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.

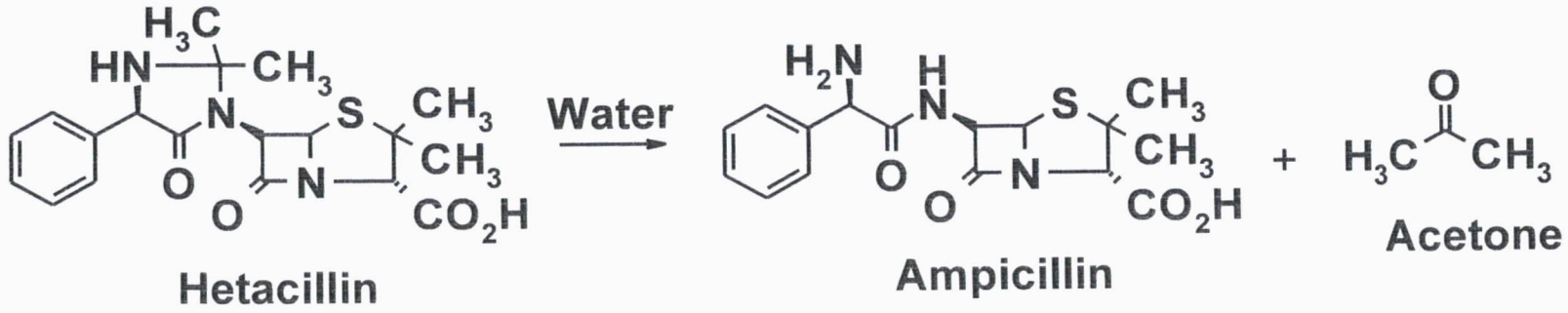
- Prodrug form of the amines:

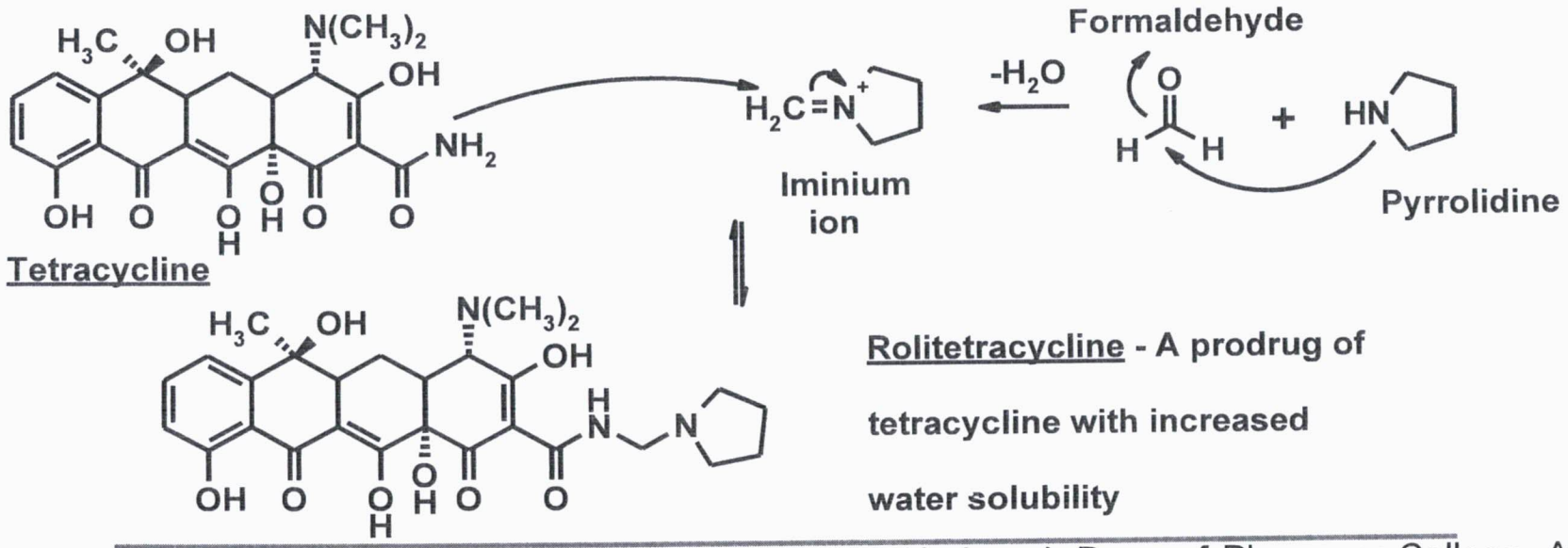
1. There have been efforts at incorporating amines into peptide linkages in which the peptide serves to increase cellular uptake by use of an amino acid transporter. The amino acids are then cleaved by specific peptidase enzymes.
2. A more common approach has been to use Mannich bases.

