Optimization of a drug structure pharmacokinetics

Drug design and development

Stages:

1) Identify target disease
2) Identify drug target
3) Establish testing procedures
4) Find a lead compound
5) Structure Activity Relationships (SAR)
6) Identify a pharmacophore
7) Drug design - optimising target interactions
8) Drug design - optimising pharmacokinetic properties
9) Toxicological and safety tests
10) Chemical development and production
11) Patenting and regulatory affairs
12) Clinical trials

1. Drug design – pharmacokinetics (drug-like prop., bioavailability)

- to improve pharmacokinetic properties of lead compound
  - to optimise chemical and metabolic stability

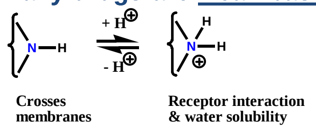
    (mouth: saliva pH 6.5-7.5, **stomach:** pH **1.5-3**, intestines: pH 6.0-6.9, blood: pH 7.35/ **digestive enzymes** (pepsine the most active in pH 1.2-2...) / **metabolic enzymes** e.g. CYP450)

  - to optimise hydrophilic / hydrophobic balance

    (solubility in blood / solubility in GIT / permeability through cell membranes / access to CNS / excretion rate)

1. 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 membranes - to avoid rapid excretion
- Drugs must have both hydrophilic and lipophilic characteristics
- Many drugs are weak bases with pK_a 6-8



NH_3 pKa 38 **NH_4^+** pKa 9

R_3NH^+, $R_2NH_2^+$, RNH_3^+ pKa 10-11

1.1 Solubility and membrane permeability

1.1.1 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

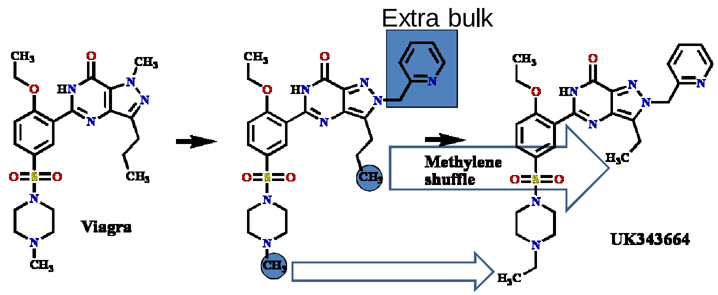
Methods:

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

1.1 Solubility and membrane permeability

1.1.1 Vary alkyl substituents

Methylene Shuffle



1.1 Solubility and membrane permeability

1.1.2 'Masking' or removing polar groups

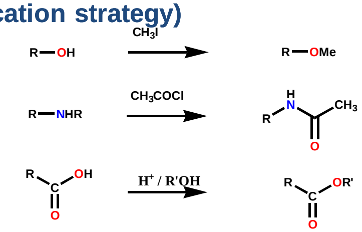
Rationale:

- 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)
- See also prodrugs

Methods:

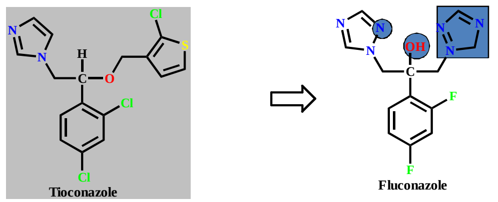


1.1 Solubility and membrane permeability

1.1.3 Adding polar groups

Rationale:

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



Antifungal agent with poor solubility - skin infections only

Systemic antifungal agent improved blood solubility

Disadvantage:

- May introduce unwanted side effects

1.1 Solubility and membrane permeability

1.1.4 Vary pK_a

Rationale:

- varying pK_a to obtain required ratio of ionised to unionised drug

Method:

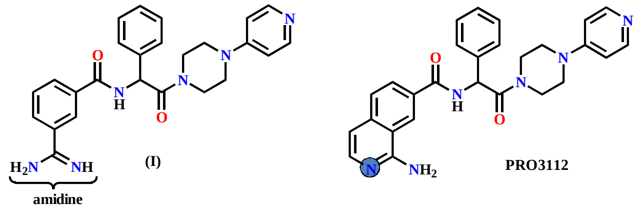
- vary alkyl substituents on amine nitrogens
- vary aryl substituents to influence aromatic amines or aromatic carboxylic acids

Disadvantage:

- May affect binding interactions

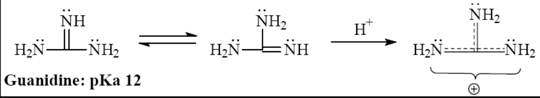
1.1 Solubility and membrane permeability

1.1.4 Vary pK_a



Antithrombotic but too basic

Decreased basicity
N is locked into heterocycle



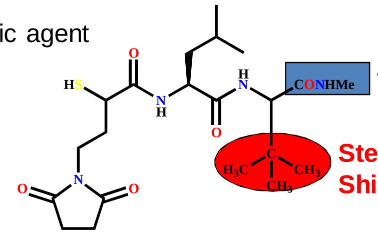
1.2 Drug stability

1.2.1 Steric Shields

Rationale:

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

Antirheumatic agent D1927



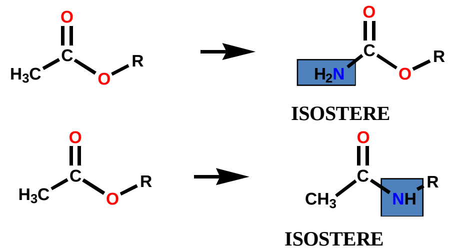
Blocks hydrolysis of terminal am

1.2 Drug stability

1.2.2 'Electronic shielding' of NH_2

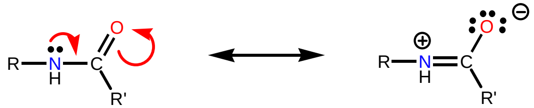
Rationale:

- **to stabilise 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**



1.2 Drug stability

1.2.2 'Electronic shielding' of NH_2

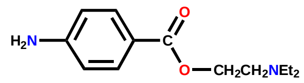


1.2 Drug stability

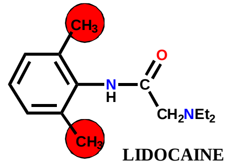
1.2.3 Stereoelectronic Effects

Rationale:

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



PROCAINE



LIDOCAINE

Local anaesthetic
(short duration)

ortho Me- groups hinder hydrolysis by esterases (**steric shields**), **amide is more stable** than ester **(electronic effect)**

1.2 Drug stability

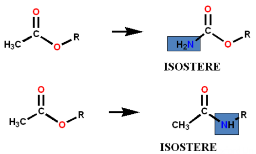
1.2.4 Bio-isosteres

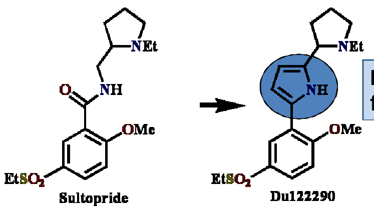
Rationale:

- replace susceptible group with a different group without affecting activity
- if bio-isostere shows improved pharmacokinetic properties

Examples:

- Amides and urethanes for esters (see ear
- Du122290 (dopamine antagonist)





Pyrrole ring => bioisostere for methylene amide

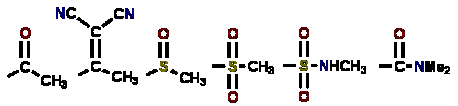
2. Hydrophobicity of the Molecule

Partition Coefficient $P = \frac{[\text{Drug in octanol}]}{[\text{Drug in water}]}$

High *P* → **High hydrophobicity**

9. Bio-isosteres

Substituent

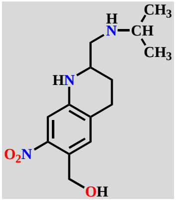


π	-0.55	0.40	-1.58	-1.63	-1.82	-1.51
σ_p	0.50	0.84	0.49	0.72	0.57	0.36
σ_m	0.38	0.66	0.52	0.60	0.46	0.35
MR	11.2	21.5	13.7	13.5	16.9	19.2

- choose substituents with similar physicochemical properties (e.g. CN, NO_2 and COMe could be bio-isosteres)
- choose bio-isosteres based on the most important physicochemical property (e.g. COMe & SOMe are similar in σ_p (0.50/0.49); SOMe and SO_2Me are similar in π)

OPTIMIZING TARGET INTERACTIONS

4.12 CASE STUDY - Development of Oxamniquine

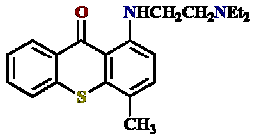


Oxamniquine

- vs schistosomiasis (bilharzia) - a water borne disease carried by snails
- 200 million sufferers in third world

bilharzióza parazitární onemocnění člověka a zvířat způsobené krevními motolicemi rodu Schistosoma (krevnička) Celosvětově je infikováno přes 200 miliónů lidí, patogenním činitelem nejsou samotní červi, nýbrž jimi nakladená vajíčka, které při své cestě ven z organismu poškozují tkáně a vyvolávají imunitní odpověď v podobě specifických zánětů (prejavuje sa horečkami, kašlem, bolestmi břicha, průjmem,

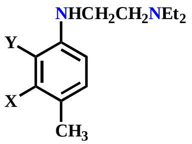
Stage 1 - Find a Lead Compound



Lucanthone

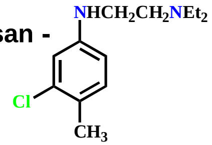
- low activity, orally inactive and slightly toxic

Stage 2 - Simplification



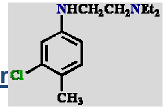
Stage 3 - Vary aromatic substituents

Mirasan -



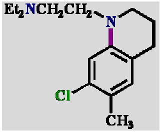
- active in mice, not in human
- electronegative Cl beneficial

Stage 4 - SAR studies

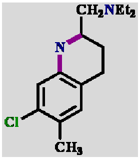


- side chain and aromatic ring are important binding gr
- both nitrogens are important
- nitrogens are on a flexible chain - conformational flexibility

Stage 5 - Rigidification



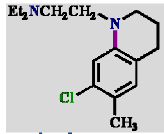
one bond 'locked', activity increases
rigidification has retained active conformation
active in monkeys, inactive in man



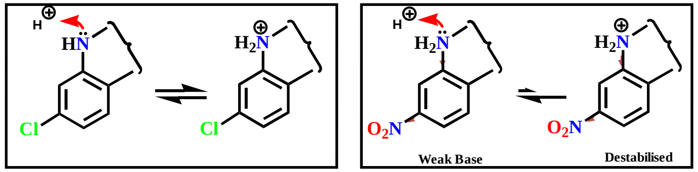
two bonds 'locked'
rigidification has retained active conformatic
activity increases in mice

Novel structures for screening.

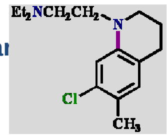
Stage 6 - Vary substituents and substituent positions on aromatic ring



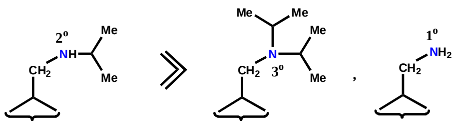
- substitution pattern on aromatic ring is essential
- electron withdrawing groups are the best for activity - replacing Cl with NO_2 increases activity
- nitro group reduces basicity of the aromatic nitrogen, structure is less easily ionised and passes through cell membranes more easily



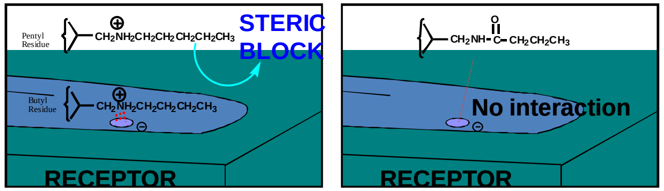
Stage 7 - Vary side chain substituents



- Secondary amine is better than primary or tertiar at the end of the chain

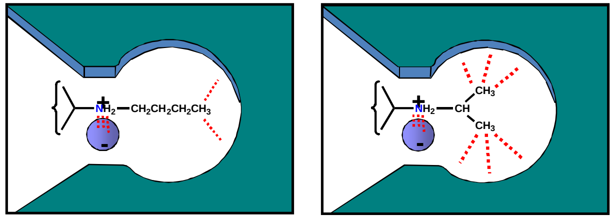


- maximum length of alkyl group on N is butyl
- acyl groups eliminate activity (implies N should be protonated for ionic interaction)

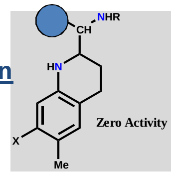


Stage 7 - Vary side chain substituents

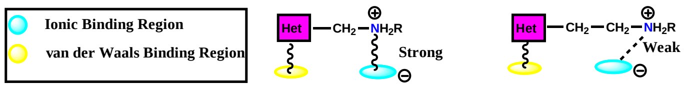
Branched alkyl groups increase activity. Implies stronger VdW interactions to bulky pocket or benefit in increased lipophilicity



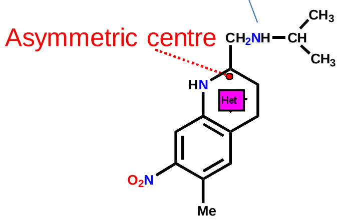
Branching on side chain eliminates activity
Prevents molecule adopting active conformation



