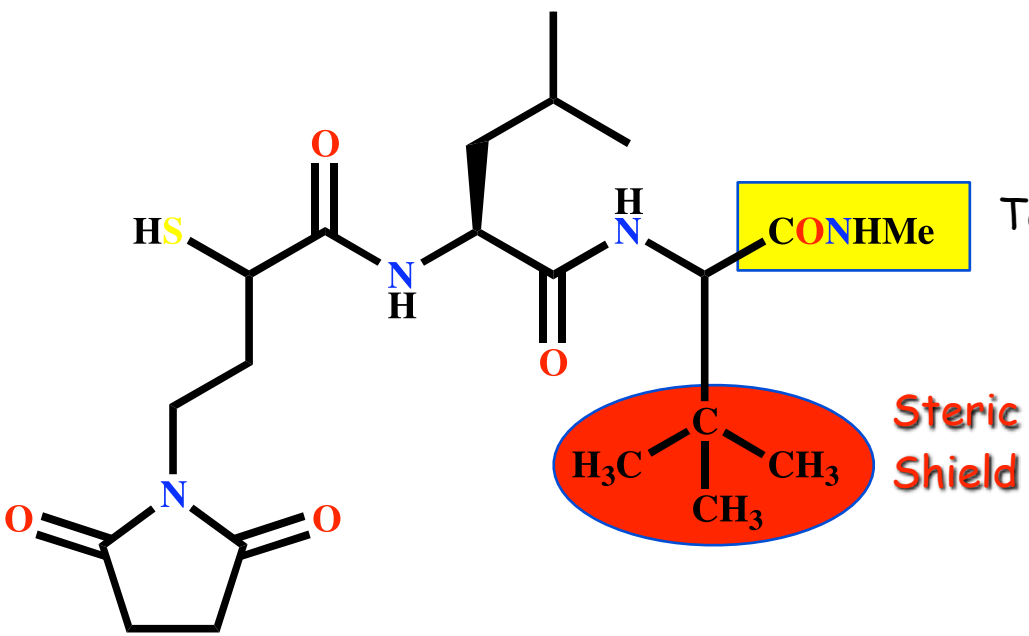
Metabolic Drug Stability

Steric Shields

Rationale:

- Used to increase chemical and metabolic stability
- Introduce bulky group as a shield
- Protects a susceptible functional group (e.g. ester) from hydrolysis
- Hinders attack by nucleophiles or enzymes

Antirheumatic agent
D1927

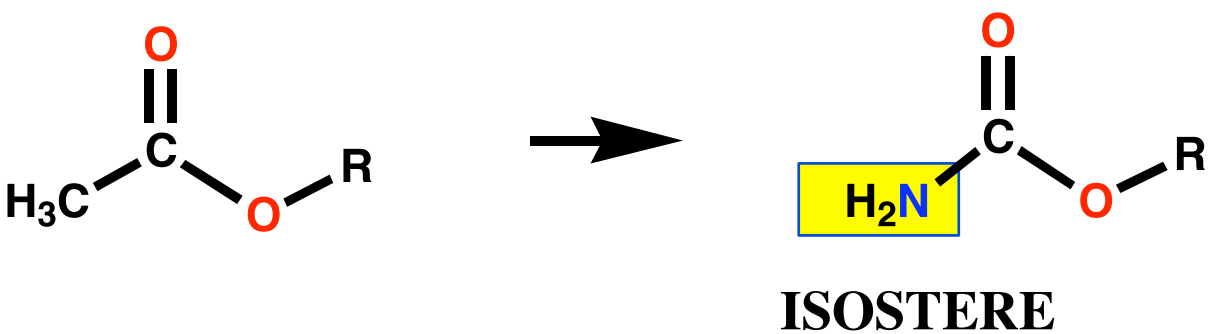


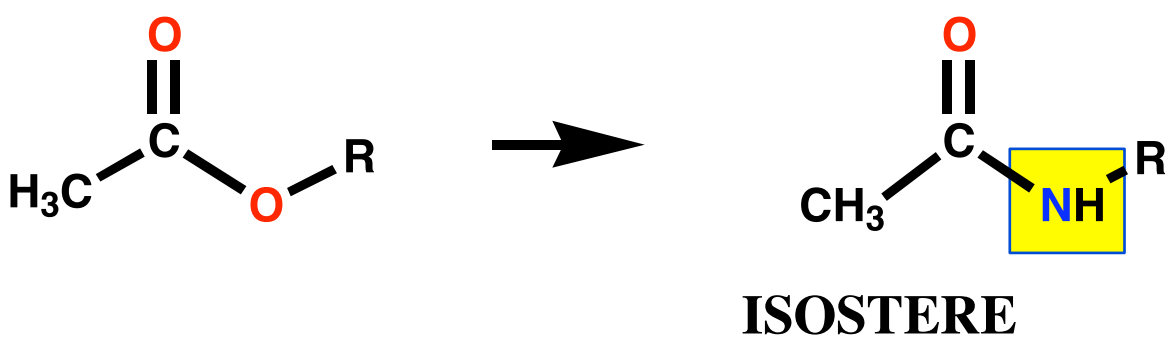
Blocks hydrolysis of terminal amide

'Electronic shielding' of NH_2

Rationale:

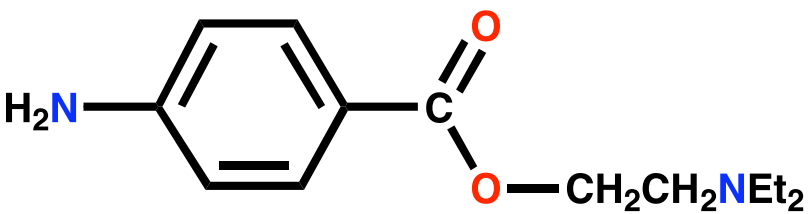
- Used to stabilize labile functional groups (e.g. esters)
- Replace labile ester with more stable urethane or amide
- Nitrogen feeds electrons into carbonyl group and makes it less reactive
- Increases chemical and metabolic stability





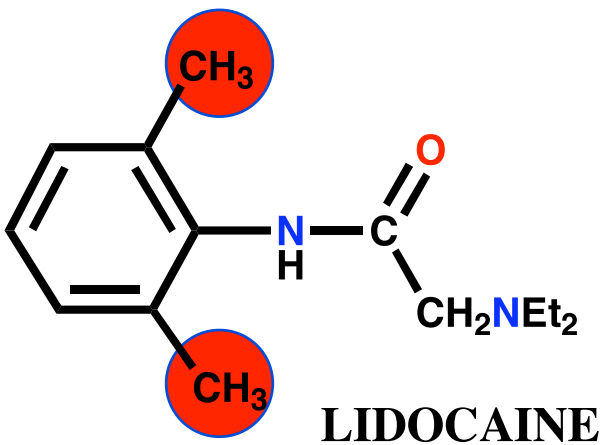
Stereoelectronic Effects

- Steric and electronic effects used in combination
- Increases chemical and metabolic stability



PROCAINE

Local anaesthetic
(short duration)

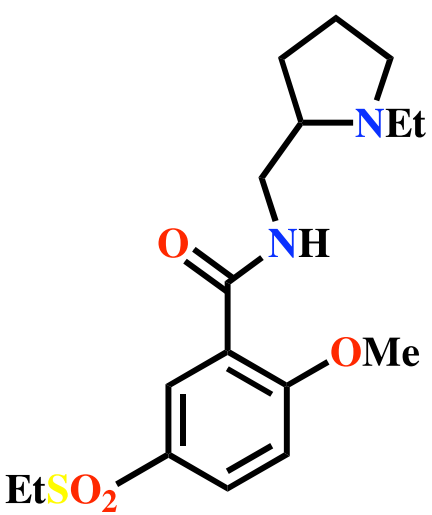


LIDOCAINE

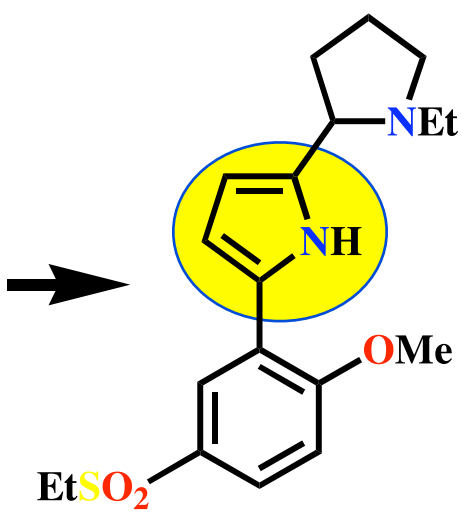
ortho methyl groups act as steric shields &
hinder hydrolysis by esterases
Amide more stable than ester
(electronic effect)

Bio-Isosteres

- Replace susceptible group with a different group without affecting activity
- Bio-isostere shows improved pharmacokinetic properties
- Bio-isosteres are not necessarily isosteres



Sultopride

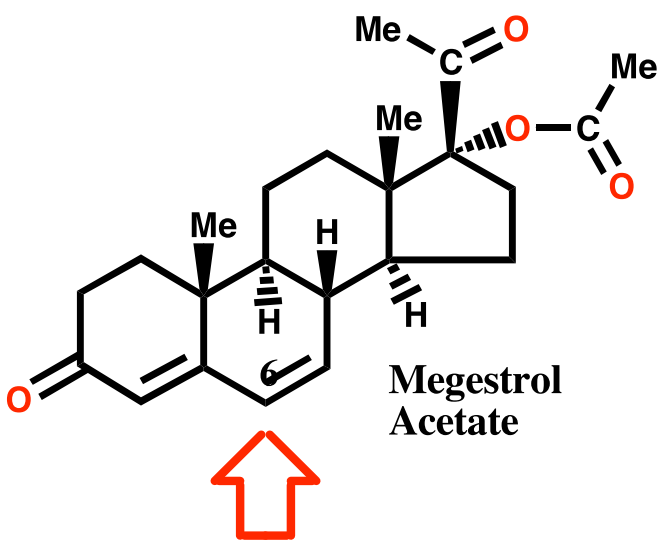


Du122290

Pyrrole ring =
bioisostere for amide

Metabolic Blockers

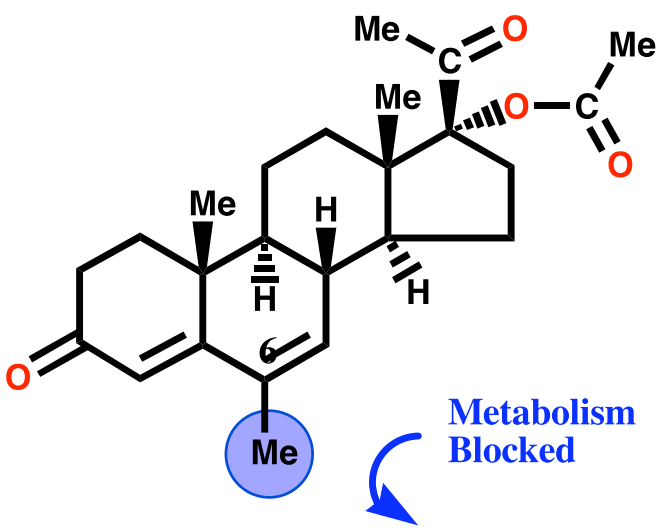
- Metabolism of drugs usually occur at specific sites. Introduce groups at a susceptible site to block the reaction
- Increases metabolic stability and drug lifetime



Megestrol Acetate

Metabolic Oxidation

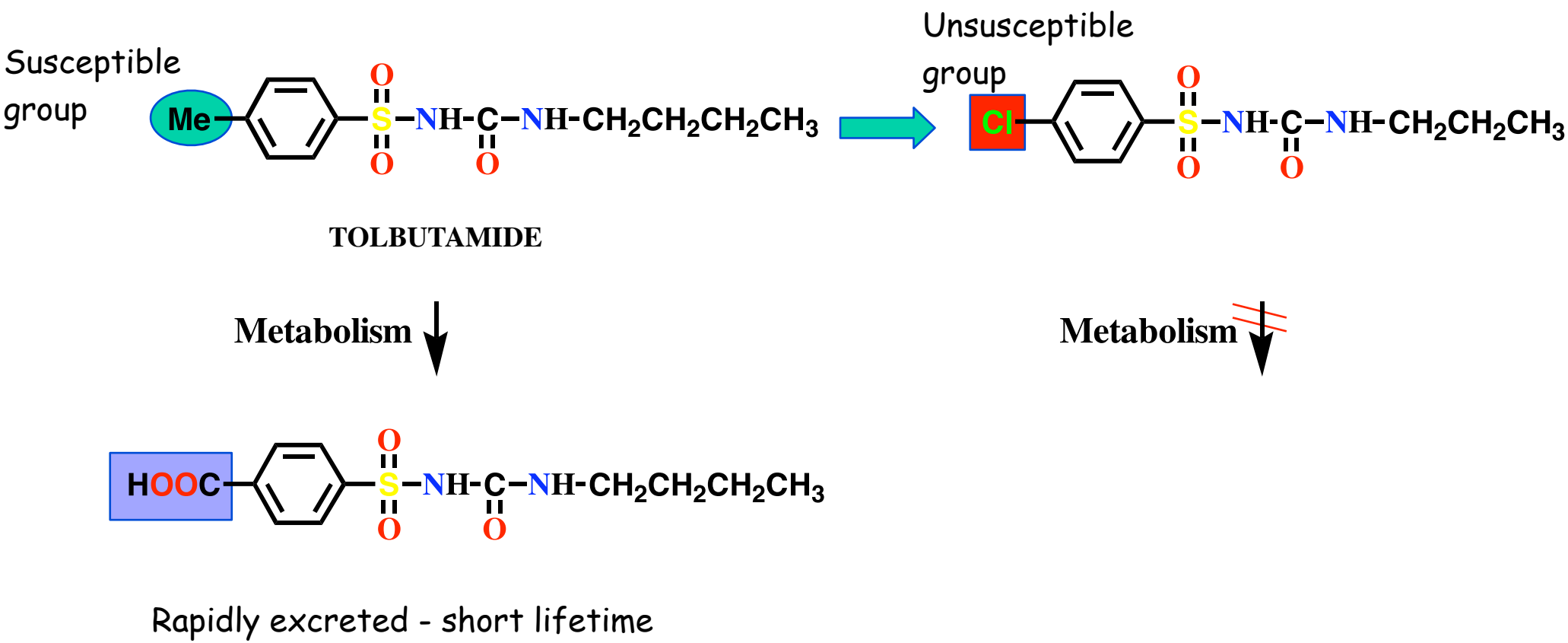
Oral contraceptive
- limited lifetime



Metabolism Blocked

Remove / replace susceptible metabolic groups

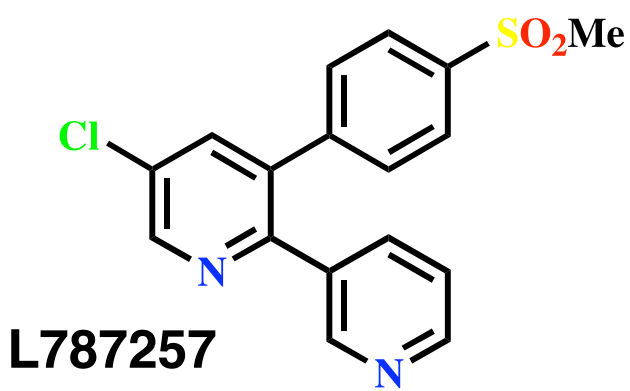
- Metabolism of drugs usually occurs at specific groups.
- Remove susceptible group or replace it with metabolically stable group [e.g. modification of tolbutamide (antibiotic)]



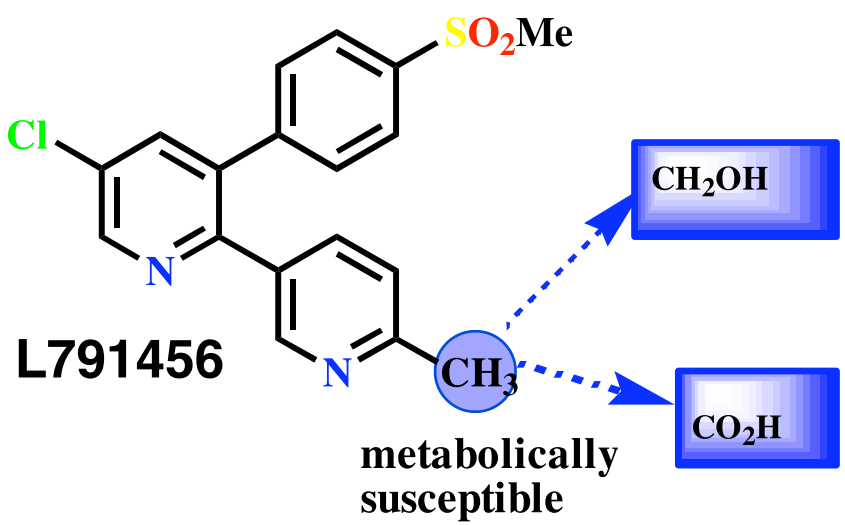
Introducing susceptible metabolic groups

