Course Weekly Outline

5th Class, 1st Semester

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Course Instructor	Assist. Prof. Dr. Monther F. Mahdi
E- mail	dmfalameri@yahoo.com
Title	***Organic Pharmaceutical Chemistry* IV**
Course Coordinator	_____
Course Objective	To give the students knowledge and experience in pro-drug as part of their medicinal and pharmaceutical field. As well as combinatorial chemistry.
Course Description	Course describes how combinatorial chemistry and HTS are being used in drug design and discovery to find new lead structures in a shorter time.
Text book	***Wilson and Gisvold Textbook of Organic Medicinal and Pharmaceutical Chemistry; Delgado JN, Remers WA, (Eds.); 10th ed., 2004.***
References	***John McCurry; Organic Chemistry.; Thomason learningInc. ; CA,USA 7th ed 2008.***
Course Assessments	Midterm test: As (20%); Quizzes: As (5%); Project: As (5%); Final exam: As (70%)

Prodrugs

- Initial definition: A pharmacologically inactive compound that is transformed by the mammalian system into an active substance by either chemical or metabolic means.

  "Albert-1958"
- "Drug Latentiation" – included later by "Harper-1959"
  - refer to drugs that were specifically designed to require bioactivation.
- Why use prodrugs?
  - Improve patient acceptability (decrease pain on injection)
  - Alter and improve absorption
  - Alter biodistribution
  - Alter metabolism
  - Alter elimination

Non-Prodrugs

- "Hard Drugs" - compounds that contain structural characteristics required for activity but are not susceptible to metabolism
  - Increased efficiency by avoiding metabolism
  - No toxic metabolites are formed
  - HOWEVER, less readily eliminated due to lack of metabolism
- "Soft Drugs" - These are the ***opposite*** of prodrugs. These compounds are designed and synthesized as ACTIVE compounds that readily undergo metabolic inactivation to nontoxic products

Conversion of Prodrugs

1. Metabolism:
   - enzyme dependant
   - Most often
2. Chemical Methods (**non-dependant**)
   - Hydrolysis or Decarboxylation
   - Less common
   - NOT patient dependant!
   - Stability/Storage issues (problem)

Prodrugs can be conveniently grouped into:

- **1. Carrier-linked prodrugs.**
  2. Bioprecursor prodrugs.
- **Carrier-linked prodrugs – drugs that are attached through a metabolically labile chemical linkage to another molecule designated as the "promoiety"**
  - **The "promoiety" alters the physical properites of the drug to increase water or fat solubility or provide site-directed delivery**
  - **Advantages:**
    - **Increased absorption**
    - **Injection site pain relief**
    - **Elimination of unpleasant taste**
    - **Decreased toxicity**
    - **Decreased metabolic inactivation**
    - **Increased chemical stability**
    - **Prolonged or shortened action**

Depending upon the nature of carrier used, the carrier-linked prodrug may further be classified into:

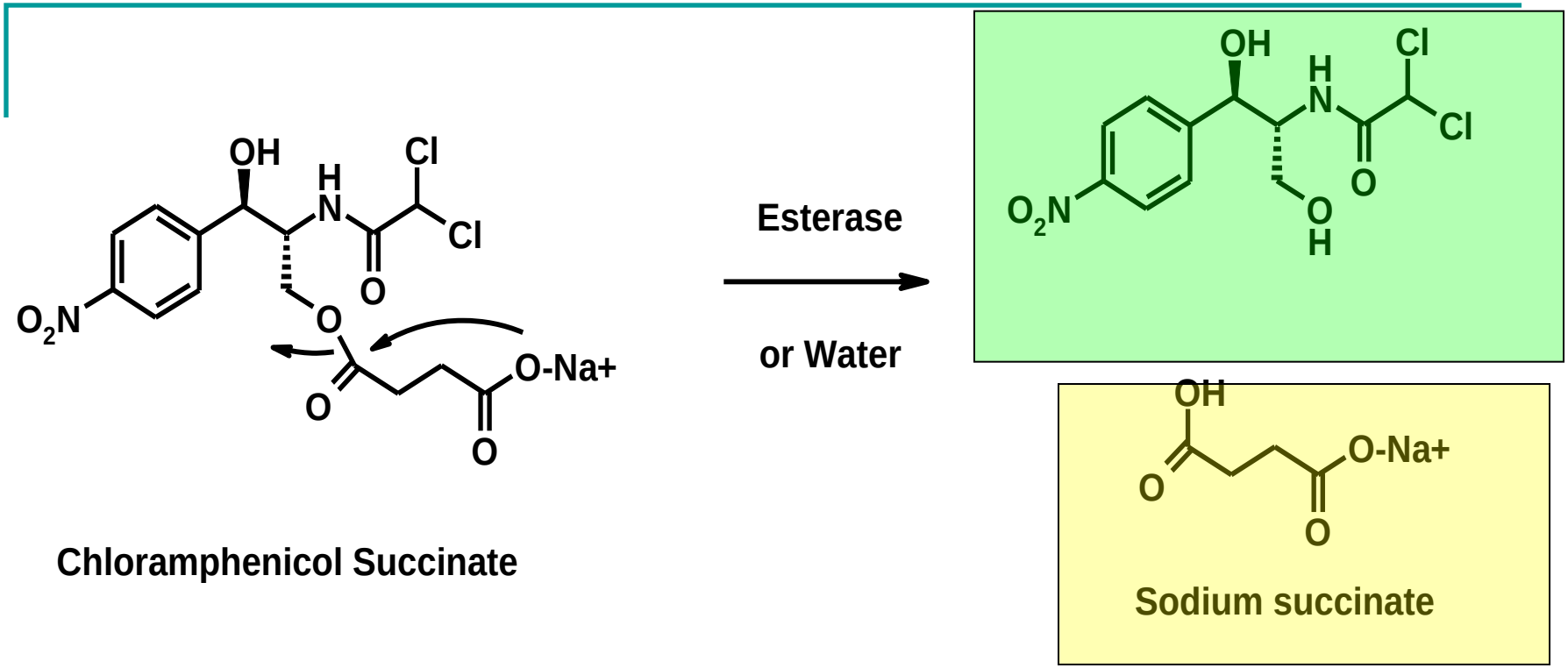
- **Double prodrugs, pro-prodrugs or cascade-latentiated prodrugs, where a prodrug is further derivatized in a fashion such that only enzymatic conversion to prodrug is possible before the latter can cleave to release the active drug.**
- **Macromolecular prodrugs, where macromolecules like polysaccharides, dextrans, cyclodextrins, proteins, peptides, and polymers are used as carriers.**
- **Site-specific prodrugs where a carrier acts as a transporter of the active drug to a specific targeted site.**
- **Mutual prodrug, where the carrier used is another biologically active drug instead of some inert molecule.**

The promoiety should be:

- Easily and completely removed after it has served its function.
- Should be nontoxic.
- Type of promoiety chosen is a function of properties desired?

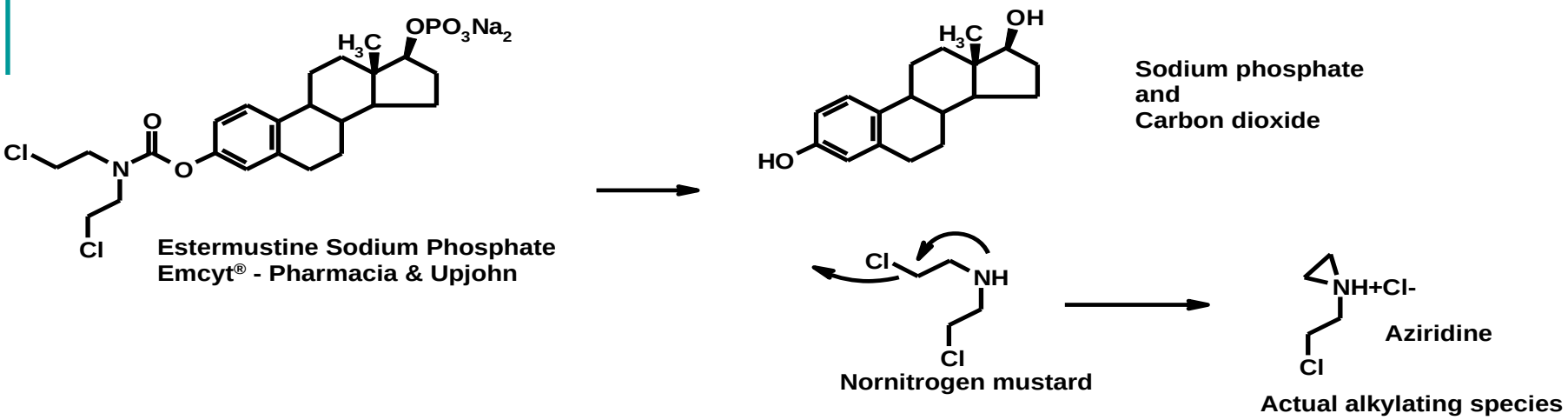
  If it is desirable to increase water solubility, then a promoiety containing an ionizable function or numerous polar functional groups is used. If, on the other hand, the goal is to increase lipid solubility or decrease water solubility, a nonpolar promoiety is appropriate.

Administration of a drug parenterally may cause pain at the site of injection, especially if the drug begins to precipitate out of solution and damage the surrounding tissue. This situation can be remedied by preparing a drug with increased solubility in the administered solvent.



- **Enzymatic and intramolecular spontaneous hydrolysis**
- **Increased water solubility, ester itself is inactive as an antibiotic**
- **Promoiety should be nontoxic and easily excreted**
- **Type of promoiety chosen is a function of properties desired**

Mutual Prodrug



- **Used for metastatic carcinoma of the prostate**
- **Promoiety also a drug!**
- **Prodrug is selectively taken up into estrogen receptor positive cells then urethane linkage is hydroylzed**
  - **17-alphaestradiol slow prostate cell growth**
  - **Nornitrogen mustard is a weak alkylating agent**

•Note that phosphorylation of the estradiol can be used to increase the water solubility, which also constitutes a prodrug modification. Both types of esters (carbamates and phosphates) are hydrolyzed by chemical or enzymatic means.

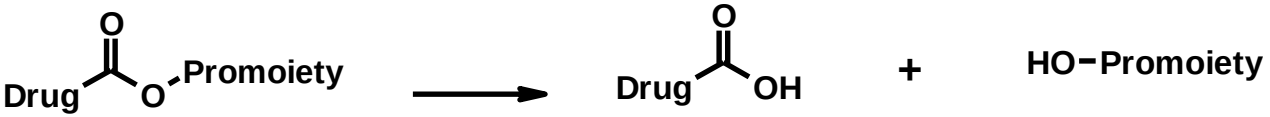
Estramustine is composed of a phosphorylated steroid (17 α -estradiol) linked to a normustard [NH(CH2CH2Cl)2] through a carbamate linkage.

The steroid portion of the molecule helps to:

- concentrate the drug in the prostate, where hydrolysis occurs to give the normustard and CO2.
- The I7 α -estradiol has an antiandrogenic effect on the prostate and thereby, slows the growth of the cancer cells.

Functional Groups in Prodrugs

- **Carboxylic acids and Alcohols:** **Most common type of prodrug**



or

- Prodrugs of agents that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester

- Ester prodrugs are the most common type of prodrug?

1. The ease with which the ester can be synthesized.
2. The ease with which the ester can be hydrolyzed to give the active drug.

- Hydrolysis of ester prodrugs are accomplished by:

1. Esterase enzymes present in plasma and other tissues e.g.

   a/ Ester hydrolase b/ Lipase

   c/ Cholesterol esterase d/ cholinesterase

   e/ acetyl cholinesterase f/ Carboxypeptidase

2. Microflora presents within the gut produce a wide variety of enzymes that can hydrolyze esters.
3. Chemical hydrolysis of the ester function may also occur to some extent.