Stage 8 - Other strategies gave no improvement
(e.g. chain extension eliminates activity)

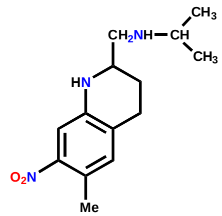


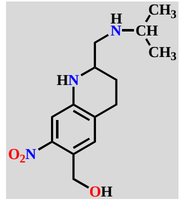
Optimum Structure



Stage 9 - Drug Metabolism Studies

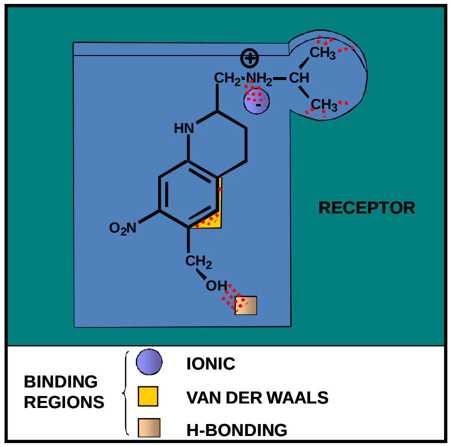
- oxidation of aromatic methyl group give oxamniquine that is an active drug
- Methyl analogue is acting as a prodrug





Oxamniquine

Stage 10 - Proposed binding interactions



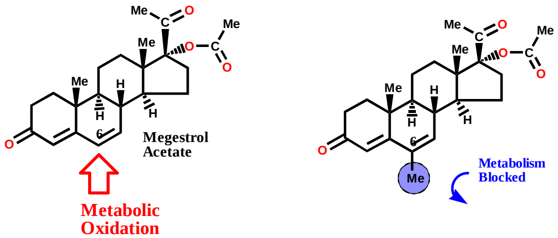
Optimization of drug stability

1.2 Drug stability

1.2.5 Metabolic blockers

Rationale:

- metabolism of drugs usually occur at specific sites
- aim is to introduce a group at a susceptible site to block metabolism
- increases drug stability and lifetime



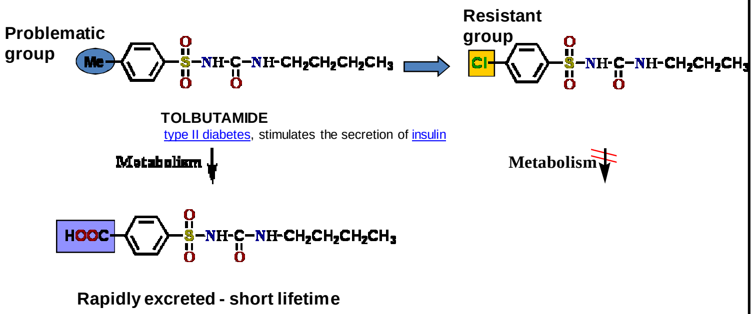
oral contraceptive (to prevent pregnancy)
- limited lifetime

1.2 Drug stability

1.2.6 Remove / replace susceptible metabolic groups

Rationale:

- metabolism of drugs usually occurs at specific groups
- Remove problematic group or replace it with metabolically stable group (e.g. modification of tolbutamide)



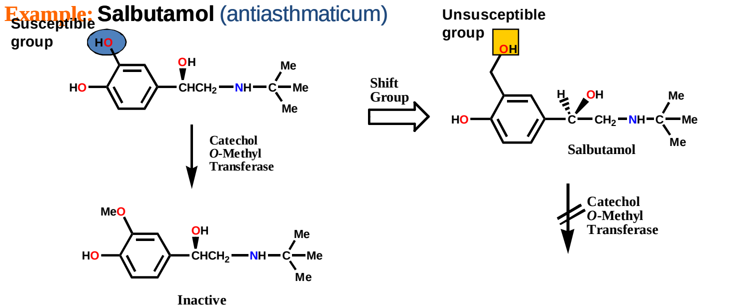
1.2 Drug stability

1.2.7 Shifting problematic group

Rationale:

- used if the metabolically susceptible group is important for binding
- shift group position to make it unrecognisable to problematic enzyme
- must still be recognisable to target

Example: **Salbutamol** (antiasthmaticum)



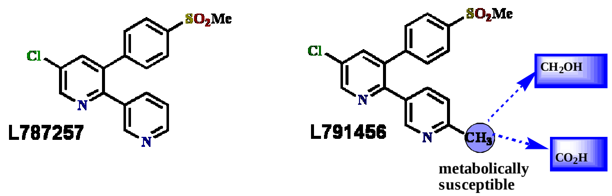
1.2 Drug stability

1.2.8 Introducing a susceptible metabolic group

Rationale:

- used to decrease metabolic stability
- used for drugs with too long lifetime in the body and cause side effects
- add group susceptible to metabolic reactions

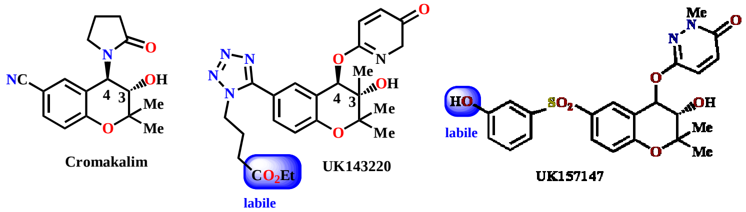
Example: **Anti-arthritic agents**



1.2 Drug stability

1.2.8 Introducing susceptible metabolic groups

Example: Anti-asthmatic agents



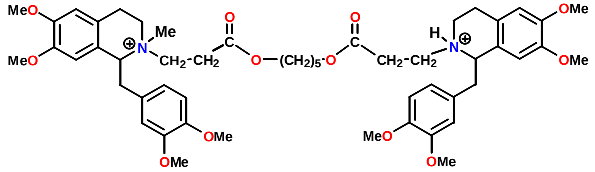
- ***Cromakalim*** (potassium channel-opening vasodilator to treat hypertension) **produces cardiovascular side effects if stays in blood supply**
- **add metabolic instabile group that compound rapidly metabolise in blood (ester is quickly hydrolysed by esterases to inactive acid or phenolic group that is quickly conjugated with sugars and eliminated from a body**

1.2 Drug stability

1.2.9 Introducing chemically susceptible groups

Rationale:
- **to decrease drug lifetime**

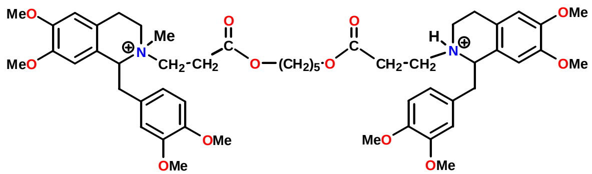
Example: **Atracurium – intravenous anesthetics**



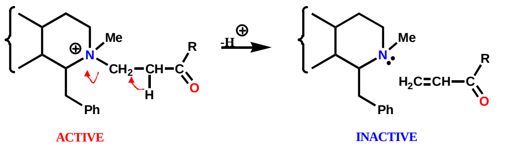
- **stable at acid pH, but unstable at blood pH = 7.3 (slightly alka**
- **in blood it self destructs by Hoffmann elimination and allows to control dose levels accurately**
- **quick recovery times after surgery**

1.2 Drug stability

1.2.9 Introducing chemically susceptible groups



Hoffmann Elimination



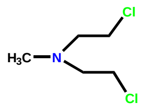
1.3 Drug targeting

1.3.1 Linking a biosynthetic building block

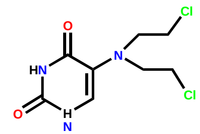
Rationale:
- **Drug 'smuggled' into cell by carrier proteins for natural building block (e.g. amino acids or nucleic acid bases)**
- **Increases selectivity of drugs to target cells and reduces toxicity to other cells**

Example:

Anticancer drugs



Non selective alkylating agent
Toxic



Uracil Mustard

- **cancer cells grow faster than normal cells and have a greater demand for nucleic acid bases**
- **drug is concentrated preferentialy in cancer cells - Trojan horse tactic**
- **nucleic acid base are alkylated by a smuggled drug**

1.3 Drug targeting

1.3.2 Linking drugs to monoclonal antibodies

Example: Anticancer agents

Rationale:

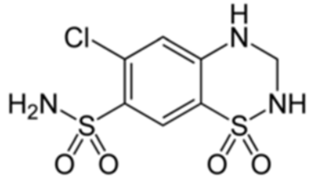
- Identify an antigen which is overexpressed on a cancer cell
- clone a monoclonal antibody for the antigen
- attach a drug or poison (e.g. ricin / protein toxin that inhibits cell protein synthesis, 0.5mg in blood is a lethal dose for human) to the monoclonal antibody
- antibody carries the inactive drug to the ca
- drug or toxine i he cancer cell

1.3 Drug targeting

1.3.3 Targeting gut infections

Rationale:

- design the antibacterial agent to be highly polar or ionise not to cross the gut wall
- drug concentrates at the site of infection (e.g. highly ionised sulfonamides)



1.3 Drug targeting

1.3.4 Targeting peripheral regions over CNS

Rationale:

- Increase polarity of the drug
- Drug is less likely to cross the blood brain barrier

The blood-brain barrier (BBB) is a membranic structure that protect the brain from chemicals in the blood, while still allowing essential metabolic function. It is composed of endothelial cells, which are packed very tightly in brain capillaries. This higher density restricts passage of substances from the bloodstream much more than endothelial cells in capillaries elsewhere in the body.

1.4 Reducing drug toxicity

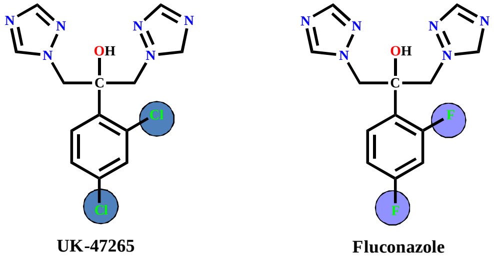
Rationale:

- Toxicity is often due to specific functional groups
- Remove or replace functional groups known to be toxic
  - aromatic nitro groups - ArNO2
  - aromatic amines – ArNH2
  - bromoarenes - ArBr
  - hydrazines – -NH-NH-
  - polyhalogenated groups
  - hydroxylamines – RNH-OH
- Vary substituents
- Vary position of substituents

1.4 Reducing drug toxicity

Example - varying substituents

- Fluconazole (Diflucan) - antifungal agent

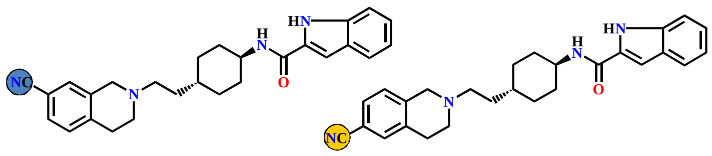


substituents varied
less toxic product

1.4 Reducing drug toxicity

Example - varying substituent position

- Dopamine antagonists



Inhibits P450 enzymes

No inhibition of P450 enzymes

Cytochrom P450: $RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$

Prodrugs and their properties

Prodrugs

Definition: inactive compounds which are converted to active compounds in the body

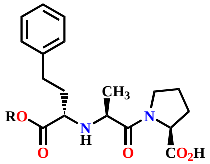
Uses:
- improving membrane permeability
- prolonging activity
- masking toxicity and side effects
- varying water solubility
- drug targeting
- improving chemical stability
- acting as 'Sleeping agents'

Prodrugs to improve membrane permeability

Esters

- used when a carboxylic acid is required for target binding
- ester masks polar and ionisable -COOH group
- to increase membrane permeability
- hydrolysed in blood by esterases
- leaving group (alcohol) should be non toxic)

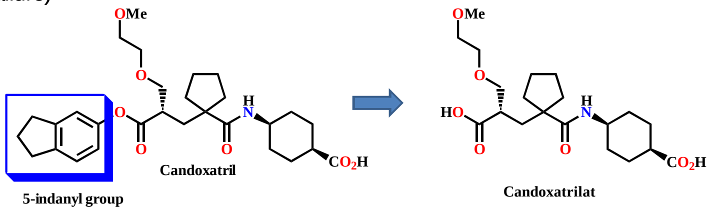
Example: Enalapril for enalaprilate (antihypertensive)



R=Et Enalapril
R=H Enalaprilit

Prodrugs to improve membrane permeability

Example: Candoxatril for Candoxatrilat (to treat a chronic heart failure)



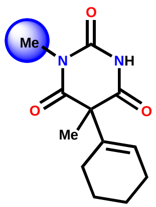
- varying the alcoholic group in ester varies the rate of hydr
- electron withdrawing groups increase rate of hydrolysis (e.g. 5-indanyl)
- leaving group (5-indanol) is non toxic

Prodrugs to improve membrane permeability

N-Methylation of amines

- to reduce polarity of amines and increase membrane permeability
- demethylated in liver recovering a drug in its active form

Example: Hexobarbitone (barbiturate, sedative effect)

