Pharmaceutical chemistry

Antineoplastic agents I

Al-Mustaqbal university

college of pharmacy

Definitions

Cancer still remains one of the most dangerous diseases in the modern world. According to the World Health Organization, it affected one person in three and caused a quarter of all deaths in the developed world during the year 2000. After heart disease, it is the largest cause of death. Cancer cells are formed when normal cells lose the normal regulatory mechanisms that control growth and multiplication. They lose the specialized characteristics that distinguish one type of cell from another (for example a liver cell from a blood cell). This is called a loss of differentiation .

The term neoplasm means new growth and is a more accurate terminology for the disease.

Treatment of cancer

➢ Most traditional anticancer drugs work by disrupting the function of DNA and are classed as cytotoxic.

➢ Some act on DNA directly; others (antimetabolites) act indirectly by inhibiting the enzymes involved in DNA synthesis.

➢ Cancer chemotherapy is now entering a new area which can be described as molecular targeted therapeutics —highly selective agents which target specific molecular targets that are abnormal or overexpressed in the cancer cell.

➢ The use of antibodies and gene therapy is another area of research which shows huge potential. Knowledge of the cell cycle is important in chemotherapy. The genetic analysis of tumours in individual patients allows the early detection and identification of cancer, as well as identifying the best treatment to be used for aparticular individual.

Drug classes

1.Alkylating Agents:

The alkylating agents are a class of drugs that are capable of forming covalent bonds with important biomolecules. The major targets of drug action are nucleophilic groups present on DNA (especially the 7-position of guanine); however, proteins and RNA among others may also be alkylated.

Alkylation of DNA is thought to lead to cell death, although the exact mechanism is uncertain. Potential mechanisms of cell death include activation of apoptosis caused by p53 activation and disruption of the template function of DNA.

The cancer cells have dysfunctional p53 so that even though the cell has been unable to replicate DNA error free, cell death via apoptosis does not occur. Cancer cells may become resistant to the effects of alkylating agents. There are several potential nucleophilic sites on DNA, which are susceptible to electrophilic attack by an alkylating agent (N-2, N-3, and N-7 of guanine, N-1, N-3, and N-7 of adenine, 0–6 of thymine, N-3 of cytosine).
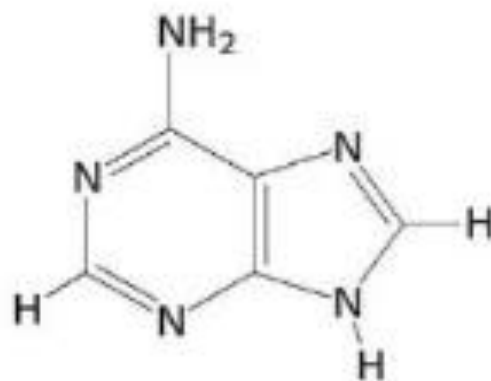
The most important of these for many alkylating agents is the N-7 position of guanine whose nucleophilicity may be enhanced by adjacent guanine residues.
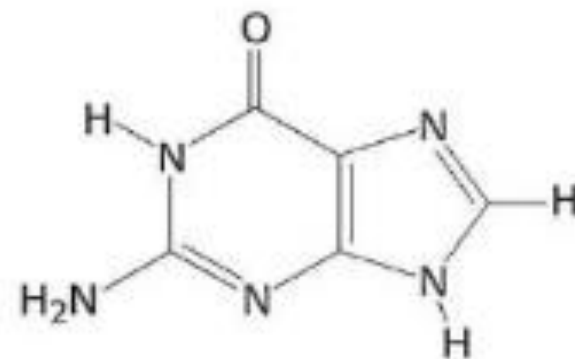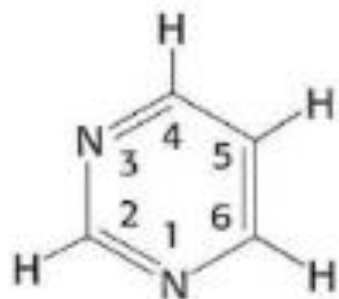
Heterocyclic Nitrogen Bases

PURINES
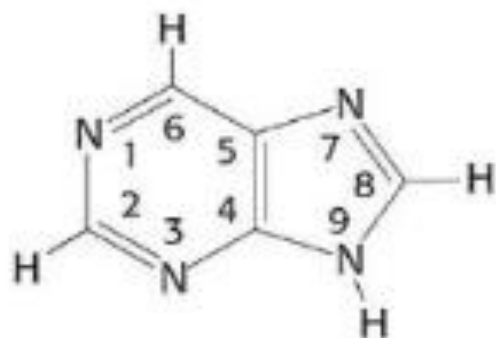
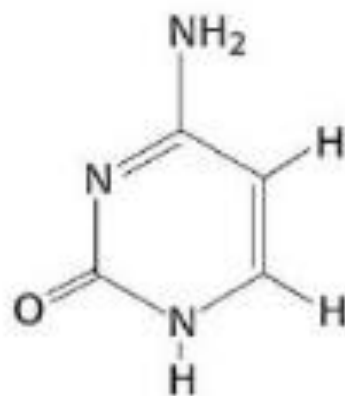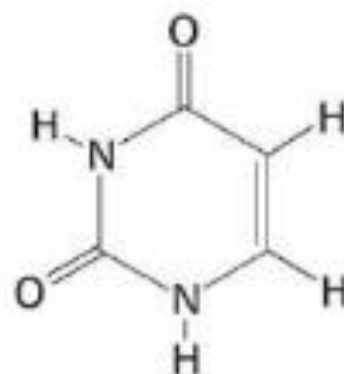**Purine**

Adenine

Guanine

PYRIMIDINES

Pyrimidine

Cytosine

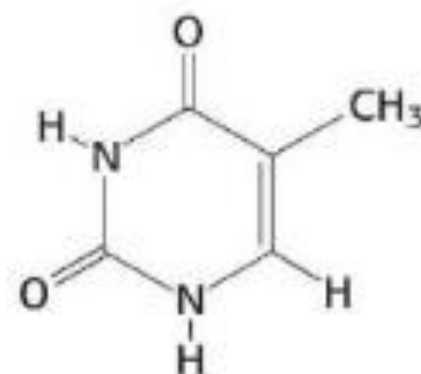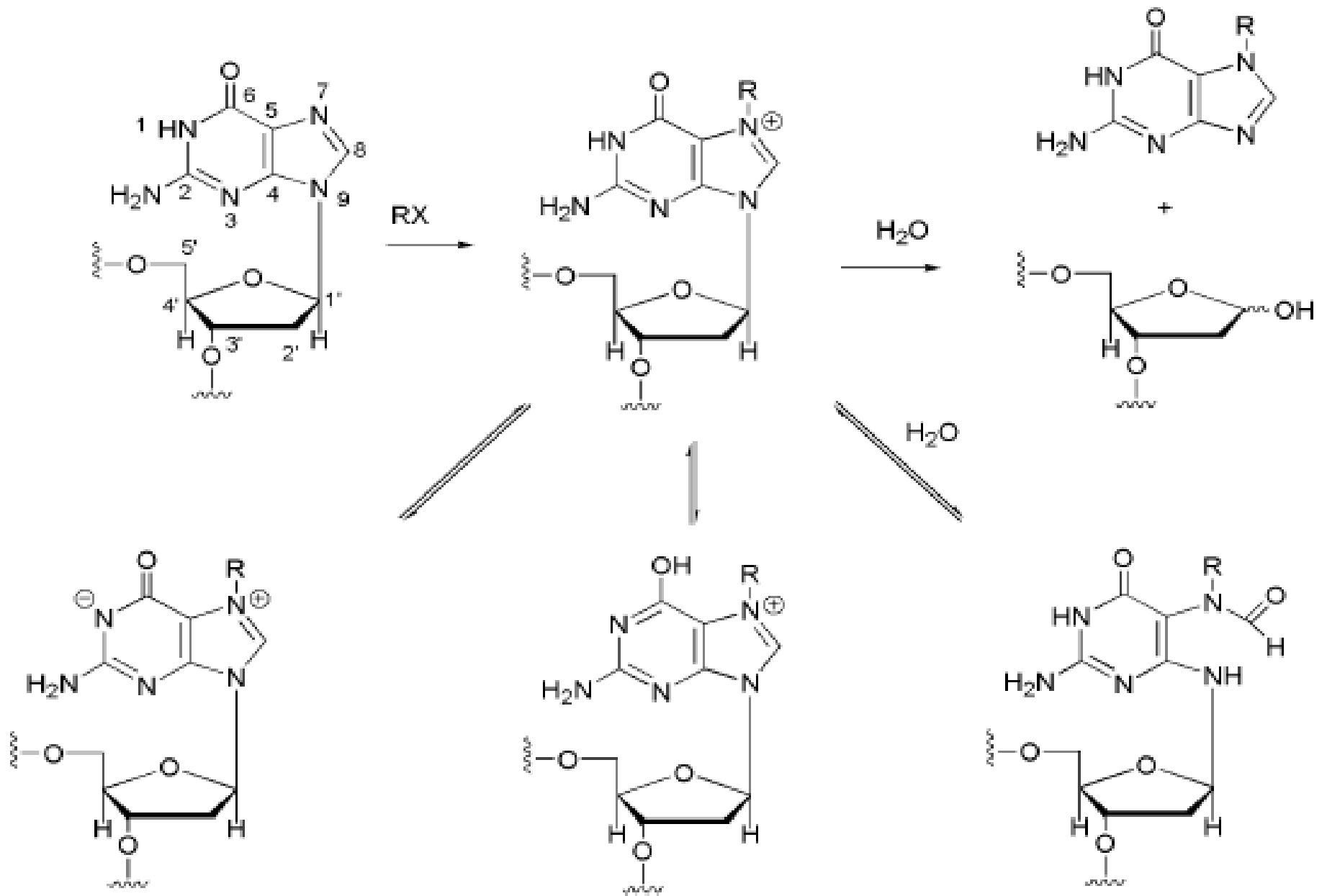**Uracil**