- Manipulation of the steric and electronic properties of the promoiety allows:

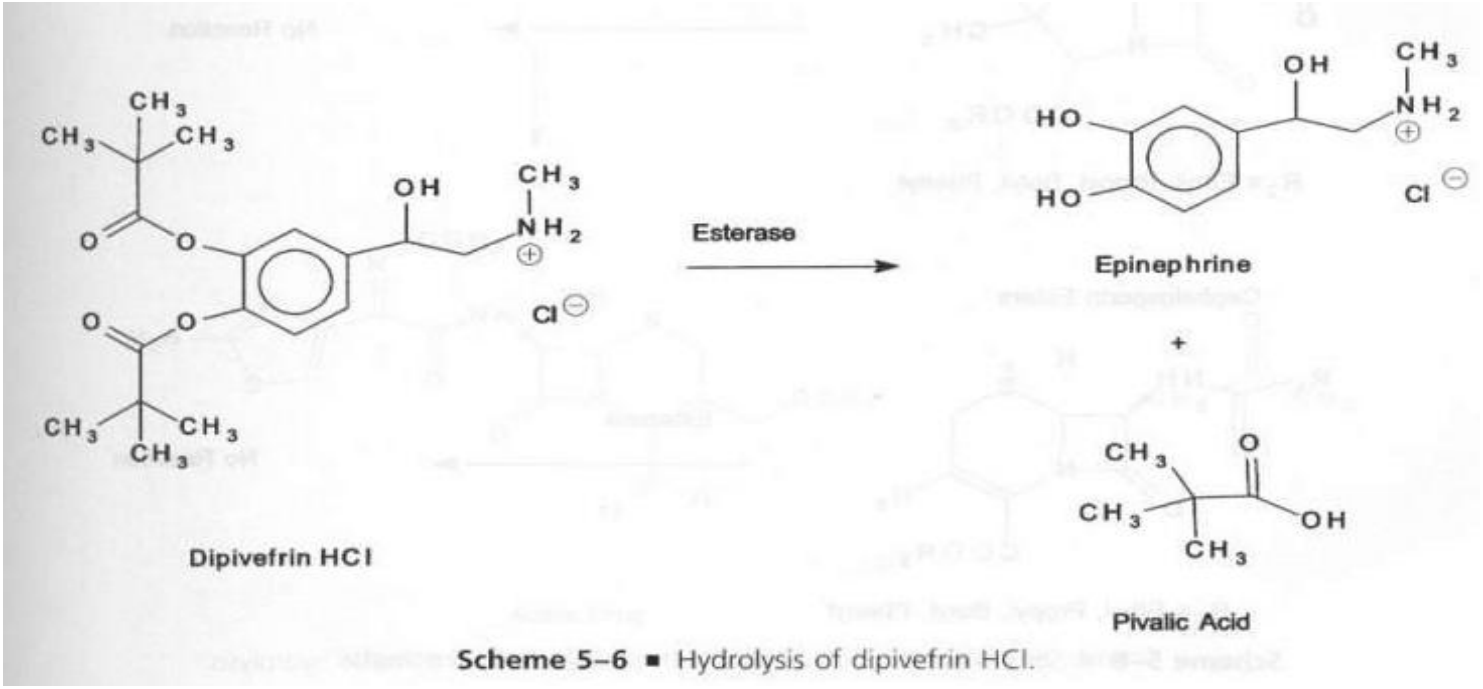
  1. Control of the rate of hydrolysis.
  2. Control of the extent of hydrolysis.
  3. Control when the active drug must be revealed at the correct point in its movement through the biological system.

- When it's desired to decrease water solubility, a nonpolar alcohol or carboxylic acid is chosen as the prodrug moiety which may yield a number of benefits including:

  1. Increased absorption, e.g. dipivefrin HCI.
  2. Decreased dissolution in the aqueous environment of the stomach.
  3. Longer duration of action.
  4. Mask an unpleasant taste of agents when given orally e.g. chloramphenicol palmitate.

Dipivefrin HCl is a prodrug of epinephrine with pivalic acid which is used in the treatment of open angle glaucoma.

- **The increased lipophilicity relative to epinephrine allows the agent, when applied, to move across the membrane of the eye easily and achieve higher intraocular concentrations. Hydrolysis of the ester functions then occurs in the cornea, conjunctiva, and aqueous humor to generate the active form, epinephrine.**



Scheme 5–6 ■ Hydrolysis of dipivefrin HCl.

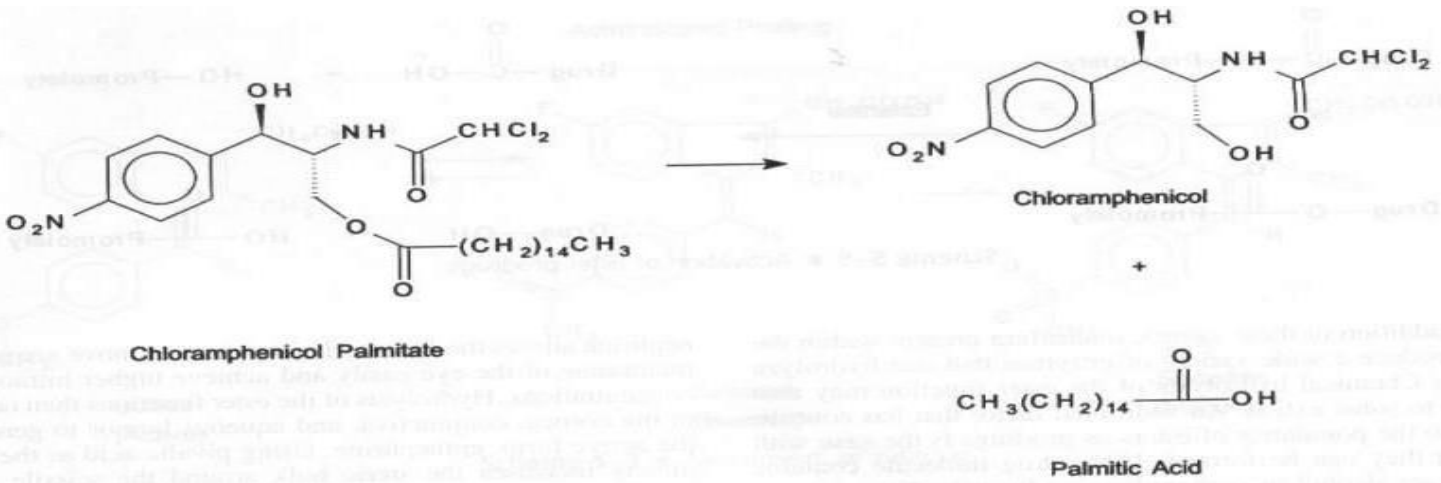
- Important of using pivalic acid in Dipivefrin HCl:

1. Increases the steric bulk around the scissile ester bond, which slows the ester hydrolysis relative to less bulky groups.

2. Protecting the catechol system as the diester prevents its oxidation and the resulting drug inactivation.

- **Chloramphenicol palmitate:**
- **Chloramphenicol has an unpleasant taste when given orally because the drug dissolves in the mouth and then is capable of interacting with taste receptors. This can present a significant problem, especially in pediatric patients, and may lead to low compliance.**
- **The hydrophobic palmitate ester does not dissolve to any appreciable extent in the mouth, so there is little chance for interaction with taste receptors.**

- The ester moiety is subsequently hydrolyzed in the GI tract, and the agent is absorbed as chloramphenicol.
- Listed below are a number of other agents that have been converted into ester prodrugs and other types of prodrugs to overcome an unpleasant taste:
- N-Acetyl sulfisoxazole
- Erythromycin estolate
- Clindamycin palmitate.

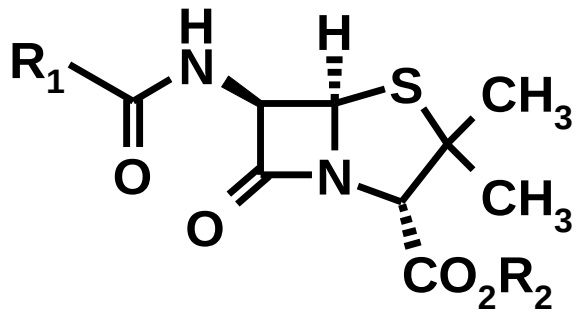


Scheme 5–7 ■ Hydrolysis of chloramphenicol palmitate.

Not all carboxylic esters are easily hydrolyzed in vivo

- Steric inhibition around the ester in some cases prevents the prodrug from being hydrolyzed.
- This is seen in the β-lactams, in which it is often desirable to increase the hydrophobicity of the agent to improve absorption or prevent dissolution in the stomach where acid-catalyzed decomposition may occur.
- Simple esters of the carboxylic acid moiety, however, are not hydrolyzed in vivo to the active carboxylate.

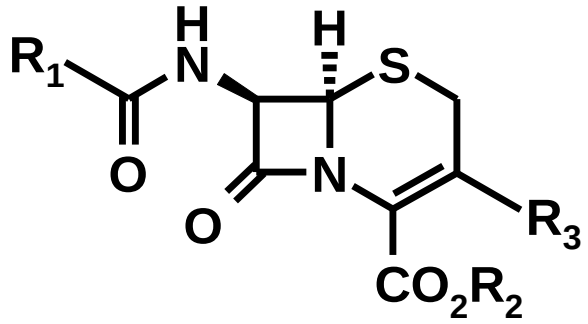
Esters Failure as Prodrugs



R_2 = ethyl, propyl, butyl, phenyl

Penicillin esters

Esterases → **NO REACTION!**



R_2 = ethyl, propyl, butyl, phenyl

Cephalosporin esters

Simple esters of β -lactams with resistance to enzymatic hydrolysis

- A solution to this problem was to use the so-called double ester approach, in which an additional ester or carbonate function is incorporated into the R_2 substituent further removed from the heterocyclic nucleus.
- Hydrolysis of such a function occurred readily, and the moiety was selected so that chemical hydrolysis of the second ester occurred quickly. This is seen in the cephalosporin cefpodoxime proxetil where a carbonate function was used.
- The carbonate is also susceptible to the action of esterase enzymes, and the unstable product undergoes further reaction to give the active carboxylate.
- This approach is frequently used to:

  1. Improve absorption.

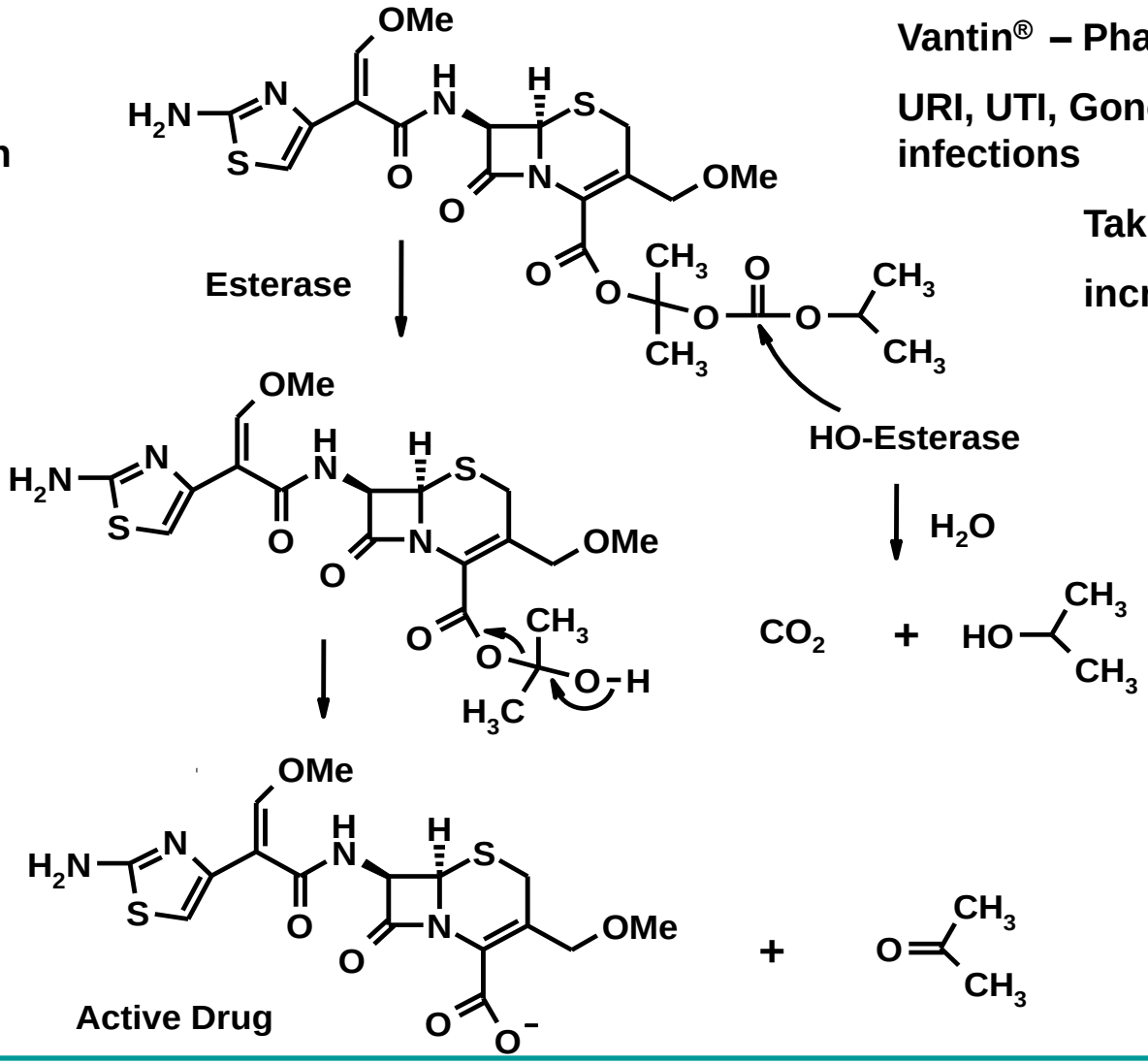
  2. Or prevent dissolution in the stomach and the subsequent acid-catalyzed decomposition of aminopenicillins and second- and third-generation cephalosporins so that these agents can be administered orally, e.g. Bacampicillin.

β-Lactam prodrug – Double esters

Why?

Increase absorption

Avoid acid catalyzed decomposition



Vantin® – Pharmacia & Upjohn

URI, UTI, Gonorrhea, skin infections

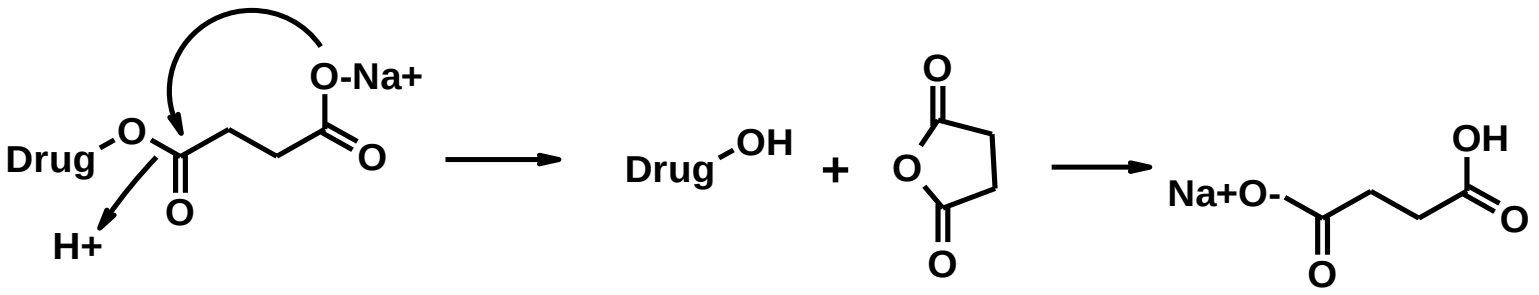
Taking with food increases absorption

- To increase the hydrophilicity of an agent, several different types of ester prodrugs have been used, including:

  1. succinates
  2. phosphates
  3. sulfonates.

- All are ionized at physiological pH and, therefore, increase the water solubility of the agents, making them more suitable for parenteral or oral administration when high water solubility is desirable.

Scheme: succinate, sulphate, and phosphate ester

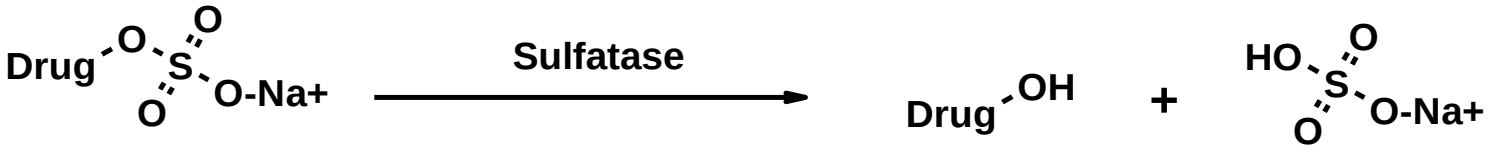


Sodium succinate prodrug

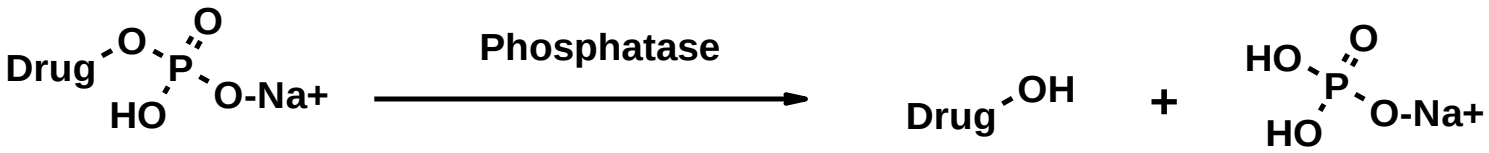
Sodium succinate

Unstable: use immediately

More stable: less prone to hydrolysis by water

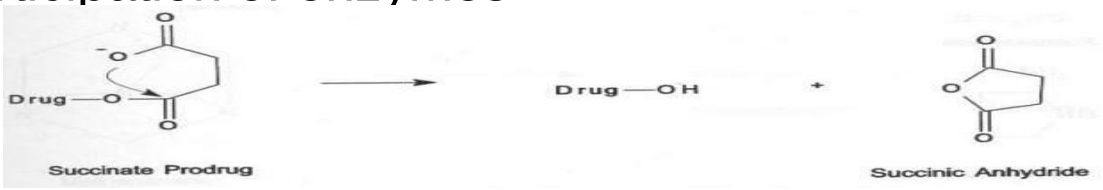


Sulfate prodrug



Phosphate prodrug

- Succinate esters containing an ionizable carboxylate are useful when rapid in vivo hydrolysis of the ester functionally is required.
- The rapid hydrolysis is related to the intramolecular attack of the carboxylate on the ester linkage, which does not require the participation of enzymes



Scheme 5-12 • Intramolecular cleavage of succinate esters.

- As a result, these agents may be somewhat unstable in solution and should be dissolved immediately prior to administration.

- The phosphates are completely ionized at physiological pH and generally hydrolyzed rapidly in vivo by phosphatase enzymes.
- Ionization of the phosphate function imparts high stability to these derivates in solution, and solutions for administration can be stored for long periods of time without hydrolysis of the phosphate.
- Such an approach has been used to produce clindamycin phosphate, which produces less pain at the injection site than clindamycin itself.
- Pain after parenteral administration is associated with local irritation caused by:

  1. Low aqueous solubility or.
  2. Highly acidic or.
  3. Highly basic solutions.

- With chindamycin phosphate, the reduction in pain is attributed to the increased water solubility of the agent.

Amines

- Derivatization of amines to give amides has not been widely used as a prodrug strategy because:

1. The high chemical stability of the amide linkage.
2. The lack of amidase enzymes necessary for hydrolysis.

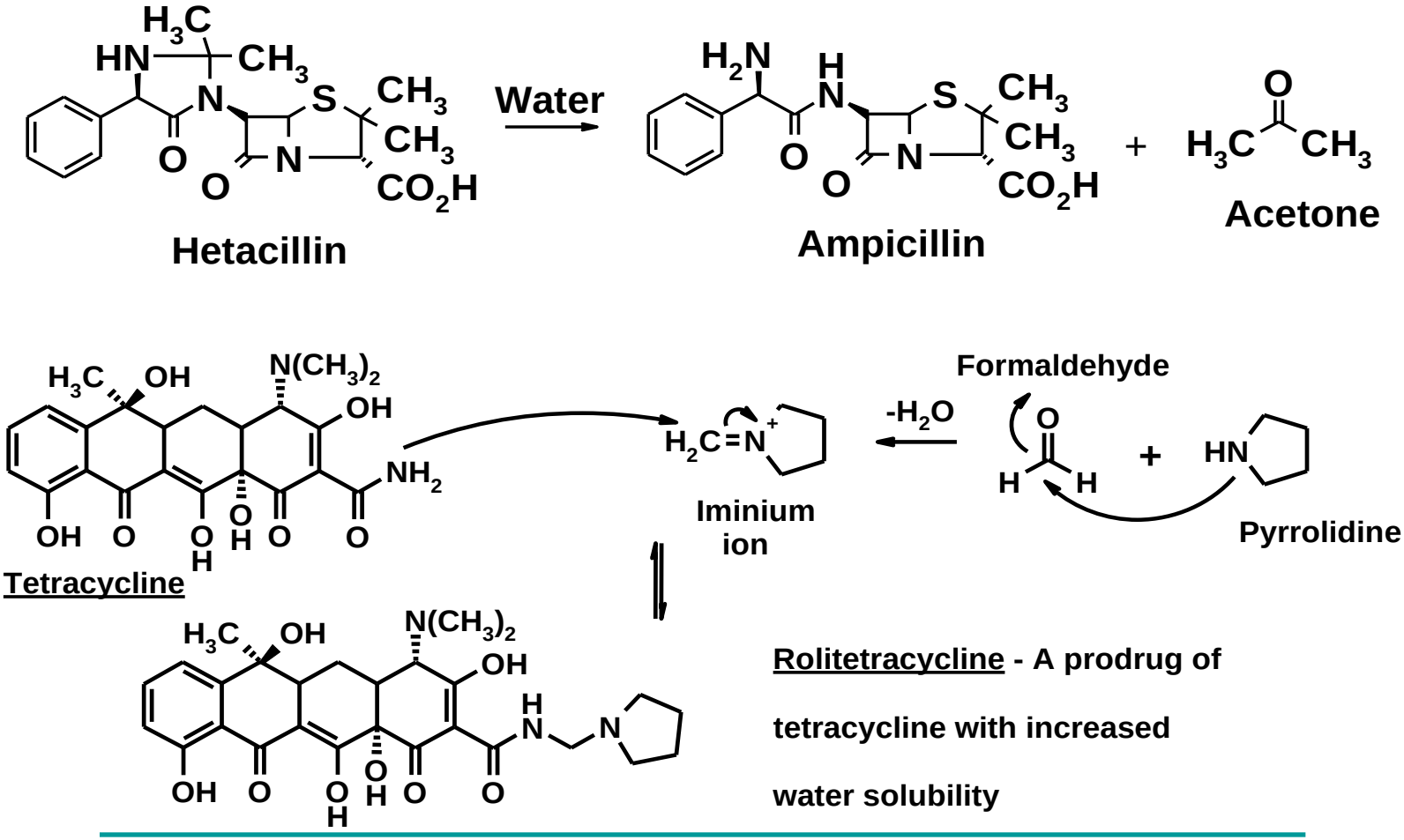
- Prodrug form of the amines:

1. There have been efforts at incorporating amines into peptide linkages in which the peptide serves to increase cellular uptake by use of an amino acid transporter. The amino acids are then cleaved by specific peptidase enzymes.
2. A more common approach has been to use Mannich bases.