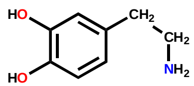


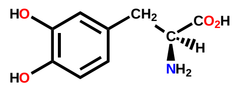
Prodrugs to improve membrane permeability

Trojan Horse Strategy

- prodrugs designed to mimic biosynthetic building block
- actively transported across cell membranes by carrier proteins

Example: Levodopa for dopamine





similar to Phe, or Tyr

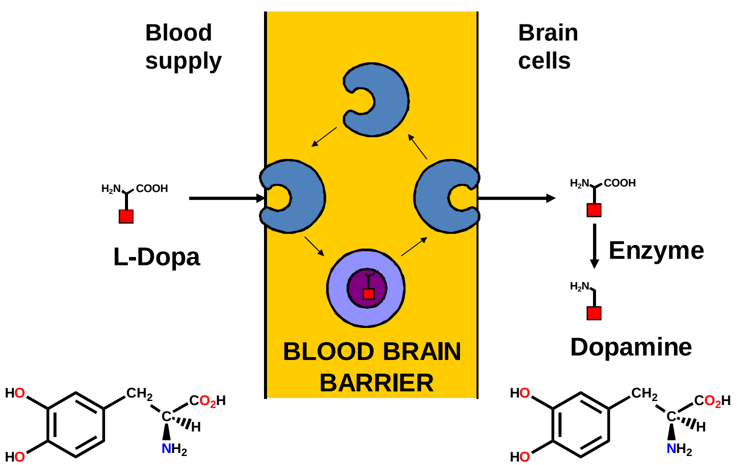
Dopamine

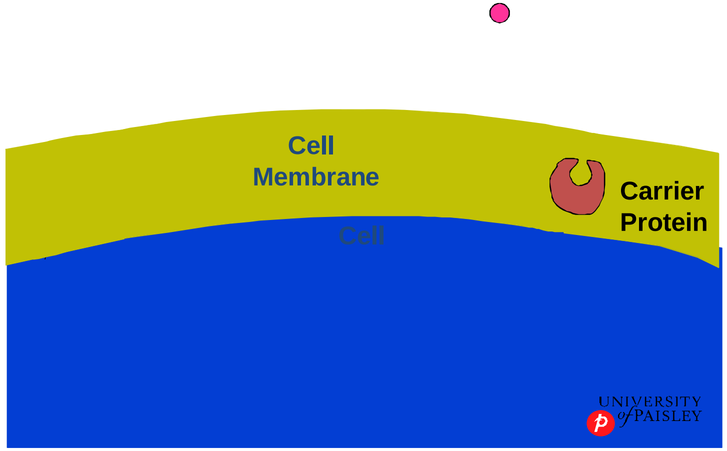
- for Parkinson's Disease treatment
- too polar to cross cell membranes and BBB

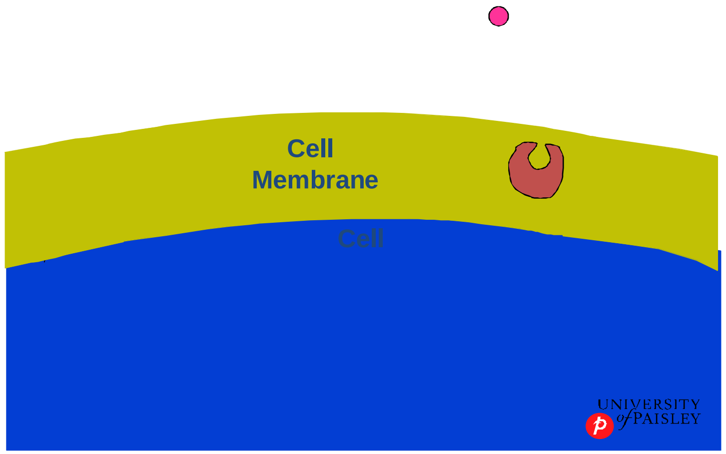
Levodopa

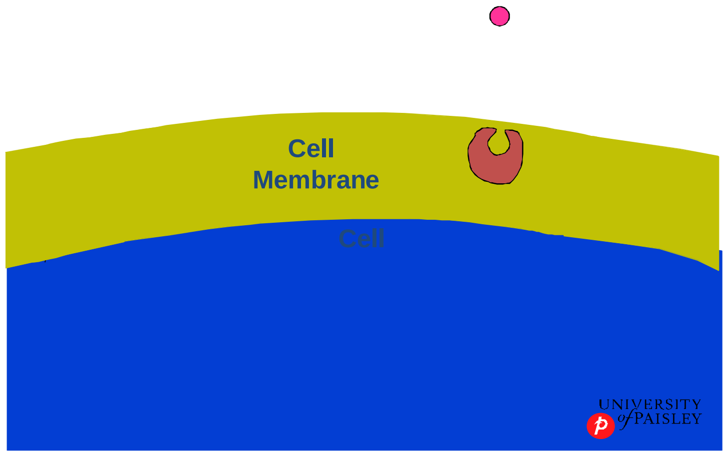
- more polar amino acid, passing cell membranes by carrier proteins for amino acids
- it decarboxylates inside a cell to dopamine

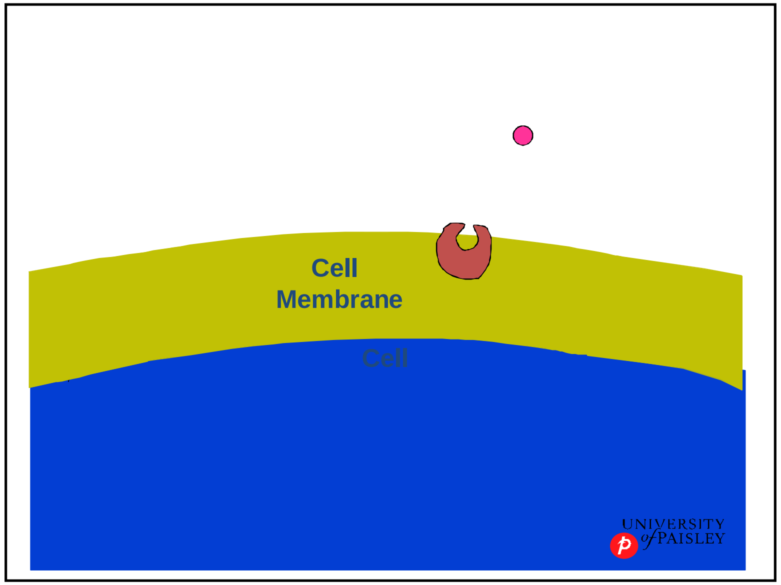
Prodrugs to improve membrane permeability