RNA

Thymine

DNA

The general mechanism for alkylation involves nucleophilic attack by –N=, -NH2, -OH, -O-PO3H of DNA and RNA, while additional nucleophiles (-SH, COOH, etc.) present on proteins may also react.

$$DNA\text{-}Nuc\text{-}H \; + \; R\text{-}X \; \xrightarrow{\text{Alkylation}} \; DNA\text{-}Nuc\text{-}R \; + \; H^{\oplus} + X^{\ominus}$$

$$H_2O \; + \; R\text{-}X \; \xrightarrow{\text{Inactivation}} \; H_2O \qquad + \; H^{\oplus} + X^{\ominus}$$

Where X = a leaving group

Scheme 10.1 • Alkylation of guanine N-7 and subsequent depurination of DNA.

Nitrogen mustards

Mustards such as mechlorethamine are classified as dialkylating agents in that one mustard molecule can alkylate two nucleophiles.

The initial acid–base reaction is necessary to release the lone pair of electrons on nitrogen, which subsequently displaces chloride to give the highly reactive aziridinium cation.

Nucleophilic attack can then occur at the aziridinium carbon and neutralize the charge on nitrogen. Mechlorethamine is highly reactive, in fact, and therefore nonselective, making it unsuitable for oral administration and necessitating direct injection into the tumor.

Alkylation of nucleophilic species by nitrogen mustards.

R=CH₃=Mechlorethamine
Hydrochloride

Base

Tissue-Nuc

Repeat
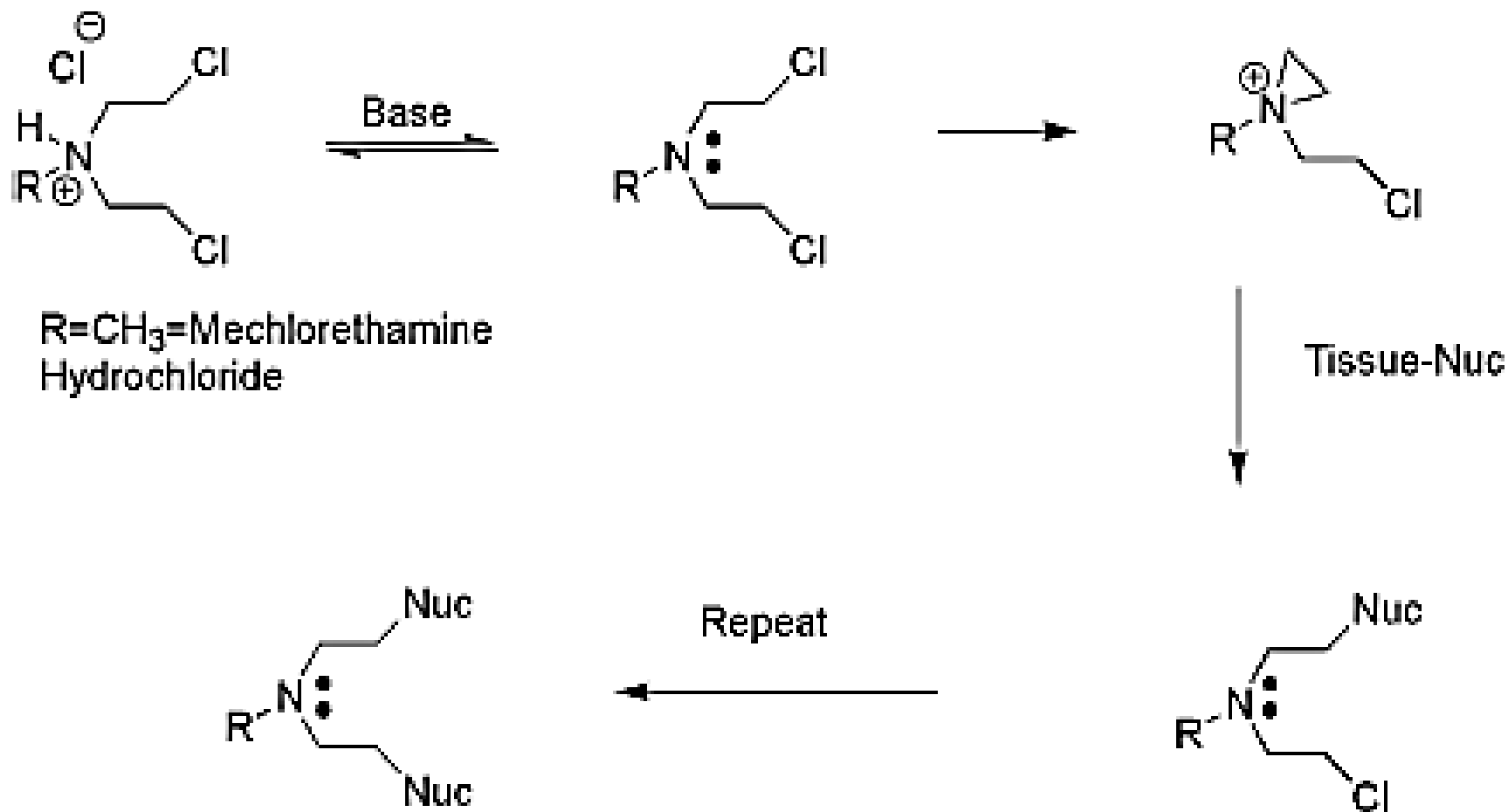
Thiosulfate inactivation of mechlorethamine.

In cases of extravasation (drug escapes from the tumor into the underlying tissue), the antidote sodium thiosulfate (Na2S2O3), a strong nucleophile, may be administered
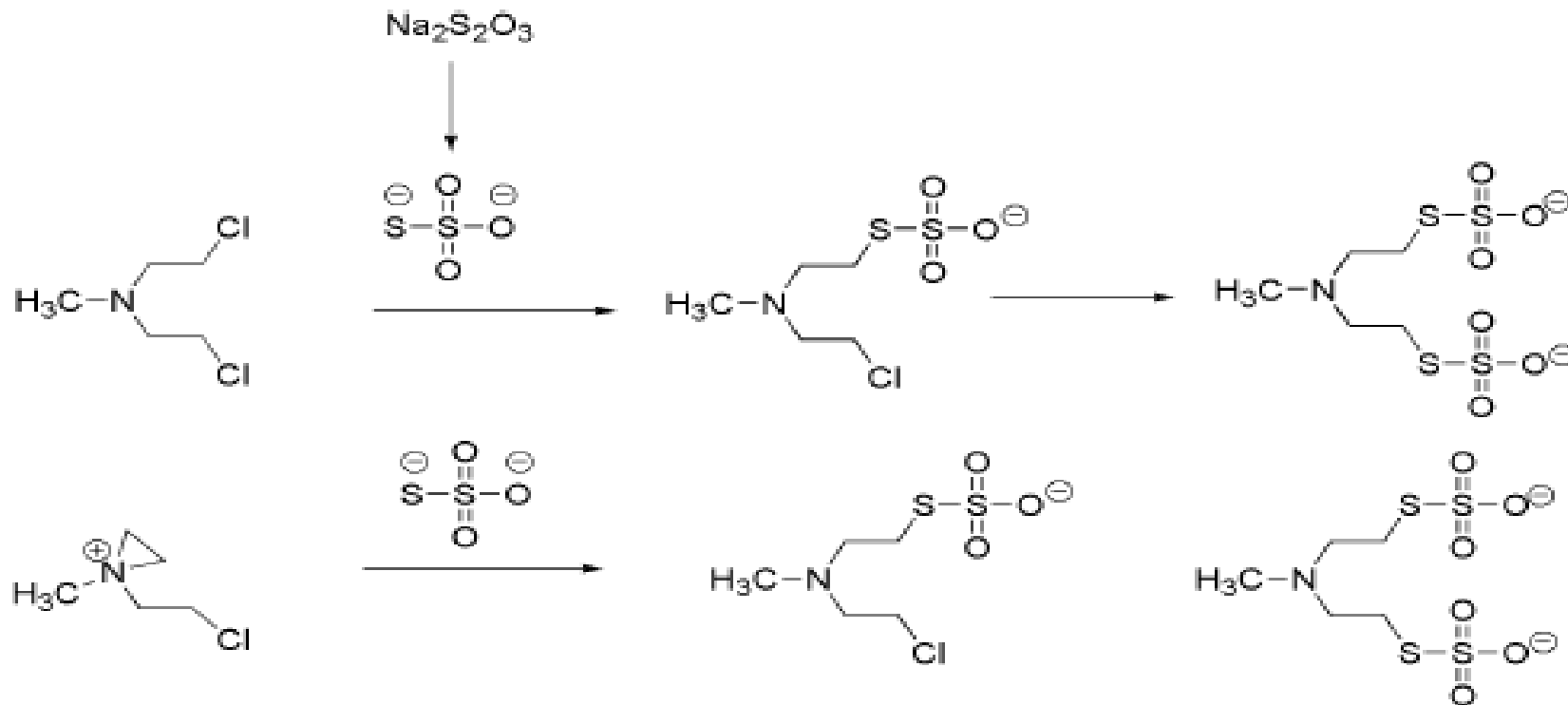
Chlorambucil and Melphalan

The lack of selectivity of mechlorethamine led to attempts to improve on the agent. One rationale was to reduce the reactivity by reducing the nucleophilicity of nitrogen, thereby slowing aziridinium cation formation.