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





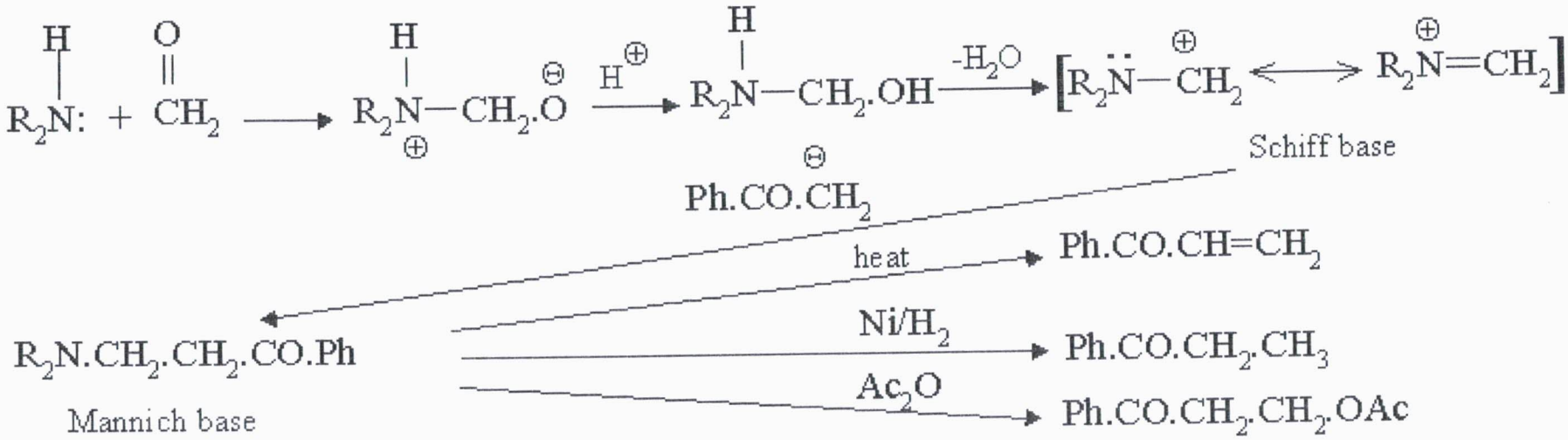
<u>Rolitetracycline</u> - A prodrug of tetracycline with increased water solubility