- Used to decrease metabolic stability and drug lifetime
- Used for drugs which 'linger' too long in the body and cause side effects
- Add groups known to be susceptible to Phase I or Phase II metabolic reactions

Anti-arthritic agents



L787257



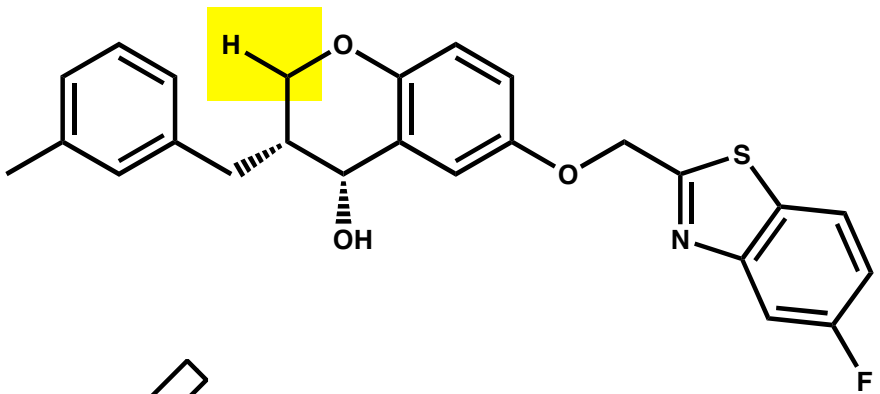
L791456

metabolically susceptible

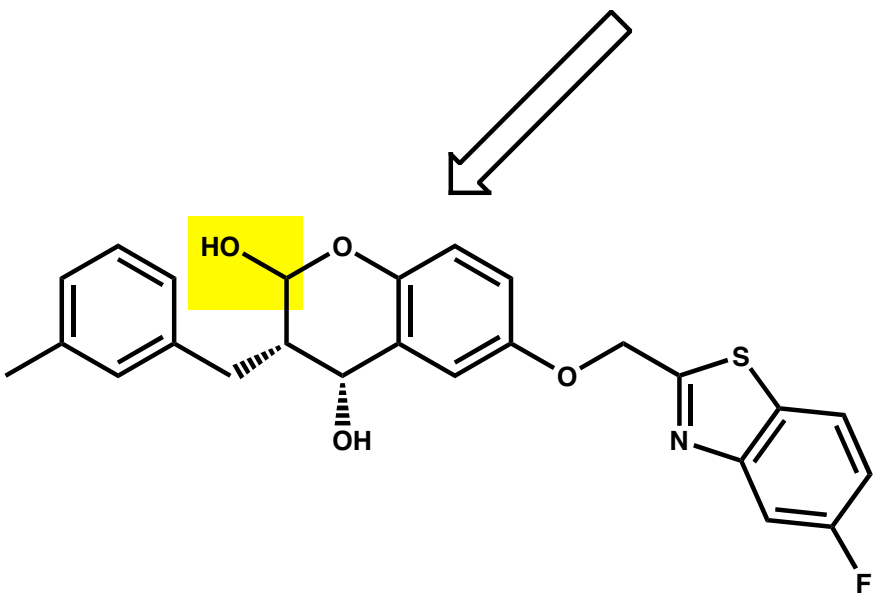
Minimizing toxicity

- stabilize compounds against metabolic activation
    - produce various isosteres
    - metabolic blocking/steric hinderance
    - modulation of compound electronics
- introduction of an alternative metabolic site
- alteration of the SAR at the activating enzyme

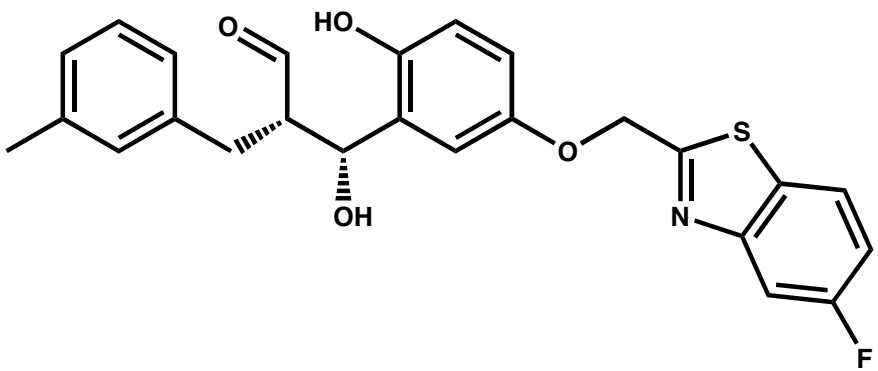
D.C. Evans, T.A. Baille, Curr. Oppin. Drug Disc. Develop. **8** (2005), 44-50



CP-85958 (Pfizer)
Liver-toxic in monkeys

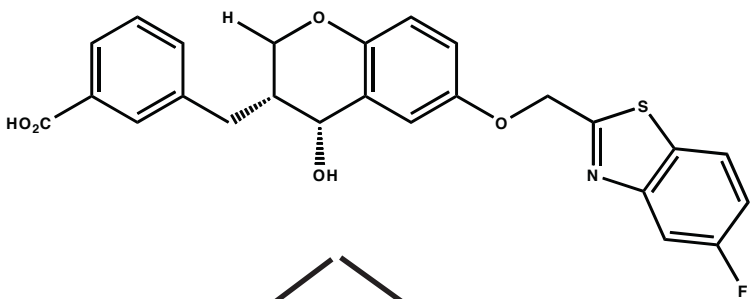


metabolized to the
corresponding lactol



ring-opening to produce
a very reactive, alkylating aldehyde

Optimization of metabolic properties in drug (lead) development



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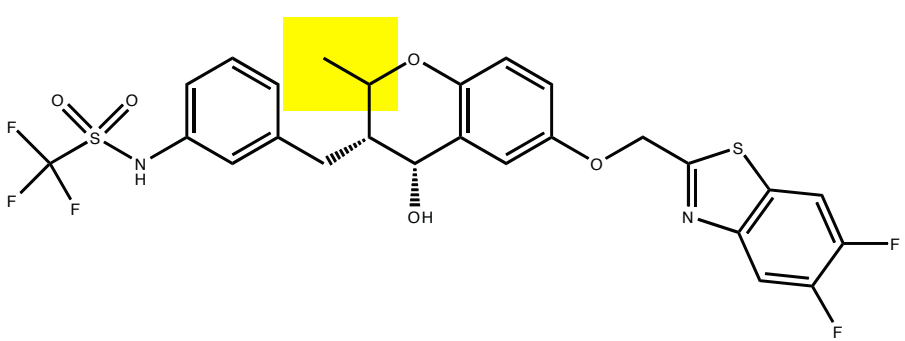
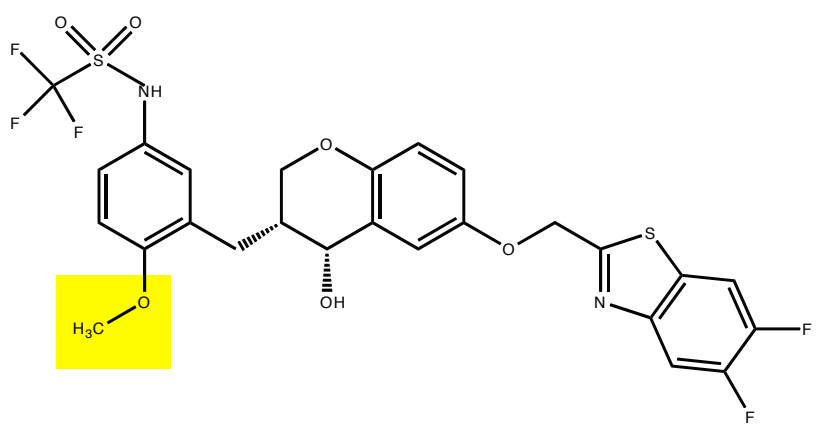


Metabolic stabilization
block lactol formation
replacement of the carboxylic acid

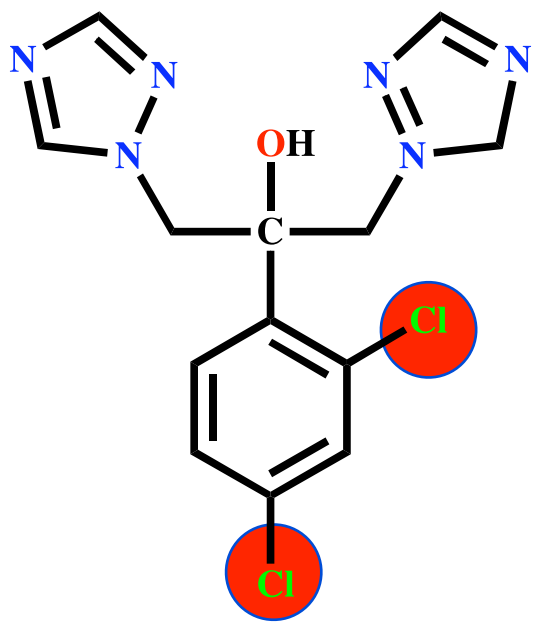


Increase metabolic lability at other site
major route: metabolic O-demethylation

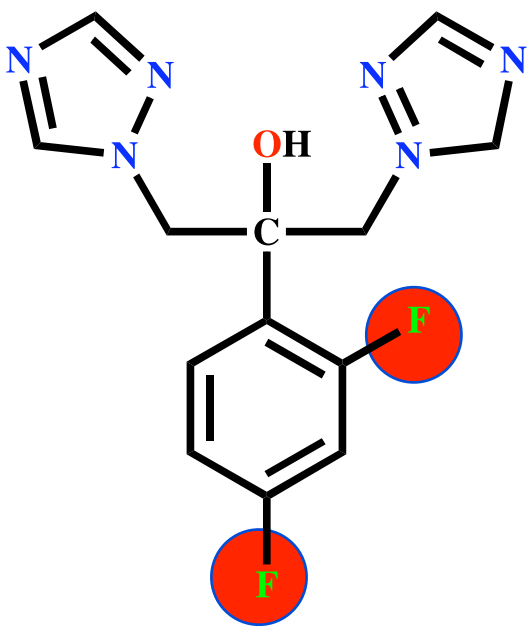
Reducing drug toxicity

Example - varying substituents

- Fluconazole (Diflucan) - antifungal agent



UK-47265

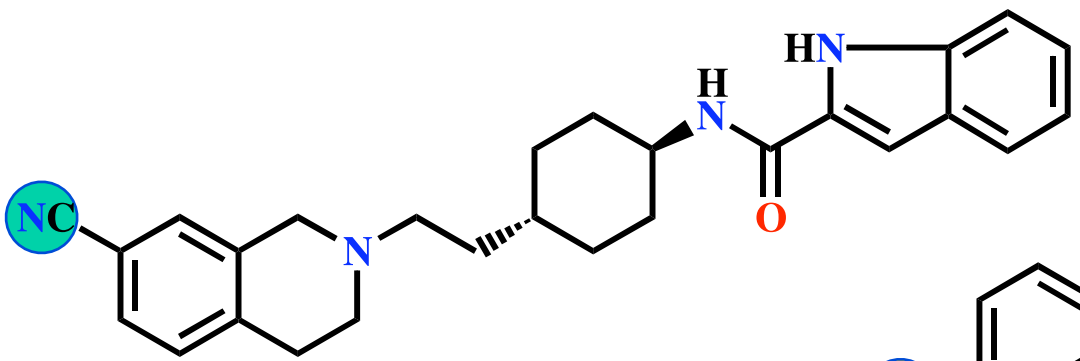


Fluconazole

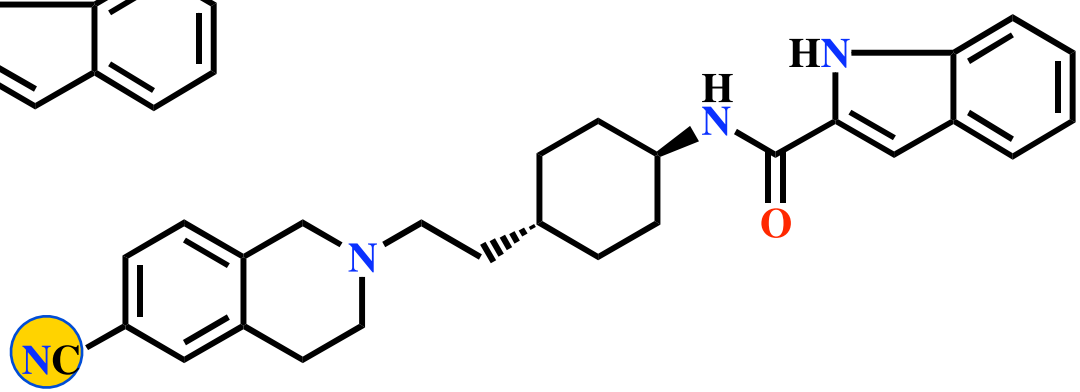
Substituents varied
Less toxic

Example - varying substituent position

- Dopamine antagonists

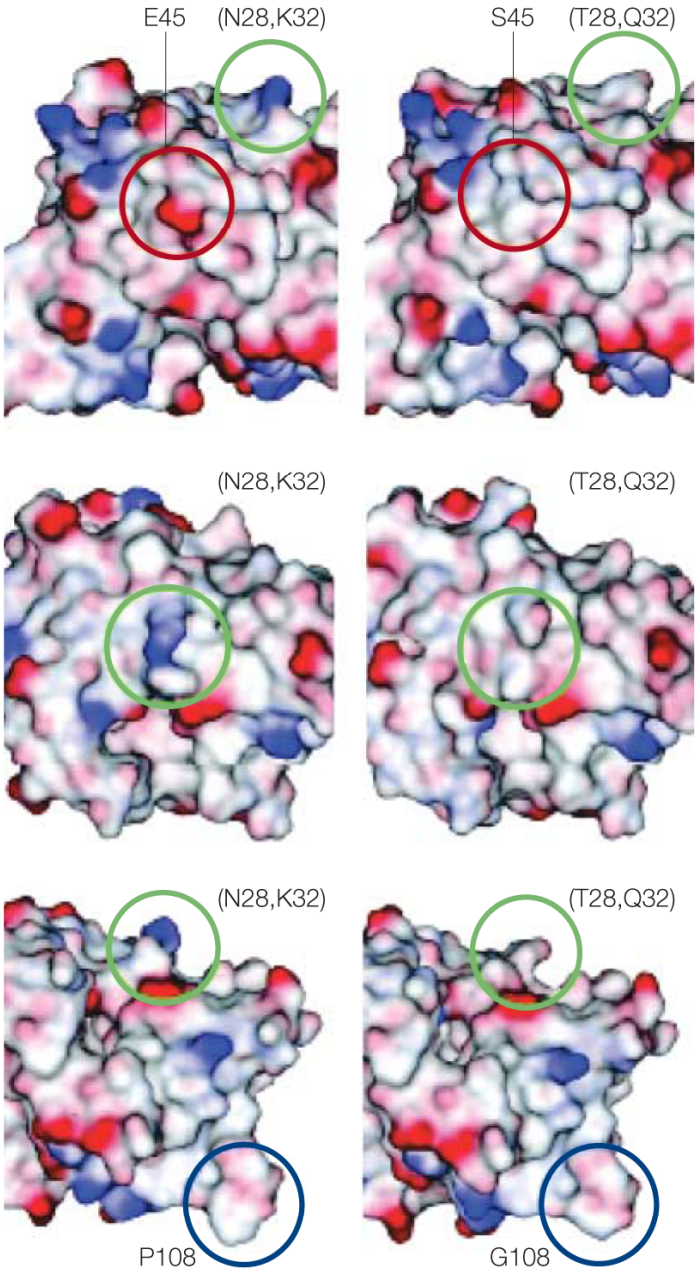


Inhibits P450 enzymes



No inhibition of P450 enzymes

Immunogenicity



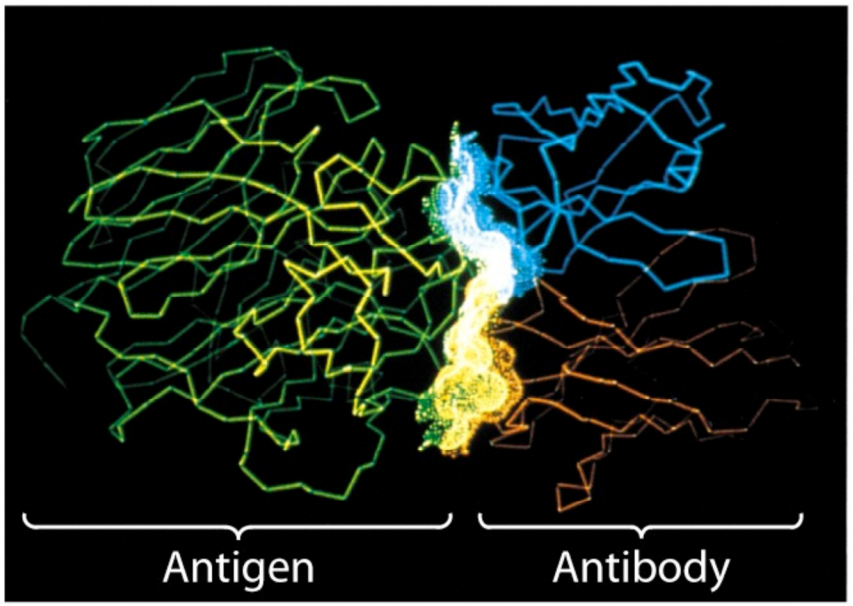
wild type mutants

- **antigen**: any substance that can be specifically bound by B-cell or T-cell receptors
- **immunogenicity**: ability of substances or invoke a humoral or cell-mediated immune response. Depends on the degree of "foreignness" or "non-selfness" (related on evolutionary distance)
- **large-size molecules are better immunogens** (larger interaction interface, more potentially immunogenic sites), also heterogeneity increases immunogenicity
- **epitopes**: parts of the molecule that bind to B-cell or T-cell receptor (antigenic determinants)

Surface residues were mutated in an allergen such significant reduction of the binding to human serum IgE occurred. Such a mutated allergen can than be used as a vaccine to produce antibodies against the wt-allergen.