This could be accomplished by replacement of the weakly electron-donating methyl group with groups that were electron withdrawing . This is seen in the case of chlorambucil and melphalan by Attachment of nitrogen to a phenyl ring.
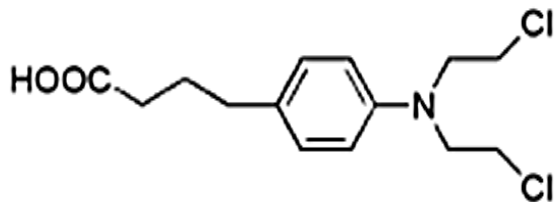
Chlorambucil
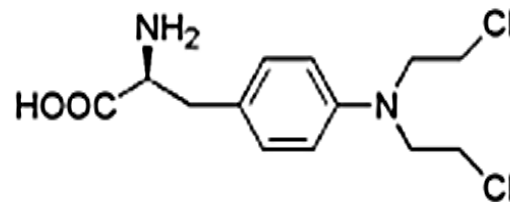
Melphalan

Reactivity was reduced such that these compounds could be administered orally. In the case of melphalan, attachment of the mustard functionality to a phenylalanine moiety was not only an attempt to reduce reactivity but also an attempt to increase entry into cancer cells by utilization of carrier mediated uptake.

Melphalan was found to utilize active transport to gain entry into cells, but selective uptake by cancer cells has not been demonstrated

Cyclophosphamide and Ifosfamide

Attachment of more highly electron-withdrawing functionalities was utilized in the case of cyclophosphamide and ifosfamide. In these cases, aziridinium cation formation is not possible until the electron-withdrawing function has been altered.

The drug could be selectively activated in cancer cells because they were believed to contain high levels of phosphoramidase enzymes.

This would remove the electron-withdrawing phosphoryl function and allow aziridine formation to occur.
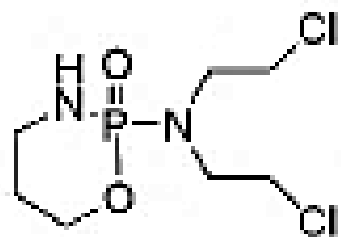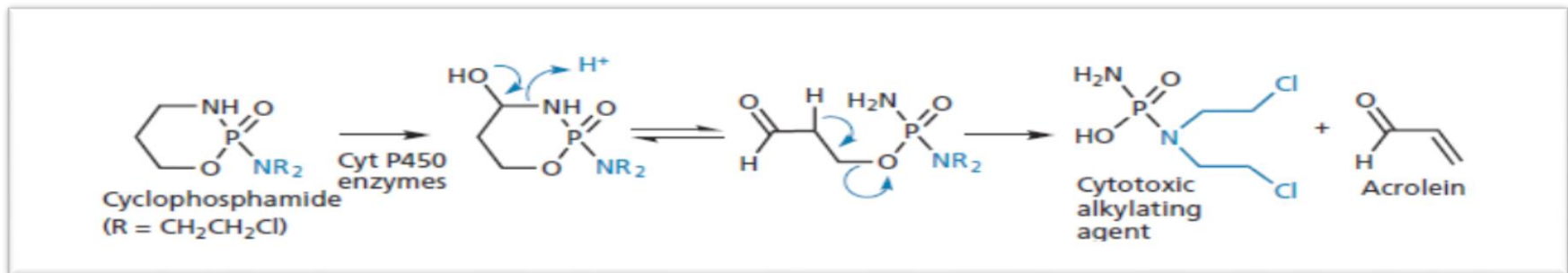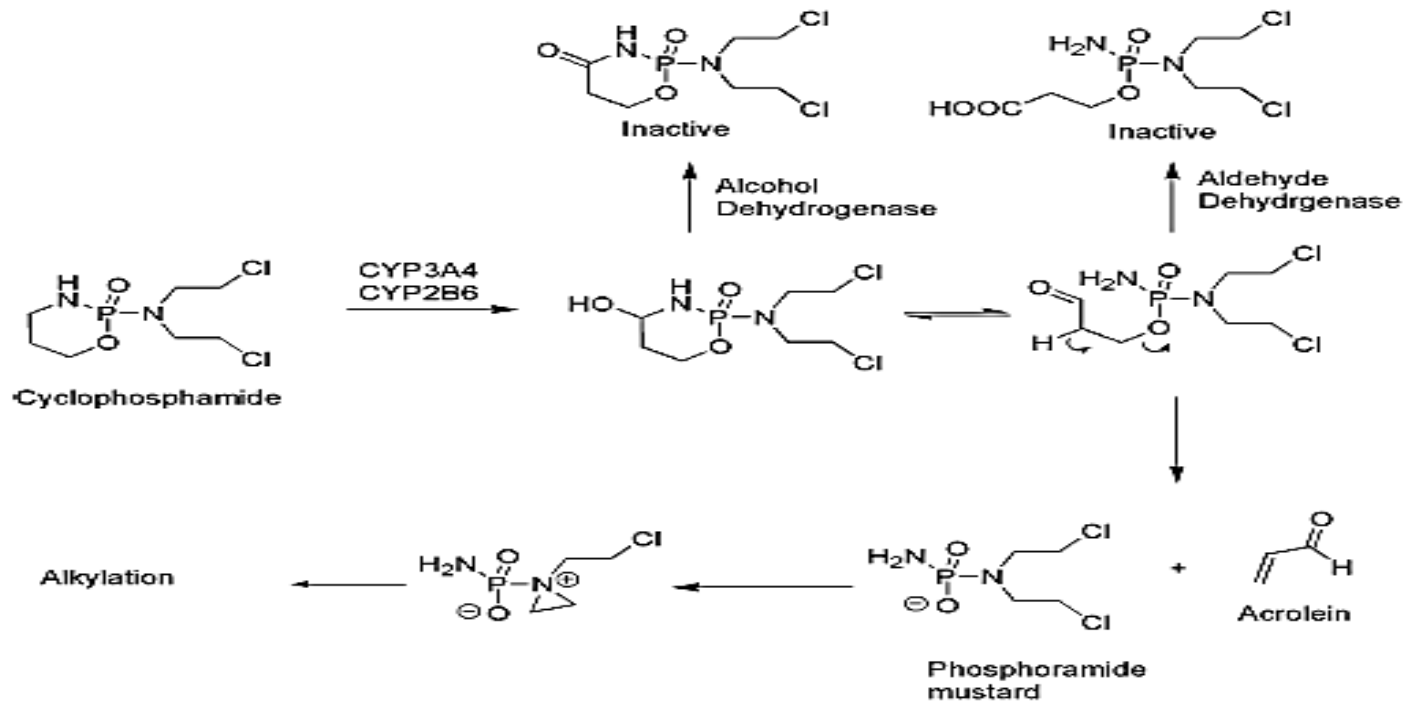

Cyclophosphamide
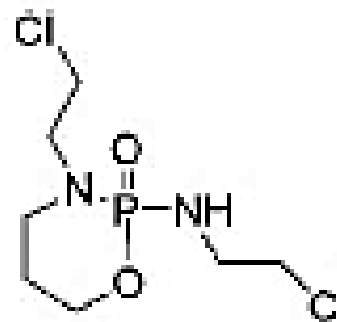
Ifosfamide

The drug was activated by cytochrome P450 (CYP) isozymes CYP2B6 and CYP3A4/5 to give a carbinolamine that could undergo ring opening to give the aldehyde.

The increased acidity of the aldehyde -hydrogen facilitates a retro-Michael decomposition

The ionized phosphoramide is now electron-releasing via induction and allows aziridinium cation formation to proceed.

To decrease the incidence of kidney and bladder toxicity, the sulfhydryl (MSH) containing agent mesna may be administered and functions to react with the electrophilic species that may be present in the kidney.