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



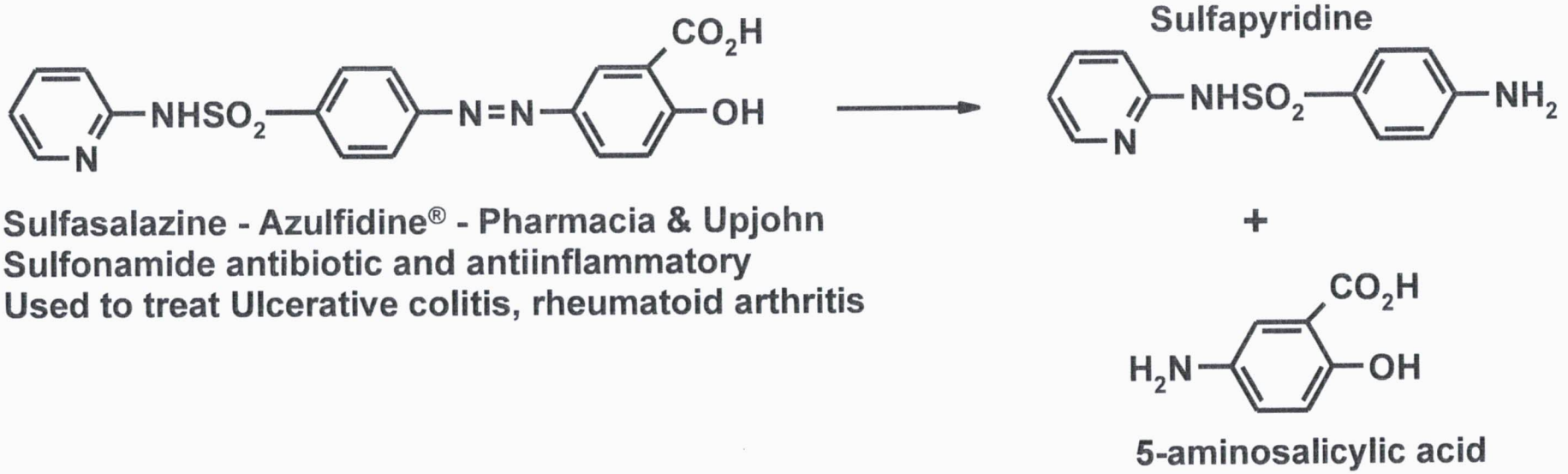
Mannich Reaction - This is nucleophilic addition reaction of an aldehyde and at least a secondary amine to produce what is known as a schiff base on protonation and elimination of a water molecule. The Schiff base is often stabilized by resonance. The addition of a carbanaion to the schiff base gives 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

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

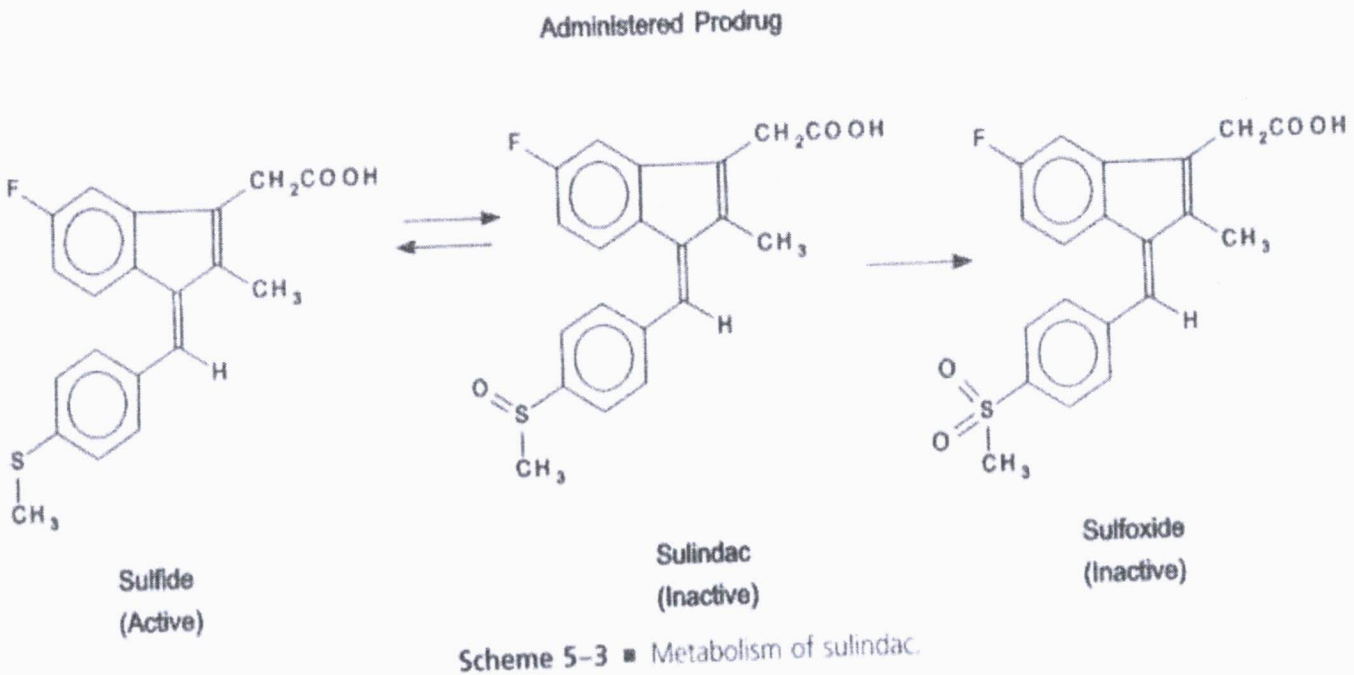
- Aldehyde and ketone derivatives
- Little clinical utility with one exception Methenamine
- These have generally involved derivatives in which the sp_2 hybridized carbonyl carbon is converted to an sp_3 hybridized carbon attached to two heteroatoms, such as oxygen, nitrogen, or sulfur.
- Under hydrolysis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