Holm, J. Immunol. 173 (2004), 5258

B-cell mediated immune response



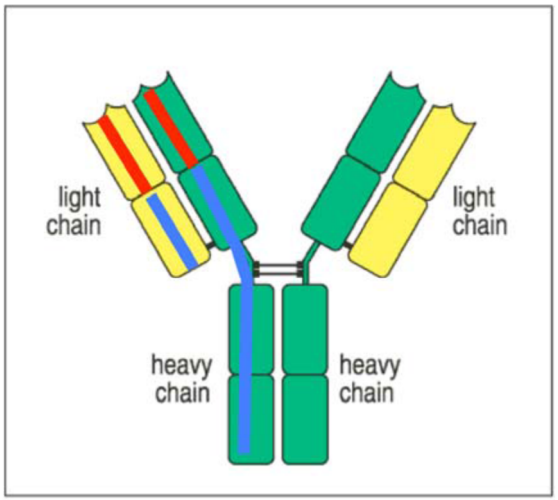


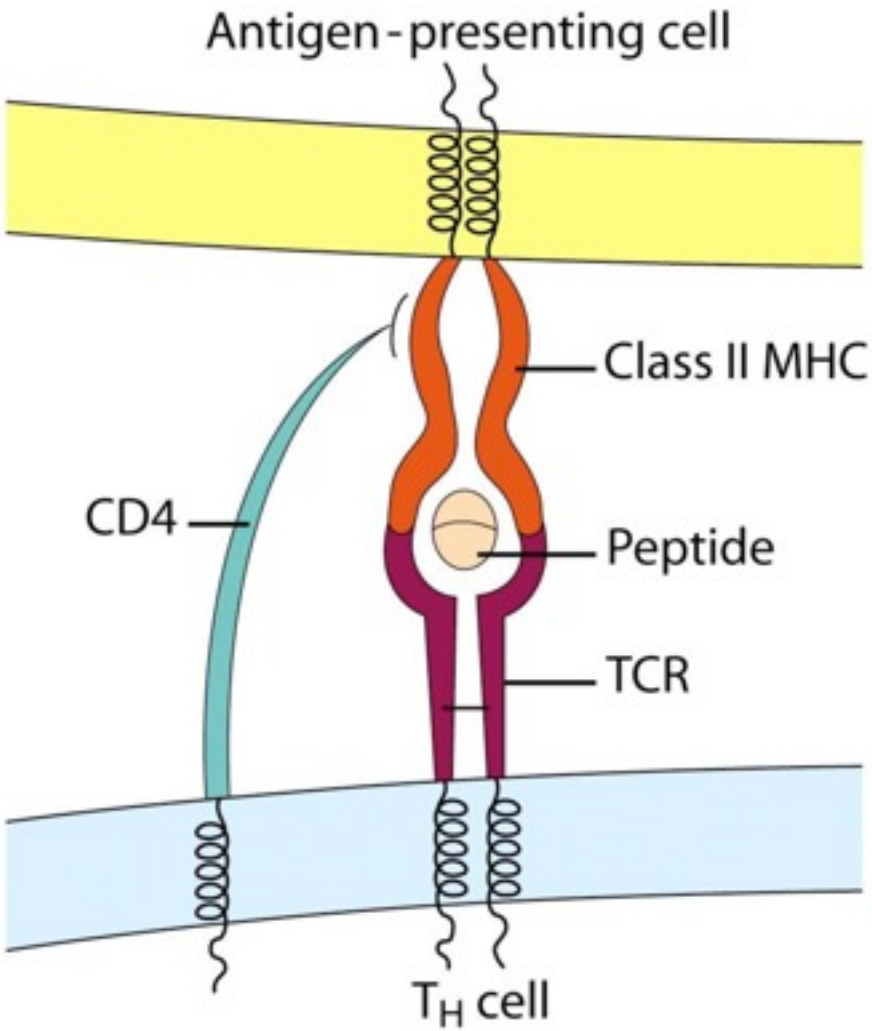
Fig 1.17 © 2001 Garland Science

Constant regions

Variable regions

- **B-cell** triggered immune reaction involves binding of antigens to antibodies, that are anchored on B-cells
- it recognizes **accessible** epitopes of peptides and proteins
- B-cell epitopes can be sequential or non-sequential, but from denatured protein only sequential epitopes will recognized

T-cell mediated immune response



- **T-cell** triggered immune reaction involves formation of antigen, T-cell receptor and MHC (major histocompatibility complex)
- it presents fragments from proteins and peptides processed by the cell (no need to be surface exposed)
- The APC-T-cell complex then triggers the immune response (production of cytokines, cell-lysing factors etc.)

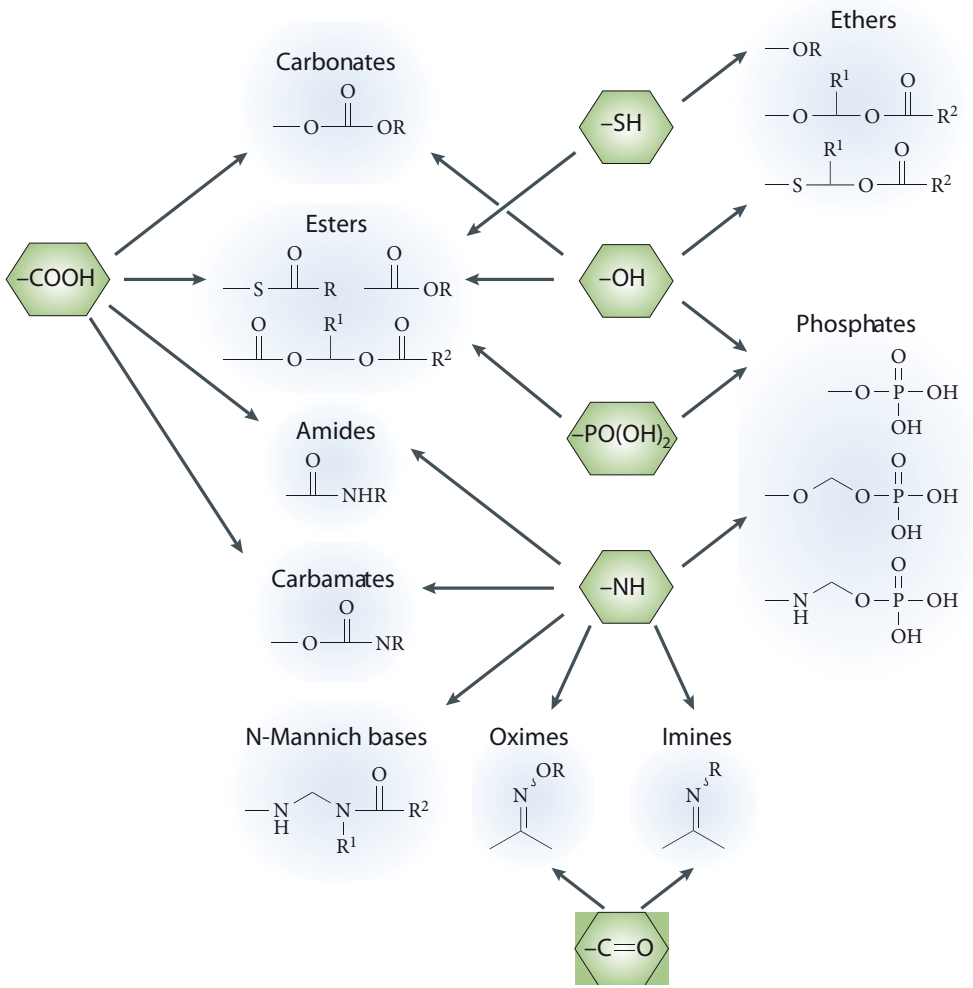
Prodrugs

Definition:

Inactive compounds which are converted to active compounds in the body.

Uses:

- Improving membrane permeability
- Prolonging activity
- Masking toxicity and side effects
- Varying water solubility
- Drug targeting
- Improving chemical stability



- **alkyl esters** enhance lipophilicity
  - readily cleaved by esterases in blood, liver and other tissues
  - simple alkyl esters are more slowly cleaved

- **phosphate ester** enhance water solubility
  - cleaved by phosphonate esterases
  - after cleavage drug may be very lipophilic and precipitate

- **carbonates and carbamates** are often enzymatically more stable than simples esters

- **amides** are only used to a smaller extend because they bioconversion using peptidases is not rapid enough

- **oximes** are prodrugs of ketones, amidines and guanidines.
  - converted by P450 enzymes
  - makes drug more lipophilic

Prodrugs to lower water solubility

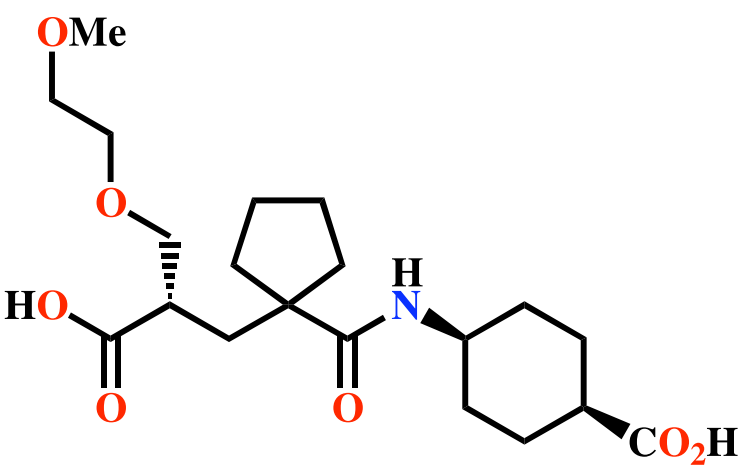
- Used to reduce solubility of foul tasting orally active drugs
- Less soluble on tongue
- Less revolting taste
- improves membrane permeability
- many nucleoside drugs are not able to cross membranes

- mask polar and ionizable groups

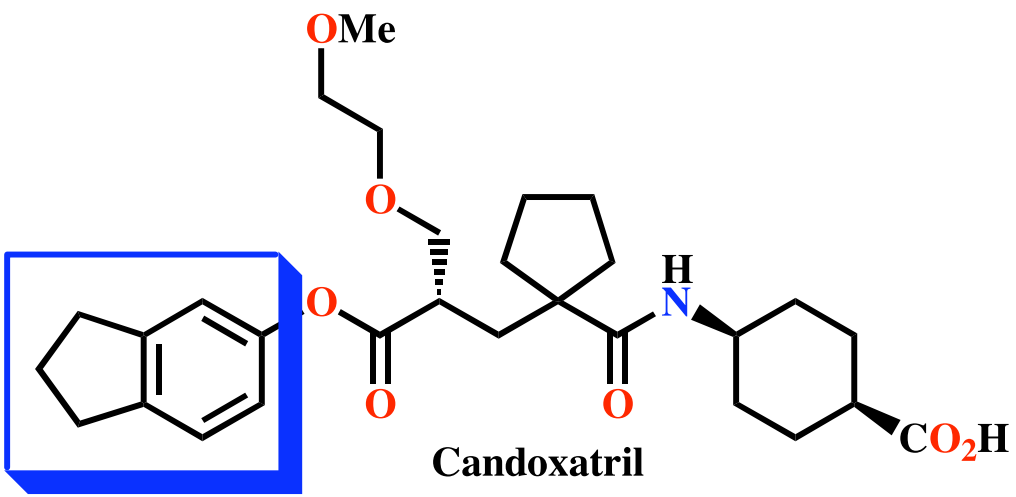
Prodrugs to improve membrane permeability

Esters

- Used to mask polar and ionizable carboxylic acids
- Hydrolyzed in blood by esterases
- Used when a carboxylic acid is required for target binding
- Leaving group (alcohol) should ideally be non toxic



Candoxatrilat



5-indanyl group

Candoxatril

Varying the ester varies the rate of hydrolysis
Electron withdrawing groups increase rate of hydrolysis (e.g. 5-indanyl)
Leaving group (5-indanol) is non toxic

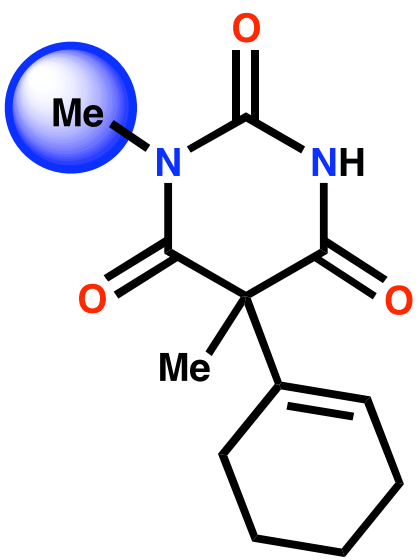
Prodrugs to improve membrane permeability

N-Methylation of amines

- Used to reduce polarity of amines
- Demethylated in liver

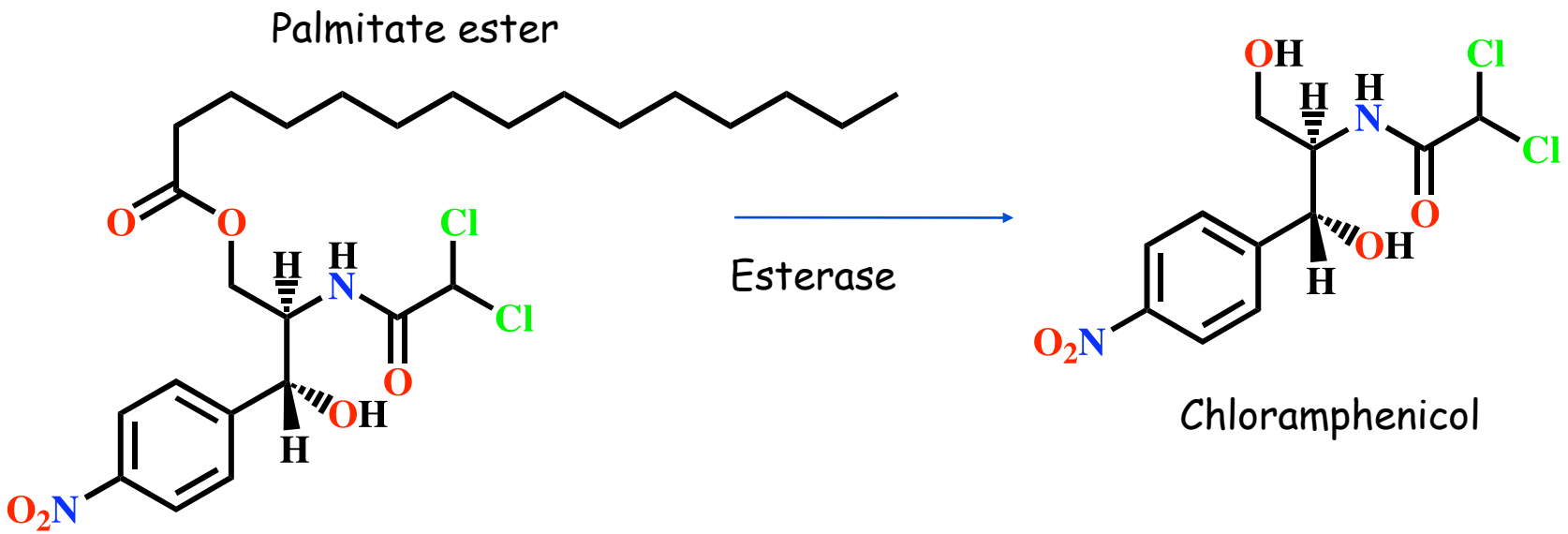
Example:

Hexobarbitone

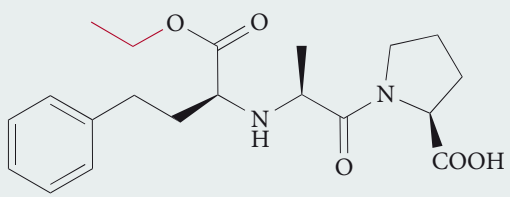
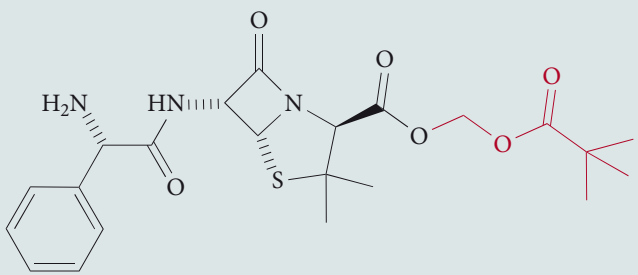
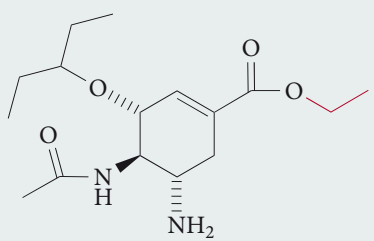
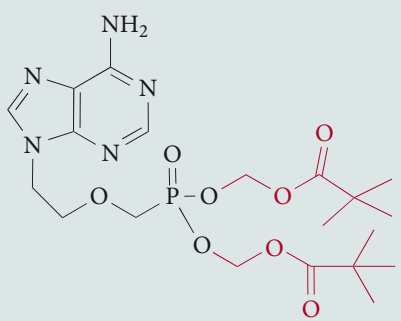


Example:

Palmitate ester of chloramphenicol (antibiotic)



Prodrugs for improved lipophilicity or permeability

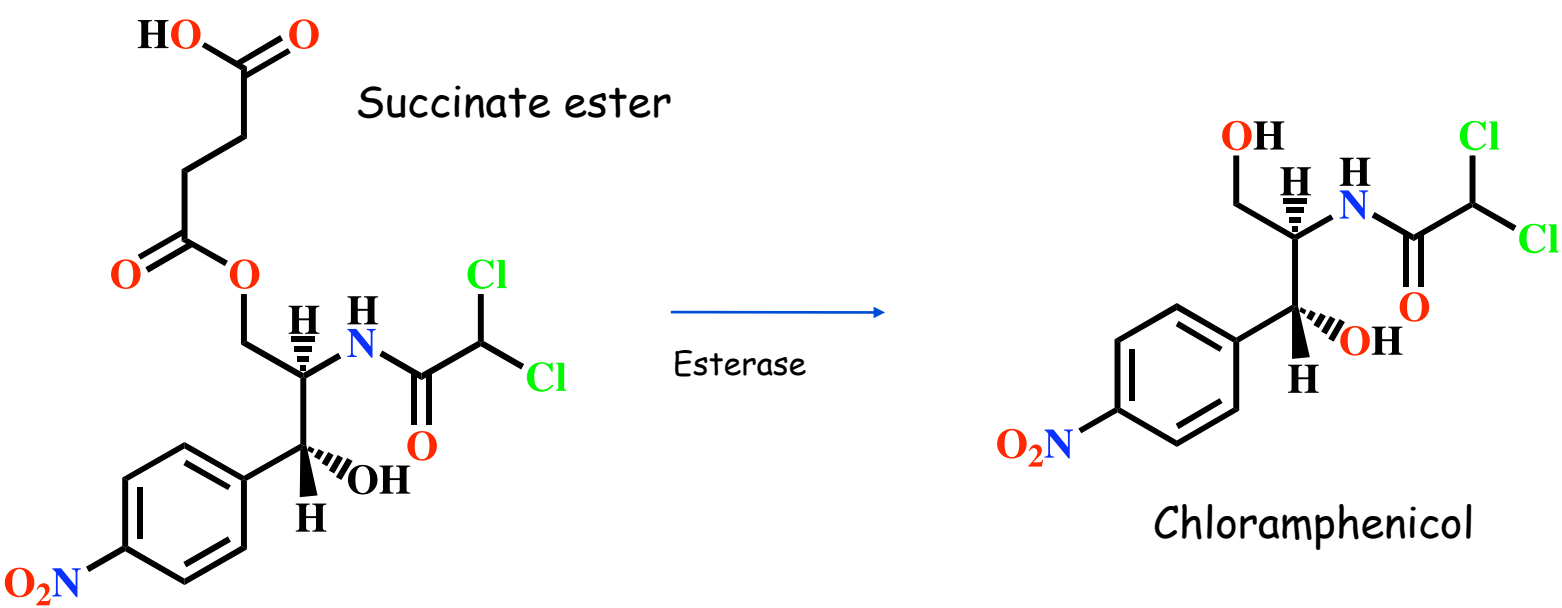
Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Enalapril (angiotensin-converting enzyme inhibitor)	Monoethyl ester of enalaprilat		• Bioconversion by esterases • The oral bioavailability of enalaprilat in humans is 36–44% • 53–74% of the administered dose is absorbed[3,172]
Pivampicillin (β-lactam antibiotic)	Pivaloylmethyl ester of ampicillin		• Bioconversion by esterases • The oral bioavailability of 32–55% for ampicillin increased to 87–94% for pivampicillin[173,174]
Oseltamivir (anti-influenza)	Ethyl ester of oseltamivir carboxylate		• Bioconversion by esterases • The oral bioavailability of less than 5% in rat and marmoset for oseltamivir carboxylate increased to 80% for oseltamivir in humans[80–82]
Adefovir dipivoxil (antiviral)	Bis-(pivaloyloxy-methyl) ester of adefovir		• Bioconversion by esterases and phosphodiesterases • The oral bioavailability of ~10% for adefovir increased to 30–45% for adefovir dipivoxil[78,79]

Prodrugs to increase water solubility

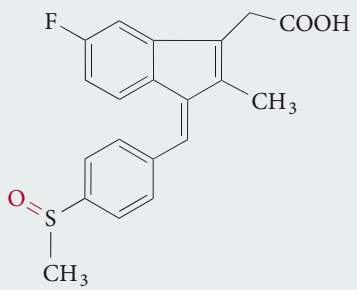
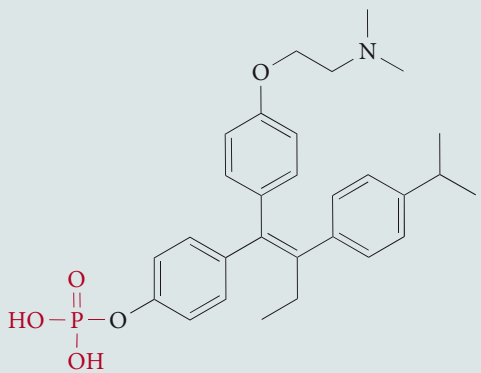
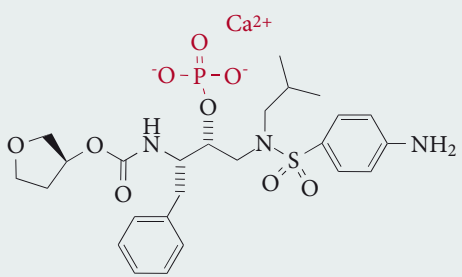
- Often used for i.v. drugs
- Allows higher concentration and smaller dose volume
- May decrease pain at site of injection

Example:

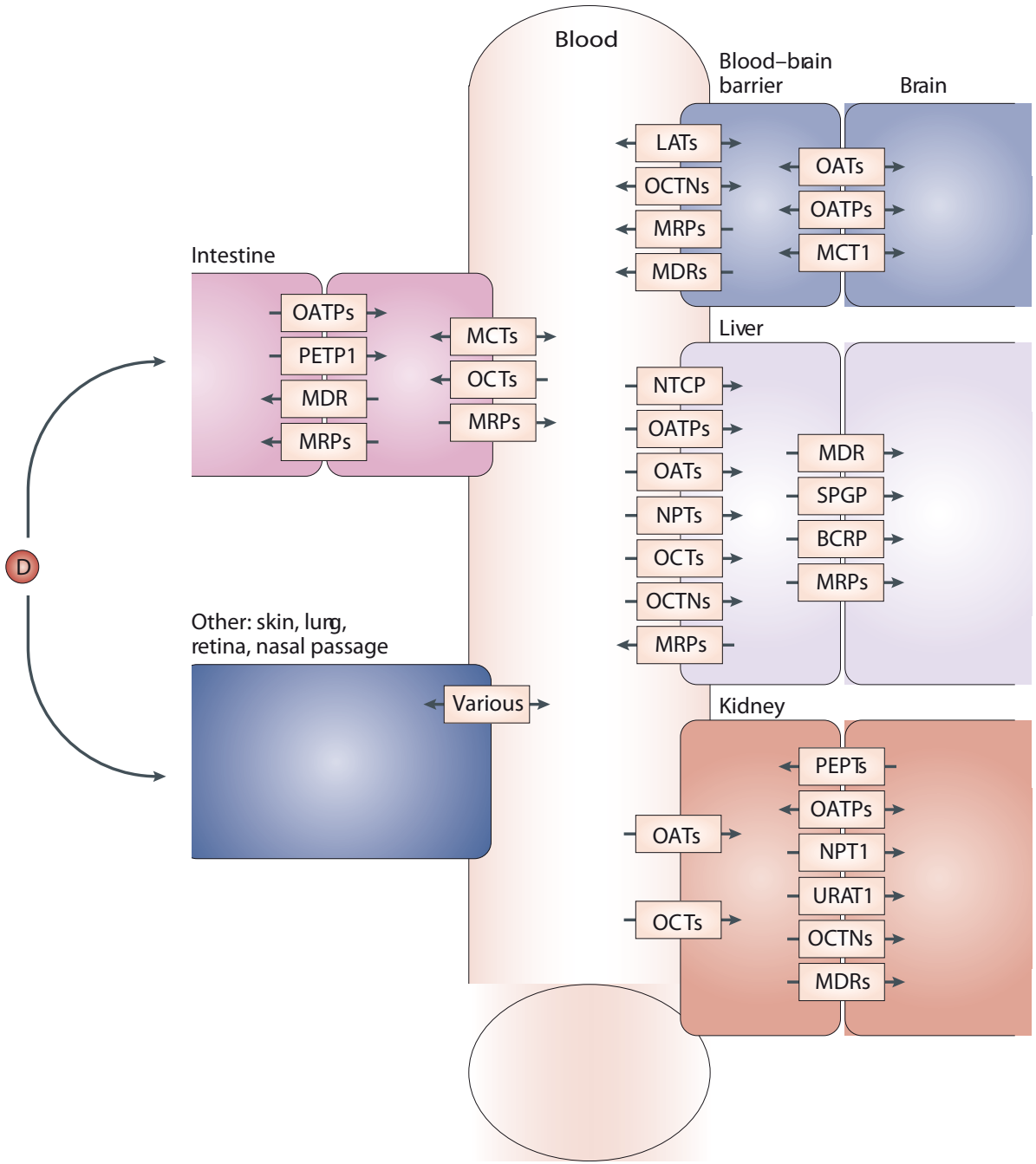
Succinate ester of chloramphenicol (antibiotic)



Prodrugs for improved aqueous solubility

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Sulindac (non-steroidal anti-inflammatory)	Oxide prodrug of sulindac sulphide		• Bioprecursor prodrug that is reduced to the active sulphide form after oral absorption • ~ 100-fold increase in aqueous solubility[62,65]
Miproxifene phosphate, TAT-59 (anticancer)	Phosphate ester of miproxifene/DP-TAT-59		• Bioconversion by alkaline phosphatases • Aqueous solubility at pH 7.4 increased by ~1,000-fold[69] • Enhanced bioavailability to 28.8% in rats and 23.8% in the dog[66] • Dose-linear pharmacokinetics in humans[69]
Fosamprenavir (antiviral)	Phosphate ester of amprenavir		• Bioconversion by alkaline phosphatases • 10-fold increased aqueous solubility • More simplified and patient compliant dosage regimen • Prolonged exclusive patent[70–72]

(Mis)using carrier to help crossing membranes



Multiple drug carriers in different tissues, all of which may need to be permeated:
BcrP, breast cancer-resistant protein (also known as ABcG2); LAts, l-type amino-acid transporters;
Mct1, monocarboxylate transporter 1 (also known as sLc16A1);
MDr, multidrug-resistant;
MrPs, multidrug-resistance-related proteins;
NPt1, sodium phosphate transporter 1 (also known as sLc17A1),
NtcP, sodium-dependent taurocholate co-transporter (also known as sLc10A1);
OAts, ornithine aminotransferases;
OAtPs, organic anion transporting polypeptides;
Octs/OctNs, organic cation transporters;
PetP1, peptide transporter 1 (also known as sLc15A1);
sPGP, sister P-glycoprotein (also known as ABcB11);
UrAt1, urate anion exchanger 1 (also known as sLc22A12)

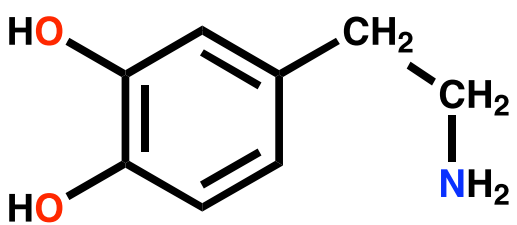
Dobson et al, Nat. Rev. Drug Discov. **7** (2008), 205.