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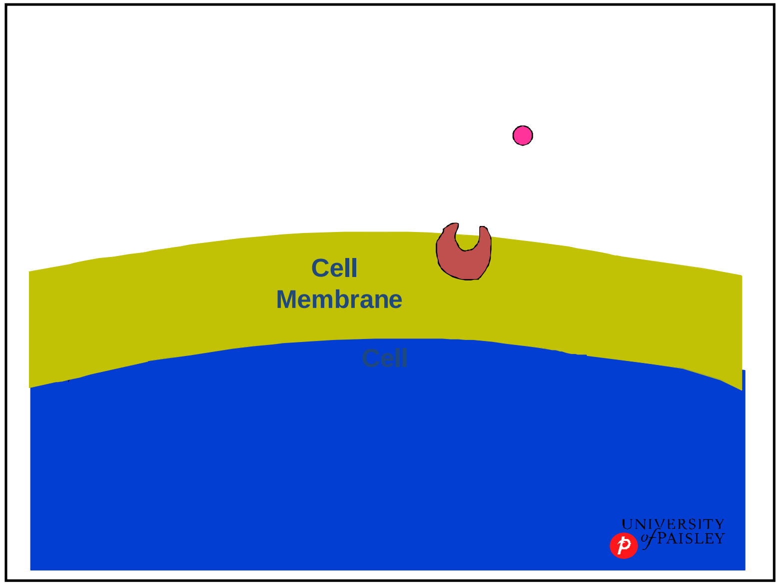
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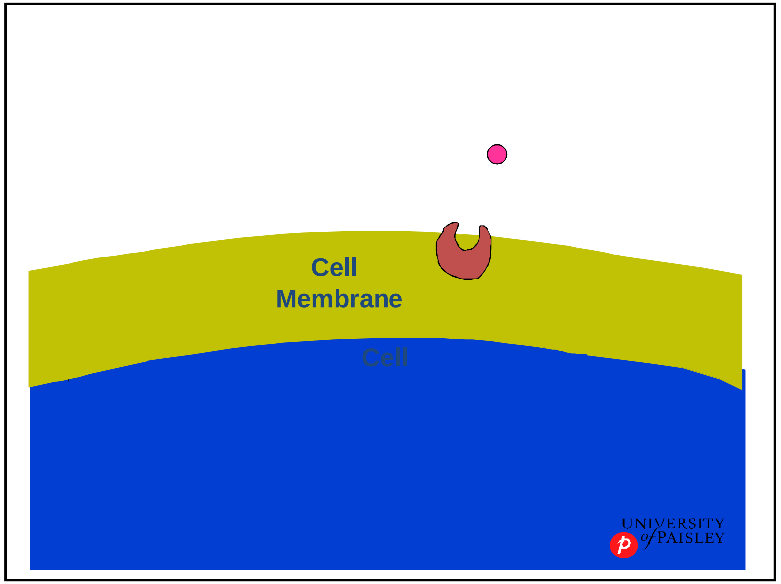
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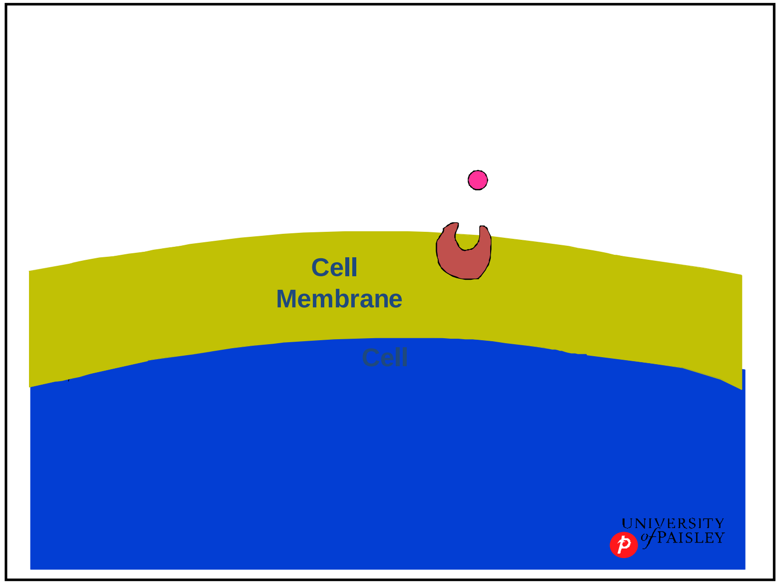
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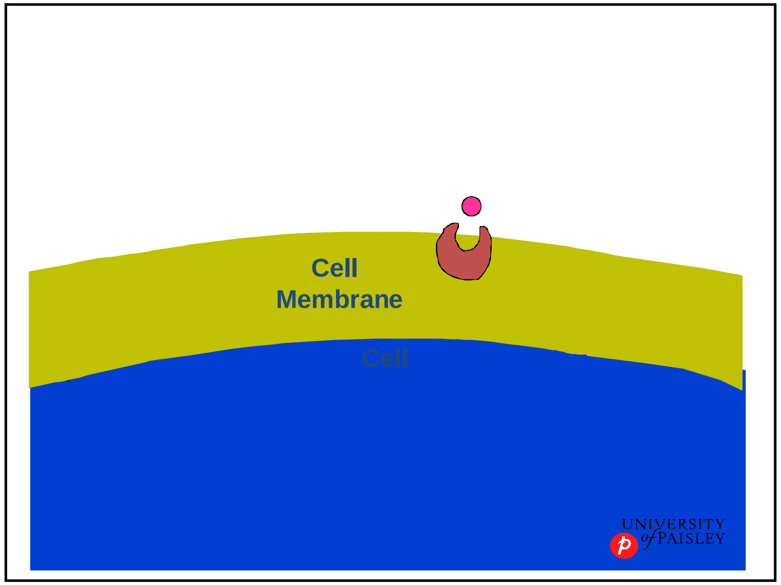
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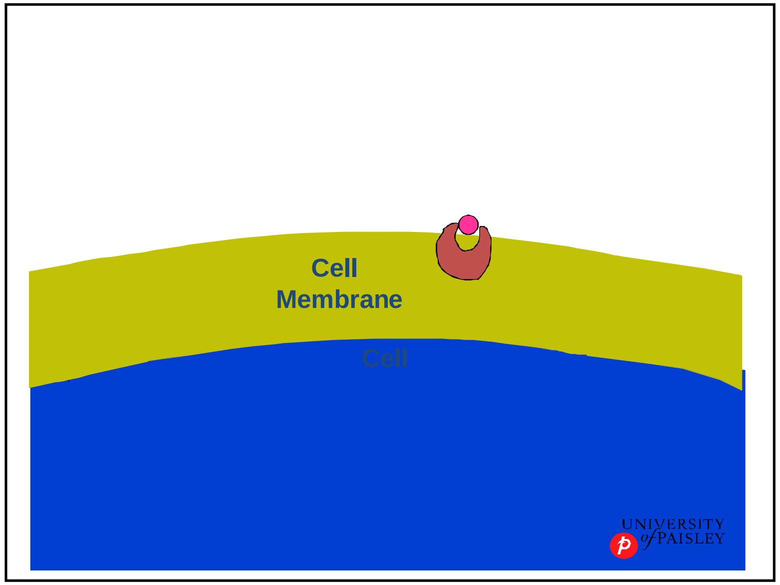
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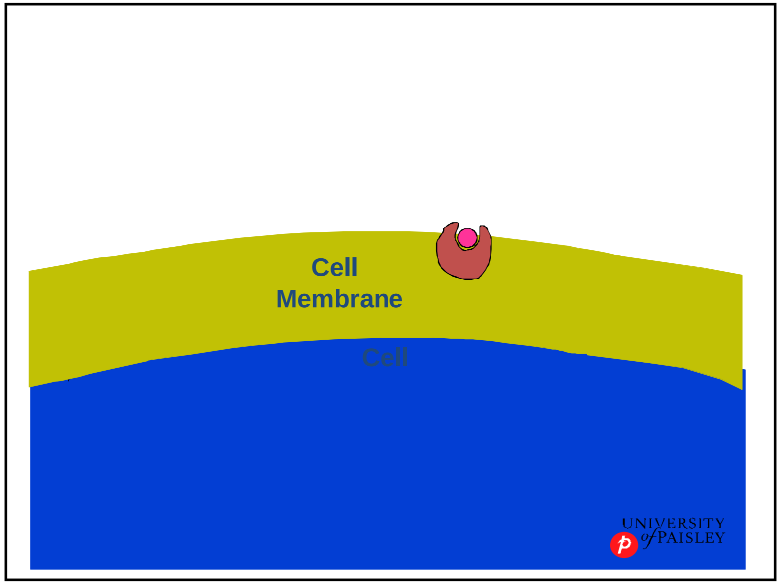
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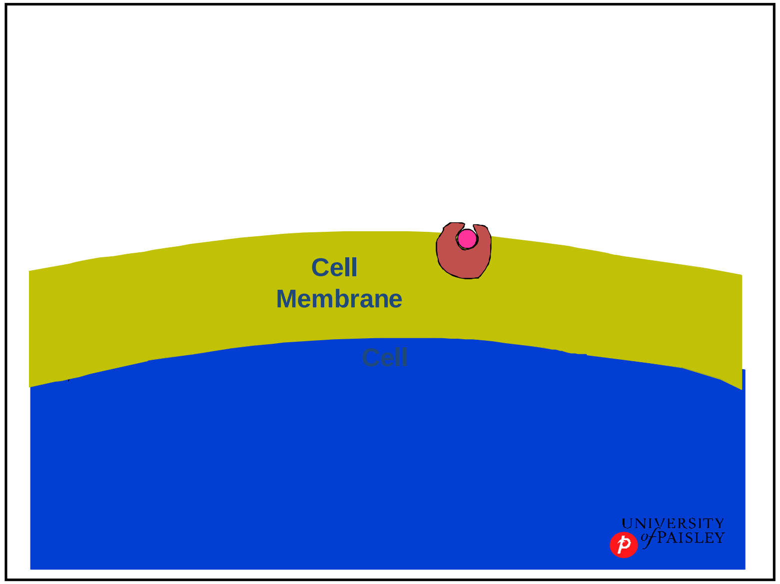
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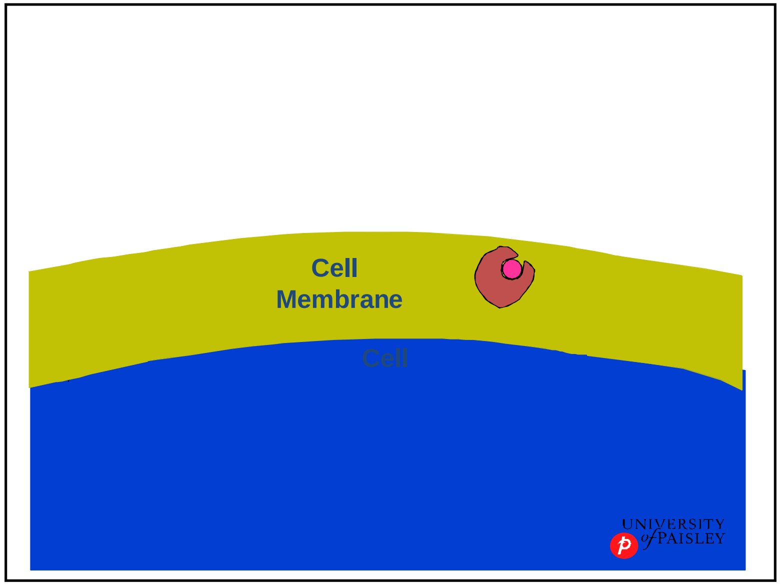
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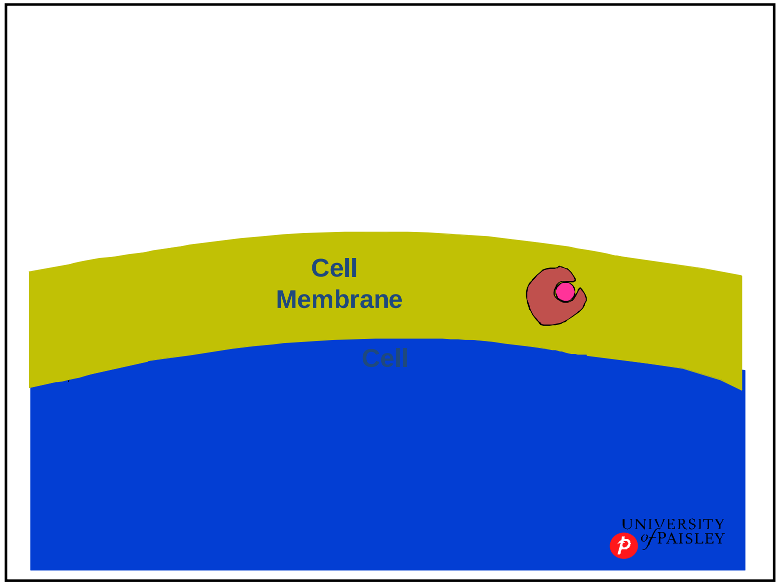
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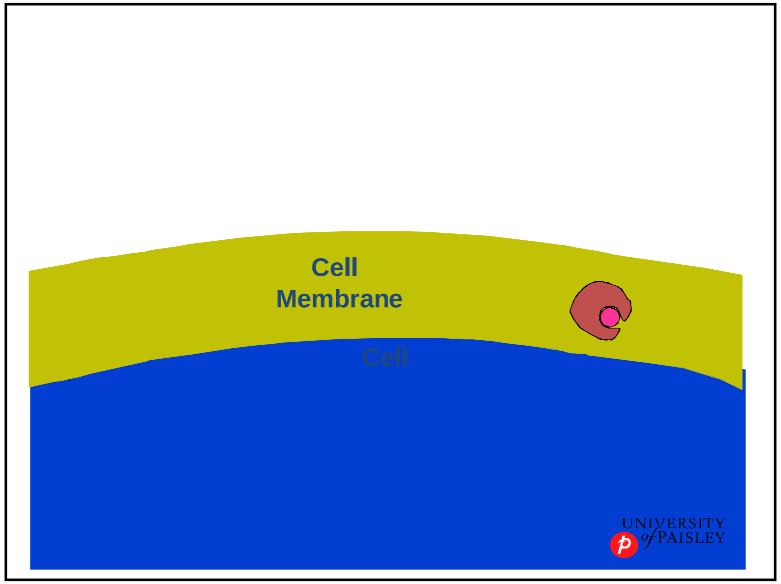
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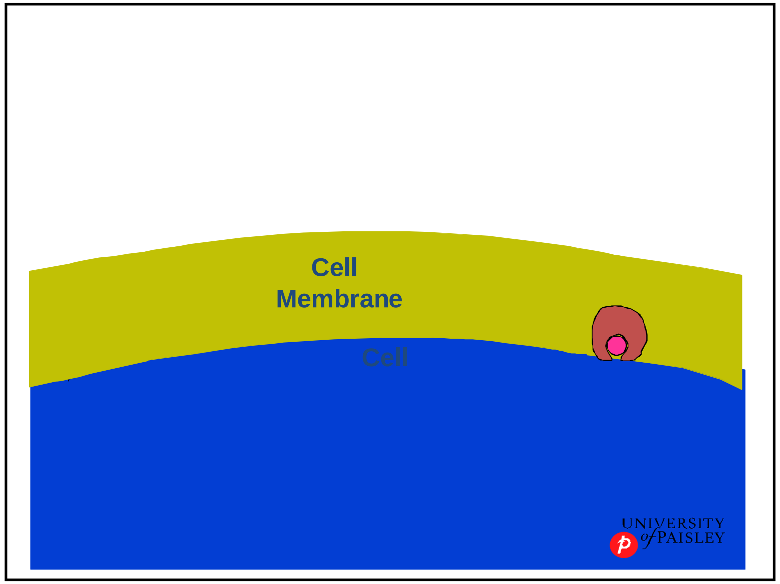
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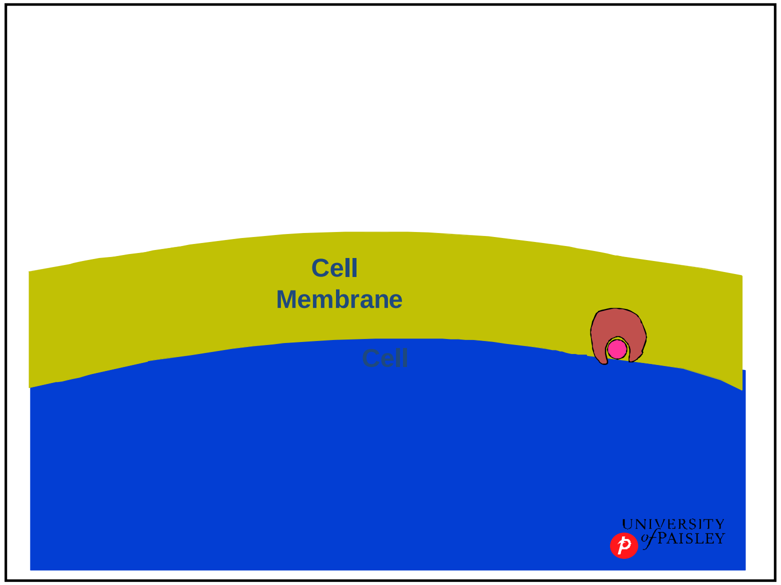
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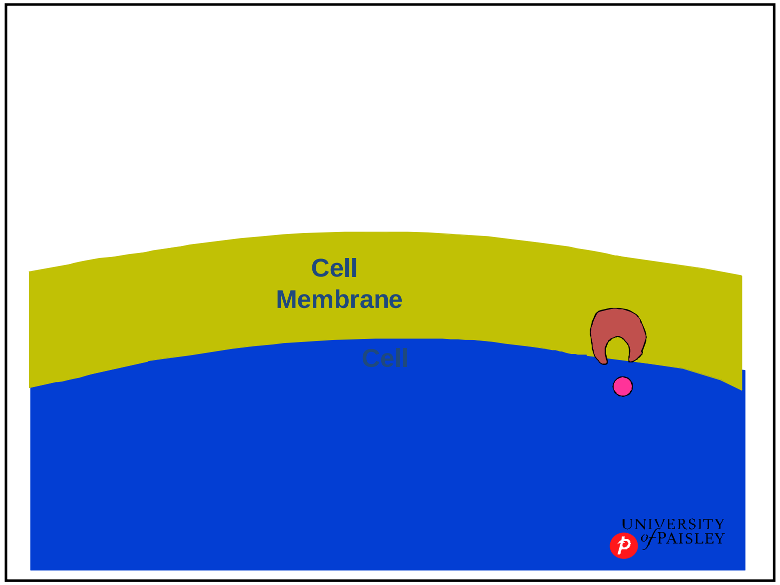
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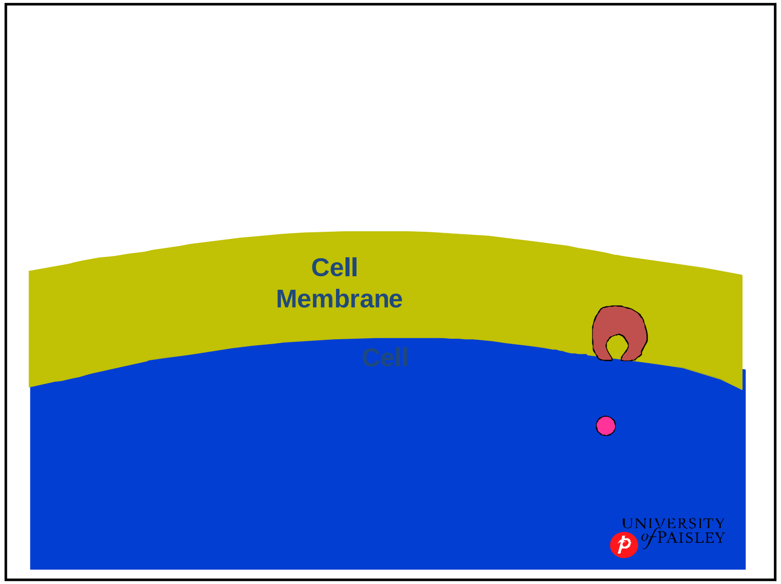
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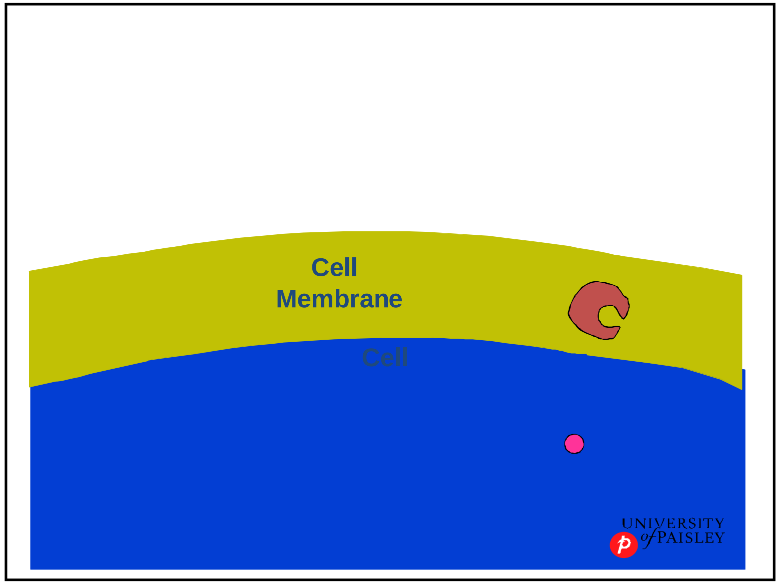
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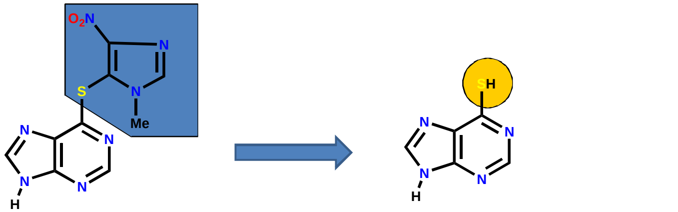
Prodrugs to prolong activity