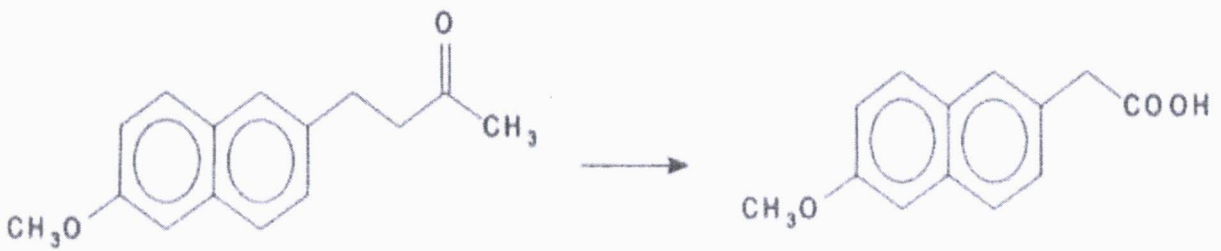
Scheme 5-3 ■ Metabolism of sulindac.

- 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.



Nabumetone
(Prodrug)

Active Form

Scheme 5–18 ■ Oxidative activation of nabumetone.

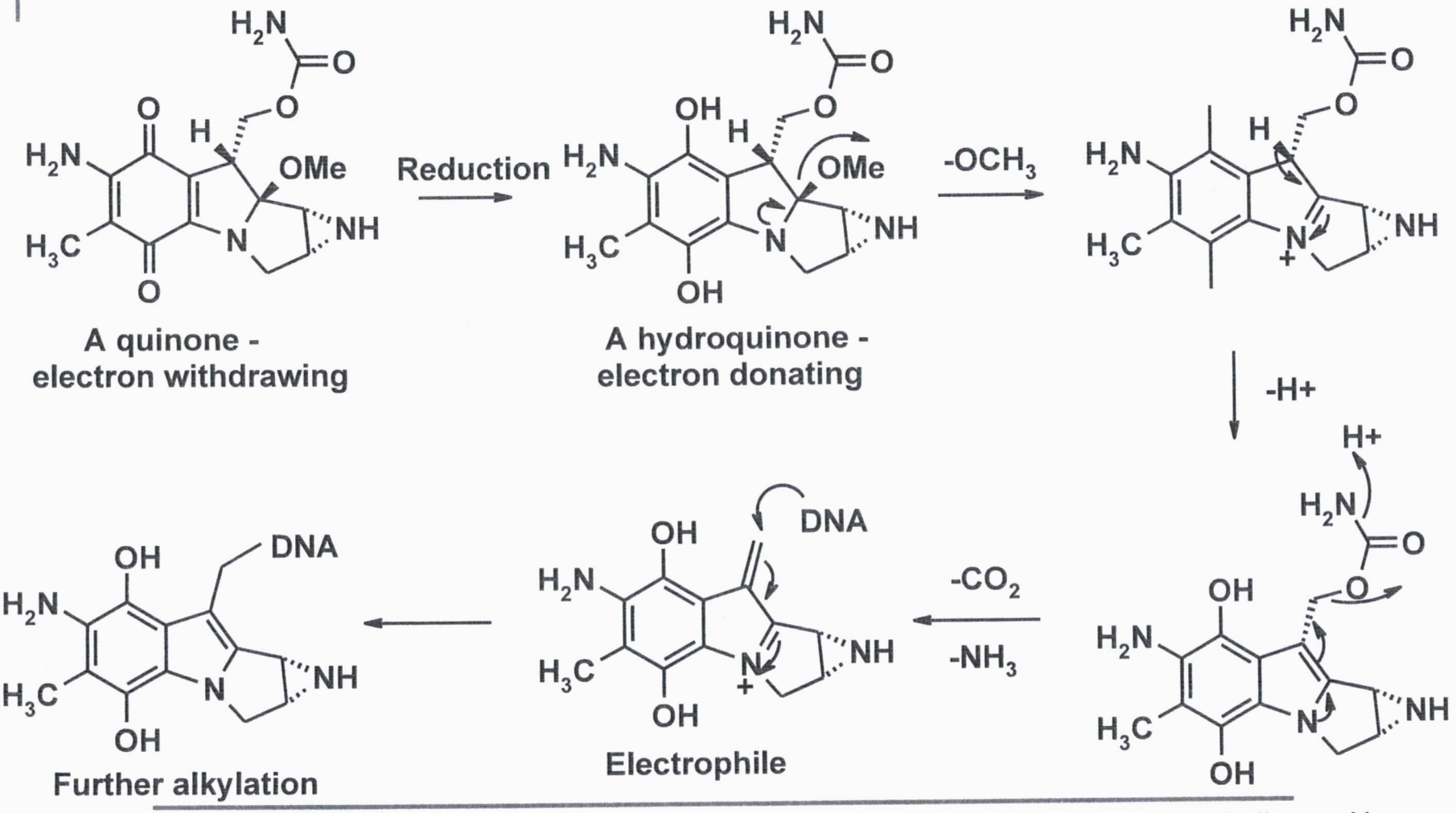
- NSAIDs produce stomach irritation. This irritation is associated in part with the presence of an acidic functionality in these agents.