Metabolic and chemical activation of cyclophosphamide

Detoxification of cyclophosphamide by mesna.
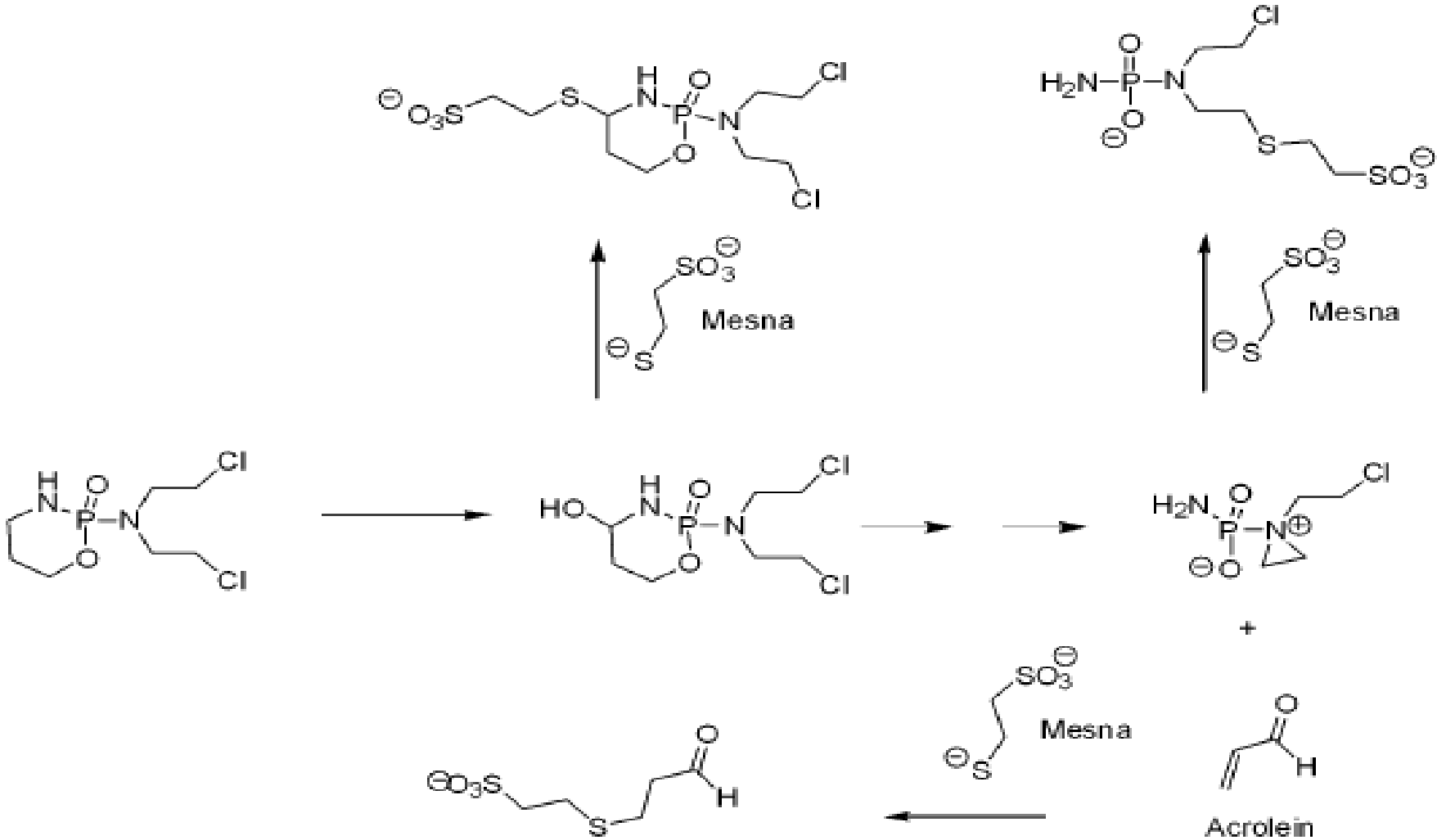
There are differences in the metabolism and activity of the agents.

➢ Both are administered as racemic mixtures as a result of the presence of a chiral phosphorus atom.

➢ There appears to be little difference in the metabolic fate of the R- and S-isomers of cyclophosphamide,

➢ In the case of ifosfamide, the R-isomer is converted to the required 4-hydroxy-ifosfamide 2 to 3 times faster than the S-isomer..

➢ The S-isomer undergoes preferential oxidation of the side chain to give N-dechloroethylation, which removes the ability of the agent to cross-link DNA and also produces the neurotoxic and urotoxic chloroacetaldehyde.

➢ An additional difference between cyclophosphamide and ifosfamide is the larger alkylating species that ultimately results after metabolic activation of ifosfamide. This results in the reactive form of ifosfamide having a higher affinity for DNA than the analogous form of cyclophosphamide and differences in the interstrand and intrastrand links that ultimately result.

Organoplatinum compounds

There are several organometallic compounds based on platinum that play a central role in many cancer treatment protocols. The first of these, cisplatin.

Less reactive platinum compounds such as carboplatin and oxaliplatin in which the leaving group was incorporated into a chelate. satraplatin is currently in clinical trials. One advantage of these agents is the possibility of oral administration.

Satraplatin has shown similar activity when given orally to that of cisplatin given by injection.

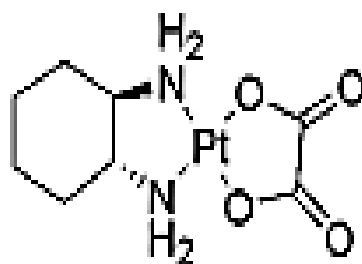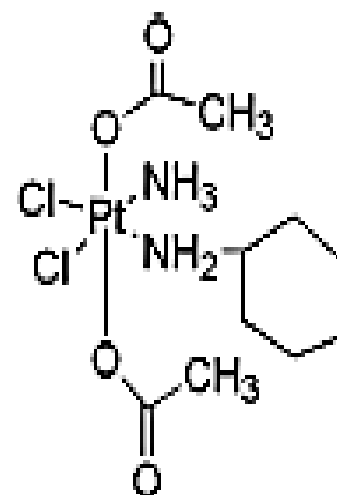# Platinum-containing anti neoplastics
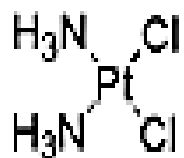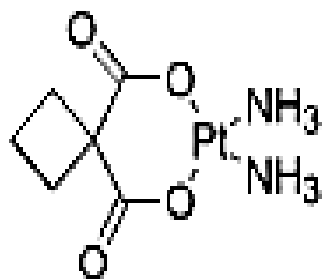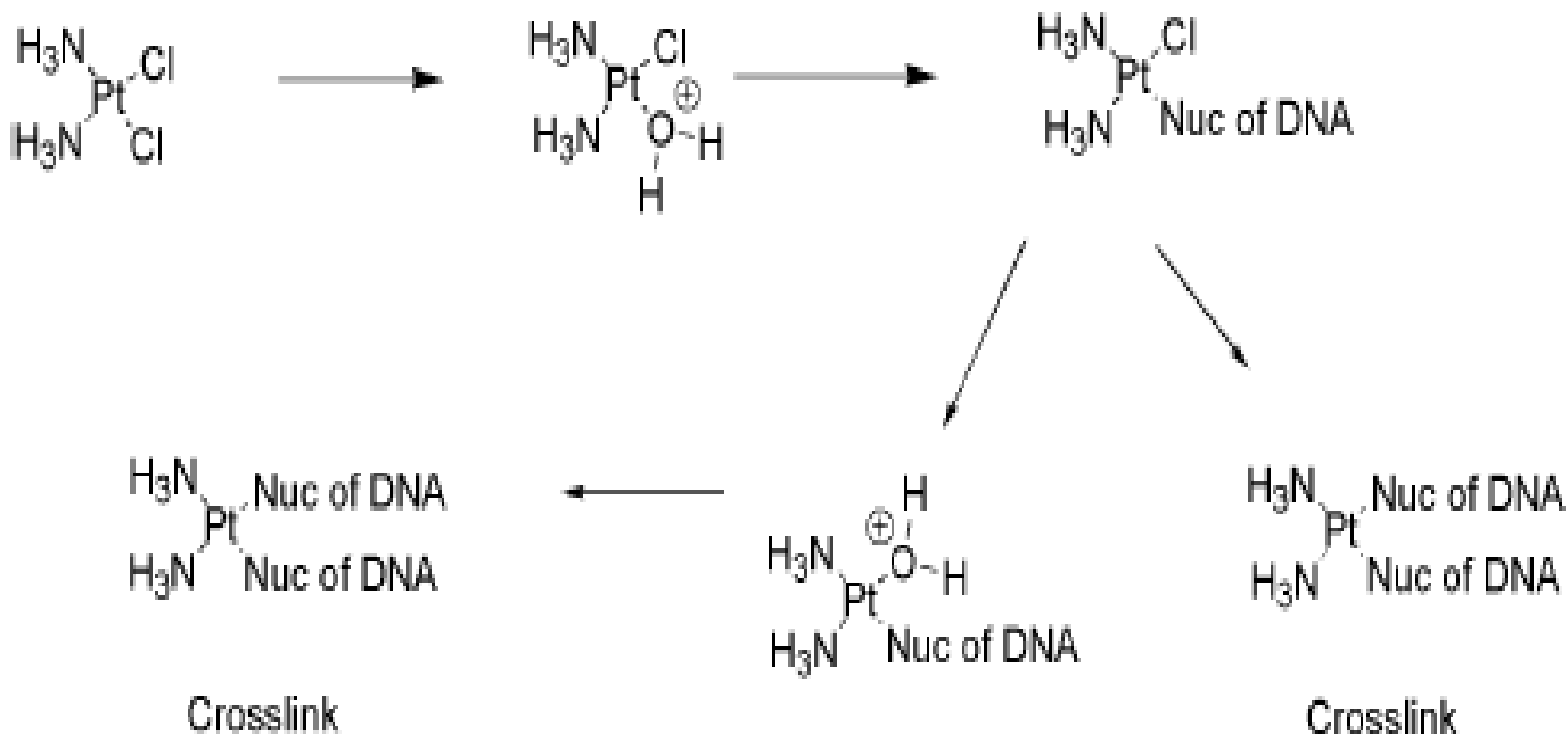