Mask polar groups

- to reduce rate of excretion

Example: Azathioprine for 6-Mercaptopurine (an immunosuppressive

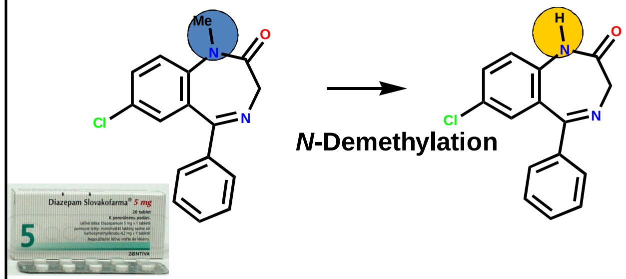


Azathioprine
- longer lifetime
- slow conversion to 6-mercaptopurine

6-Mercaptopurine (a drug)
- short lifetime - eliminates too quickly

Prodrugs to prolong activity

Example: Valium for nordazepam (an active metabolite of valium)



Valium / Diazepam

Nordazepam

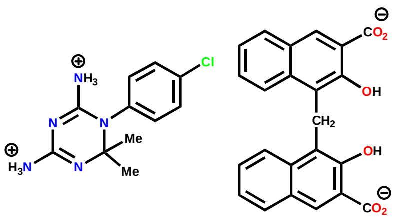
Diazepam (marketed as Valium by *Hoffmann-La Roche*), is a prodrug used for **treating anxiety, insomnia, seizures, alcohol withdrawal and muscle spasms.** Nordazepam is an active metabolite of diazepam

Prodrugs to prolong activity

Adding a hydrophobic group

- hydrophobic drug concentrates in fat tissue
- slow removal of hydrophobic group ensures slow release into blood supply

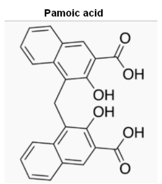
Example: Cycloguanil palmoate (antimalarial drug)



Cycloguanil

Pamoate

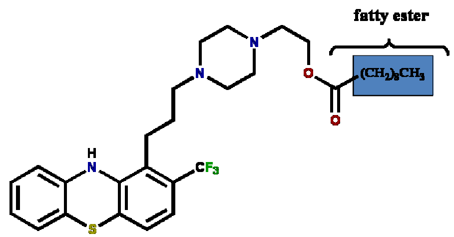
Lipophilic



Prodrugs to prolong activity

Adding a hydrophobic group

Example: hydrophobic esters of antipsychotic fluphenazine

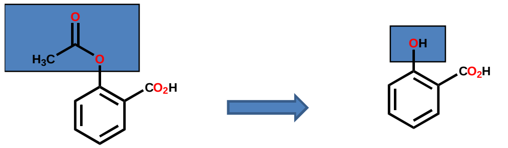


- administred by intramuscular injection
- drug concentrates in fatty tissues
- slowly releases into the blood supply
- rapidly hydrolyses in the blood

Prodrugs to mask toxicity and side effects

- mask groups responsible for toxicity / side effects
- used when groups are important for activity and can not be removed

Example: Aspirin for salicylic acid

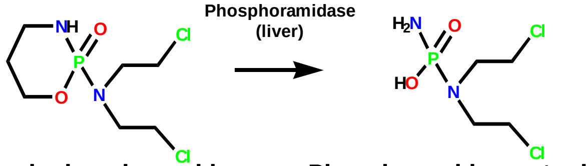


Aspirin
- phenolic group is masked by ester
- hydrolysed in body

Salicylic acid
- analgesic, but causes stomach ulcers due to phenol group

Prodrugs to mask toxicity and side effects

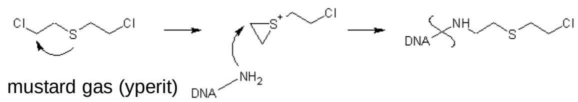
Example: Cyclophosphoramide for phosphoramide (anticancer agent)



Cyclophosphoramide
- a non toxic oral drug

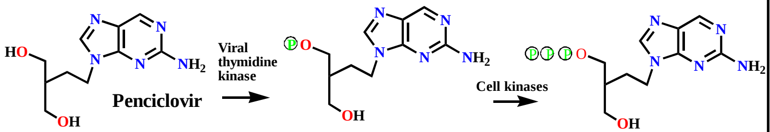
Phosphoramide mustard
- alkylating agent

first used in World War I by the German army against British soldiers near Ypres in July 1917



Prodrugs to mask toxicity and side effects

Example: antiviral drug against herpes virus infection

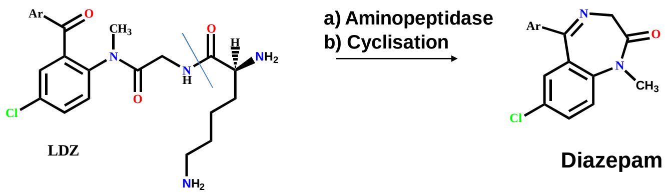


prodrug **drug**

Hu-thymidine kinases phosphorylate substrate less rapidly than does the **viral** thymidine kinase, so the **active triphosphate is present at much higher concentrations in virally infected cells** than in uninfected cells. The **activated drug binds to viral DNA polymerase with a much higher affinity** than to human DNA polymerases. Therefore **penciclovir exhibits negligible cytotoxicity to healthy cells**.

Prodrugs to mask toxicity and side effects

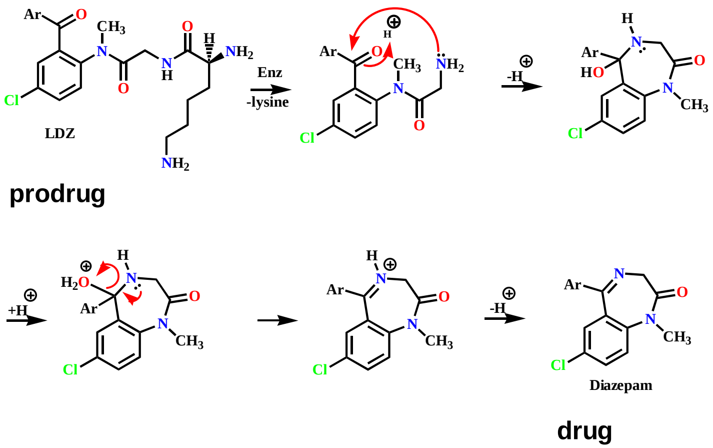
Example: LDZ for diazepam



Diazepam

- **avoids drowsy (sleepy) side effects of diazepam**

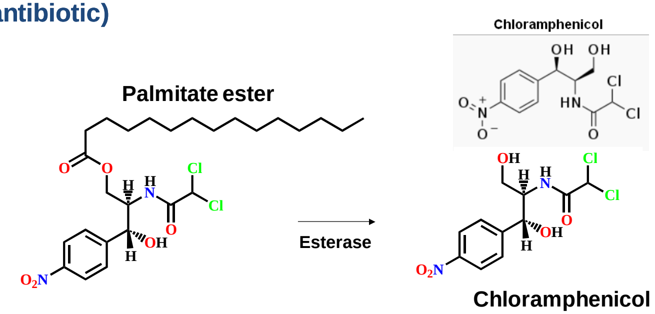
1.5.3 Prodrugs to mask toxicity and side effects



Prodrugs to lower water solubility

- used to reduce solubility of foul tasting orally active drugs
- less soluble on tongue, less revolting taste

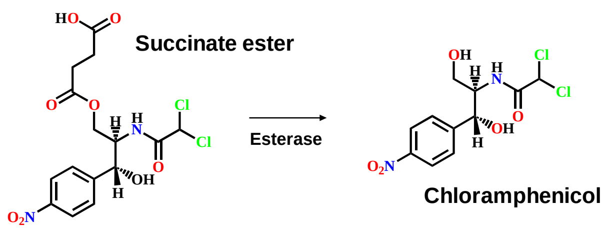
Example: Palmitate ester of chloramphenicol (antibiotic)



Prodrugs to increase water solubility

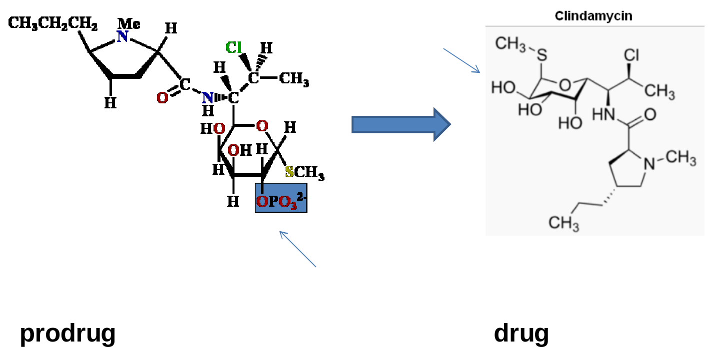
- allows higher concentration (smaller dose volume often for i.v. drugs)
- may decrease pain at site of injection

Example: Succinate ester of chloramphenicol (antibiotic)



1.5.5 Prodrugs to increase water solubility

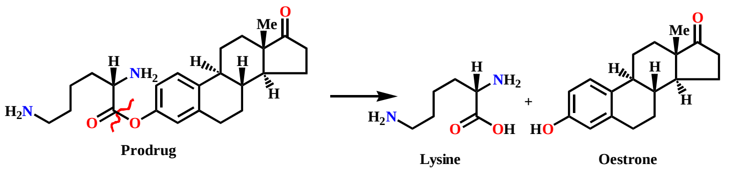
Example: phosphate ester of clindamycin (antibacterial)



- less painful on injection

Prodrugs to increase water solubility

Example: Lysine ester of oestrone



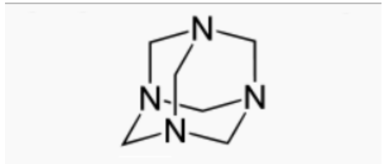
prodrug **drug**

- is an estrogenic hormone secreted by the ovary as well as adipose tissue
- **Lysine ester of oestrone is better absorbed orally than oestrone**
- **Increased water solubility prevents formation of fat globules in gut**
- **better interaction with the gut wall**

Prodrugs used to target drugs

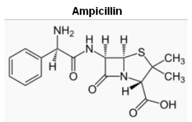
Example: Hexamine (also called hexamethylenetetramine)