Mannich bases result from the reaction of two amines with an aldehyde or ketone

Amine derivatives as prodrugs

- Amides not used due to high stability
- **<u>Most common amine derivative used</u>** is a Mannich Base prodrug



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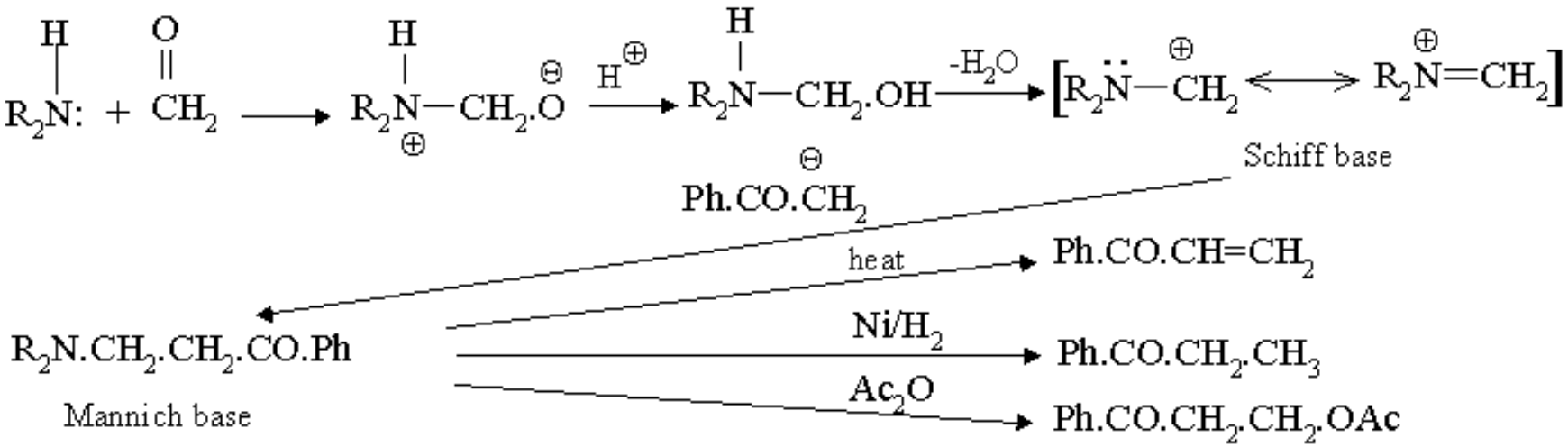
As seen with hetacillin, the effect of forming the Mannich base is to

1. lower the basicity of the amine and, thereby,
2. increase lipophilicity and absorption.

- When nitrogen is present in an amide linkage, it is sometimes desirable to use the amide nitrogen as one of the amines necessary to form a Mannich base.
- This approach was used with the antibiotic tetracycline—the amide nitrogen was allowed to react with formaldehyde and pyrrolidine to give the Mannich base rolitetracycline.
- In this case, addition of the basic pyrrolidine nitrogen introduces an additional ionizable functionality and increases the water solubility of the parent drug. The Mannich base hydrolyzes completely and rapidly in aqueous media to give the active tetracycline.

Mannich Base Chemistry



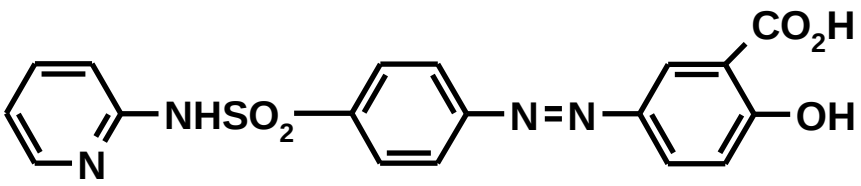
<u>Mannich Reaction</u> - This is nucleophilic addition reaction of an aldehyde and at least a secondary amine to produce what is known as a schiff base on protonation and elimination of a water molecule. The Schiff base is often stabilized by resonance. The addition of a carbanaion to the schiff base gives another base called the Mannich base. The Mannich base formed can readily eliminate the secondary amine to give the synthetic usefulness of the reaction, but when primary amines or ammonia are used the hydrogen on nitrogen atom can participate in a further reaction to give more complex products.

3- Azo linkage

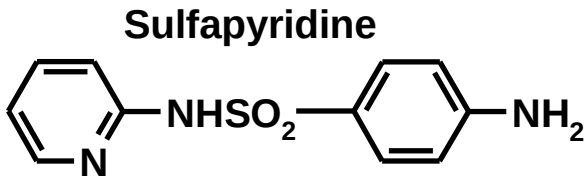
- Amines have occasionally been incorporated into an azo linkage to produce a prodrug.
- e.g. sulfasalazine, which is used in the treatment of ulcerative colitis. The azo linkage is broken in the gut by the action of azo reductases produced by microflora.
- This releases the active agent, amino salicylic acid, which has an anti-inflammatory effect on the colon, and sulfapyridine. As shown in this Scheme:

Azo Prodrugs

- Bacterial reductases → reductive cleavage
  - Release of 2 amine compounds
  - Occurs in ***colon*** → discourages small intestine systemic absorption
  - Concentrates the drug at the desired site of action

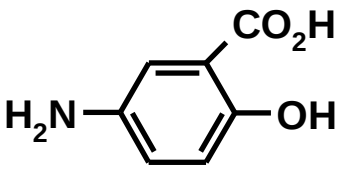


Sulfasalazine - Azulfidine® - Pharmacia & Upjohn
Sulfonamide antibiotic and antiinflammatory
Used to treat Ulcerative colitis, rheumatoid arthritis



Sulfapyridine

+



5-aminosalicylic acid

The advantage of this prodrug approach is:

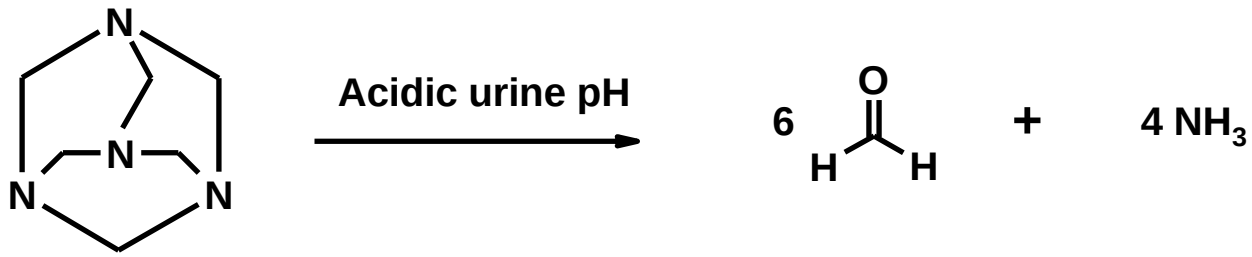
- Cleavage of the azo linkage and generation of aminosalicylic acid prior to absorption prevents the systemic absorption of the agent.
- Helps concentrate the active agent at the site of action.

Carbonyl prodrugs

- Aldehyde and ketone derivatives
- Little clinical utility with one exception Methenamine
- These have generally involved derivatives in which the sp_2 hybridized carbonyl carbon is converted to an sp_3 hybridized carbon attached to two heteroatoms, such as oxygen, nitrogen, or sulfur.
- Under hydrolysis conditions, these functionalities are reconvened to the carbonyl compounds.

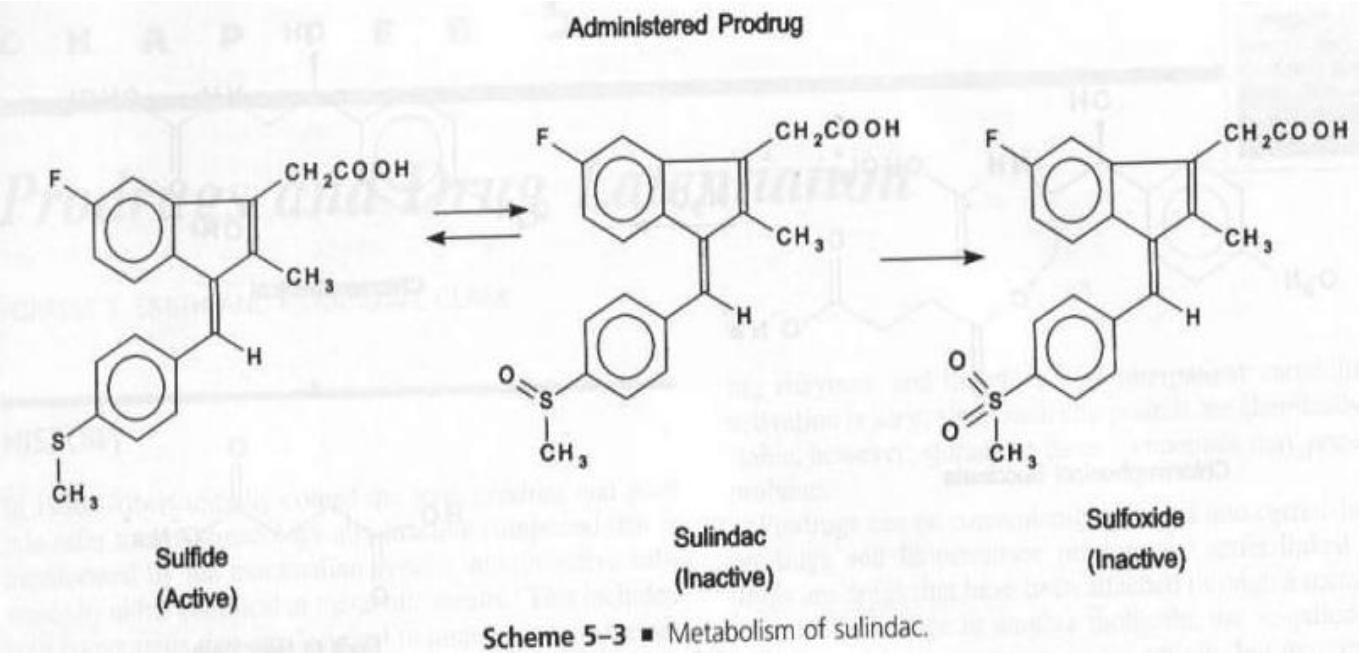
Methenamine releases formaldehyde in the urine, which acts as an antibacterial agent. The agent is administered in enteric-coated capsules to protect it from premature hydrolysis in the acidic environment of the stomach.

In the urine, where the acidic pH catalyzes the chemical hydrolysis to give formaldehyde. Use of this prodrug approach prevents the systemic release of formaldehyde and reduces toxicity.



BIOPRECURSOR PRODRUGS:

- Bioprecursor prodrugs do not contain a carrier or promoiety but rather contain a latent functionality that is metabolically or chemically transformed to the active drug molecule.
- The types of activation often involve:
- Oxidative activation, commonly seen since a number of endogenous enzymes can carry out these transformations.
- reductive activation
- phosphorylation, and
- In some cases chemical activation.
- Phosphorylation has been widely exploited in the development of antiviral agents, and many currently available agents depend on this type of activation.
- The nonsteroidal anti-inflammatory drug (NSAID) Sulindac is inactive as the sulfoxide and must be reduced metabolically to the active sulfide.