Cisplatin   Carboplatin   Oxaliplatin   Satraplatin

Mechanism of cisplatin activation and formation of DNA adducts.
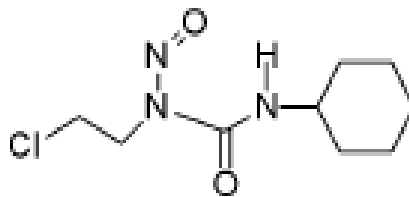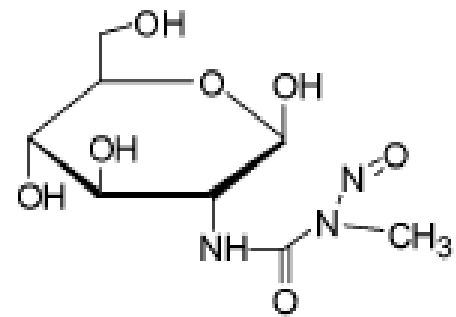
Nitrosoureas

Compound was based on the idea that its chemical decomposition was leading to the formation of diazomethane (CH_2N_2) and subsequent alkylation of DNA, this led to the nitrosoureas, where it was found that activity could be enhanced by attachment of a 2-haloethyl substituent to both nitrogens
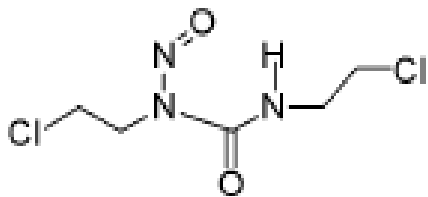
Carmustine (BCNU)   Lomustine (CCNU)   Streptozocin

These compounds are reasonably stable at pH 4.5 but undergo both acid and base catalyzed decomposition at lower and higher pH, respectively.

There are several pathways of decomposition that are possible for these compounds, but the one that appears to be most important for alkylation of DNA involves:

Abstraction of the NH proton, which is relatively acidic (pKa 8–9). Rearrangement to give an isocyanate and a diazohydroxide. The diazohydroxide, upon protonation followed by loss of water, yields a diazo species that decomposes to a reactive carbocation.

The isocyanate functions to carbamylate proteins and RNA, whereas the carbocation is believed to be the agent responsible for DNA alkylation.

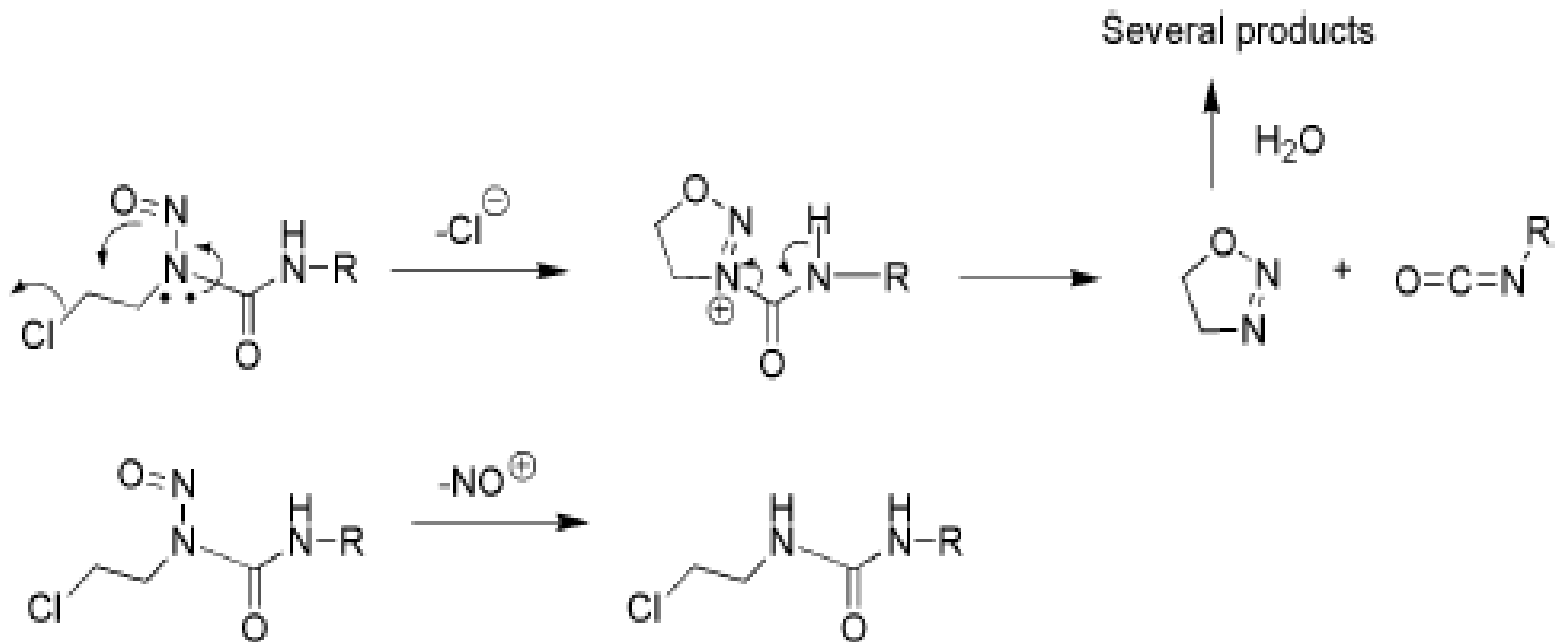# Nitrosoureas: Pathways of decomposition and DNA alkylation
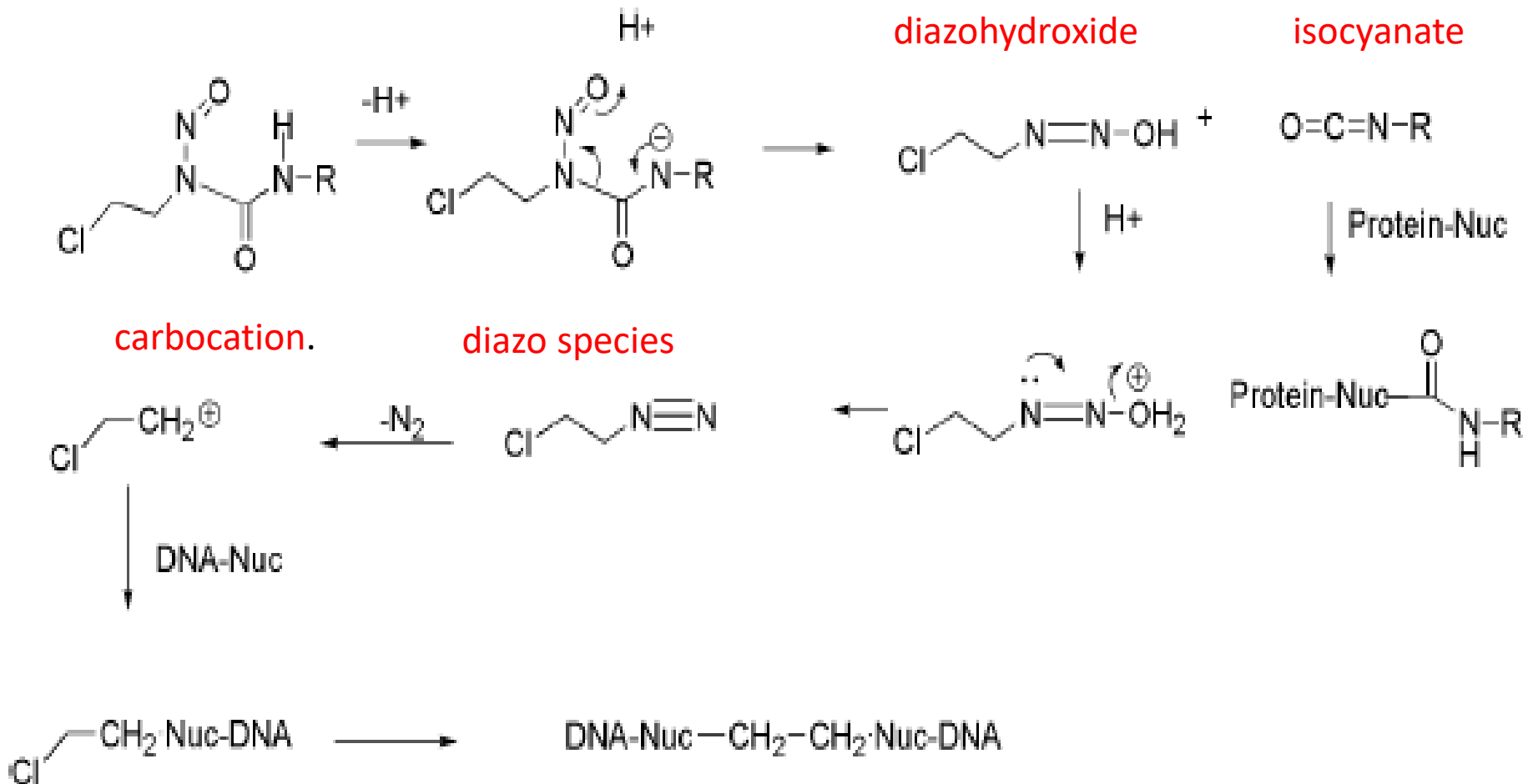
Detoxification pathways of the nitrosoureas

Detoxification pathways of the nitrosoureas are also possible and can play a role in resistance to this group of agents.