Hexamethylenetetramine

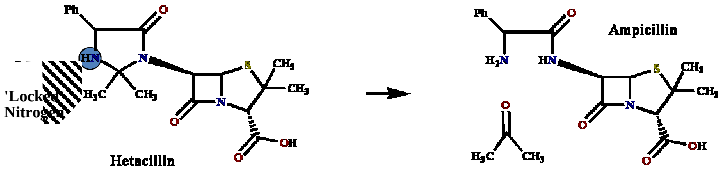


- **stable and inactive at pH > 5, stable in blood** pH = 7.3
- **used for urinary infections where pH < 5 and degrades to formaldehyde (antibacterial agent)**

Prodrugs to increase chemical stability



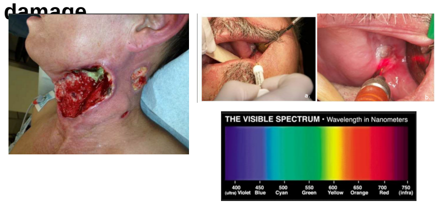
Example: Hetacillin for ampicillin (antibiotic)

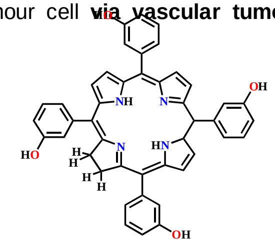


- **ampicillin is** chemically **unstable** in solution **due to the α-NH_2 group attacking the β-lactame ring**
- **NH in heteracillin is locked up within a heterocyclic ring**

Prodrugs activated by external influences - sleeping agents

Example: Photodynamic therapy (PDT) of advanced head and neck cancer – Foscan requires oxygen and laser light of 652 nm for activation. The aim of the treatment is **reduce symptoms by shrinking the cancer**. This is called **palliative treatment**. It will not cure the cancer. Foscan **uptake is** mediated to tumour cell **via vascular tumor damage**





- **inactive prodrug accumulates in cells** (4 days between injection of Foscan® into the bloodstream and activation with laser light)
- **activated by light - method of targeting tumour cells**
- **Foscan is excited and reacts there with oxygen to produce toxic singlet oxygen causing cell destruction**

Drug- synergism

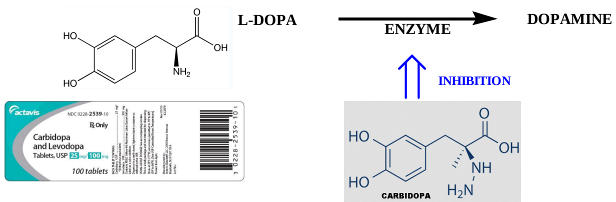
Drug- synergism

Definition: **drug which have a benefical effect on the activity or pharmacokinetic properties of another drug**

Sentry (guard) Drugs

Definition: **a drug that is added to 'protect' another drug**

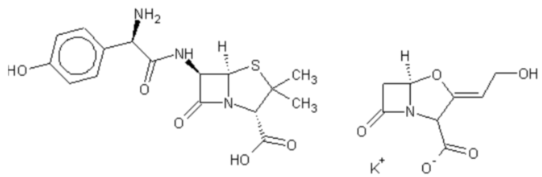
Example: **Carbidopa**



- **Carbidopa protects L-dopa in peripheral blood by inhibiting enzyme decarboxylase**
- **Carbidopa is polar and does not cross the BBB, therefore it has no effect on the decarboxylation of L-Dopa to dopamine in the CNS**
- **smaller doses of L-dopa can be administered => less side effects**

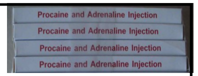
Sentry (guard) Drugs

Example: **Clavulanic acid** is a **beta-lactamase inhibitor** combined with penicillin group antibiotics **to overcome certain types of antibiotic resistance**. Beta-lactamase otherwise inactivates most of penicillins.



Augmentin: **amoxicillin combined with potassium**

Localising drug to a target area

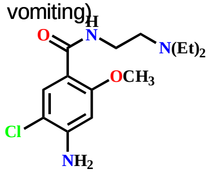


Example: Adrenaline + procaine (more powerful local anaesthetic)

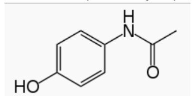
- adrenaline constricts blood vessels at the injection area, therefore procaine is localised at the injection area

Increasing absorption

Example: **Paracetamol + Metoclopramide**
(analgesic + drug to treat nausea and vomiting)



Paracetamol (acetaminophen)



Paracetamol inhibuje COX-2 (cyklooxygenázu 2) v hypotalame (antipyretický účinok) a nepriamo pôsobí na sérotonínové 5-HT3-receptory v mieche (analgetický účinok). Nástup účinku do 30 min.

- administered together with analgesics for the fast treatment of migraine. Metoclopramide increases gastric motility and causes faster absorption of analgesics

---

- **Optimization and scaling up synthesis**
- **Product characterisation**
- **IP protection**

Drug design and development

Stages:

1) Identify target disease
2) Identify drug target
3) Establish testing procedures
4) Find a lead compound
5) Structure Activity Relationships (SAR)
6) Identify a pharmacophore
7) Drug design- optimising target interactions
8) Drug design - optimising pharmacokinetic properties
9) Preclinical trials
10) Chemical development and process development
11) Patenting and regulatory affairs
12) Clinical trials

Note: Stages 9-11 are usually carried out in parallel

Preclinical trials

Drug Metabolism

identification of drug metabolites in test animals and determination of properties of metabolites

Toxicology

acute and chronic toxicity by *in vitro* and *in vivo* tests

Pharmacology

determination selectivity of action at drug target

Formulation

stability tests
methods of delivery (administration)

Chemical Development

Definition:

Development of a synthesis suitable for large scale drug production up to 100 kg.

Priorities:
- to optimise the final synthetic step and the purification procedures
- to define the product specifications
- to produce a product that consistently passes the purity specifications
- to produce a high quality product in high yield using a synthesis that is cheap and efficient
- to produce a synthesis that is safe and environmentally friendly with a minimum number of steps

Chemical Development

Phases:
- Synthesis 1kg of an active compound for initial preclinical testing (often by a scale up of the original synthesis)
- synthesis of 10kg for toxicological studies, formulation and initial clinical trials
- synthesis of 100kg for clinical trials

Notes:
- chemical development is more than just scaling up the original synthesis
- different reaction conditions or synthetic routes are often required
- time period can be up to 5 years
- need to balance long term aims of developing a large scale synthesis versus short term aim need for batches for preclinical trials
- the product produced by the fully developed route must meet the defined specifications

The initial synthesis

Origin
- the initial synthesis was designed in the research lab

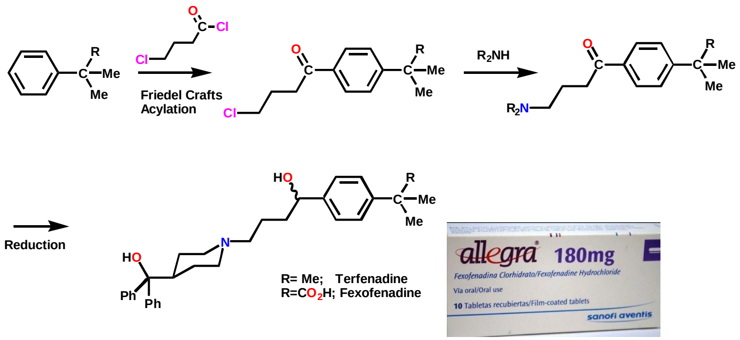
Priorities of the original synthesis
- to synthesise as many different compounds as quickly as possible in order to identify active compounds
- yield and cost are low priorities
- usually done on small scale

Likely problems related to the original synthesis
- the use of hazardous starting materials and reagents
- experimental procedures which are impractical on large scale
- the number of reaction steps involved
- yield and cost

Scale up
- original synthesis may be scaled up for the first 1 kg of product but is then modified or altered completely for larger quantities

The initial synthesis

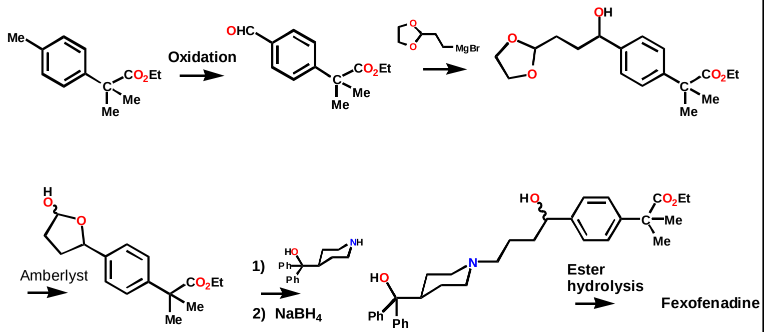
The initial synthesis of fexofenadine (anti-asthmatic, Allegra)



- fexofenadine synthesised by the same route used for terfenadine
- unsatisfactory since the Friedel Crafts reaction gives the *meta* isomer as well
- requires chromatography to remove *meta* isomer

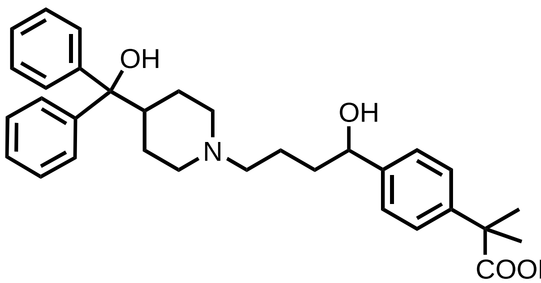
The initial synthesis

Revised synthesis of fexofenadine



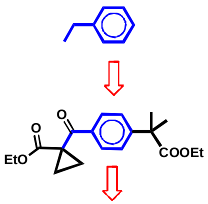
- **more practical and efficient synthesis using easily available starting materials**
- **no side isomers, no chromatography is required for purification**

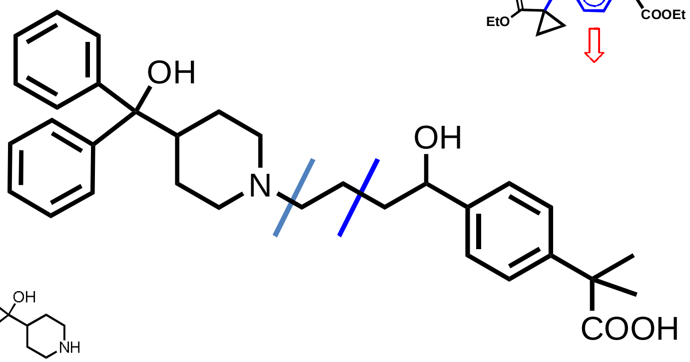
Allegra - Fexofenadine

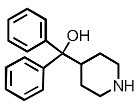


Histamine H_1-receptor antagonist. Used in the treatment of allergic rhinitis. Worldwide 38th best selling prescription drug ($1.08 bn) (Aventis)

unusual disconnection approach



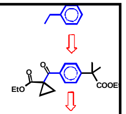


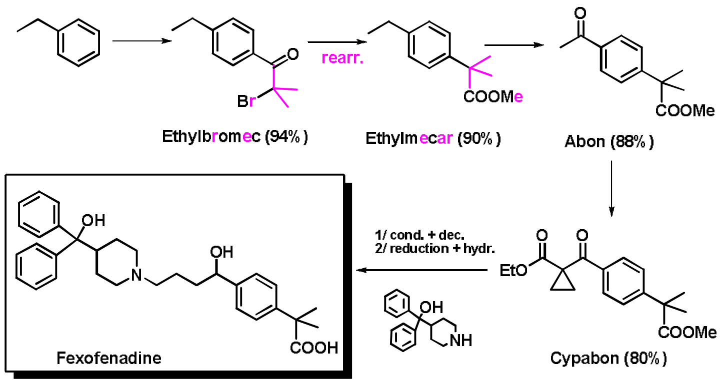


AZACYCLONOL

A. BOHÁČ

Novel Synthesis of Fexofenadine





A. BOHÁČ

Optimisation of reactions

Aims:

- to optimise the yield and purity of product from each reaction step

Notes:

- maximum yield does not necessarily mean maximum purity (may need to accept less than the maximum yield to achieve an acceptable purity)
- need to consider cost and safety for each reaction step

Factors to optimise:

- temperature, reaction time, stirring rate, pH, pressure, catalysts, order and rate of addition of reactants and reagents, purification procedure

Optimisation of reactions

Temperature

- optimum temperature is the temperature at which the rate of reaction is maximised with a minimum of side reactions
- increasing the temperature increases the reaction rate
- increasing the temperature may increase side reactions and amount of impurities
- compromise is often required

Optimisation of reactions

Pressure

- increased pressure (> 5 000 atm) accelerates reactions where the transition state occupies a smaller volume than the starting materials
- useful if increased heating causes side reactions

Examples of reactions accelerated by pressure

- esterifications; amine quaternisation; hydrolysis of esters; Claisen and Cope rearrangements; nucleophilic substitutions; Diels Alder reactions

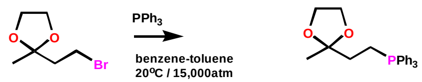
Example: esterification of acetic acid with ethanol

- proceeds 5 times faster at 2 000 atm than at 1 atm
- proceeds 26 times faster at 4 000 atm

Optimisation of reactions

Pressure

Example 1:



- no reaction at 20 °C and 1 atm
- decomposition at 80°C and 1 atm
- good yield at 20 °C and 15 000 atm

Example 2:

- hydrolysis of chiral esters by base with heating may cause racemisation
- can be carried out at room temperature with pressure instead

Optimisation of reactions

Reaction time

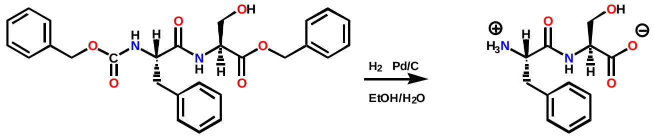
- optimum reaction time is the time required to get the best yield consistent with high purity
- monitor reactions to find the optimum time using TLC, GC, HPLC, IR, NMR
- if reaction goes to completion, optimum time is often the time required to reach completion
- if reaction reaches equilibrium, optimum time is often the time required to reach equilibrium
- however optimum time may not be the same as the time to reach completion or equilibrium if side reactions take place
- excess reaction times increase the chances of side reactions and the formation of impurities
- reaction times greater than 15 h should be avoided (costly at production level)

Optimisation of reactions

Solvent

- is important to outcome yield and purity
- should normally be capable for dissolving reactants and reagents, insolubility of a product in solvent may improve yields by shifting an equilibrium reaction to its products. This may be a problem by reaction with solid catalyst.