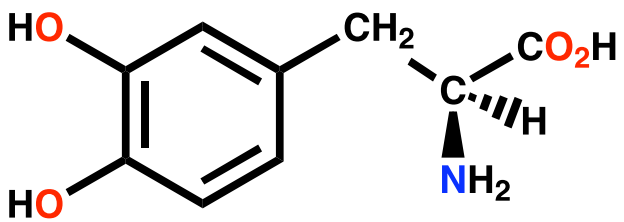
Prodrugs to improve membrane permeability

Trojan Horse Strategy

- Prodrug designed to mimic biosynthetic building block
- Transported across cell membranes by carrier proteins

Example: Levodopa for dopamine





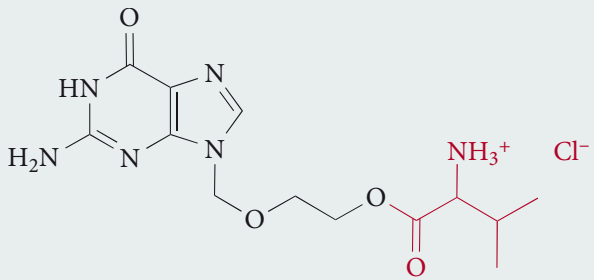
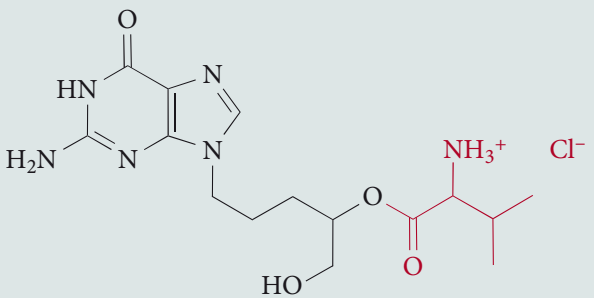
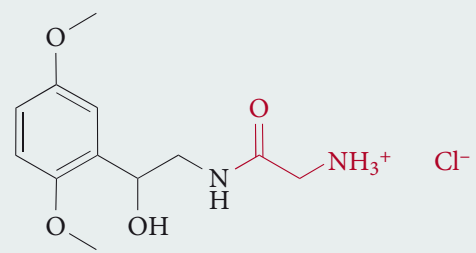
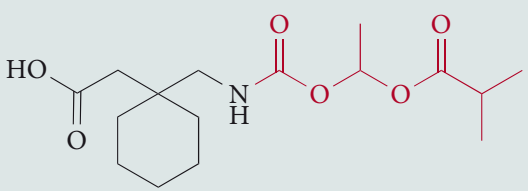
Dopamine

- Useful in treating Parkinson's Disease
- Too polar to cross cell membranes and BBB

Levodopa

- More polar but is an amino acid
- Carried across cell membranes by carrier proteins for amino acids
- Decarboxylated in cell to dopamine

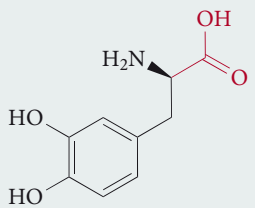
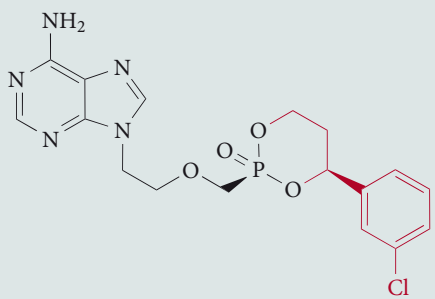
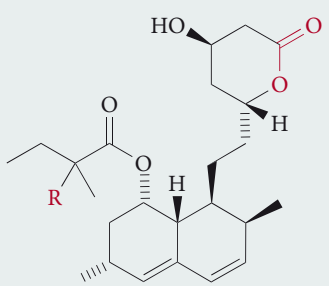
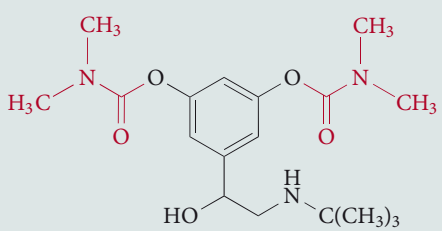
Prodrugs to exploit carrier-mediated absorption

Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Valacyclovir (antiviral)	L-Valyl ester of acyclovir		• Bioconversion by valacyclovir hydrolase (valacyclovirase) • Transported predominantly by hPEPT1 • Oral bioavailability improved from 12–20% (acyclovir) to 54% (valacyclovir)[90–92,182]
Valganciclovir (antiviral)	L-Valyl ester of ganciclovir		• Bioconversion by intestinal and hepatic esterases • Transported predominantly by hPEPT1 • Oral bioavailability improved from 6% (ganciclovir) to 61% (valganciclovir)[183,184]
Midodrine (vasopressor)	Glycyl amide of desglymidodrine		• Bioconversion by unknown peptidase • Transported by hPEPT1 • Oral bioavailability improved from 50% (desglymidodrine) to 93% (midodrine)[94]
XP13512 (restless leg syndrome, neuropathic pain)	Isobutanoyloxy-ethoxy carbamate of gabapentin		• Bioconversion by esterases • Transported by both MCT1 and SMVT • Oral bioavailability improved from 25% (gabapentin) to 84% (XP13512) in monkeys[98,99]

hPEPT1, human peptide transporter 1 (also known as SLC15A1); MCT1, monocarboxylic acid transporter 1 (also known as SLC16A1); SMVT, sodium-dependent vitamin transporter (also known as SLC5A6).

Other prodrug mechanisms

Prodrugs for other purposes

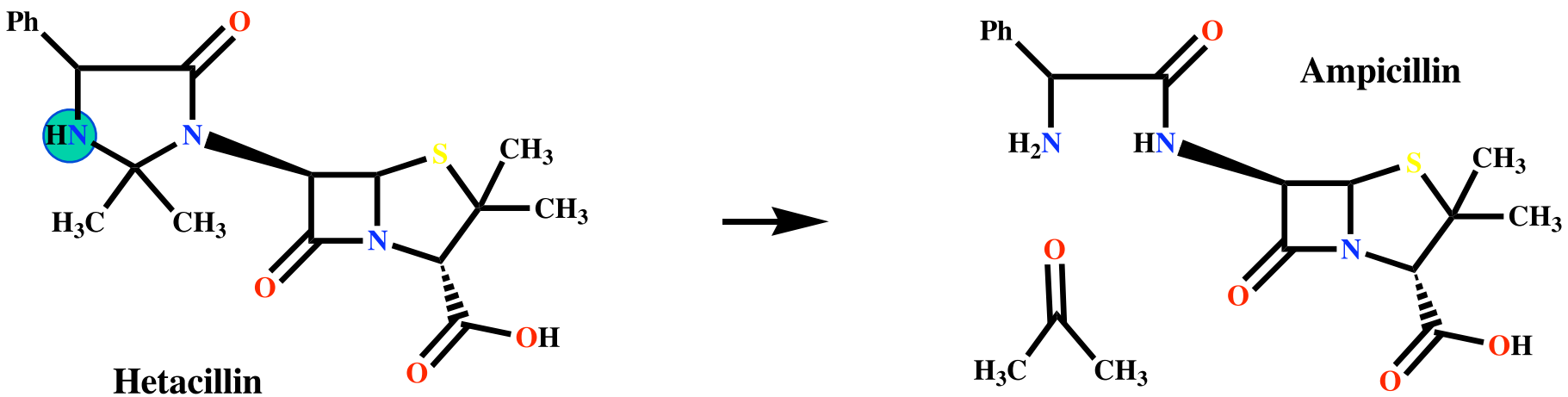
Prodrug name (therapeutic area)	Functional group	Structure	Prodrug strategy
Levodopa (Parkinson's disease)	Carboxylic acid of dopamine		• Crosses the blood–brain barrier and enters the brain by using LAT1 • Is decarboxylated to dopamine by aromatic amino-acid decarboxylase[136,137]
Pradefovir mesylate (antiviral)	2-(3-chlorophenyl)-[1,3,2]dioxaphosphinane of adefovir		• Undergoes cytochrome P450-catalyzed oxidation to adefovir predominantly in the liver[154,155,187]
Simvastatin, R=CH_3; lovastatin, R=H (hypercholesterolaemia)	Inactive lactone forms		• Bioprecursor prodrugs that are converted into the active hydroxyl acid forms in the liver[156,157,188]
Bambuterol (asthma)	Bisdimethylcarbamate of terbutaline		• Prolongs duration of drug action • Undergoes cascade of hydrolysis and oxidation reactions to terbutaline[163,165]

LAT1, type 1 L-type amino-acid transporter.

Prodrugs to increase chemical stability

Example:

Hetacillin for ampicillin

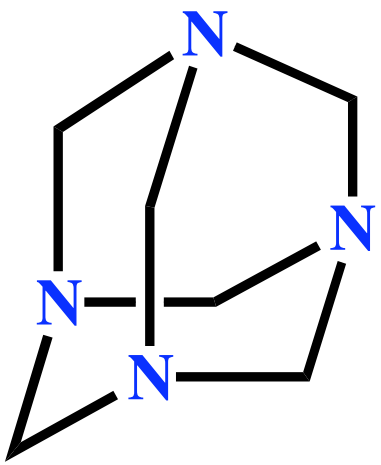


- Ampicillin is chemically unstable in solution due to the α-NH_2 group attacking the β-lactase ring
- 'N' in heteracillin is locked up within a heterocyclic ring

Prodrugs used to target drugs

Example:

Hexamine



- Stable and inactive at pH>5
- Stable at blood pH
- Used for urinary infections where pH<5
- Degrades at pH<5 to form formaldehyde (antibacterial agent)

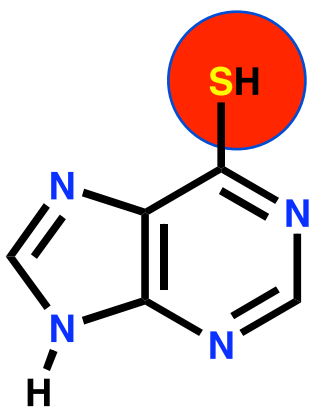
Prodrugs to prolong activity

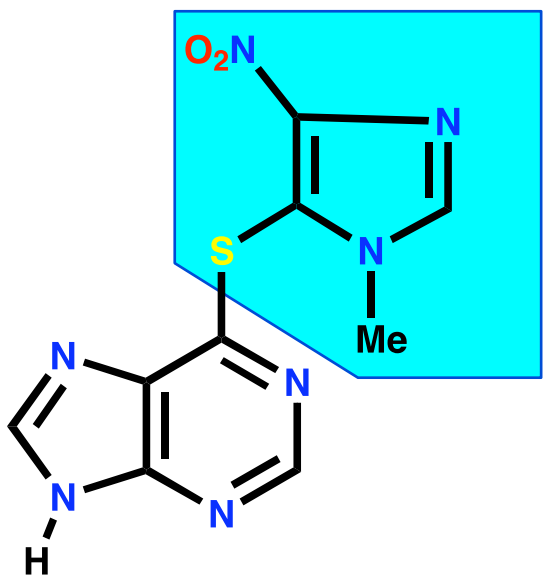
Mask polar groups

- Reduces rate of excretion

Example:

Azathioprine for 6-mercaptopurine





6-Mercaptopurine

(suppresses immune response)

- Short lifetime - eliminated too quickly

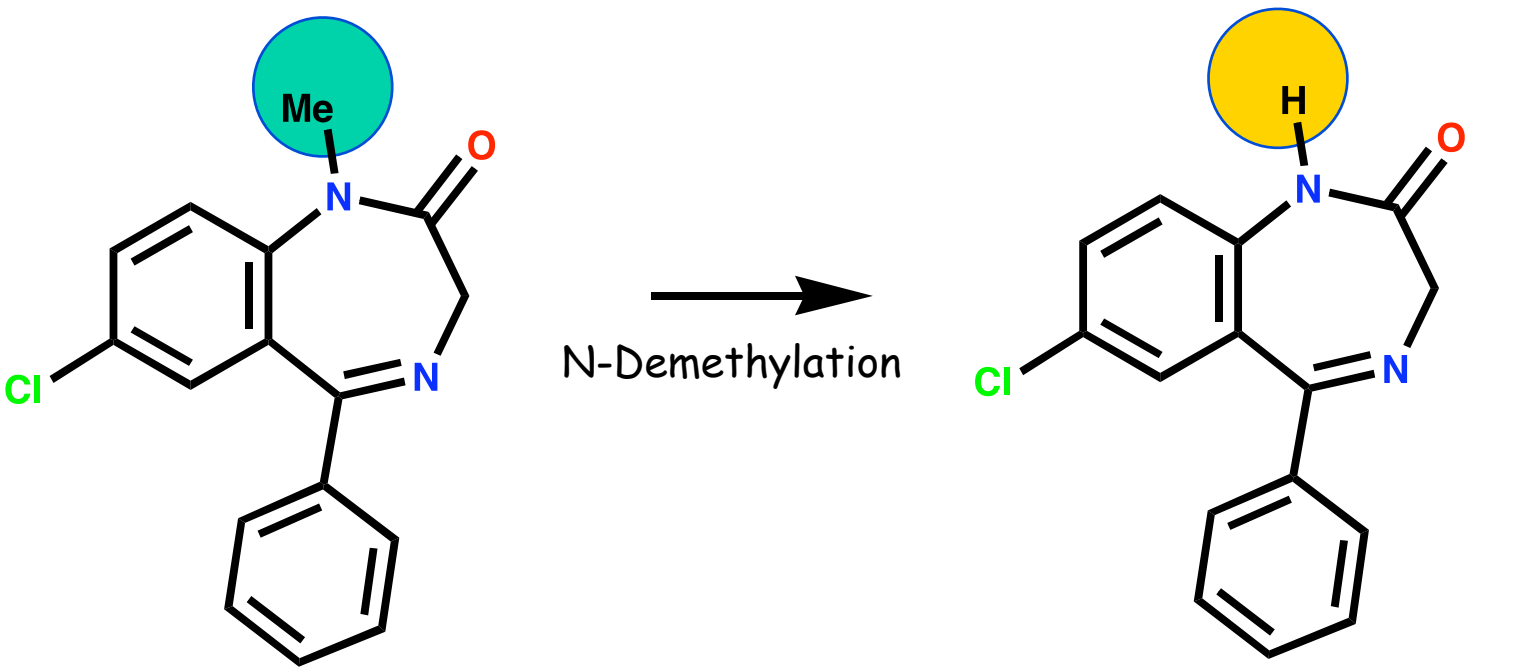
Azathioprine

- Slow conversion to 6-mercaptopurine
- Longer lifetime

Prodrugs to prolong activity

Example:

Valium for nordazepam



Valium

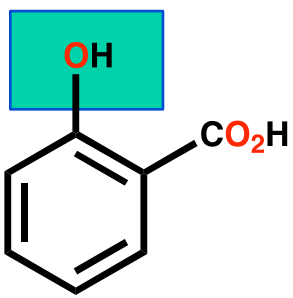
Nordazepam

Prodrugs to mask toxicity and side effects

- Mask groups responsible for toxicity/side effects
- Used when groups are important for activity

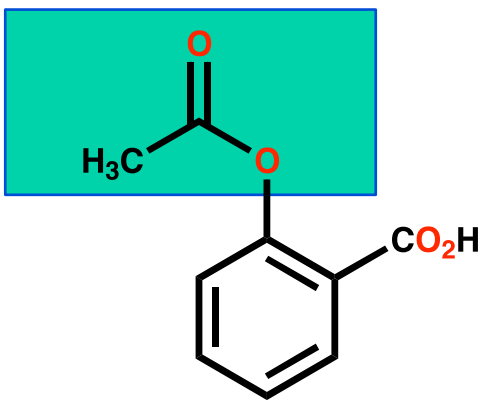
Example:

Aspirin for salicylic acid



Salicylic acid

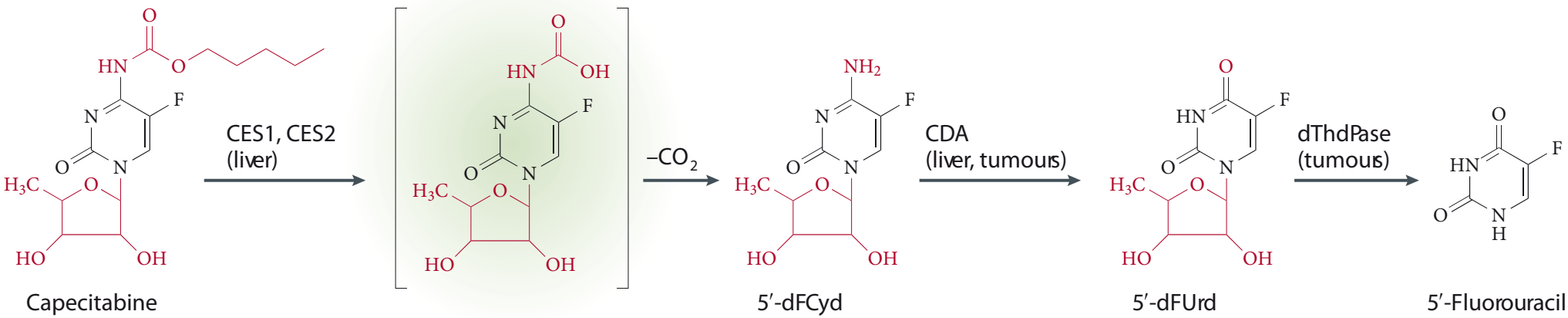
- Analgesic, but causes stomach ulcers due to phenol group



Aspirin

- Phenol masked by ester
- Hydrolyzed in body

Capecitabine requires multiple steps for activation



- human carboxylesterases 1 and 2 in the liver cleave the ester bond of the carbamate
- it is followed by fast spontaneous decarboxylation
- cytidine deaminase in the liver and in tumor convert the amine into a carbonyl moiety
- finally tymidine phosphorylase liberates the active drug fluoruracil

Problems in the usage of peptides/proteins as Drugs

- instability of proteins/peptides in the gastrointestinal tract due to proteolysis
- low permeability across membranes due to the high molecular mass and the high polar surface
- inefficient to pass the blood-brain barrier

Potentially immunogenic protein modifications

Modification	Effect
Engineered modifications	
Amino-acid sequence	Human versus analogues and non-human proteins
Chemical modification	Acylation, PEGylation
Pharmaceutical formulation	Lyophilization, micro-encapuslation
Unwanted modifications during processing, production and storage	
Chemical degradation	Deamidation, oxidation
Physical degradation	Denaturation, aggregation, fibrillation, misfolding

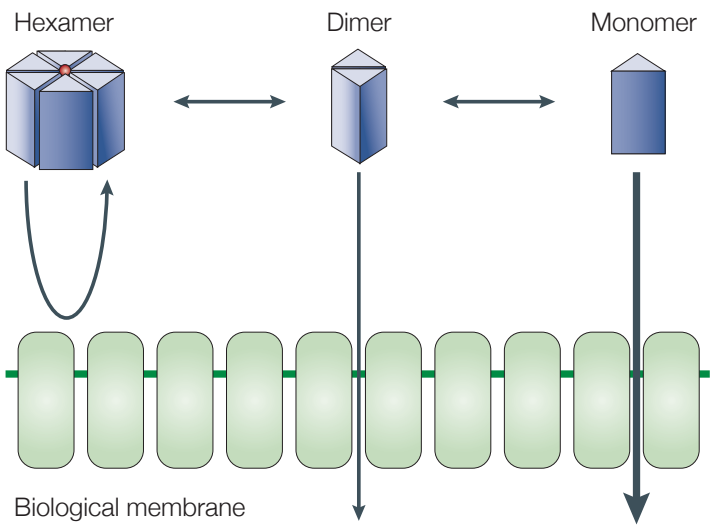
PEG, polyethylene glycol.