The first of these involves dechlorination, which is facilitated by CYP participation.

The second route involves denitrosation

2.Antimetabolites

Most antimetabolites are effective cancer chemotherapeutic agents via interaction with the biosynthesis of nucleic acids.

Therefore, several of the useful drugs used in antimetabolite therapy are purines, pyrimidines, folates, and related compounds.

The antimetabolite drugs may exert their effects by several individual mechanisms involving enzyme inhibition at active, allosteric, or related sites. The purine and pyrimidine antimetabolites are often compounds incorporated into nucleic acids and the nucleic acid polymers (DNA, RNA, etc.).

The antifolates are compounds designed to interact at cofactor sites for enzymes involved in the biosynthesis of nucleic acid bases.

A. Pyrimidine Drugs (uracil derivatives)

The pyrimidine derivative 5-fluorouracil (5-FU) was designed to block the conversion of uridine to thymidine.

The normal biosynthesis of thymidine involves methylation of the 5-position of the pyrimidine ring of uridine.

The replacement of the hydrogen at the 5-position of uracil with a fluorine results in an antimetabolite drug, leading to the formation of a stable covalent ternary complex composed of 5-FU, thymidylate synthase (TS), and cofactor (a tetrahydrofolate species).

The metabolic activation (anabolism) of 5-FU required to produce the anticancer effects accounts for no more than 20% of the administered amount of drug in most patients.

❑ Catabolic inactivation via the normal pathways for uracil consumes the remaining approximate 80% of the dose. The major enzyme of pyrimidine catabolism is dihydropyrimidine dehydrogenase (DPD), and 5-FU is a substrate for this enzyme.

❑ Variability in the levels of DPD activity among the patient population is a major factor in the bioavailability of 5-FU.

❑ low bioavailability of 5-FU as a result of the catabolic efficiency of DPD and other enzymes has lead to the

development of unique dosing routes and schedules as well as the development of prodrug forms of 5-FU

➢ TS is responsible for the reductive methylation of deoxyuridine monophosphate (dUMP) by 5,10-methylenetetrahydrofolate to yield dTMP and dihydrofolate. Because thymine is unique to DNA, the TS enzyme system plays an important role in replication and cell division.

➢ The tetrahydrofolate cofactor species serves as both the one-carbon donor and the hydride source in this system.

- ❑ Attempts at chemical modification of 5-FU to protect from catabolic events have produced several prodrug forms, which are converted via in vivo metabolic and/or chemical transformation to the parent drug 5-FU.

- The carbamate derivative of 5-deoxy-5-fluorocytidine is known as capecitabine, and it is converted to 5-FU through a series of activation steps.

- The tetrahydrofuran derivative tegafur is slowly converted to 5-FU but requires quite high doses to reach therapeutic plasma concentrations.

- Esters of the N-hydroxymethyl derivative of tegafur show greater anticancer activity than tegafur.
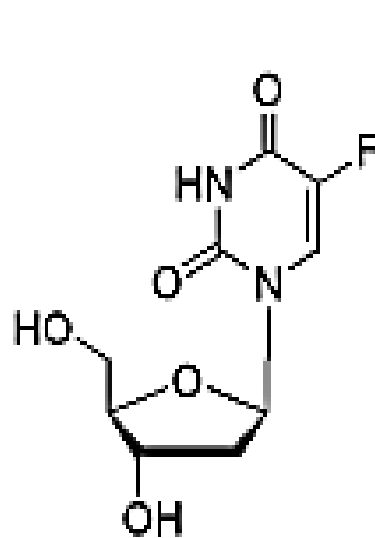
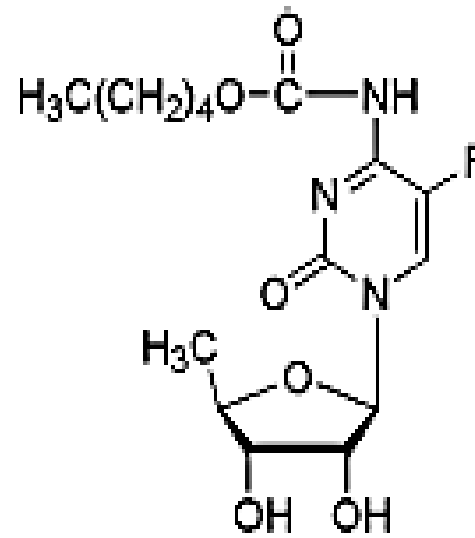# Metabolic activation of capecitabine to 5-FU.