- 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

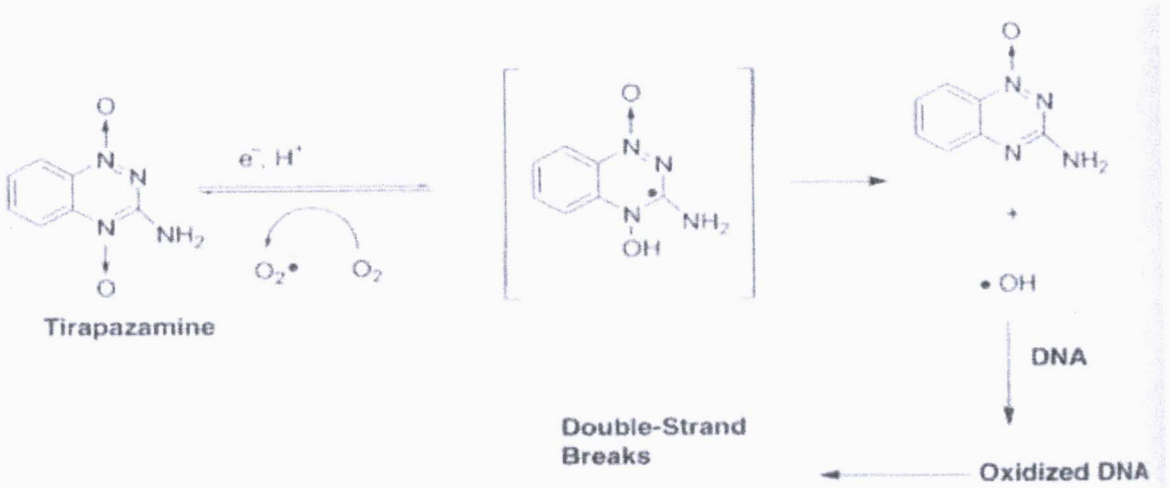


- Mitomycin C contains a quinone functionality that undergoes reduction to give a hydroquinone. This is important because of the differential effect of the quinone and hydroquinone on the electron pair of the nitrogen.
- Whereas the quinone has an electron-withdrawing effect on this electron pair, the 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.