Approaches to improve pharmacokinetics of proteins

- Co-administration of protease inhibitors
- Encapsulation, coatings or other delivery methods (Liposomes)
- Cell-membrane permeabilization: Addition of fatty acids, bile salts surfactants and Aspirin
- Modifications of tight junctions: Co-addition of certain toxins or polymeric materials (dangerous, because that may allow passage of potential harmful compounds from the gut into systemic circulation)
- Receptor-mediated endocytosis: Vitamin B12 receptor, Fc receptor (no size limit)
- Usage of membrane-transporters: Covalent link recognizing epitope for bile acid transporter, di- and tripeptide transporter, glucose transporter (small cargos)
- Increase of drug lipophilicity: Addition of functional groups by conjugating
- labile lipid attachments (e.g. to Lys sidechains, Insulin)

Protein Modification

- Insulin: Developed a form that is monomeric and can more easily get into the systemic circulation after subcutaneous injection. This was achieved by various mutations that stabilized the monomeric form (insulin lispro (Eli Lilly); Novorapid, (NovoNordisk))

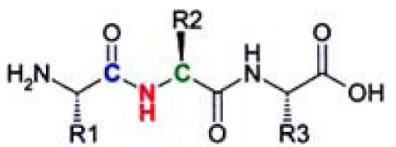


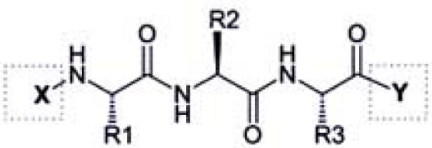
- other examples: human interleukin-2 (IL-2) was converted into the desglycosylated form (Proleukin, Chiron, a des-alanyl interleukin).

Preventing protein misfolding during storage

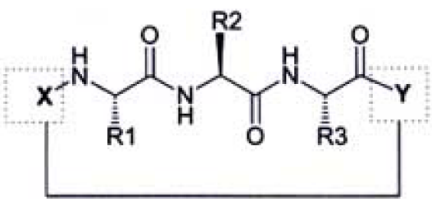
- Protein misfolding is influenced by shear/shaking , temperature, pH and protein concentration to a large extend
- Irreversible aggregation by disulfide-shuffling, stable hydrophobic association
- Fibrillation may cause toxicity of proteins that are otherwise not harmful
- Often a few "gatekeeper" residues are involved in forming aggregates and removing these can tip the balance towards a stable monomeric form
- small molecules that can from hydrogen bonds with beta-strands can prevent fibrillation
- addition of sugars or salts that tend to be excluded from protein surfaces and hence favor compacts states
- amino acids such as Arg or Glu (50mM) that neutralize opposite charges
- polyols, PEGs and other polymers that sterically hinder protein-protein interactions
- addition of detergents and other amphiphiles to reduce the effects from shear
- addition of cyclodextrins to remove aggregation (used for insulin, growth hormones (these bind to unfolded states, in particular to aromatic residues preventing their aggregation) (caution: some cyclodextrins may extract membrane components)
- sometimes lyophilization can reduce problems due to long-term storage

Modifications of peptides to increase plasma lifetimes and BBB passage

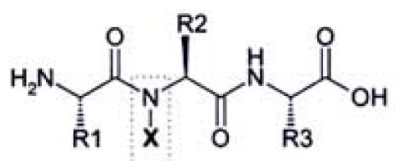




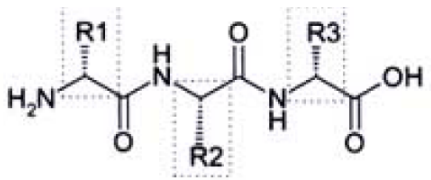
endgroup modification



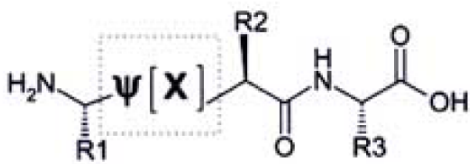
cyclization



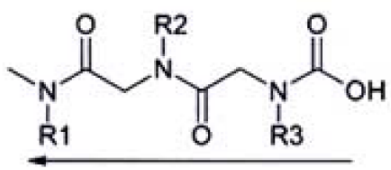
N-alkylation



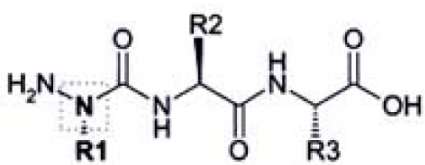
D-amino acids



Amid-bond surrogate



Peptoid



Aza peptide

- modification of backbone: Esters, Ketones
- Increase BBB passage into the CNS:

  Reduce size of molecule (< 500), coupling to a lipid carrier such as triglyceride or a liposome, increase of lipophilicity (acylation, methylation etc.)

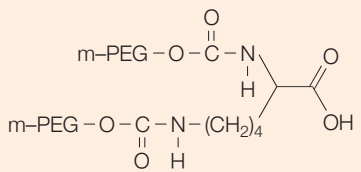
  conversion into prodrug adding a lipid with a brain-specific lipase cleavage site

  usage of endogenous transport mechanism (requires certain sequence modifications, e.g. introduction of cationic charges or modification with polyamines, glycosylation to facilitate transport with glucose transporters, coupling to cell-penetrating peptides (penetratins)

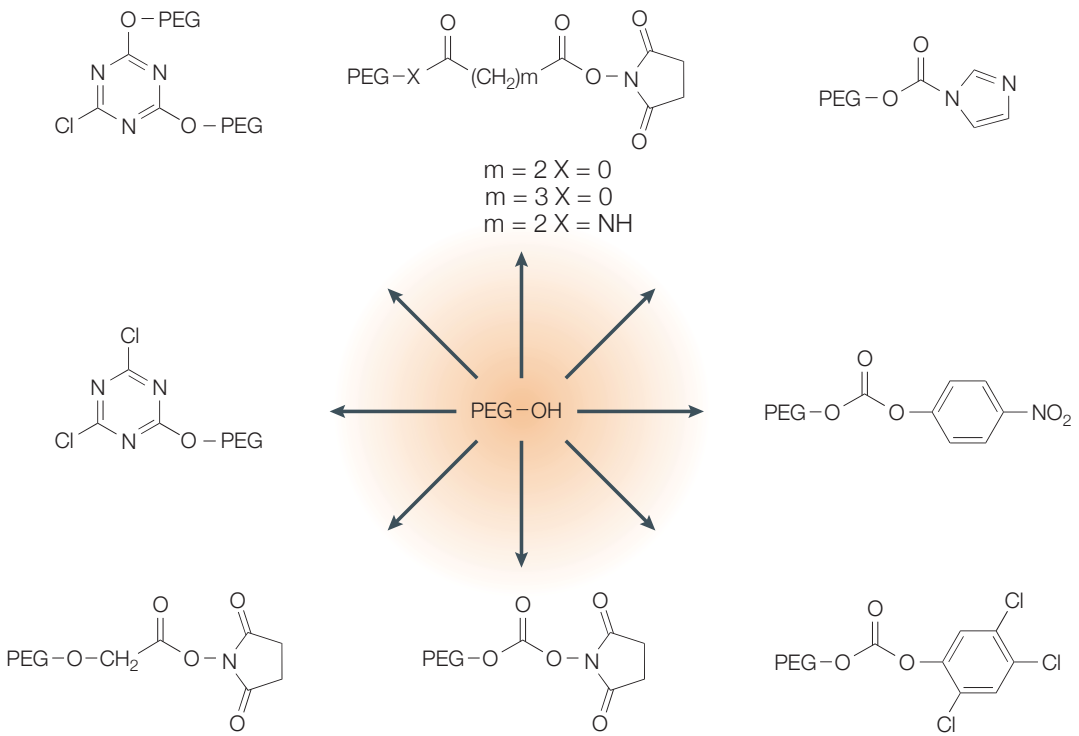
Other Protein Modifications

- Acetylation
  - Attachment of acetyl groups or fatty acid groups to surface residues can increase the affinity to serum albumin so that degradation is retarded and circulation time is increased
  - more efficient for small proteins
  - examples: insulin, glucagon-like peptide 1, interferon-α, desmopressin
- PEGylation
  - reduces plasma clearance by reducing the metabolic rate and receptor-mediated uptake from system circulation by increasing the size (reduced renal clearance)
  - shields antigenic and immunogenic epitopes and thereby reduces immunogenic reactions
  - unfortunately, product heterogeneity is large (difficult product quality control, difficult approval).

PEGylation

Linear PEG-OH	$H-(OCH_2CH_2)_n-OH$
Linear m–PEG-OH	$CH_3-(OCH_2CH_2)_n-OH$
Branched m–PEG_2	

different types of PEG

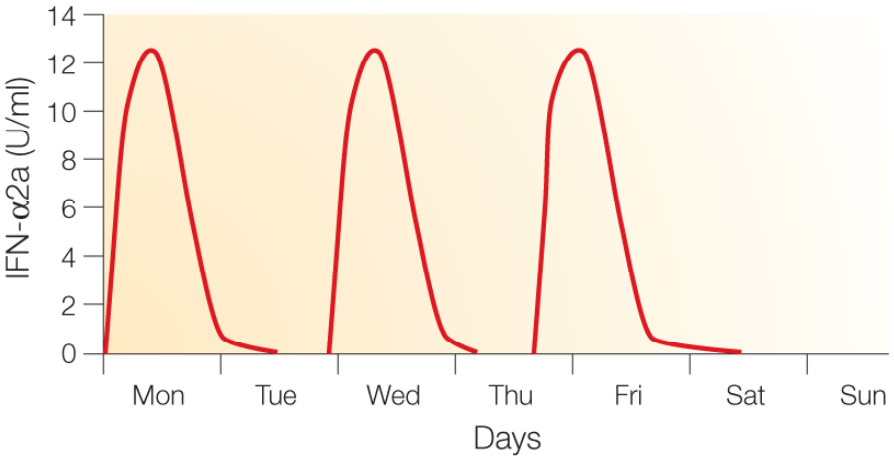


activation reagents for pegylation

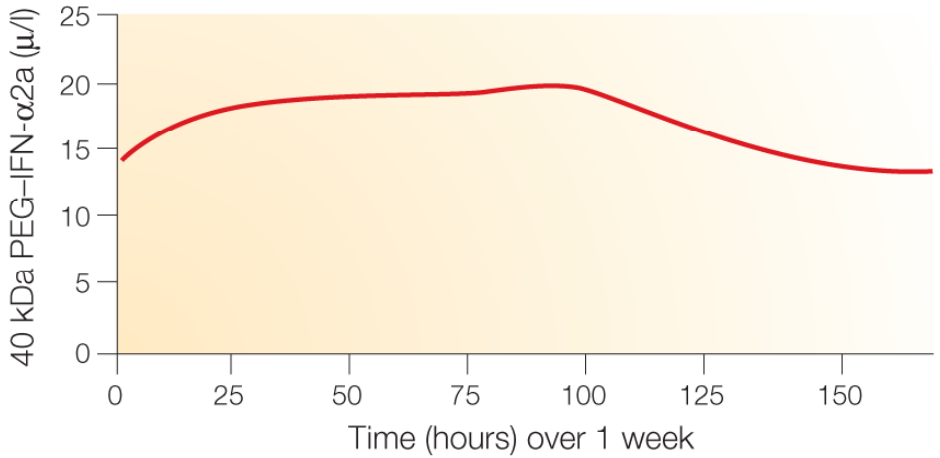
- proteins are pegylated to reactive side-chains, e.g. the free N-terminus or the ε amino group of lysines. Possibly also with thiol groups of Cys residues
- the PEG part is highly solvated and largely increases the solubility of the complex
- polymer nanoparticles have been demonstrated to pass the blood-brain barrier
- targets: proteins,peptides, small-molecule drugs, antibodies, ab-fragments

Pharmacokinetics of PEGylated drugs

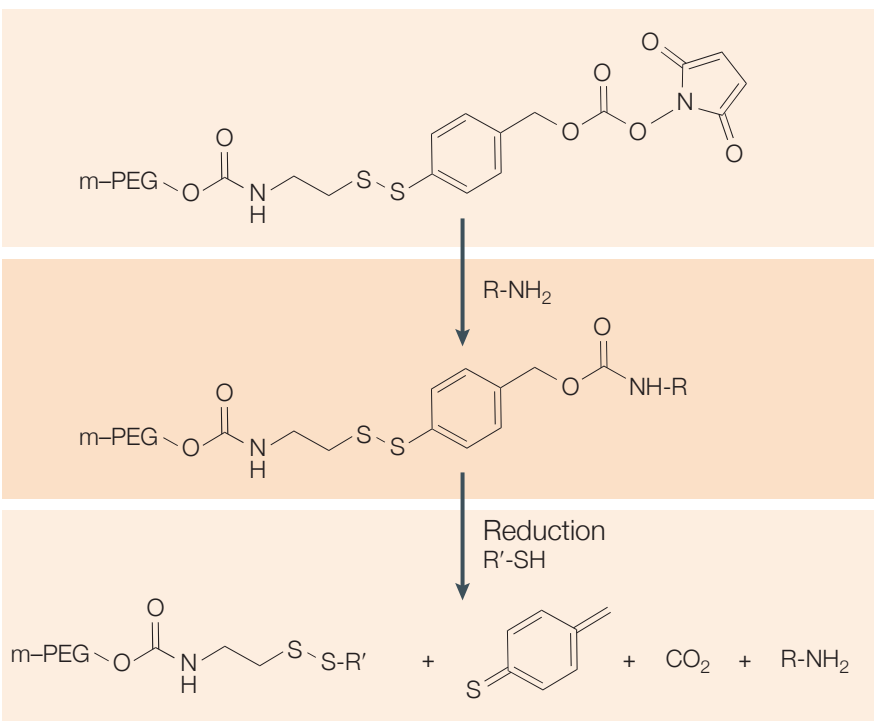
Plasma levels of interferon after subcutaneous injection



interferon α 2a



40kDa-PEG-IFN- α 2a



- Special linker allow controlled release of the drug from the PEG attachment, e.g. when using the p- or -o-disulfide of benzyl urethan.
Inside the endosomal compartment of a cell the mildly reducing conditions release the cargo