Capecitabine

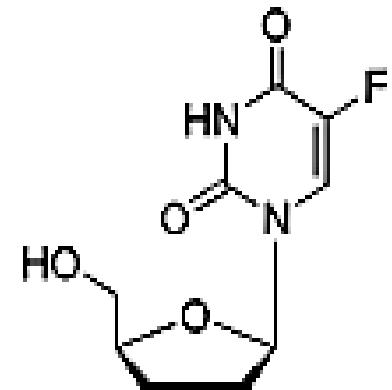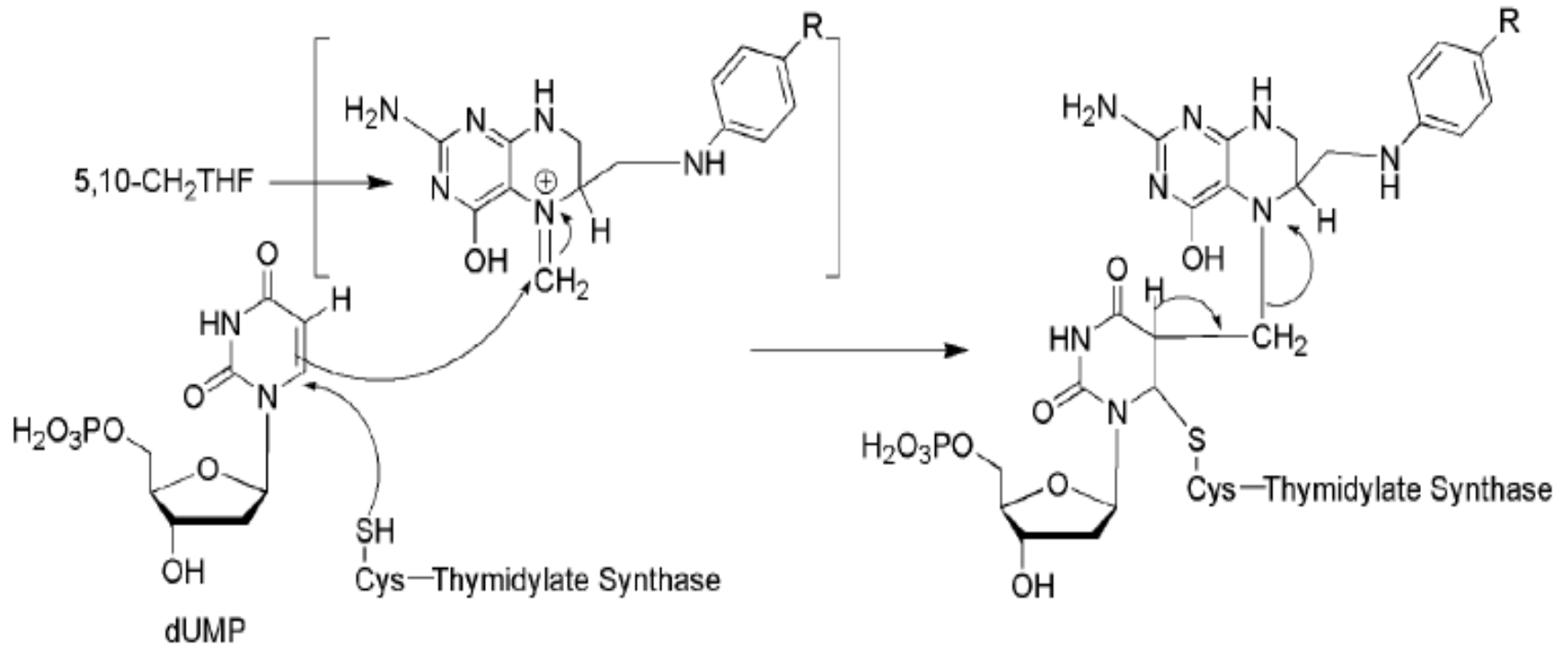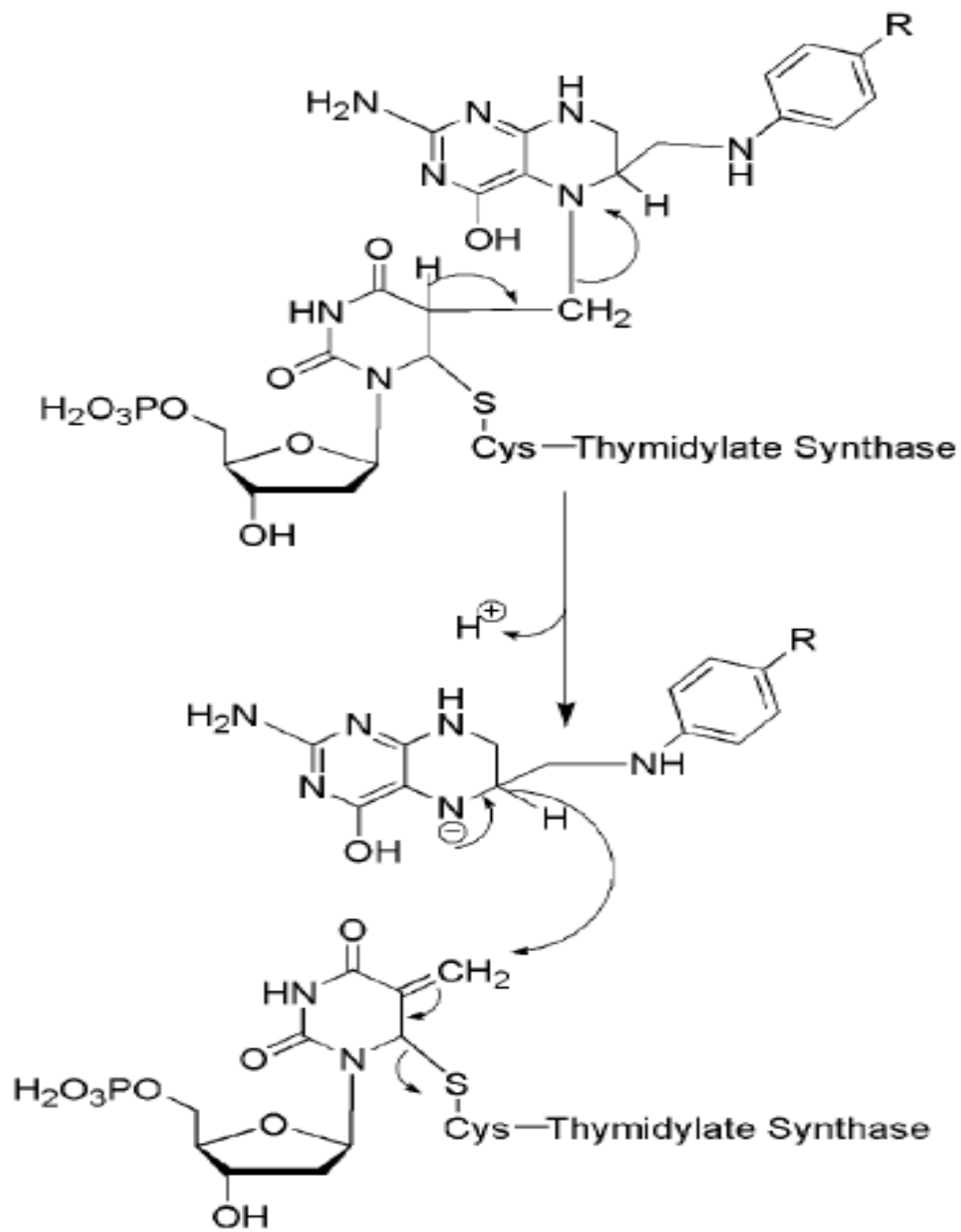5-FU
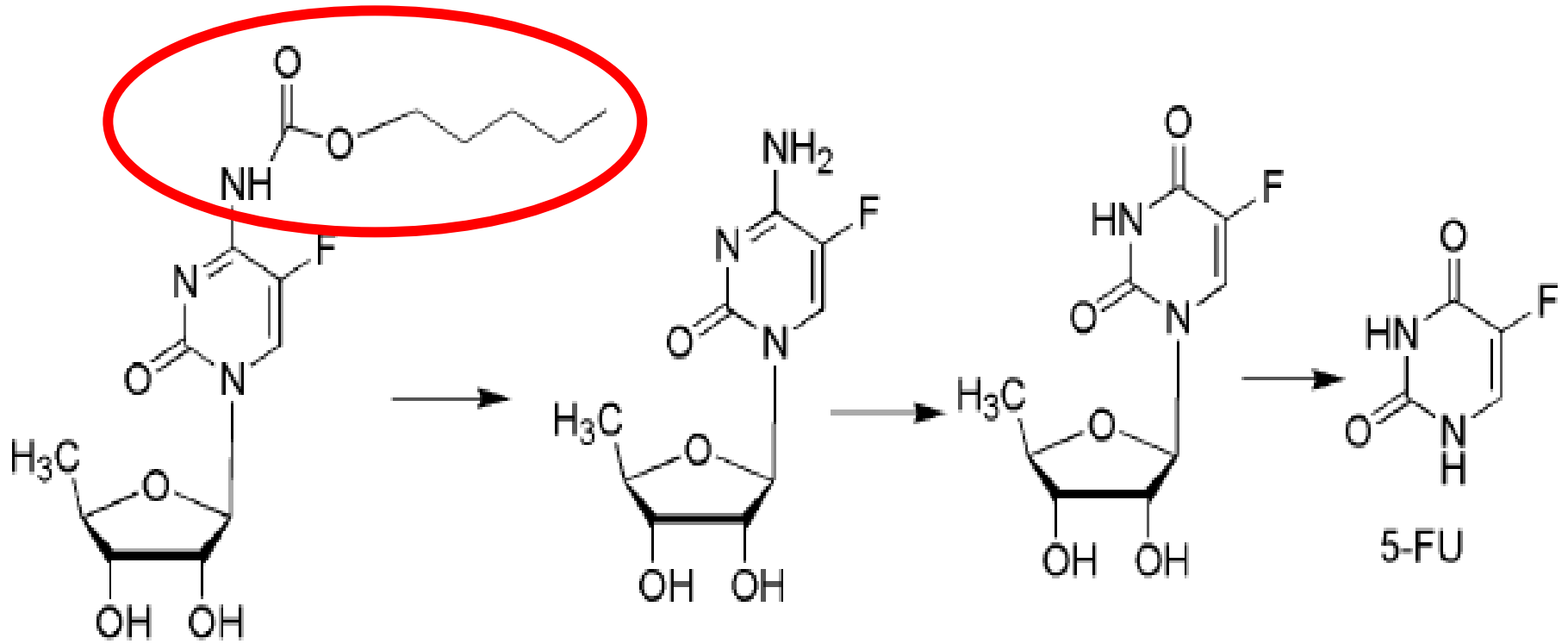
Pyrimidine Drugs
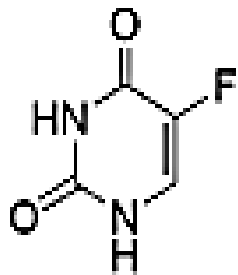
5-Fluorouracil

Floxuridine

Capecitabine

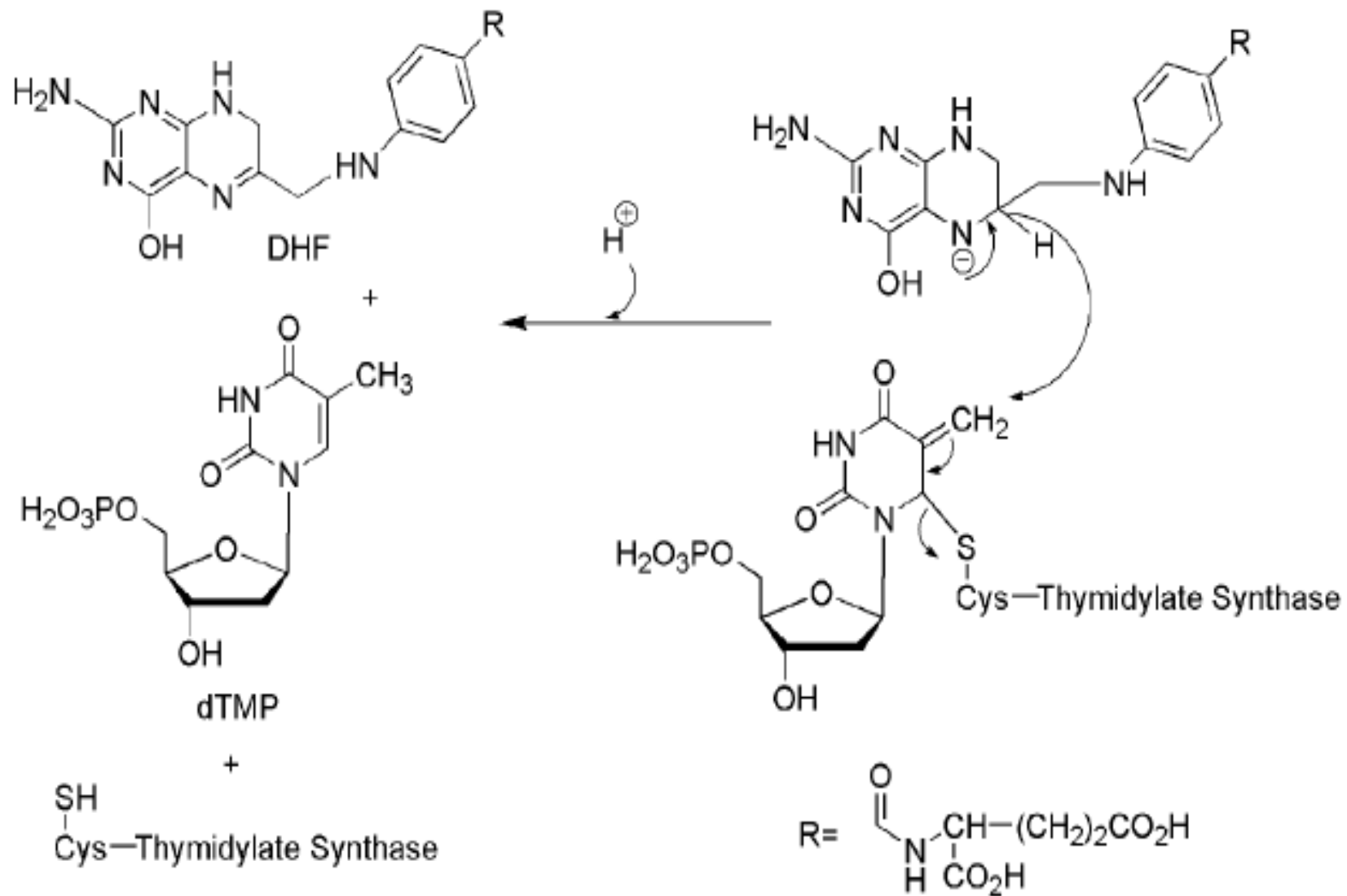Tegafur

Biochemical conversion of uridine to thymidine.