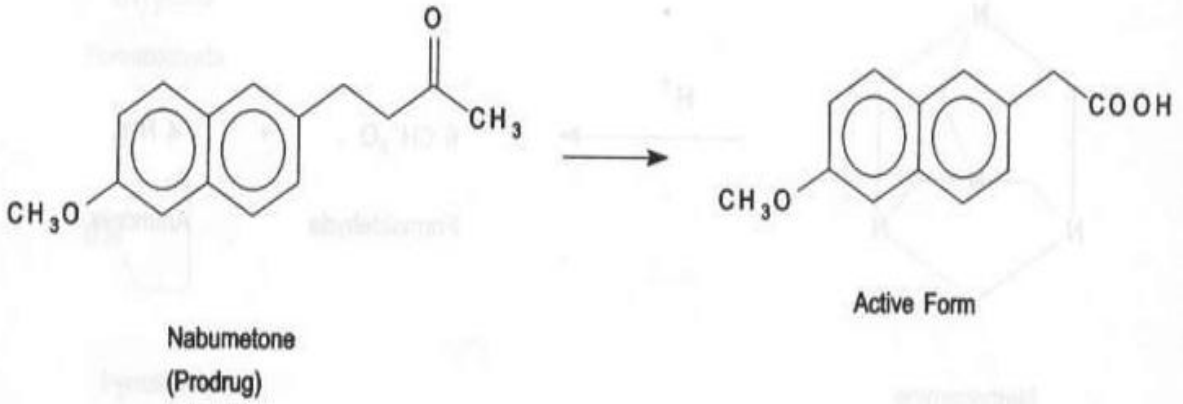
Scheme 5–3 ■ Metabolism of sulindac.

- Administration of the inactive form has the benefit of reducing the gastrointestinal (CIT) irritation associated with the sulfide.

The problems associated with bioprecursor prodrugs approach:

- Participation of alternate metabolic paths that may inactivate the compound. In this case, after absorption of sulindac, irreversible metabolic oxidation of the sulfoxide to the sulfone can also occur to give an inactive compound.
- Although seen less frequently, some prodrugs rely on chemical mechanisms for conversion of the prodrug to its active form.
- Metabolite generated after chemical hydrolysis some times toxic (it must be nontoxic and easily removed after it has performed its function).

A good example of a prodrug that requires oxidative activation is the NSAID nabumetone.



Scheme 5–18 ■ Oxidative activation of nabumetone.

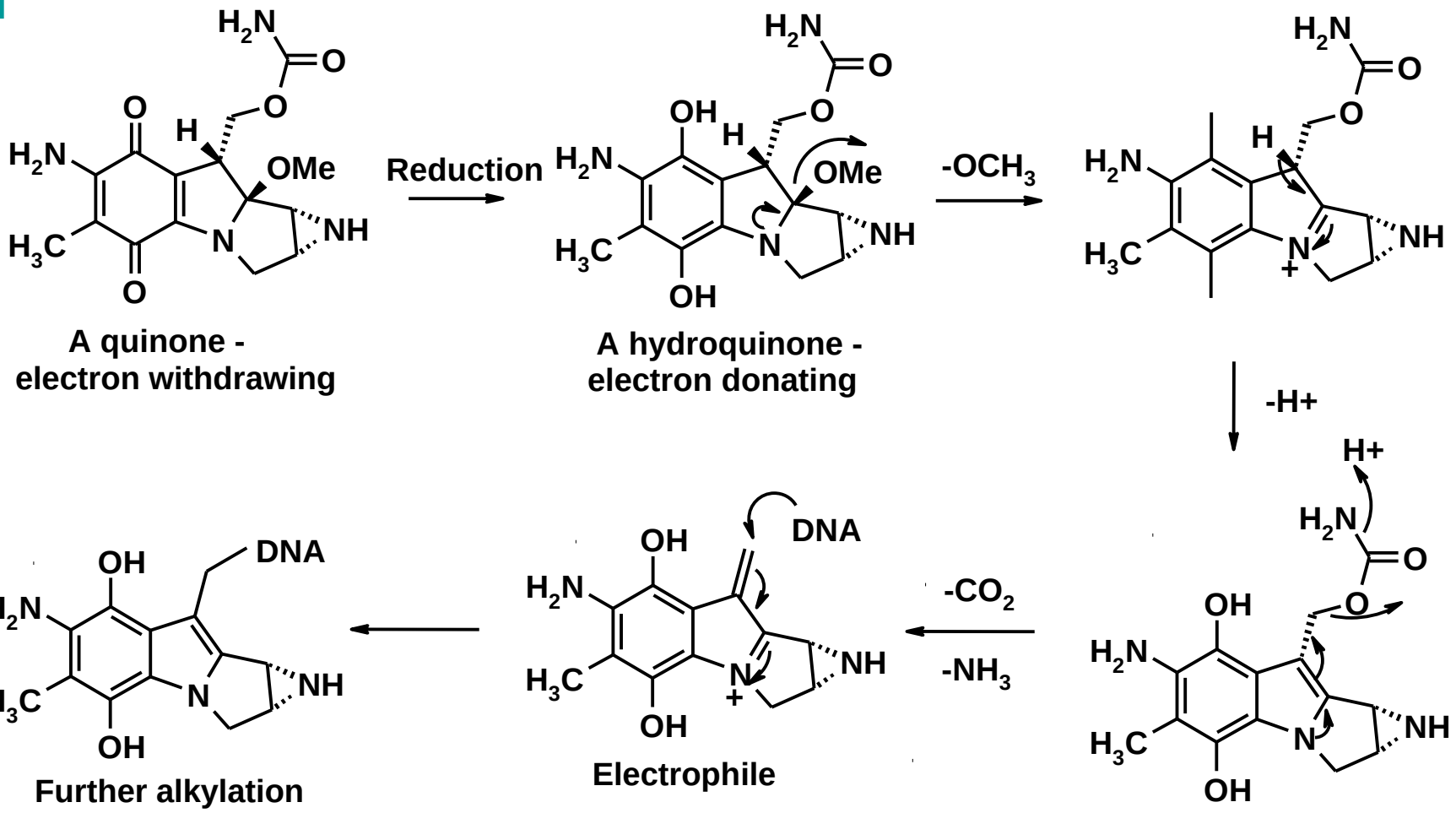
- NSAIDs produce stomach irritation. This irritation is associated in part with the presence of an acidic functionality in these agents.

- The carboxylic acid functionality commonly found in these agents is un-ionized in the highly acidic environment of the stomach. As a result, these agents are more lipophilic in nature and may pass into the cells of the gastric mucosa.
- The intracellular pH of these cells is more basic than that of the stomach lumen, and the NSAID becomes ionized. This results in backflow of H+ from the lumen into these cells, with concomitant cellular damage.
- This type of damage could be prevented if the carboxylic acid function could be eliminated from these agents: this functional group is required for activity, however. Nabumetone contains no acidic functionality and passes through the stomach without producing the irritation normally associated with this class of agents. Subsequent absorption occurs in the intestine, and metabolism in the liver produces the active compound as shown in above Scheme.

- This approach, however, did not completely eliminate the gastric irritation associated with nabumetone, since it is due only in part to a direct effect on the stomach. Inhibition of the target enzyme, cyclooxygenase, while having an anti-inflammatory effect, also results in the increased release of gastric acid, which irritates the stomach.
- Reductive activation is occasionally seen as a method of prodrug activation but, because there are fewer reducing enzymes, is generally less common than oxidative activation.
- One of the best known examples of reductive activation is for the antineoplastic agent mitomycin C. which is used in the treatment of bladder and lung cancer as shown in the following Scheme

Bioprecursor Prodrugs

Reduction example - Mitomycin C - Mutamycin® - Bristol Myers
Adenocarcinoma of the stomach and pancreas

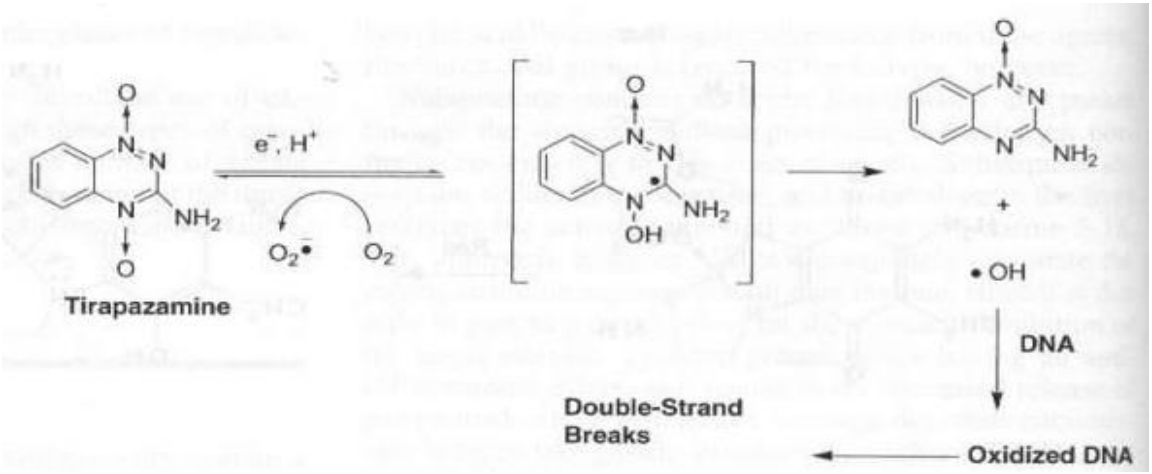


- Mitomycin C contains a quinone functionality that undergoes reduction to give a hydroquinone. This is important because of the differential effect of the quinone and hydroquinone on the electron pair of the nitrogen.
- Whereas the quinone has an electron-withdrawing effect on this electron pair, the hydroquinone has an electron-releasing effect, which allows these electrons to participate in the expulsion of methoxide and the subsequent loss of the carbamate to generate a reactive species that can alkylate DNA.

Important notes:

- The cascade of events that leads to an alkylating active drug species is initiated by the reduction of the quinone functionality in mitomycin C.
- The selectivity of mitomycin for hypoxic cells is minimal, however, the selectivity is determined in part by the reduction potential of the quinone, which can be influenced by the substituents attached to the ring.
- In an effort to modify the reduction potential of mitomycin C. various analogues have been prepared and tested for antineoplastic activity in slow-growing solid tumors that are poorly vascularized. In these tissues with a low oxygen content it was thought that reductive metabolism might be more prevalent than in normal tissues, so the agents would be selectively activated and, therefore, selectively toxic.
- A much more selective agent Tirapazamine is reported to be 100 to 200 times more selective for hypoxic cells than for normal cells.