Commercial pegylated drugs

Modified proteins and protein-delivery systems approved for marketing

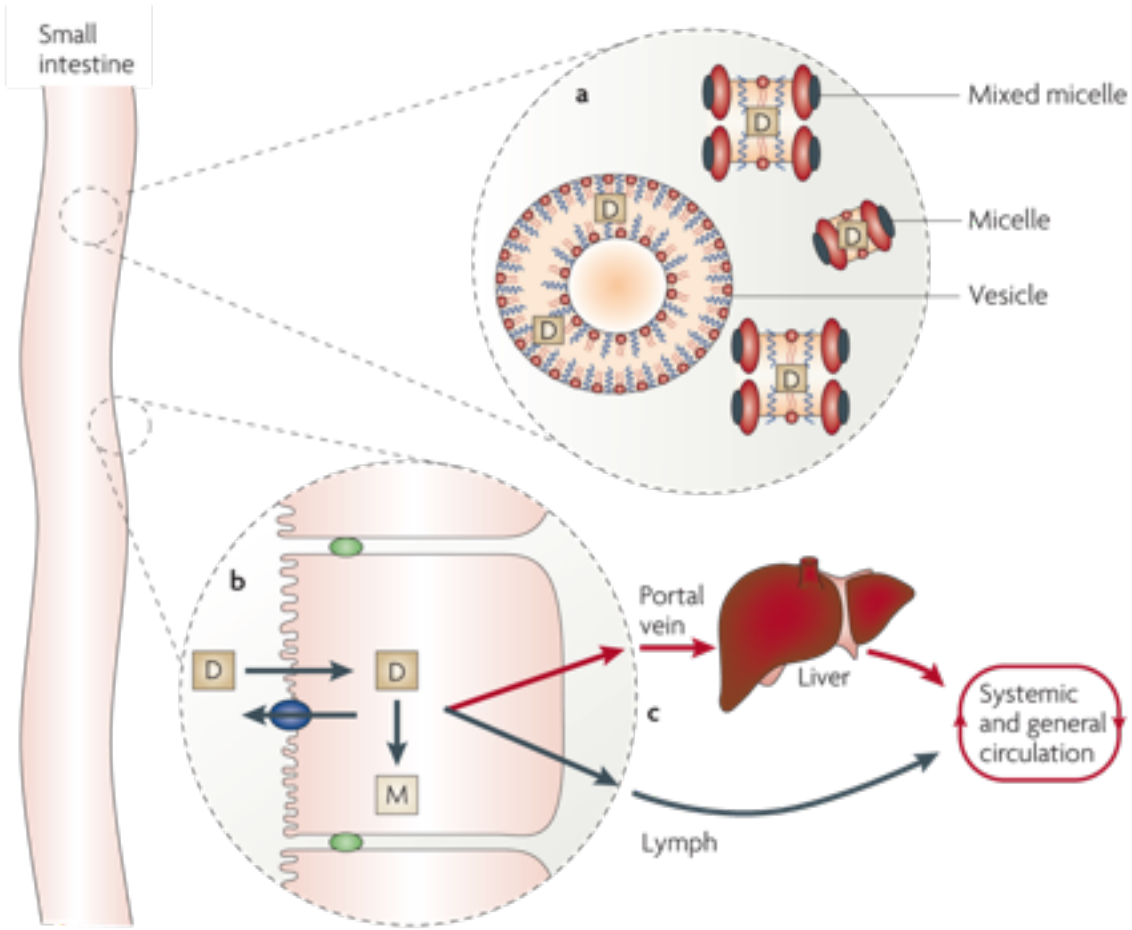
Product (company)	Drug	Modification/delivery system	Administration route
Proleukin (Chiron)	Aldesleukin	Analogue	Intravenous
Humalog (Eli Lilly)	Insulin lispro	Analogue	Subcutaneous
NovoRapid (Novo Nordisk)	Insulin aspart	Analogue	Subcutaneous
Neulasta (Amgen)	PEGinterferon α-2a	Mono-pegylated	Subcutaneous
Pegasys (Roche)	Pegfilgrastim	Mono-pegylated	Subcutaneous
Somavert (Pharmacia)	Pegvisomat	Multi (4–6)-pegylated	Subcutaneous
Levemir (Novo Nordisk)	Insulin detemir	Mono-acylated	Subcutaneous
Nutropin Depot (Genentech)	Human growth hormone	PLGA microspheres	Subcutaneous
InductOs (Wyeth (MDT))	Bone morphogenic protein 2	Absorbable collagen sponge	Implanatable medical device

PLGA, poly(lactide-co-glycolic acid)

DDS Systems on the commercial market

Drug or therapeutic agent (trade name), manufacturer(s)	Indication	Year of approval
Liposomal amphotericin B (AmBisome), Gilead, Fujisawa	Fungal infections	1990 (Europe), 1997
	Leishmaniasis	2000
PEG-adenosine deaminase (Adagen), Enzon	Severe combined immunodeficiency disease	1990
Styrene maleic acid and neocarzinostatin copolymer in Ethiodol (SMANCS/Lipiodol, Zinostatin stimalamer), Yamanouchi	Hepatocellular carcinoma	1993 (Japan) 1996 (Japan)
Stealth (PEG-stabilized) liposomal doxorubicin (Doxil/Caelyx), ALZA, Schering Plough	Kaposi's sarcoma	1995
	Refractory ovarian cancer	1999
	Refractory breast cancer	2003 (Europe, Canada)
Liposomal cytosine arabinoside (DepoCyt), SkyePharma	Lymphomatous meningitis	1999
	Neoplastic meningitis	Phase IV
Denileukin diftitox or interleukin 2–diptheria toxin fusion protein (ONTAK), Seragen	Cutaneous T-cell lymphoma	1999
Liposomal doxorubicin (Myocet), Elan	Metastatic breast cancer in combination with cyclophosphamide	2000 (Europe)
Gemtuzumab ozogamicin or anti-CD33–linked calicheamicin (Mylotarg), Wyeth-Ayerst	$CD33^+$ relapsed acute myeloid leukemia	2000
Liposomal verteporfin (Visudyne), QLT, Novartis	Wet macular degeneration in conjunction with laser treatment	2000 2001 2003 (Japan)
PEG-interferon α-2b (PEG-Intron), Enzon, Schering-Plough	Hepatitis C	2001
PEG–granulocyte colony stimulating factor or pegfilgrastim, (Neulasta), Amgen	Reduction of febrile neutropenia associated with chemotherapy	2002
^{90}Y-ibritumomab tiuxetan or ^{90}Y anti-CD20 (Zevalin), IDEC	Relapsed or refractory non-Hodgkin's lymphoma	2002
^{131}I-tositumomab (anti-CD20) (Bexxar), Corixa, GlaxoSmithKline	$CD20^+$ relapsed non-Hodgkin's lymphoma	2003

Lipid-Assisted Delivery

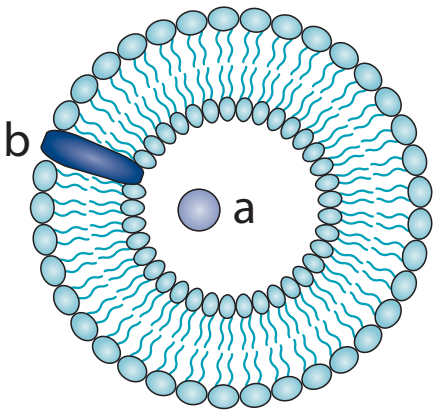


Lipids can solubilize drugs in the intestinal compartment by incorporating them into micelles, mixed micelles or vesicles

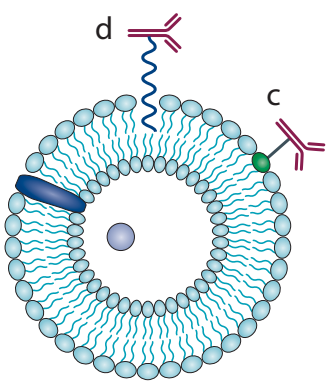
Lipids can altering the pathway portal vein vs. lymphatic system and hence may thereby reduce first pass metabolism in the liver

Lipids can interfere with the enterocyte-based transport and the involved metabolic processes, potentially changing drug uptake, efflux or formation of metabolites

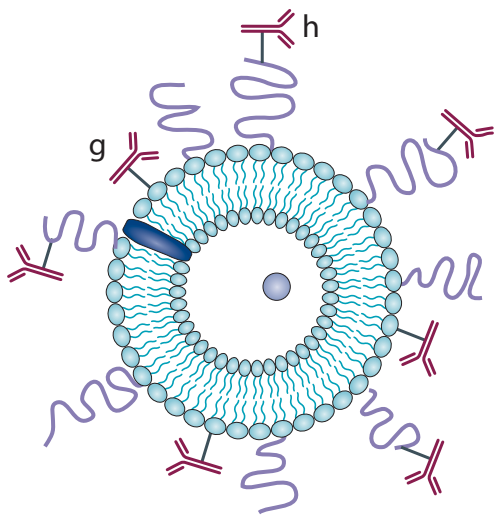
Liposome as (smart) delivery systems



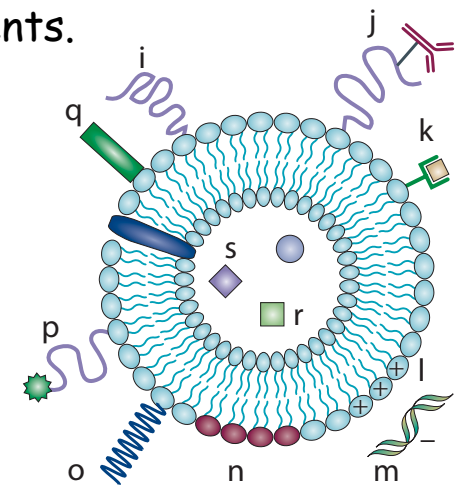
- Liposomes are spherical aggregates of lipids, that can accommodate hydrophilic drugs in the aqueous compartment and hydrophobic drugs in the liposomal membrane
- Liposomes are biocompatible
- Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions,yet do not cause undesirable side reactions.
- Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.



antibody-target immunoliposomes



liposomes with surface-grafted PEG for protection and antibody for targeting



Liposome with protective polymer (i) or targeting ligands such as antibody (j), a diagnostic label (k), positively charged lipids (l) allowing for the complexation with DNA (m), stimuli-sensitive lipids (n) or polymers (o), cell-penetrating peptides (p), viral components (q). In addition to a drug, liposome can loaded with magnetic particles (r) for magnetic targeting or with colloidal gold or silver particles (s) for electron microscopy.

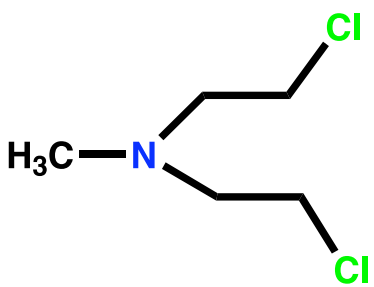
Drug Targeting

Linking a biosynthetic building block

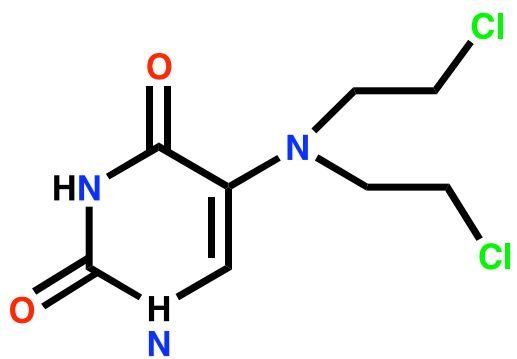
- Drug 'smuggled' into cell by carrier proteins for natural building block (e.g. amino acids or nucleic acid bases)
- Increases selectivity of drugs to target cells and reduces toxicity to other cells

Example:

Anticancer drugs



Non selective alkylating agent
Toxic



Uracil Mustard

- Alkylating group is attached to a nucleic acid base
- Cancer cells grow faster than normal cells and have a greater demand for nucleic acid bases
- Drug is concentrated in cancer cells - Trojan horse tactic

Linking drugs to monoclonal antibodies

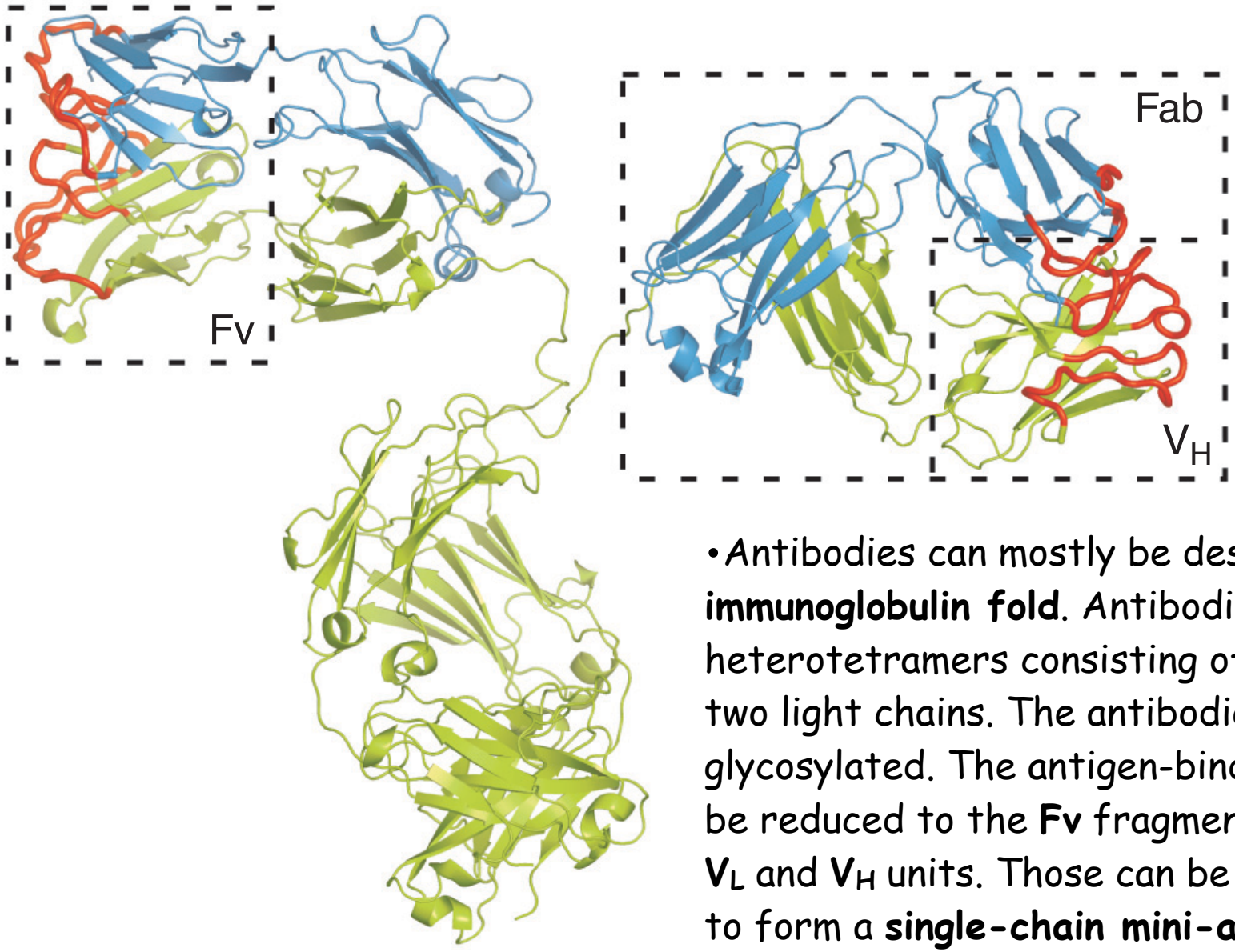
Example:

Anticancer agents

Rationale:

- Identify an antigen which is overexpressed on a cancer cell
- Clone a monoclonal antibody for the antigen
- Attach a drug or poison (e.g. ricin) to the monoclonal antibody
- Antibody carries the drug to the cancer cell
- Drug is released at the cancer cell