DHF

+

dTMP

+

SH
|
Cys—Thymidylate Synthase

$H^{\oplus}$

H_2O_3PO

Cys—Thymidylate Synthase

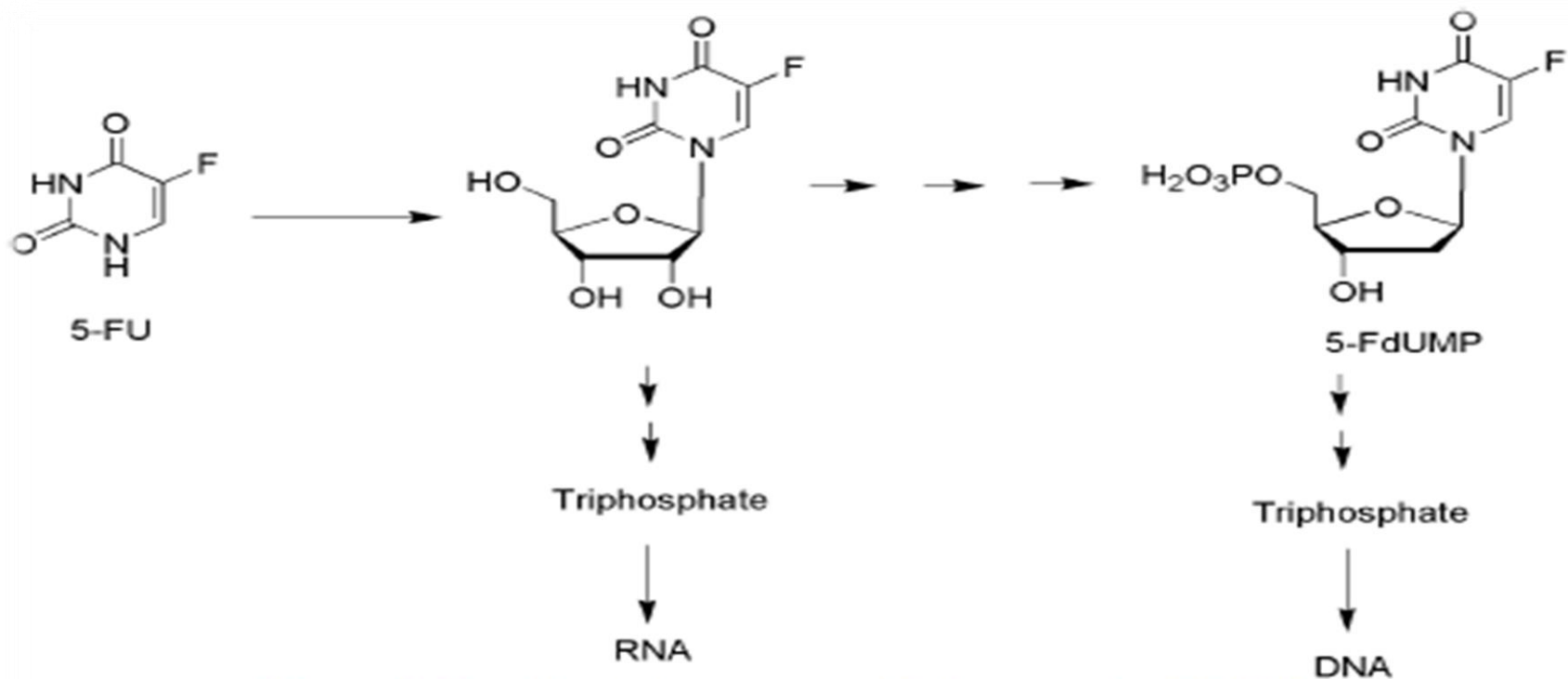$R= $ formyl-NH-CH-$(CH_2)_2CO_2H$ with CO_2H

5-Fluorouracil is activated by conversion to the corresponding nucleotide species, 5-fluoro-2-deoxyuridylic acid.

The resulting 5-fluoro-2-deoxyuridylic acid is a powerful inhibitor of thymidylate synthetase , the enzyme that converts 2-deoxyuridylic acid to thymidylic acid.

Scheme 10.18 • Metabolic activation of 5-fluorouracil to 5-FdUMP.

In the normal process, this step is followed by the elimination of dihydrofolate from the ternary complex, regeneration of the active enzyme species, and the product thymidine.

Cytarabine and gemcitabine(cytosine derivatives)

Pyrimidine analogs as antimetabolites for cancer therapy have been developed based on the cytosine structure as well.

Modification of the normal ribose or deoxyribose moiety has produced useful drug species such as cytarabine (ara-C) and gemcitabine. Cytosine arabinoside (ara-C or cytarabine) is simply the arabinose sugar instead of ribose, and the only difference in structure is the epimeric hydroxyl group at the 2-position of the pentose sugar.

Mechanism of action may include a slowing of the DNA chain elongation reaction via DNA polymerase or cellular inefficiencies in DNA processing or repair after incorporation.

Gemcitabine is the result of fluorination of the 2`- position of the sugar moiety. After its anabolism to diphosphate and triphosphate metabolites, it inhibits ribonucleotide reductase and competes with 2-deoxycytidine triphosphate for incorporation into DNA.

The mechanism of action for gemcitabine is likely similar to that of ara-C including alteration of the rate of incorporation into DNA as well as the rate of DNA processing and repair.

Modification of the pyrimidine ring has also been explored for the development of potential anticancer drugs based on antimetabolite theory.

Several pyrimidine nucleoside analogs have one more or one less nitrogen in the heterocyclic ring. They are known as azapyrimidine or deazapyrimidine nucleosides. 5-Azacytidine is an example of a drug in this category

Anticancer drugs based on pyrimidine and related compounds

Gemcitabine   Cytarabine   5-Azacytidine   Decitabine
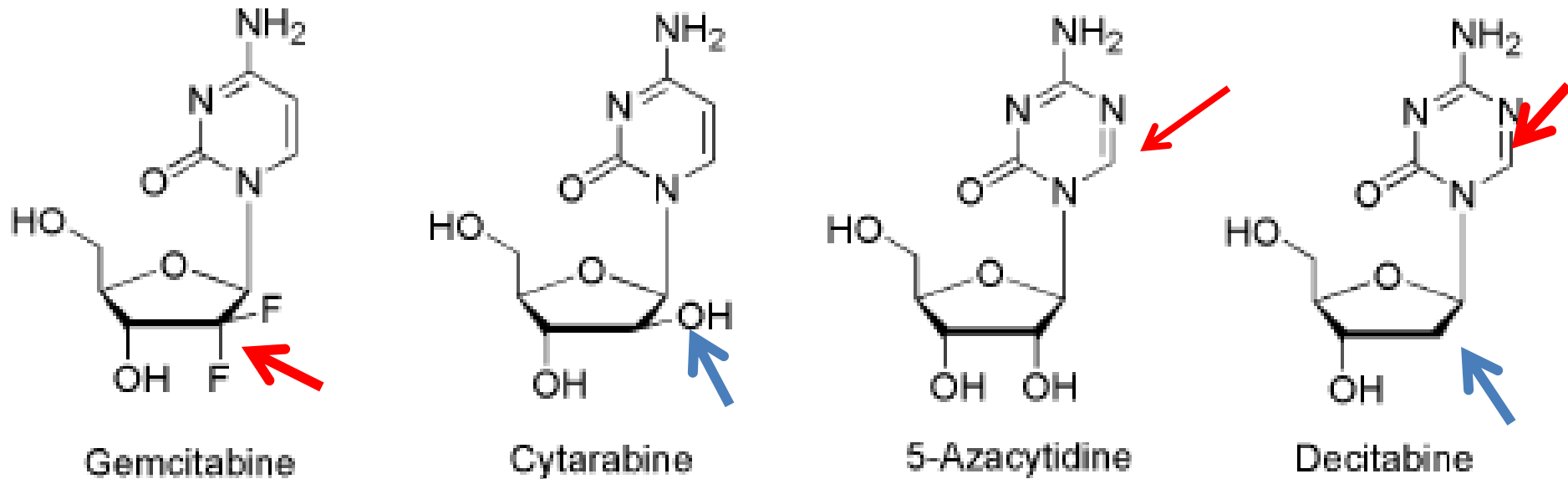
The mode of action of this compound is complex involving reversible inhibition of DNA methyl transferase, and this lack of methylated DNA activates tumor suppressor genes. In certain tumor systems, it is incorporated into nucleic acids, which may result in misreading or processing errors.