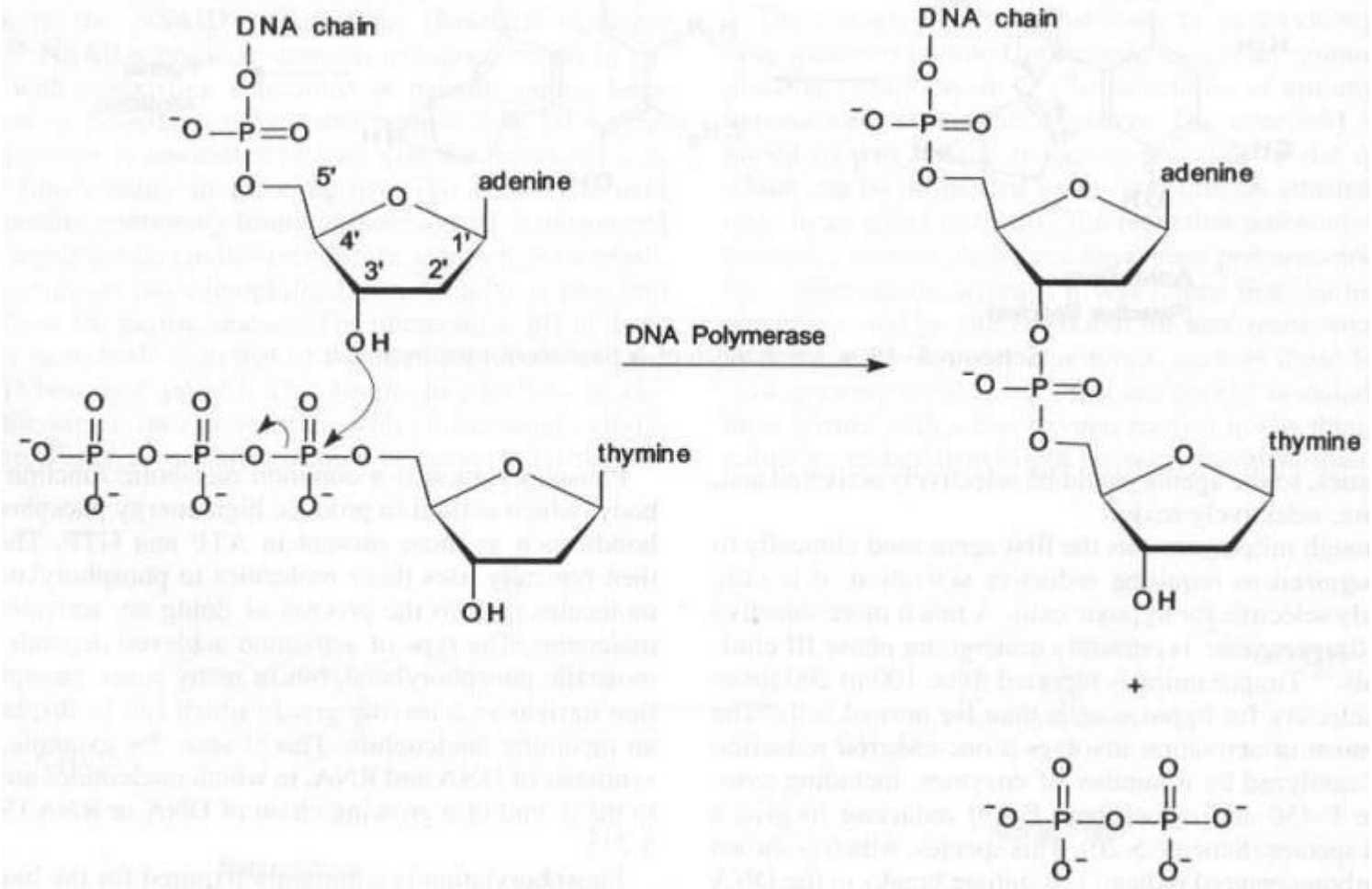
- The mechanism of activation involves a one-electron reduction that is catalyzed by a number of enzymes, including cytochrome P.450 and cytochrome P-450 reductase to give a radical species as shown in the following scheme:



- This species, which is shown as a carbon-centered radical, can initiate breaks in the DNA chain under hypoxic conditions. Under aerobic conditions, hydroxide radical is formed, which can initiate chain breaks.

Phosphorylation

- is a common metabolic function of' the body, which is used to produce high-energy phosphodiester bonds such as those present in ATP and GTP. The body then typically uses these molecules to phosphorylate other molecules and, in the process of doing so, activates these molecules.
- Phosphorylalion introduces a leaving group, which can be displaced by an incoming nucleophile. This is seen, for example, in the synthesis of DNA and RNA, in which nucleotides are added to the 3' end of a growing chain of DNA or RNA as shown in the following scheme:

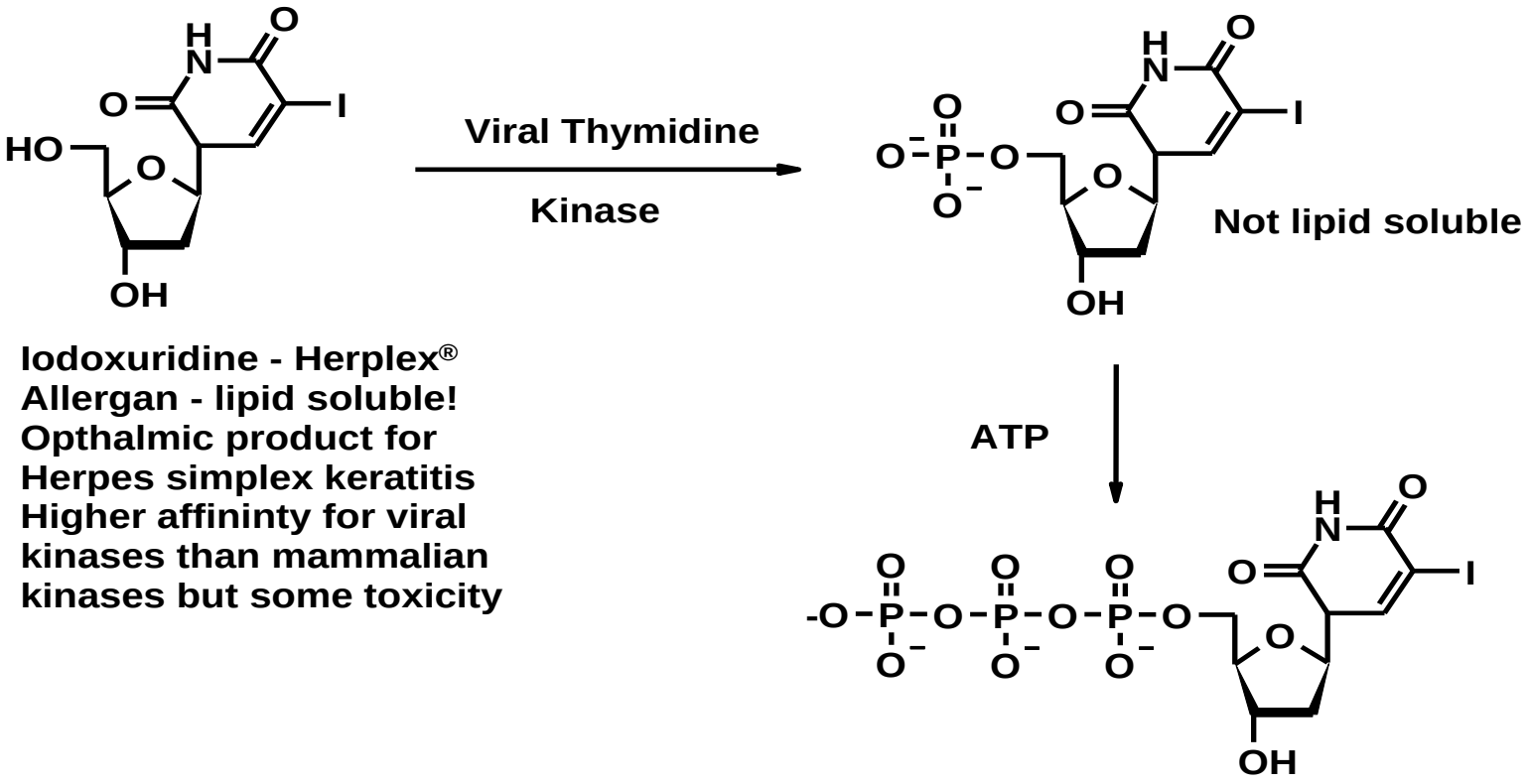


Scheme 5–21 ■ DNA synthesis.

- Phosphorylation is commonly required for the bioactivation of antiviral agents. These agents are commonly nucleosides, which must be converted to the nucleotides to have activity. Most often, antiviral agents disrupt the synthesis or function of DNA or RNA, which is generally accomplished by conversion to the triphosphate.
- Since normal cells are also involved in the synthesis of DNA and RNA, compounds have been sought that would be converted to the triphosphates, the active form, in greater amounts in infected cells than in normal cells.
- Therefore, nucleosides that have higher affinity for the viral kinase enzymes than the mammalian kinases are desirable and have greater selective toxicity.
- This can be seen in the prodrug idoxuridine, which was the first agent to show clinical effectiveness against viruses as shown in the following scheme:

Bioprecursor Prodrugs

Phosphorylation example –



Iodoxuridine - Herplex®
Allergan - lipid soluble!
Opthalmic product for
Herpes simplex keratitis
Higher affininty for viral
kinases than mammalian
kinases but some toxicity

TWO mechanisms of action: 1. Inhibits DNA polymerase 2. Incorporated into DNA affording incorrect base pairing and template activity

(Note: Thymine contain CH3 instead of I)

- The nucleoside enters the cell, where it is phosphorylated.
- In virally infected cells, this phosphorylation is accomplished preferentially by viral thymidine kinase, because the idoxuridine is a better substrate for the viral enzyme than for the corresponding mammalian enzyme.
- Therefore, the drug is activated to a greater extent in the virally infected cells and achieves some selective toxicity, although this selectivity is rather low, and there is significant toxicity to normal cells.
- Once the drug has been phosphorylated to the triphosphate stage, it can inhibit DNA synthesis in a number of ways, including:
  - Inhibition of viral DNA polymerase.
  - Incorporation into DNA, which results in incorrect base pairing that, disrupts the ability of DNA to function as a template for DNA and RNA synthesis.

Advantages of the prodrug idoxuridine

- Achieves some selective toxicity.
- Increased cell penetration. The prodrug can easily enter the cell via active transport mechanisms, whereas the active nucleotides are unable to use this process and are too polar to cross the membrane via passive diffusion.

CHEMICAL DELIVERY SYSTEMS:

- The knowledge gained from drug metabolism and prodrug studies may be used to target a drug to its site of action.
- Site-specific chemical delivery requires that the prodrug reaches the target site and that the enzymatic or chemical process exists at the target site for conversion of the prodrug to the active drug.

Many factors are involved in the relative success of site-specific drug delivery, including:

- Extent of target organ perfusion; since high metabolic activity occurs in highly perfused tissues such as liver and kidney, delivery to these organs has a natural advantage.
- Rate of conversion of prodrug to active drug in both target and non target sites; On arrival at the target site, the prodrug should be selectively converted to drug relative to its rate of conversion at non target sites.
- Input/output rates of prodrug and drug from the target sites. It is highly desirable to have the active drug, once formed, migrate from the target site at a slow rate.

Aims of site-specific drug delivery:

- Increased therapeutic effectiveness.
- Limited side effects.

- Other than chemical drug delivery, many carrier systems have been evaluated for drug delivery, including:
- proteins, Polysaccharides, liposomes, emulsions, cellular carriers (erythrocytes and leukocytes), Magnetic control targeting, and implanted mechanical pumps.
- What is the Basic Goal?
  - Protect a non-specific biological environment from a drug
  - Protect a drug from a non-specific biological environment
  - Especially evaluated for drugs with a narrow therapeutic window especially anti-cancer agents

General notes:

- Site-specific drug delivery has been evaluated extensively for drugs with narrow therapeutic windows, such as many of the anticancer drugs.
- The target sites include cancer cells, GI tract, kidney and urinary tract, bacterial cells, viral material, ocular tissue, and the blood—brain barrier.

Examples of site-specific drug delivery:

- The prodrug methenamine can be considered a site-specific chemical delivery system for the urinary tract antiseptic agent formaldehyde. The low pH of the urine promotes the hydrolysis of methenamine to formaldehyde, the active antibacterial agent. The rate of hydrolysis increases with increased acidity (decreased pH), and this can be promoted by administration of urinary pH-lowering agents or by diet. The pH of the plasma is buffered to about 7.4, and the rate of hydrolysis is low, preventing systemic toxicity from formaldehyde.
- The antiviral drugs, such as idoxuridine these drugs serve as substrates for phosphorylating enzymes found in viruses, and the phosphorylated species is the active antiviral agent. The active phosphorylated species is incorporated into viral DNA, disrupting viral replication and, thus, producing the antiviral effect.