Example



- poor yield in ethanol - product precipitates and coats the catalyst
- poor yield in water - reactant poorly soluble
- quantitative yield in ethanol-water (1:1)

Optimisation of reactions

Solvent

- should have a suitable boiling point if one wishes to heat the reaction at a constant temperature (heating to reflux)
- should be compatible with the reaction being carried out
- solvents are classed as polar (EtOH, H_2O, acetone) or nonpolar (PhMe, $CHCl_3$)
- polar solvents are classed as protic (EtOH, H_2O) or aprotic (DMF, DMSO)
- protic solvents are capable of H-bonding (HBD)
- the polarity and the H-bonding ability of the solvent may affect the reaction

Optimisation of reactions

Solvent

Example:

- protic solvents (e.g. EtOH) give higher rates for S_N1 reactions (not for S_N2), they aid departure of anion in the rate determining step
- dipolar aprotic solvents (e.g. DMSO) are better for S_N2 reactions

S_N2 reaction

- aq EtOH: reaction time 1-4 d (24-96 h)
- DMSO: reaction time 1-2 h, DMSO solvates selectively cations but leaves anions relatively unsolvated, therefore the nucleophile is more reactive

Optimisation of reactions

Concentration

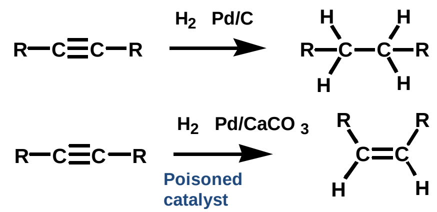
- High concentration (small volume of solvent) favours increased reaction rate but may increase chance of side reactions
- Low concentrations (large volume of solvent) are useful for exothermic reactions (solvent acts as a 'heat sink')

Optimisation of reactions

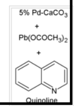
Catalysts

- a catalyst increases a rate at which reactions reach equilibrium
- classed as heterogeneous or homogeneous
- choice of catalyst can influence type of product obtained and yield

Examples:



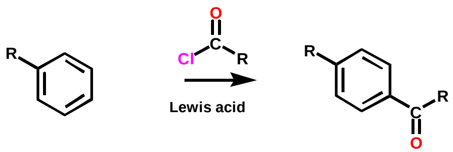
Lindlar



Optimisation of reactions

Catalysts

Example:



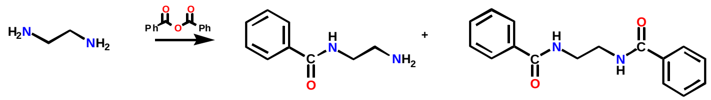
vary Lewis acid catalysts (e.g. $AlCl_3$ or $ZnCl_2$) to optimise yield and purity

Optimisation of reactions

Excess reactant

- shifts equilibrium to products if reaction is thermodynamically controlled
- excess reactant must be cheap, readily available and easily separable from product
- may also affect outcome of reaction

Example:



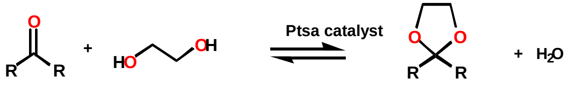
- Excess diamine is used to increase the proportion of mono-acylated product

Optimisation of reactions

Removing a product

- removing a product (e.g. precipitation, destillation) elevates yield in case of an equilibrium reaction

Example:



removing water by distillation shifts an equilibrium towards product

Optimisation of reactions

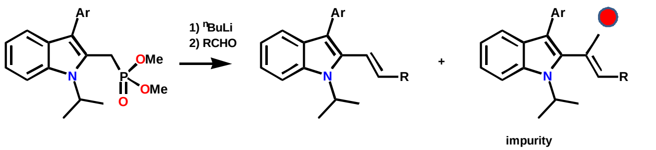
Methods of addition

- adding one reagent slowly to another one helps to control the temperature of highly exothermic reactions
- stirring rates may be crucial to prevent localised regions of high compound or temperature concentrations
- dilution of reactant or reagent in solvent before addition to the reaction mixture helps to prevent localised areas of high concentration
- order of addition may influence the reaction outcome and yield

Optimisation of reactions

Methods of addition

Example



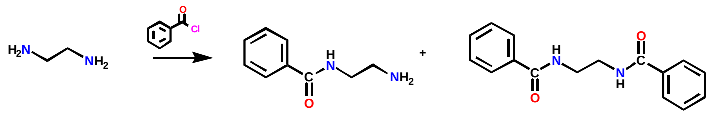
- Impurity is formed when BuLi is added to the phosphonate (the phosphonate anion reacts with unreacted phosphonate that is a donor of electrophilic Me group)
- No impurity is formed if the phosphonate is added to butyl lithium
  (in this case, no unreacted fosfonate is present)

Optimisation of reactions

Reactivity of reagents and reactants

Less reactive reagents may affect the outcome of the reaction

Example



- a mixture of mono and diacylated products 50 : 50 is obtained even when benzoyl chloride is added to the excess of diamine
- using less reactive benzoic anhydride gives a ratio of mono to diacylated product of 95 : 5

Scaling up a reaction

Priorities
cost, safety and practicality

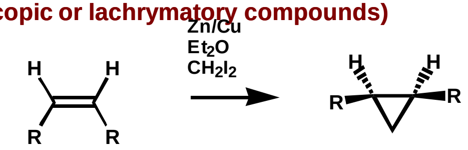
Factors to consider
reagents, reactants and intermediates, solvents, side products, temperature, catalysts, procedures, physical parameters, purification

Scaling up a reaction

Reagents

- reagents used in the initial synthesis are often unsuitable due to their cost or hazards
- hazardous byproducts may be formed from certain reagents (e.g. mercuric acetate can form mercury)
- reagents may be unsuitable on environmental grounds (e.g. smell)
- reagents may be unsuitable to handle on large scale (e.g. hygroscopic or lachrymatory compounds)

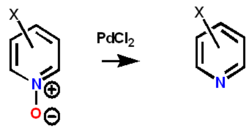
Example:

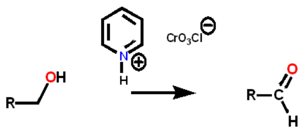


- Zn/Cu amalgam is too expensive for scale up
- can be replaced with zinc powder

Scaling up a reaction

Reagents





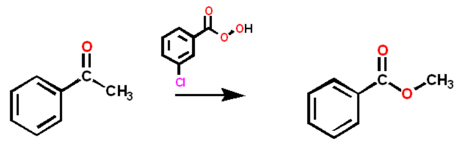
- reactions above should be avoided for scale up
- palladium chloride and pyridinium chlorochromate are both carcinogenic
- synthetic route will be rejected by regulatory authorities (FDA, EMA) if carcinogenic reagents are used near the end of the synthetic route

Scaling up a reaction

Reagents

Choice may need to be made between cost and safety

Example:



- m-chloroperbenzoic acid (MCPBA) is preferred over cheaper peroxide reagents for the Baeyer-Villiger oxidation since MCPBA has a higher decomposition temperature and is safer to use

Scaling up a reaction

Reactants and intermediates

- starting materials should be cheap and readily available
- hazards of starting materials and intermediates must be considered (e.g. diazomethane or diazonium salts are explosive)
- may have to alter synthesis to avoid hazardous intermediates

Scaling up a reaction

Solvents

- solvents must not be excessively flammable or toxic
- many solvents used in research labs are unsuitable for scale up due to flammability, cost, toxicity etc. (e.g. Et_2O, $CHCl_3$, dioxane, benzene, HMPA (hexamethylphosphoric triamide))
- concentrations currently used in research labs are relatively low
- the concentration of reaction is normally increased during scale up to avoid large volumes of solvent (solvent:solute ratio 5:1 or less)
- increased concentrations means less solvent, less hazards, greater economy and increased reaction rate
- changing solvent can affect outcome or yield
- not feasible to purify solvents on production scale

Scaling up a reaction

Solvents

Properties of solvents

- Ignition temperature - temperature at which solvent ignites (CS_2 over 102 °C)
- Flash point - temperature at which vapours of the solvent ignite in the presence of an ignition source (spark or flame, CS_2 over -42 °C)
- vapour pressure - measure of a solvent's volatility
- vapour density - measure of whether vapours of the solvent rise or creep along the floor

Scaling up a reaction

Solvents

Hazardous solvents

- solvents which are flammable at a low solvent/air mixture and over a wide range of solvent/air mixtures (e.g. Et_2O has a flammable solvent/air range of 2-36 %, is heavier than air and can creep along plant floors to ignite on hot pipes)
- solvents with a flash point less than -18 °C (e.g. Et_2O and CS_2)

Scaling up a reaction

Solvents

Alternatives for common research solvents

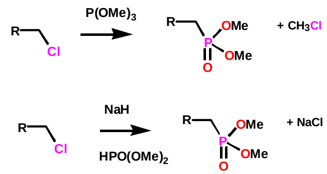
- **dimethoxyethane for Et_2O**
- **(less flammable, higher b.p. and higher heat capacity)**
- **t-butyl methyl ether (cheaper, safer and does not form peroxides) for Et_2O**
- **heptane for pentane and hexane (less flammable)**
- **ethyl acetate for chlorinated solvents (less toxic)**
- **toluene for benzene (less carcinogenic)**
- **xylene for benzene (less carcinogenic)**
- **tetrahydrofuran for dioxane (less carcinogenic)**

Scaling up a reaction

SIDE PRODUCTS

- **reactions producing hazardous side products are unsuitable for scale up**
- **may need to consider different reagents**

Example



- **preparation of a phosphonate produces methyl chloride (gaseous, toxic and an alkylating agent, trimethyl phosphite stinks)**
- **sodium dimethyl phosphonate is used instead since it results in the formation of**

Scaling up a reaction

TEMPERATURE

must be practical for reaction vessels in the production plant

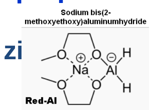
Scaling up a reaction

PROMOTERS

- **certain chemicals can sometimes be added at a catalytic level to promote reactions on large scale**
- **may remove impurities in commercial solvents and reagents**

Example 1

- **RedAl is used as a promoter in cyclopropanation reaction with zinc**
- **removes zinc oxides from the surface of the zi**
- **removes water from the solvent**
- **removes peroxides from the solvent**



It is a safer substitute for LAH

Example 2

- **MeMgI is used as a promoter for the Grignard reaction**

Scaling up a reaction

EXPERIMENTAL PROCEDURES

some experimental procedures carried out on small scale
may be impractical on large scale

Examples

scraping solids out of flasks
concentrating solutions to dryness
Rotary evaporators
vacuum ovens to dry oils
chromatography for purification
drying agents (e.g. sodium sulphate)
addition of reagents within short time
use of separating funnels for washing and extracting

Scaling up a reaction

EXPERIMENTAL PROCEDURES

Some alternative procedures suitable for large scale synthe

- Drying organic solutions
  - add a suitable solvent and azeotrope off the water
  - extract with brine
- Concentrating solutions
  - carried out under normal distillation conditions
- Purification
  - crystallisation preferred
- Washing and extracting solutions
  - stirring solvent phases in large reaction vessels
  - countercurrent extraction

Scaling up a reaction

PHYSICAL PARAMETERS

may play an important role for a reaction outcome and yield

Parameters involved
- stirring efficiency
- surface area to volume ratio of reactor vessel
- rate of heat transfer
- temperature gradient between the centre of the reactor and the walls

SYNTHETIC PROCESS DEVELOPMENT

PROCESS DEVELOPMENT

DEFINITION

Development of the overall synthetic route to make it suitable for the production site and can produce batches of product <u>in ton quantities with consistent yield and purity</u>

PRIORITIES

- minimising number of reaction steps
- exploitation of convergent syntheses
- minimising number of technical operations
- safety - chemical hazards
- safety - reaction hazards
- minimising number of purification steps
- environmental issues
- cost