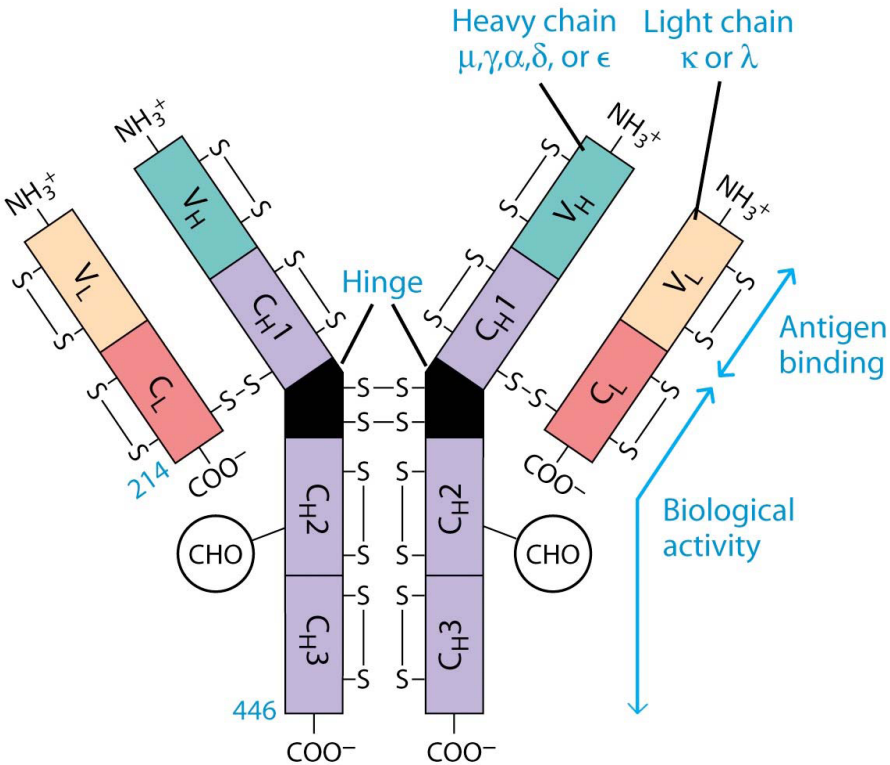
Antibodies

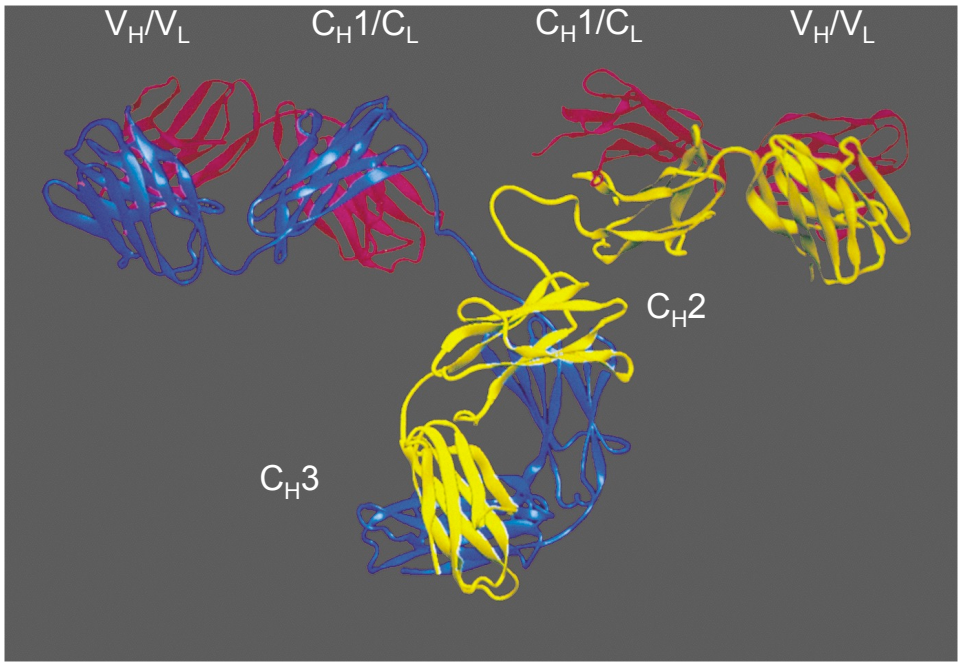


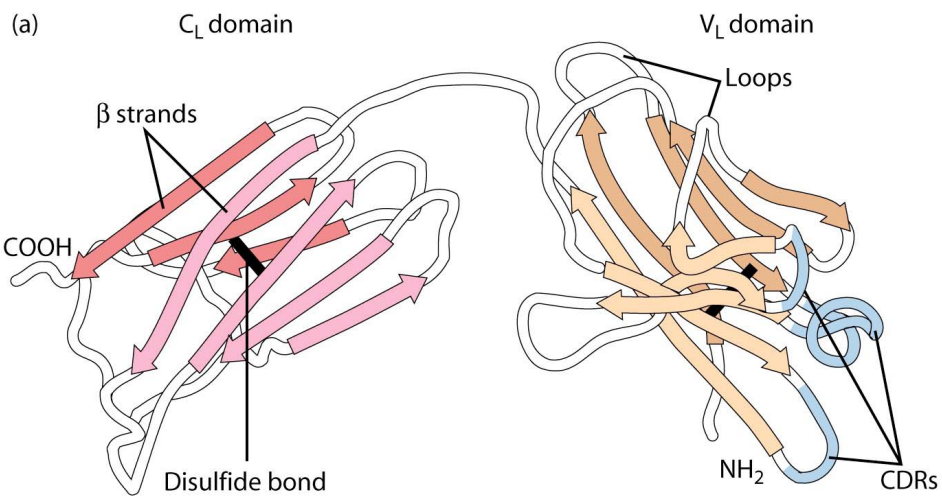
•Antibodies can mostly be described by the **immunoglobulin fold**. Antibodies are heterotetramers consisting of two heavy and two light chains. The antibodies are heavily glycosylated. The antigen-binding molecule can be reduced to the **Fv** fragment consisting of the V_L and V_H units. Those can be covalently linked to form a **single-chain mini-antibody** scFv.

•Complete antibodies have long serum half-lives. Fab and scFv fragments that lack the Fc region have short serum half-lives. This can be improved by pegylation, that also reduces immunogenicity.

Antibody Structure

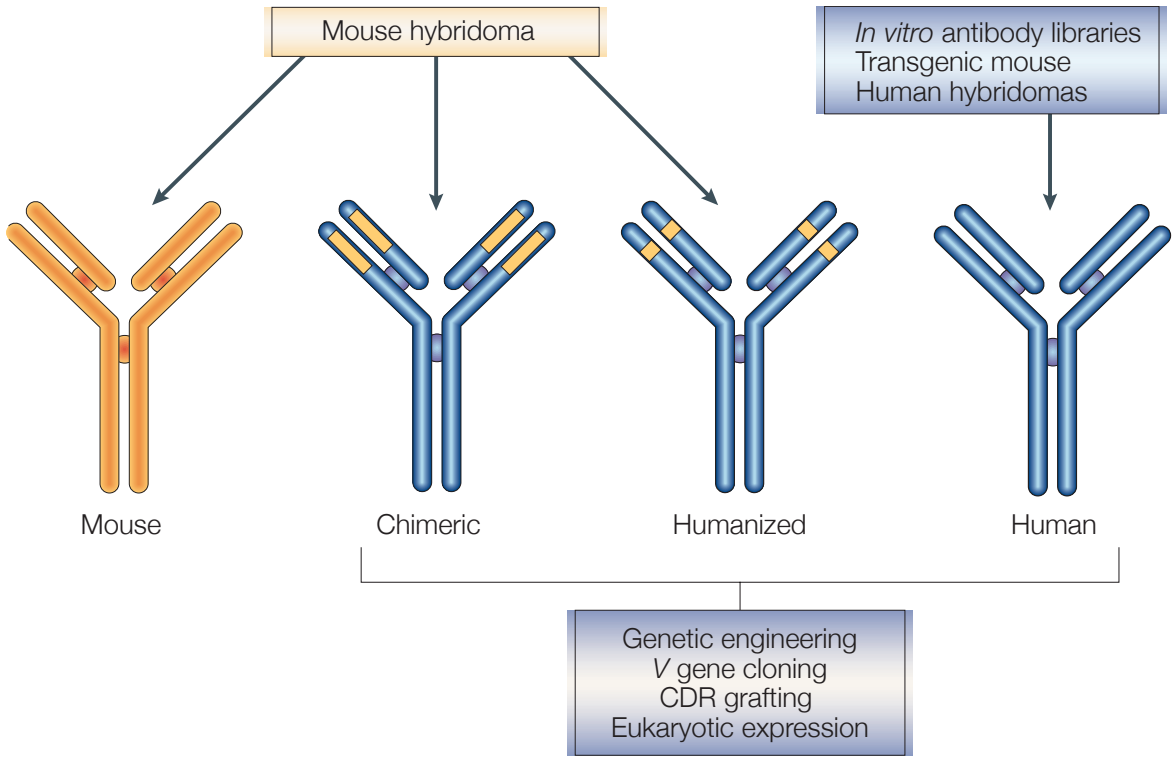






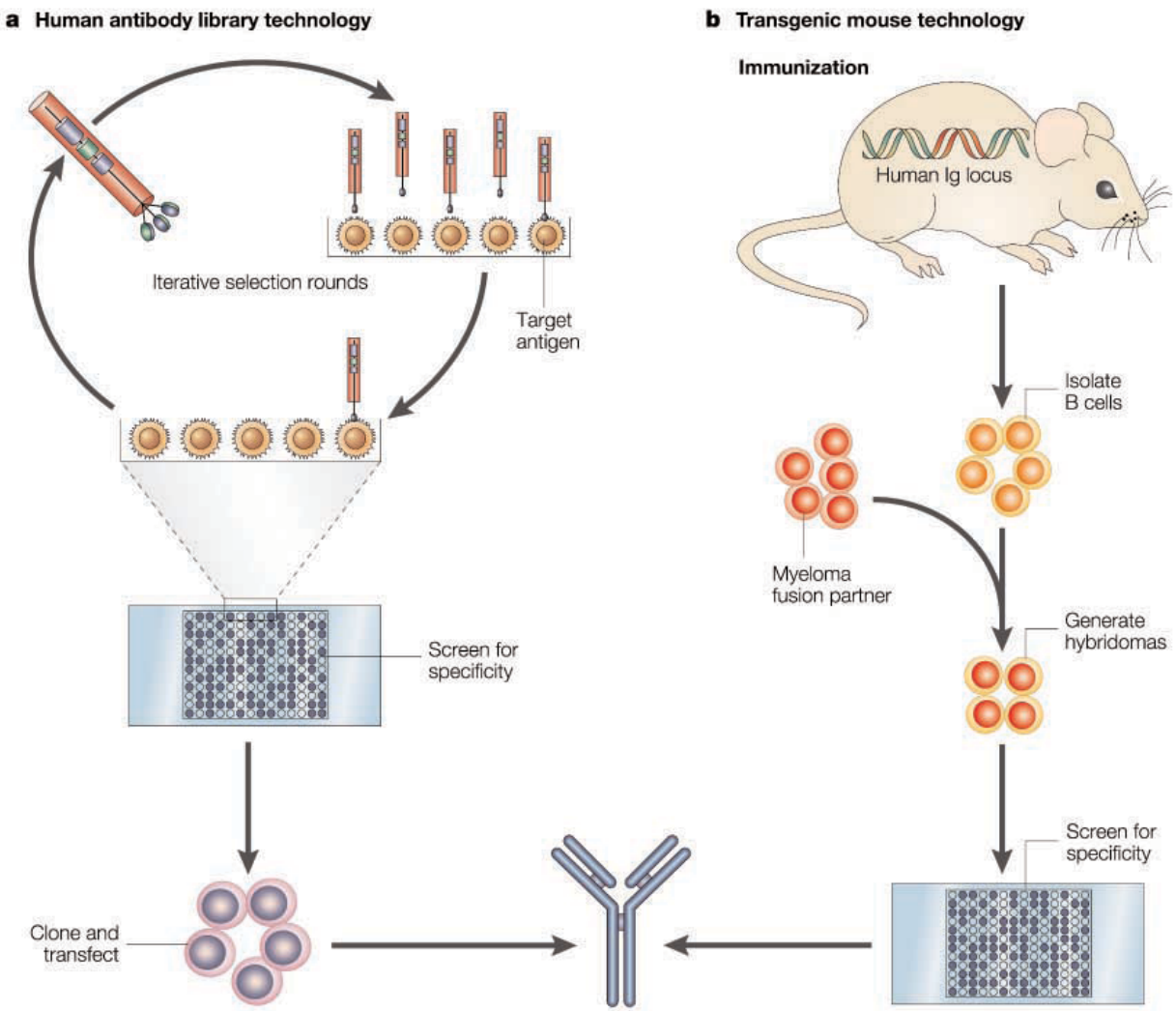
•antibodies are made of a heavy chain and a light chain, that are linked together by a disulfide bond. The antigen recognizing element is located in the complementary-determining regions (CDRs) of the VH elements

Avoiding Immune Response: Humanizing Antibodies



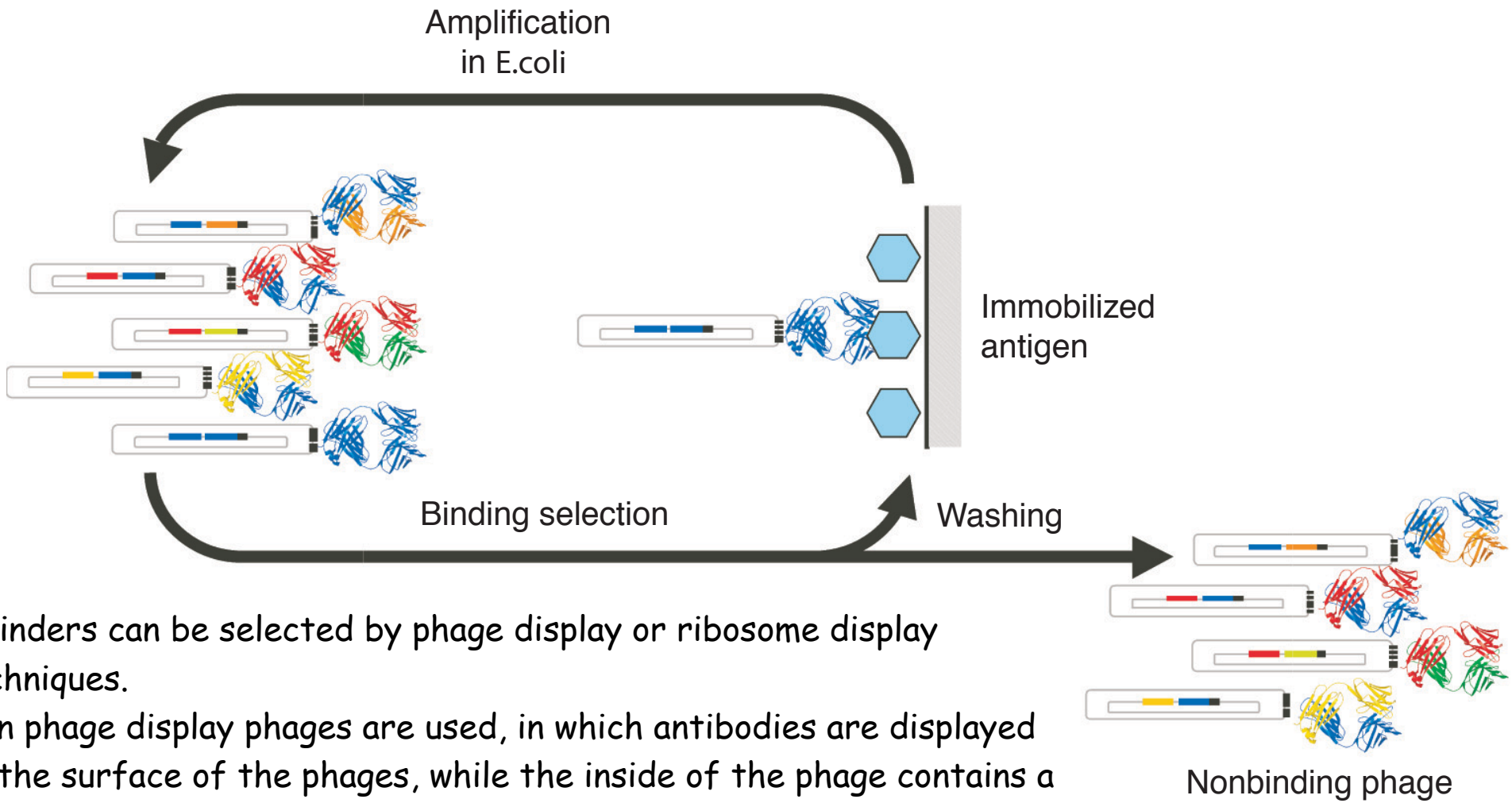
- Antibodies produced in mouse are potentially highly immunogenic in human
- In order to reduce immunicity chimeric antibodies have been developed constructed by taking taken human constant and mouse variable regions
- Immunicity could be further reduced by grafting mouse CDRs onto human antibodies

Avoiding Immune Response: Using in-vitro and in-vivo human ab techniques



- synthetic human antibody libraries can be constructed, in which the CDR loops are varied, and binders can be selected from those.
- Alternatively, transgenic mice can be used, in which the human gene for antibody production is contained, producing human antibodies in mice.

Selecting Binders by Phage Display



- Binders can be selected by phage display or ribosome display techniques.
- In phage display phages are used, in which antibodies are displayed on the surface of the phages, while the inside of the phage contains a plasmid encoding for the antibody DNA.
- Large libraries can be produced and screened for binder (e.g. by affinity chromatography) and amplified in E.Coli and used for further selections for improved binders.
- Good binders can finally be isolated as individual clones and sequenced.

Targets of Antibodies

Target	Agent
Vascular endothelial growth factor	Bevacizumab
Lymphocyte function-associated antigen 1	Efalizumab
Epidermal growth factor receptor	Cetuximab
Human epidermal growth factor receptor 2	Trastuzumab
Immunoglobulin E (IgE)	Omalizumab
CD-3	Muromonab-CD3
CD-20	Rituximab, ibritumomab tiuxetan, ^{131}I-tositumomab
CD-33	Gemtuzumab
CD-52	Alemtuzumab
F protein of RSV subtypes A and B	Palivizumab
CD-25	Basiliximab, daclizumab
Tumour-necrosis factor-α	Adalimumab, infliximab
Glycoprotein IIb/IIIa receptor	Abciximab
α4-Integrin subunit	Natalizumab