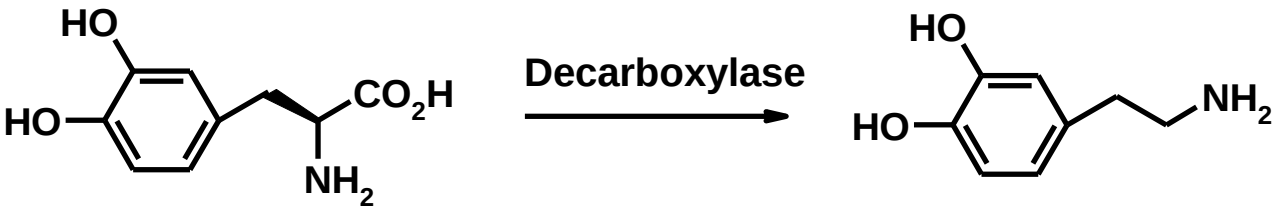
- The relative physicochemical properties of prodrug and its phosphorylated derivative suggest an appropriate input/output ratio for site specificity.
- The reduces any human toxicity that might be associated with this drug is due to:
- A/ phosphorylation is accomplished preferentially by viral thymidine kinase
- B/ increased polarity and viral retention of the active phosphorylated species likely.

The amino acid drug L-dopa

- It can be considered a site specific chemical delivery system that delivers the drug dopamine to the brain.
- The brain has an active transport system that operates to incorporate L amino acids into the central nervous system (CNS), and L-dopa is transported into the brain in this manner.
- Once across the blood—brain barrier, L-dopa undergoes decarboxylation, as shown in the following Scheme to yield the active metabolite, dopamine.

Chemical Delivery Systems

- Example: L-Dopa or Levodopa – Anti-Parkinsonism agent
  - Larodopa® – Roche and Dopar® - Procter & Gamble



Dopamine

- Brain has a specific transport system for L-amino acids
- Dopamine does not cross the blood brain barrier efficiently, is rapidly metabolized by oxidative deamination, and can cause peripheral side effects

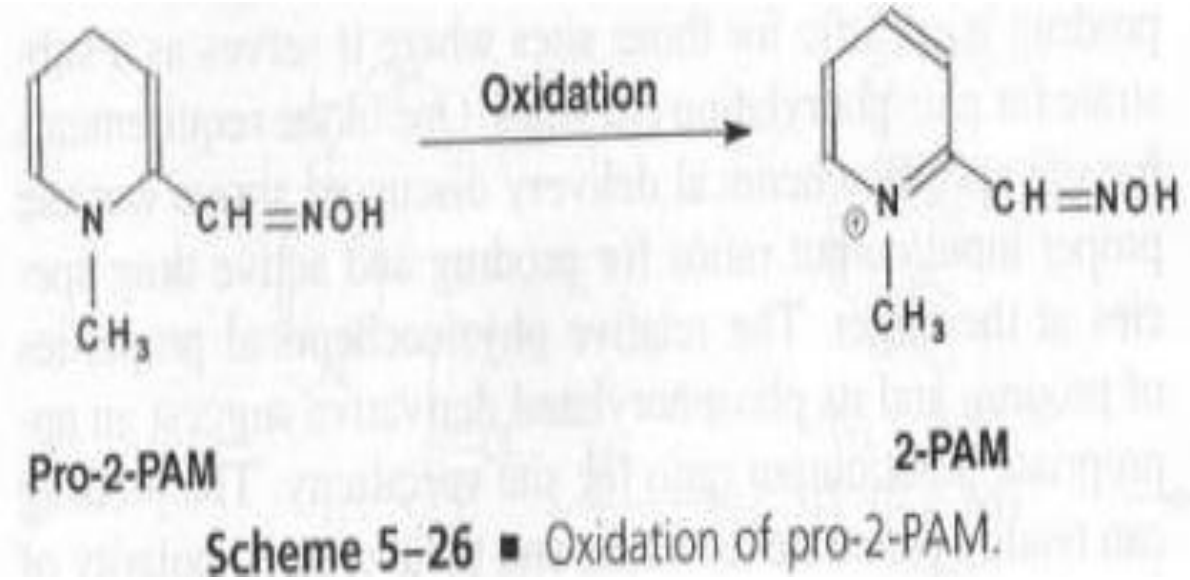
Direct systemic administration of dopamine does not produce significant levels of the drug in the brain because of:

- A/ its high polarity and poor membrane permeability
- B/ its facile metabolic degradation by oxidative deamination.
- Dopamine formed on the inside of the blood—brain barrier is held there, however, because of the poor membrane permeability of this drug.
- Although some specificity for brain tissue is achieved by this delivery method, peripheral side effects of L.-dopa are the direct result of decarboxylation to dopamine in other organ systems.
- In this case, the enzyme activating system is not localized at the target site, and its presence in other tissues and organs leads to undesirable side effects.

(pro-2- PAM)

- Another example of the chemical delivery of a drug to the brain and CNS is the prodrug form of 2-PAM (pro-2- PAM),
- an important antidote for the phosphate and carbamate acetyl cholinesterase inhibitors used in insecticides and nerve gases.
- The polar properties of 2-PAM, a permanent cationic species,
- A/ prevent this drug from being absorbed following oral administration
- B/ restrict the drug from access to the brain, even after IV administration.

- Pro-2-PAM is a dihydropyridine derivative that undergoes metabolic and chemical oxidation to yield the active drug 2-PAM as showing in the following scheme:

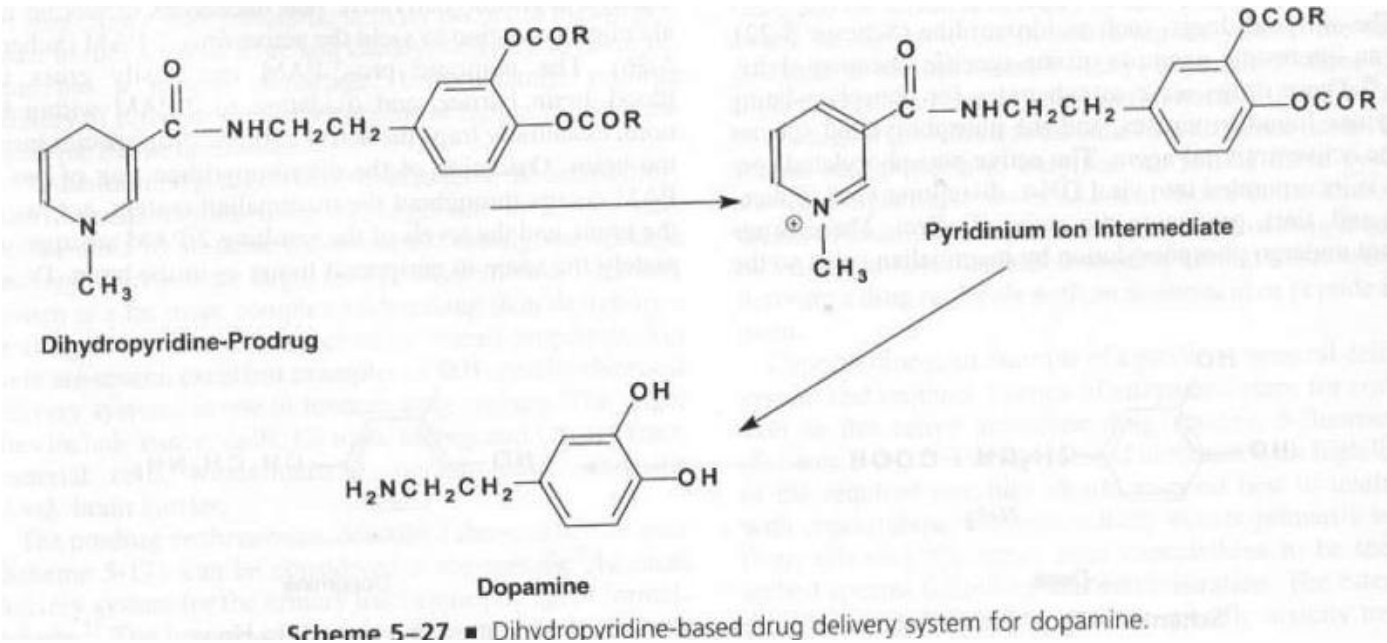


Scheme 5–26 ■ Oxidation of pro-2-PAM.

- The nonionic pro-2-PAM can easily cross the blood—brain barrier, and oxidation to 2-PAM within the brain essentially traps the active cationic drug species inside the brain.
- Oxidation of the dihydropyridine ring of pro-2-PAM occurs throughout the mammalian system, not just in the brain, and the levels of the resulting 2-PAM are approximately the same in peripheral tissue as in the brain.
- Note: IV administration of pro-2-PAM, however, yields brain levels of 2-PAM that are approximately 10 times higher than those achieved by IV administration of the parent drug.

- The facile oxidation of the dihydropyridine ring system has been extensively investigated as a general process for chemical delivery of a number of drugs to the CNS.
- This process is a multistep procedure involving:
- A/ delivery of the drug—dihydropyridine derivative to the brain via facile diffusion across the blood—brain barrier,
- B/ followed by oxidation to the quaternary pyridine cation, which is trapped in the brain.
- C/ the drug is then released from the pyridine cation by a second metabolic/chemical event.

- A number of functional groups can be added to the dihydropyridine to facilitate the derivatization of various functional groups found in CNS drugs.
- Since many CNS drugs are amines, amides of dihydropyridine carboxylic acids are often prepared and used to
- deliver the drugs across the blood—brain barrier into the brain.
- serve to protect the amines from metabolic degradation before they reach the target site.
- The dihydropyridine derivative of a dopamine ester, shown in the following Scheme; has access to the CNS via passive absorption of the tertiary amine, which on oxidation restricts the resulting pyridinium amide to the brain. Amide hydrolysis then delivers the active form of the drug at or near its site of action.

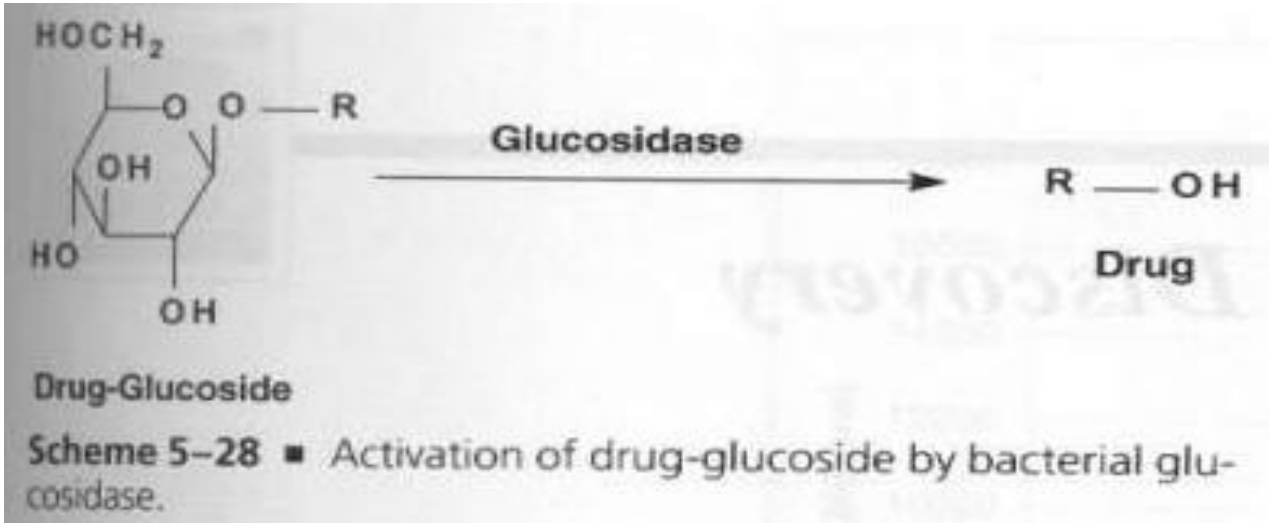


Scheme 5–27 ■ Dihydropyridine-based drug delivery system for dopamine.