PROCESS DEVELOPMENT

NUMBER OF REACTION STEPS

minimising number of reaction steps may increase the overall yield

requires a good understanding of synthetic organic chemistry

PROCESS DEVELOPMENT

CONVERGENT SYNTHESES

- to prepare a product in two synthetic pathways and then link them together is preferable over linear synthesis because of higher overall yield

LINEAR SYNTHESIS

e.g. 10 step synthesis, each yielding 80%

A → B → C → D → E → F → G → H → I → J → K

Overall yield =10.7% assuming an 80% yield per reaction

CONVERGENT SYNTHESIS

L → M → N → O → P → Q

R → S → T → U → V

Q + V → K

Overall yield = 26.2% from L assuming an 80% yield per reaction
Overall yield from R = 32.8%

PROCESS DEVELOPMENT

NUMBER OF OPERATIONS

- minimise number of operations to increase the overall yield
- avoid isolation and purification of the intermediates
- keep intermediates in solution for transfer from one reaction vessel to another one
- use a solvent which is common to a series of reactions in the process

Example

Alcohol $\xrightarrow{SOCl_2}$ Alkyl halide $\xrightarrow{PPh_3}$ Wittig reagent

an alkyl halide is not isolated, but is transferred in solution to the next reaction vessel for the subsequent Wittig reaction

PROCESS DEVELOPMENT

SAFETY - CHEMICAL HAZARDS

- to assess the potential hazards of all chemicals, solvents, intermediates and residues in the process
- to introduce proper monitoring and controls to minimise the risks

PROCESS DEVELOPMENT

Main hazards

Toxicity -

- compounds must not have an LD_{50} less than 100 mg/kg (teaspoon)

Flammability

- avoid high risk solvents
- medium risk solvents require precautions to avoid static electricity

Explosiveness

- dust explosion test - determines whether a spark ignites a dust cloud of the compound
- hammer test - determines whether dropping a weight on the compound produces sound or light

Thermal instability -

- reaction process must not use temperatures higher than decomposition temperatures

PROCESS DEVELOPMENT

SAFETY - REACTION HAZARDS

- assess the potential hazards of all reactions
- carefully monitor any exothermic reactions (!induction effect)
- control exothermic reactions by cooling and/or the rate at which reactants are added
- the rate of stirring can be crucial and must be monitored
- autocatalytic reactions are potentially dangerous

PROCESS DEVELOPMENT

PURIFICATIONS

- keep the number of purifications to a minimum to enhance the overall yield
- chromatography is often impractical
- ideally, purification should be carried out by crystallising only the final product of the process
- crystallisation conditions must be controlled to ensure consistent purity, crystal form and size
- crystallisation conditions must be monitored for cooling rate and stirring rate
- crystals which are too large may trap solvent
- crystals which are too fine may clog up filters
- hot filtrations prior to crystallisation must be done at least 15 ^{o}C above the crystallisation temperature

PROCESS DEVELOPMENT
ENVIRONMENTAL ISSUES

- chemicals should be disposed safely or recycled on environmental and economic grounds
- solvents should be recycled and re-used
- avoid mixed solvents - difficult to recycle
- avoid solvents with low b.p. to avoid escape into the atmosphere
- water is the preferred solvent
- spent reagents should be made safe before disposal
- use catalysts whenever relevant
- use 'clean' technology whenever possible (e.g. electrochemistry, photochemistry, ultrasound, microwaves)

PROCESS DEVELOPMENT
COST

- keep cost to a minimum
- maximise the overall yield
- minimise the cost of raw materials
- minimise the cost of labour and overheads by producing large batches on each run

SPECIFICATIONS

Definition
Specifications => definition a product's properties and purity
all batches must pass the predetermined specification limits

Troubleshooting
necessary if any batches fail the specifications
identify any impurities present and their source
identify methods of removing impurities or preventing their formation

Sources of Impurities
impure reagents and reactants
reaction conditions
order of reagent addition

SPECIFICATIONS
PROPERTIES AND PURITY

- melting point, colour of solution, particle size, crystal polymorphism, pH, chemical and stereochemical purity
- impurities present are defined and quantified
- residual solvents present are defined and quantified
- acceptable limits of impurities and solvents are defined
- acceptable limits are dependent on toxicity (e.g. ethanol 2%, methanol 0.05%)
- carcinogenic impurities must not be present in final stages of synthesis

SPECIFICATIONS

IMPURITIES

- isolate, purify and identify all impurities
  (HPLC, NMR, MS spectroscopy)
- identify the source of any impurity
- alter the the reaction conditions or purification at the final stage

SPECIFICATIONS

PURIFICATIONS

- introduce a purification to remove any impurities at the end of the reaction sequence or after the problematic reaction
- methods of purification - crystallisation, distillation, precipitation of impurity from solution, precipitation of product from solution

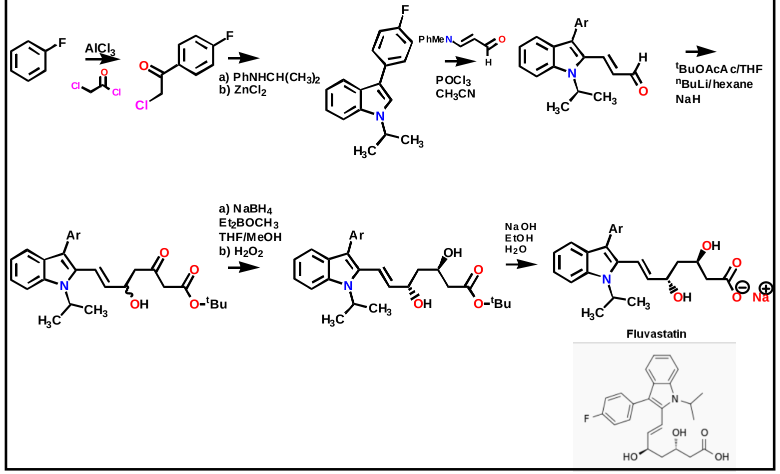
SPECIFICATIONS

IMPURE REAGENTS / REACTANTS

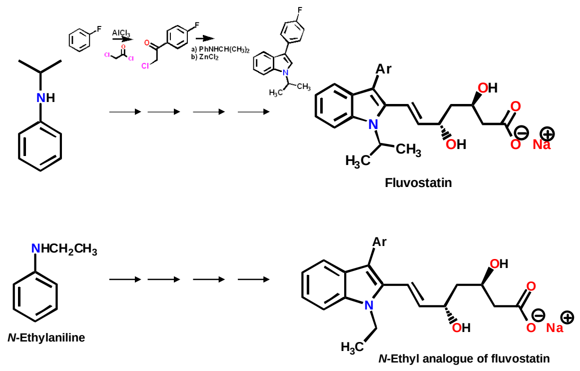
- commercially available reagents or reactants can contain impurities
- impurities introduced early on in the synthetic route may survive the synthetic route and contaminate the product
- an impurity at an early stage of the synthetic route may undergo the same reactions as the starting material and contaminate the final product

SPECIFICATIONS

Example Synthesis of fluvastatin (antihypercholesterolemic drug)



SPECIFICATIONS

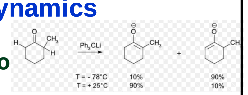


Impurity **Final impurity**

SPECIFICATIONS

REACTION CONDITIONS

- **vary the reaction conditions to minimise any impurities**
  (e.g. solvent, catalyst, ratio of reactants and reagents)
- **consider reaction kinetics and thermodynamics**
  heating favours the thermodynamic product
  rapid addition of reactant favours the kinetic pro



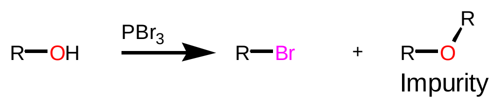
- **consider sensitivity of a reagent to air and to oxidation**
  N-butyllithium oxidises in air to lithium butoxide
  benzaldehyde oxidises to benzoic acid
  consider using fresh reagents or a nitrogen atmosphere

SPECIFICATIONS

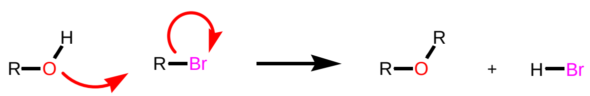
ORDER OF ADDITION

order in which reagents are added may result in impurities

Example



Mechanism of impurity formation



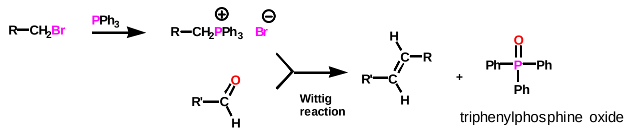
impurity occurs when PBr_3 is added to the alcohol but not when the alcohol is added to PBr_3

SPECIFICATIONS

TROUBLESOME BY-PRODUCTS

- **by-products formed in some reactions may be difficult to remove**
- **change the reagent or reaction to get less difficult by-products**

Example - Wittig reaction

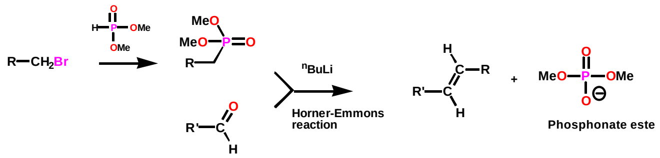


by-product = triphenylphosphine oxide (requires chromatography to remove)

SPECIFICATIONS

TROUBLESOME BY-PRODUCTS

Horner-Emmons reaction – an alternative synthesis

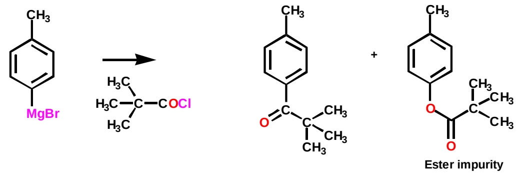


by-product phosphonate ester can be remove easily by washing with water

SPECIFICATIONS

CHANGING A SYNTHESIS

Example- Grignard synthesis

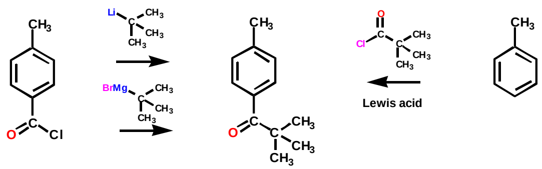


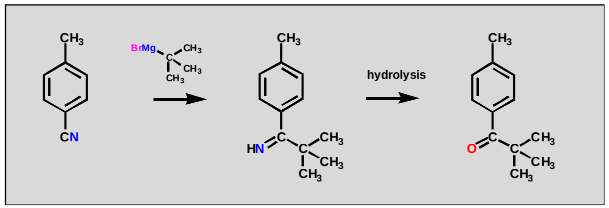
- ester impurity is formed by oxidation of Grignard reagent to a phenol which can react with acyl chloride reagent
- avoidable by adding Grignard reagent to the acid chloride but…not easy on large scale due to the air sensitivity and poor solubility of the Grignard reagent

SPECIFICATIONS

CHANGING SYNTHESIS

different routes to the same product





SPECIFICATIONS

INORGANIC IMPURITIES

- the final product must be checked for inorganic impurities (e.g. metal salts)
- deionised water may need to be used if the desired compound is a metal ion chelator or is isolated from water

PATENTING AND REGULATORY AFFAIRS

PATENTING

- carried out as soon as a potentially useful drug is identified
- carried out before preclinical and clinical trials
- several years of patent protection are lost due to the trials
- cannot specify the exact structure that is likely to reach market,
  therefore patent a group of compounds rather than an individual
  structure

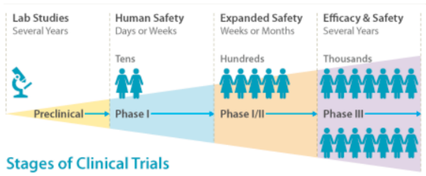
PATENTING AND REGULATORY AFFAIRS

REGULATORY AFFAIRS

- a drug before reach a marked must be approved by regulatory bodies
  Food and Drug Administration (FDA) http://www.fda.gov/
  European Medicines Agency (EMA) http://www.ema.europa.eu/ema/

- GLP - Good Laboratory Practice
- GMP - Good Manufacturing Practice
- GCP - Good Clinical Practice

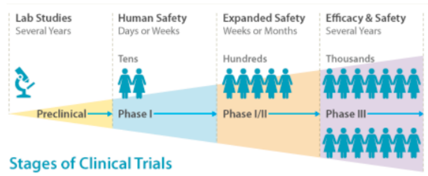
CLINICAL TRIALS



Phase 1
- carried out on healthy volunteers
- useful in establishing dose levels
- useful for studying pharmacokinetics, including drug metal

Phase 2
- carried out on patients
- carried out as double blind studies
- demonstrates whether a drug is therapeutically useful
- establishes a dosing regimen
- identifies side effects

CLINICAL TRIALS



Phase 3
- carried out on a larger number of patients
- establishes statistical proof for efficacy and safety

Phase 4 – post marketing phase
- carried out after a drug reaches the market
- studies long term effects when a drug is used chronically
- identifies unusual side effects