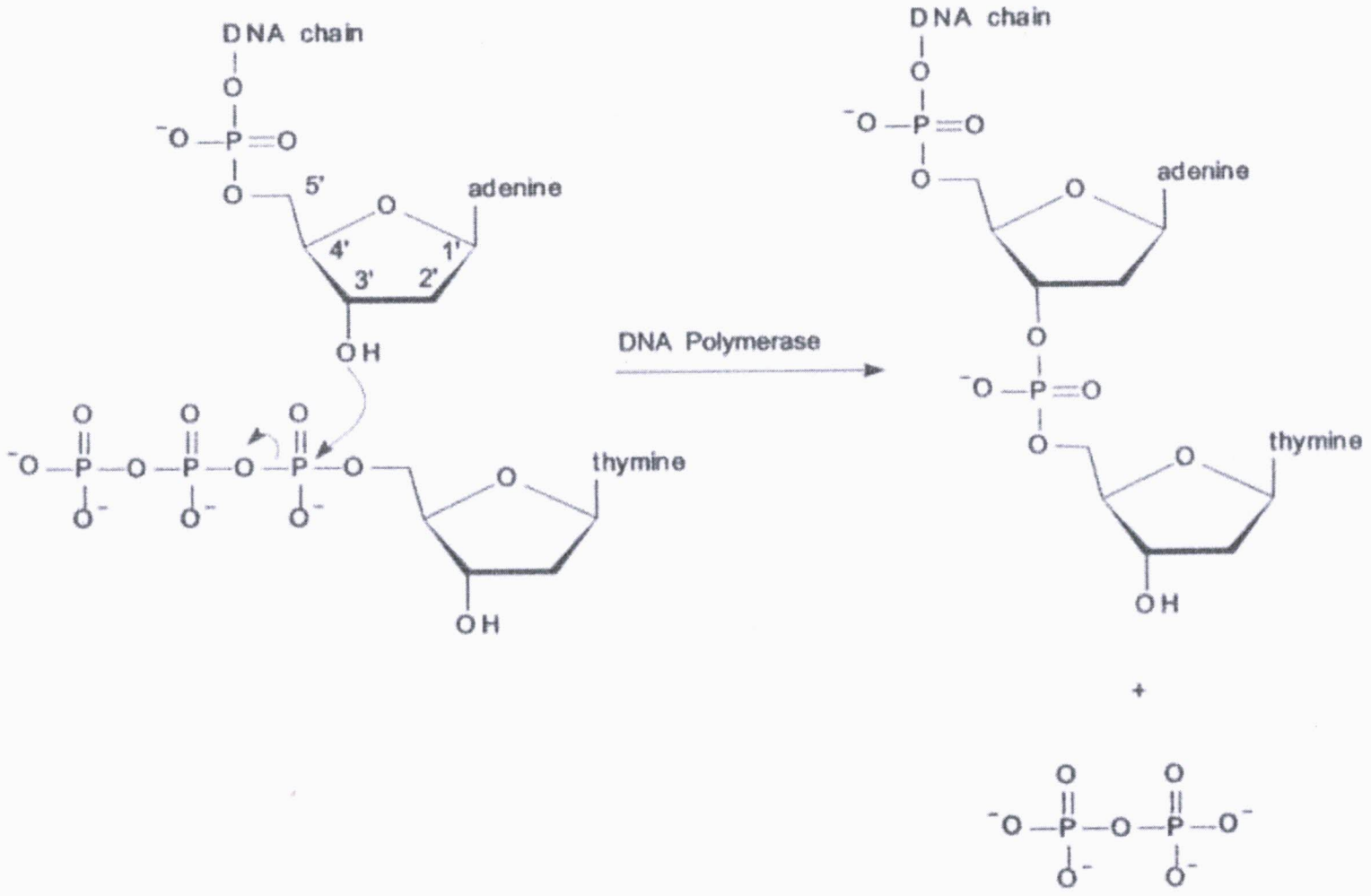
- The mechanism of activation involves a one-electron reduction that is catalyzed by a number of enzymes, including cytochrome P.450 and cytochrome P-450 reductase to give a radical species as shown in the following scheme:



- 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:

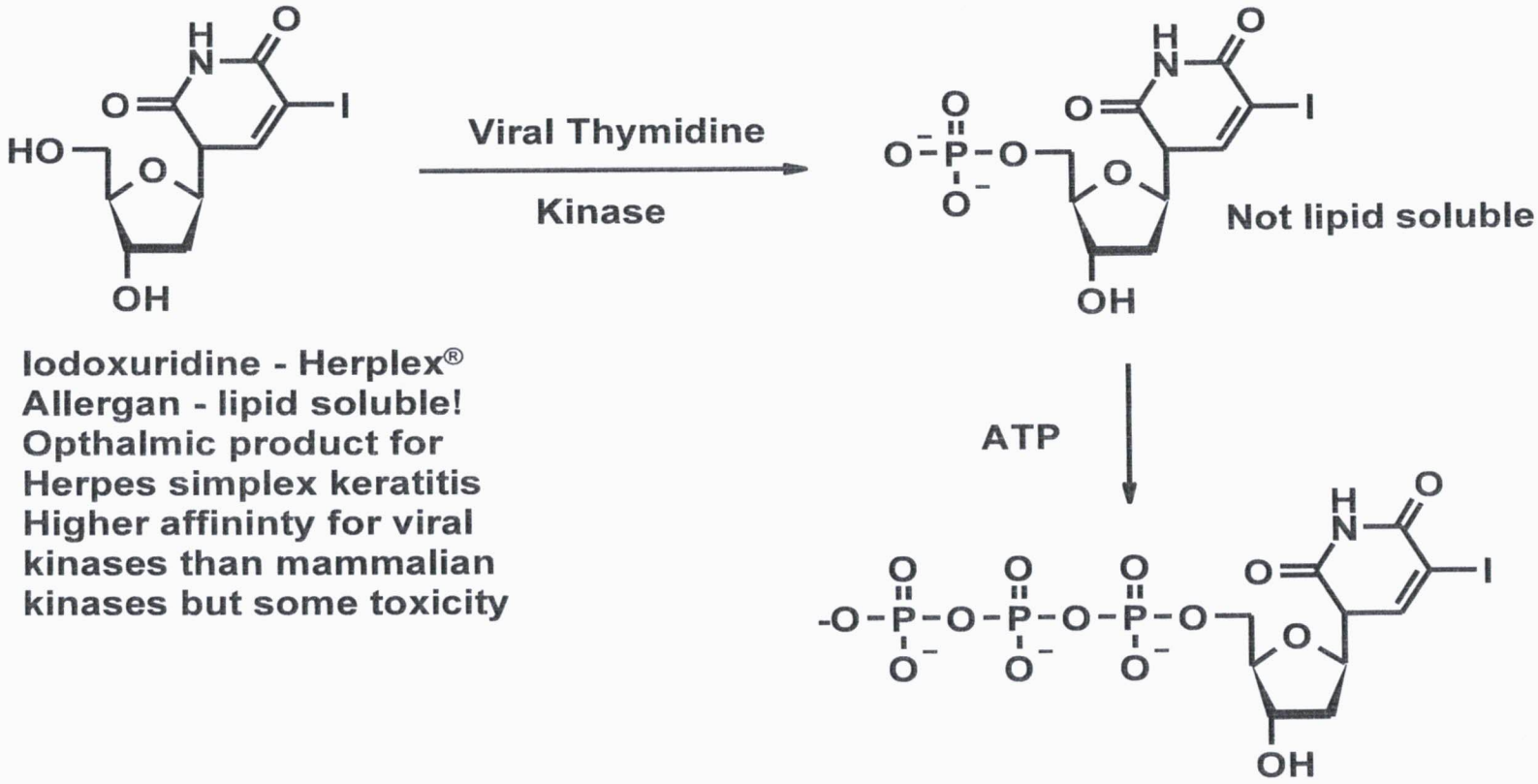


Scheme 5–21 ■ DNA synthesis.

- 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.