- The amide hydrolysis step may be slower than the dihydropyridine oxidation step, and thus a reservoir of pyridinium amide precursor may be available for conversion to the active drug species.

The delivery of drugs to the colon and lower GI tract

- The delivery of drugs to the colon and lower GI tract has taken advantage of the unique enzymatic processes found in colon bacteria. The glucosidase activity of these bacteria allows hydrolysis of glucoside derivatives of drugs in the colon and provides higher concentrations of active drug.
- A number of steroid drugs as shown in the following scheme: demonstrate increased effectiveness in the lower GI tract following administration as their glucoside derivatives.



Scheme 5–28 ■ Activation of drug-glucoside by bacterial glucosidase.

- The polar glucoside derivatives of the steroids are not well absorbed into the bloodstream from the GI tract and remain available to serve as substrates for the bacteria that are found primarily in the human colon.

- Many enzymatic systems show higher activity in tumor cells than in normal tissue because of the higher growth rates associated with tumor tissue.
- Peptidases and proteolytic enzymes are among those systems showing higher activity in and near tumor cells.
- Thus, one means of attempting to produce higher rates of drug incorporation into tumors than in surrounding normal tissue involves deriving a drug molecule with an amino acid or peptide fragment.
- Note: the presence of the enzymes in normal tissue prevents the possibility of complete site specificity for these agents.

Polymeric prodrugs:

- Polymers, including **biopolymers**, are made of repetitive units called monomers.
- **Biopolymers** are polymers produced by living organisms. Cellulose, starch, chitin, proteins, peptides, DNA and RNA are all examples of biopolymers, in which the monomeric units, respectively, are sugars, amino acids, and nucleotides.
- Polymers are used as carriers for the delivery of drugs, proteins, targeting moieties, and imaging agents.
- Several polymers have been successfully utilized in clinical research:
- 1. poly(ethylene glycol) (PEG),
- 2. N-(2-hydroxypropyl)methacrylamide (HPMA),
- 3. poly(lactide-co-glycolide) (PLGA) copolymers

polymeric prodrug

- A conjugation of a drug with a polymer forms so-called ‘polymeric prodrug’.
- Based on the site and the mode of action, polymer conjugates possess either ‘tuned’ degradable or non-degradable bonds.
- Polymeric prodrugs have several advantages over their low molecular weight precursors. The main advantages include:
- 1. An increase in water solubility of low soluble or insoluble drugs, and therefore, enhancement of drug bioavailability.

- 2. Protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking.
- 3. An improvement in pharmacokinetics.
- 4. A reduction in antigenic activity of the drug leading to a less pronounced immunological body response.
- 5.The ability to provide passive or active targeting of the drug specifically to the site of its action.
- 6. The possibility to form an advanced complex drug delivery system, which, in addition to drug and polymer carrier, may include several other active components that enhance the specific activity of the main drug.

- Depending on the nature and site of action of a drug, either homopolymers, or graft or block polymers are being extensively used in bioconjugates.
- Due to their higher molecular weight, polymers are known to dominate the physical properties of the bioconjugated moiety.
- Along with the polymer, the physico-chemical properties of the drug or biomolecule to be conjugated are equally important.

- The following properties of the drug molecules make it suitable as an ideal candidate to form the polymeric conjugate:
- 1. Lower aqueous solubility.
- 2. Instability at varied physiological pHs.
- 3.Higher systemic toxicity, and
- 4. Reduced cellular entry

- Numerous polymeric prodrugs are in clinical phases and several others have been approved e.g. liposomal _ Amphotericin B & PEG_Adenosine deaminase.
- Covalent conjugation of biomolecules, e.g. protein drugs to synthetic polymers, particularly poly (ethylene glycol) (PEG) does:
- 1. Increase their plasma stability.
- 2. Reduces protein immunogenicity and
- 3. Can increase therapeutic index.

Successful bioconjugation depends upon:

- The chemical structure.
- Molecular weight.
- Steric hindrance and
- The reactivity of the biomolecule as well as the polymer.

- In order to synthesize a bioconjugate, both chemical entities need to posses a reactive or functional groups such as –COOH, – OH, –SH or –NH2.
- However, the presence of multiple reactive groups makes the task a bit complex. Therefore, the synthetic methodology to form a conjugate involves either protection or deprotection of the groups.

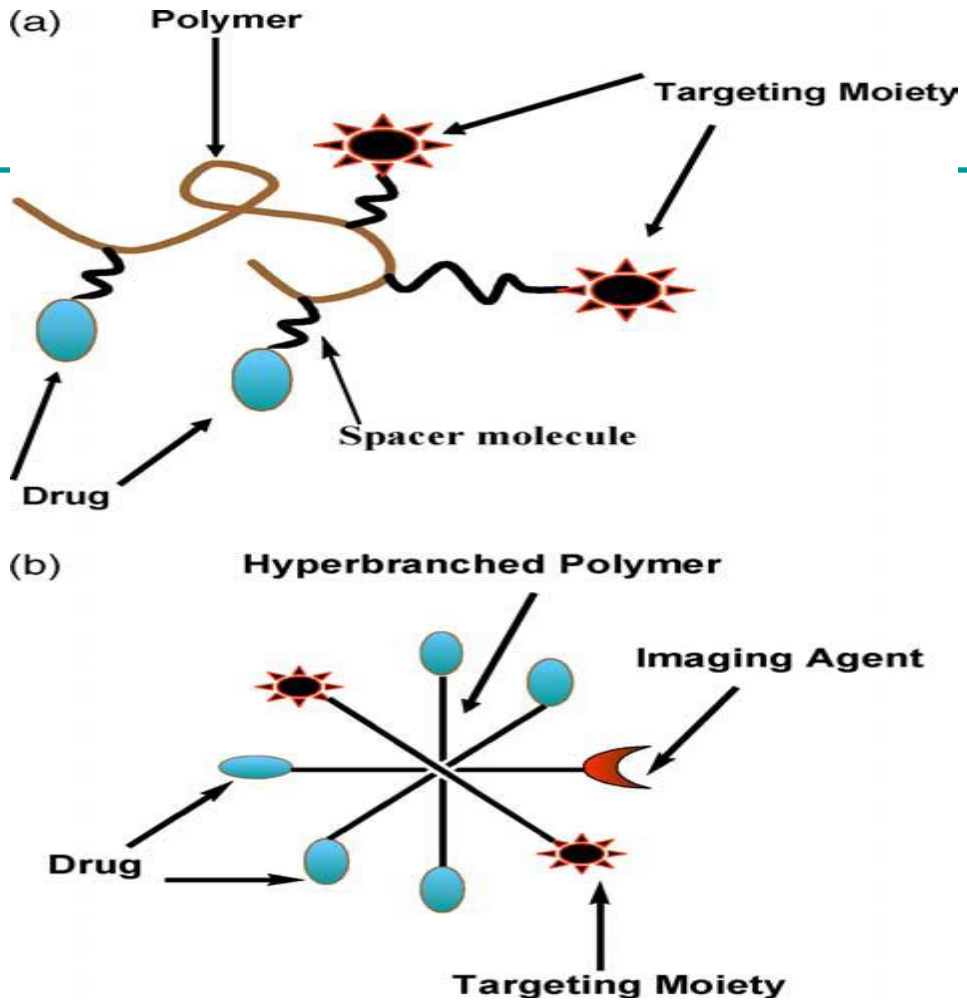
- Many of the most commonly used strategies involve use of coupling agents such as dicyclohexyl carbodiimide (DCC) or use of N-hydroxysuccinimide esters.
- Chemical conjugation of drugs or other biomolecules to polymers and its modifications can form stable bonds such as ester, amide, and disulphide.
- Covalent bonds (e.g. ester or amide) are stable and could deliver the drug at the targeted site, but such bonds may not easily release targeting agents and peptides under the influence of acceptable environmental changes.
- In the past, most of the polymeric prodrugs have been developed for the delivery of anticancer agents. High molecular weight prodrugs containing cytotoxic components have been developed to decrease peripheral side effects and to obtain a more specific administration of the drugs to the cancerous tissues.
- Polymer–drug conjugates can therefore be tailored for activation by extra- or intracellular enzymes releasing the parent drug in situ.

Design and synthesis of polymeric prodrugs:

- The most complete realization of the prodrug approach is possible by the use of an advanced type of prodrug—the drug delivery system (DDS).
- Such a system can be constructed not only to target a desired organ as a whole, its cells or specific organelles inside certain cells but also to release a specified amount of the drug at specific times.

- Three major types of polymeric prodrugs are currently being used:
- 1. Prodrugs that are broken down inside cells to form active substance or substances.
- 2. Prodrugs that are usually the combination of two or more substances. Under specific intracellular conditions, these substances react forming an active drug.
- 3. Prodrugs that are include three components: a targeting moiety, a carrier, and one or more active component(s).

- In general, an ideal polymeric prodrug model consists mainly of a combination of one or more components:
- (a) A polymeric backbone as a vehicle,
- (b) One or more drugs of the biological active components,
- (c) Spacer for hydrolysis of the biomolecule and versatility for conjugation,
- (d) An imaging agent and
- (e) Targeting moiety (Fig. 1a and b).



- Fig. 1. Schematic presentation for (a) polymeric prodrug with targeting agent and (b) hyperbranched polymer conjugate with targeting and imaging agent.

- The drug delivery carrier can be either biocompatible or an inert biodegradable polymer.
- The drug is coupled directly or via a spacer arm onto the polymer backbone.
- Selection of the spacer arm is critical as it opens the possibility of controlling the site and the rate of release of the active drug from the conjugates either by hydrolysis or by enzymatic degradation.
- The most challenging aspect of this protocol is the possibility of altering the body distribution and the cellular uptake by cell-specific or non-specific uptake enhancers.

- The polymers selected for preparing macromolecular prodrugs can be categorized according to:
- 1. Chemical nature (vinylic or acrylic polymers, polysaccharides, poly (a-amino acids), etc.,
- 2. Biodegradability,
- 3. Origin (either natural polymers or synthetic polymers) and
- 4. Molecular weight (oligomers, macromers and polymers).

Polymeric drug delivery system (PDDS)

- Modification of a polymer to form a conjugate with a biomolecule depends upon two interrelated chemical reactions:
- (1) Reactive functional groups present in the polymer and
- (2) Functional groups present on the biological component.
- In general, most of the biomolecules such as ligands, peptides, proteins, carbohydrates, lipids, polymers, nucleic acid and oligonucleotide possess combinations of these functional groups. Selection of a suitable method, process, and reagents are crucial for successful chemical conjugation.

- The following are common strategies adapted to obtain a polymeric drug delivery system as biologically active prodrug conjugates:
- 1. N-hydroxysuccinimide (NHS) ester and coupling methods, due to their higher reactivity at physiological pH makes NHS a choice for amine coupling reactions in bioconjugation synthesis.
- 2. Incorporation of spacers in prodrug conjugates; various spacers have been incorporated along with the polymers and copolymers to decrease the crowding effect and steric hindrance.
- The incorporation of a spacer arm can enhance ligand–protein binding and has application in prodrug conjugates and in biotechnology.

- For example, amino acid spacers such as glycine, alanine, and small peptides are preferred due to their chemical versatility for covalent conjugation and biodegradability.
- 3. Carbodiimide coupling reactions or zero lengths cross-linkers;
- Coupling and condensation reactions are unique to obtain chemical conjugates involving drugs or other biocomponents with polymers. The smallest possible reagents for bioconjugate synthesis are called zero length cross-linkers