Cancer

- The American Cancer Society defines cancer as a group of diseases characterized by **uncontrolled growth**, and the spread of abnormal cells that left untreated may lead to death.
- Related to this definition is the term ***neoplasia***, which is the uncontrolled growth of **new** tissue, the product of which is known as a *tumor*, and these tumors may be either malignant or benign..

Introduction

- Malignant tumors have the capability of invading surrounding tissues and moving to distant locations in the body in a process known as ***metastasis***; characteristics that benign tumors do not possess.
- Treatment of malignant tumors or cancer has generally involved initially surgical removal followed by radiation and/or chemotherapy, if necessary. In those cases where complete surgical removal is not feasible, radiation and chemotherapy become the only available options.
- The term ***chemotherapy***, in the strictest sense, refers to drugs that are used to kill cells and includes both antibiotics and agents used in the treatment of cancer, but it is often used to refer exclusively to anticancer agents also known as ***antineoplastics***.
- Traditional chemotherapy has been based on the principle of **selective toxicity**; however, this has been difficult to achieve in the case of cancer cells because these cells utilize the biochemical pathways used by normal cells.
- In many cases, the agents have attempted to exploit the increased proliferative rates of cancer cells compared with normal cells. This has been difficult to achieve even in a relative sense because, in part, of the fact that not all normal tissue is slowly proliferative and, conversely, not all cancer cells are highly proliferative.

Cancer molecular biology

- Increased knowledge of intercellular and intracellular communication has led to the development of several newer agents that have shown some effectiveness in treating several cancers, especially when used in combination with more traditional agents.
- These have included several monoclonal antibodies that target the overproduction of growth factor receptors and Tyrosine Kinase inhibitors that target the transduction process involved in growth factor stimulation
- You have to refer to the youtube lectures in your moodle.

Cancer Types

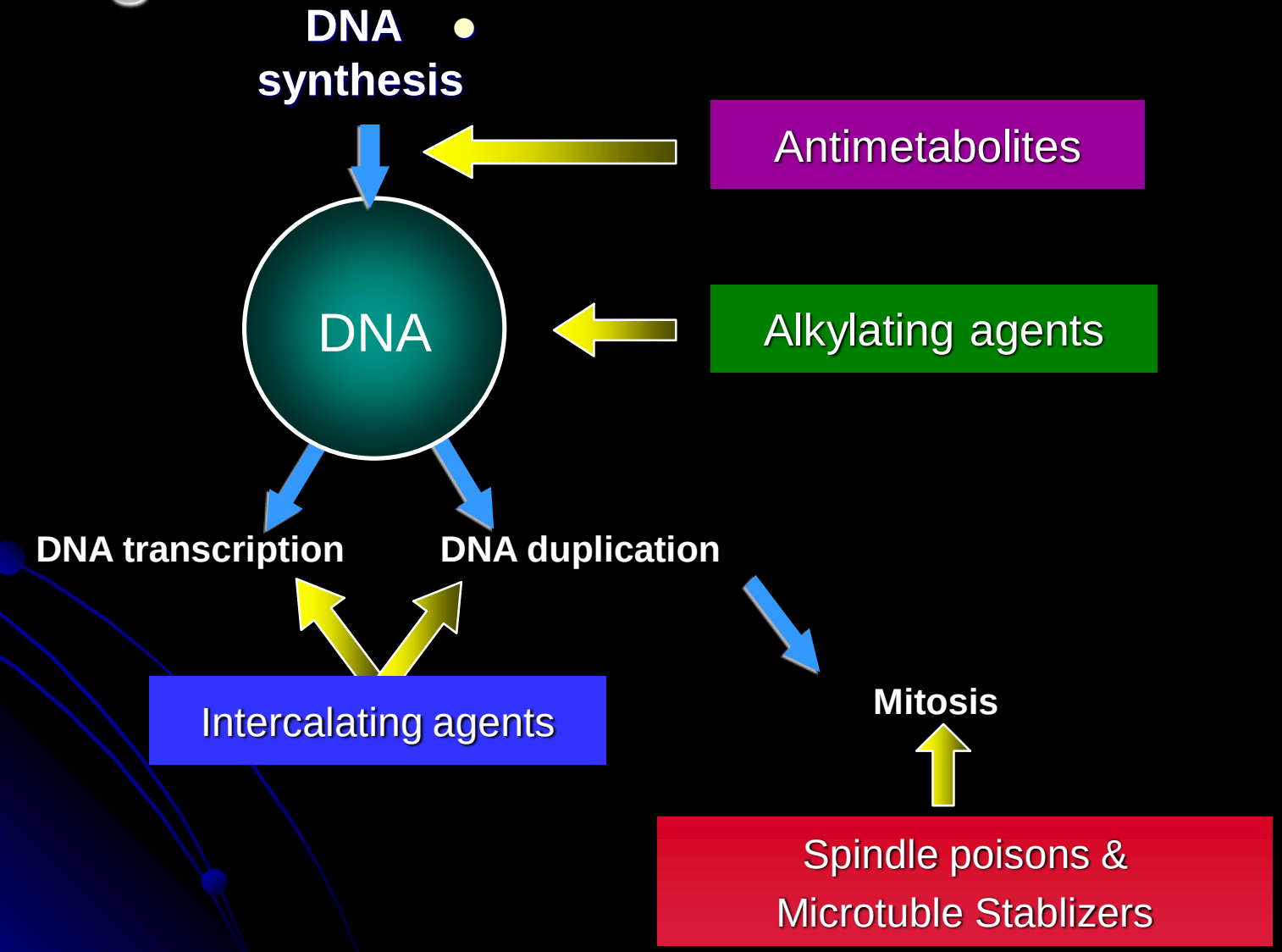
categorized based on the functions/locations of the cells from which they originate:

- **Carcinoma: a tumor derived from epithelial cells, those cells that line the surface of our skin and organs (80-90% of all cancer cases reported)**
- **Sarcoma: a tumor derived from muscle, bone, cartilage, fat or connective tissues.**
- **Leukemia: a cancer derived from white blood cells or their precursors.**
- **Lymphoma: a cancer of bone marrow derived cells that affects the lymphatic system.**
- **Myelomas: a cancer involving the white blood cells responsible for the production of antibodies (B lymphocytes).**

Antineoplastic Agents

- **Alkylating agents** — Carboplatin, cyclophosmamide, melphalan, thiotepa
  (Form bonds with nucelic acids and proteins)
- **Antimetabolites** — Methotrexate, fluorouracil, gemcitabine
  (similar to metabolites involved in nucelic acid synthesis)
- **Natural Products** — doxorubicin, docetaxel, vinolbine, topotecan
  (anti tumour antibiotics,mictotubule stabilizer, mitotic inhibitor, topoisomerase inhibiotor)
- **Endocrine agents** — Anastrozole, tamoxifen, prednisolone, goserelin
  (Aromatase inhibitors, oestrogen antagonist, corticosteroids, LHRH agonist)
- **Molecularly targeted agents** — Retinoids, trastuzumab, gefitinib
  (gene expression, monoclonal antibody, tyrosine kinase inhibitor)
- **Biologic response modifiers** — Interferon, thalidomide, filgrastim

Sites of Action of Cytotoxic Agents – Cellular Level



Antineoplastic Agents

Alkylating agents	Topoisomerase inhibitors	Antimetabolites	Molecularly targeted
busulfan	dactinomycin	cytarabine	erlotinib
carboplatin	daunomycin	clofarabine	imatinib
carmustine	doxorubicin	fludarabine	sorafenib
cisplatin	etoposide	gemcitabine	sunitinib
cyclophosphamide	etoposide phosphate	mercaptopurine	tretinoin
dacarbazine	idarubicin	methotrexate	Herceptin
ifosfamide	irinotecan	nelarabine	**Miscellaneous**
lomustine	liposomal daunomycin	thioguanine	arsenic trioxide
mechlorethamine	liposomal doxorubicin	**Tubulin binders**	asparaginase
melphalan	mitoxantrone	docetaxel	bleomycin
oxaliplatin	teniposide	ixabepilone	dexamethasone
procarbazine	topotecan	vinblastine	hydroxyurea
temozolomide		vincristine	mitotane
thiotepa		vinorelbine	PEG-asparaginase
		paclitaxel	prednisone

Problems with chemotherapy

- **Treatments are non-specific, attack healthy cells as well as normal cells since cancer cells are derived from normal cells.**
- **Cancers can develop resistance: for example with platinum-drugs, cancer cells became resistant by many ways:**
  - **Decreased drug uptake/increased efflux**
  - **Enhanced tolerance of DNA adducts**
  - **Enhanced repair of DNA adducts**
  - **Increased drug deactivation by intracellular glutathione**

Ideal cytotoxic drugs should:

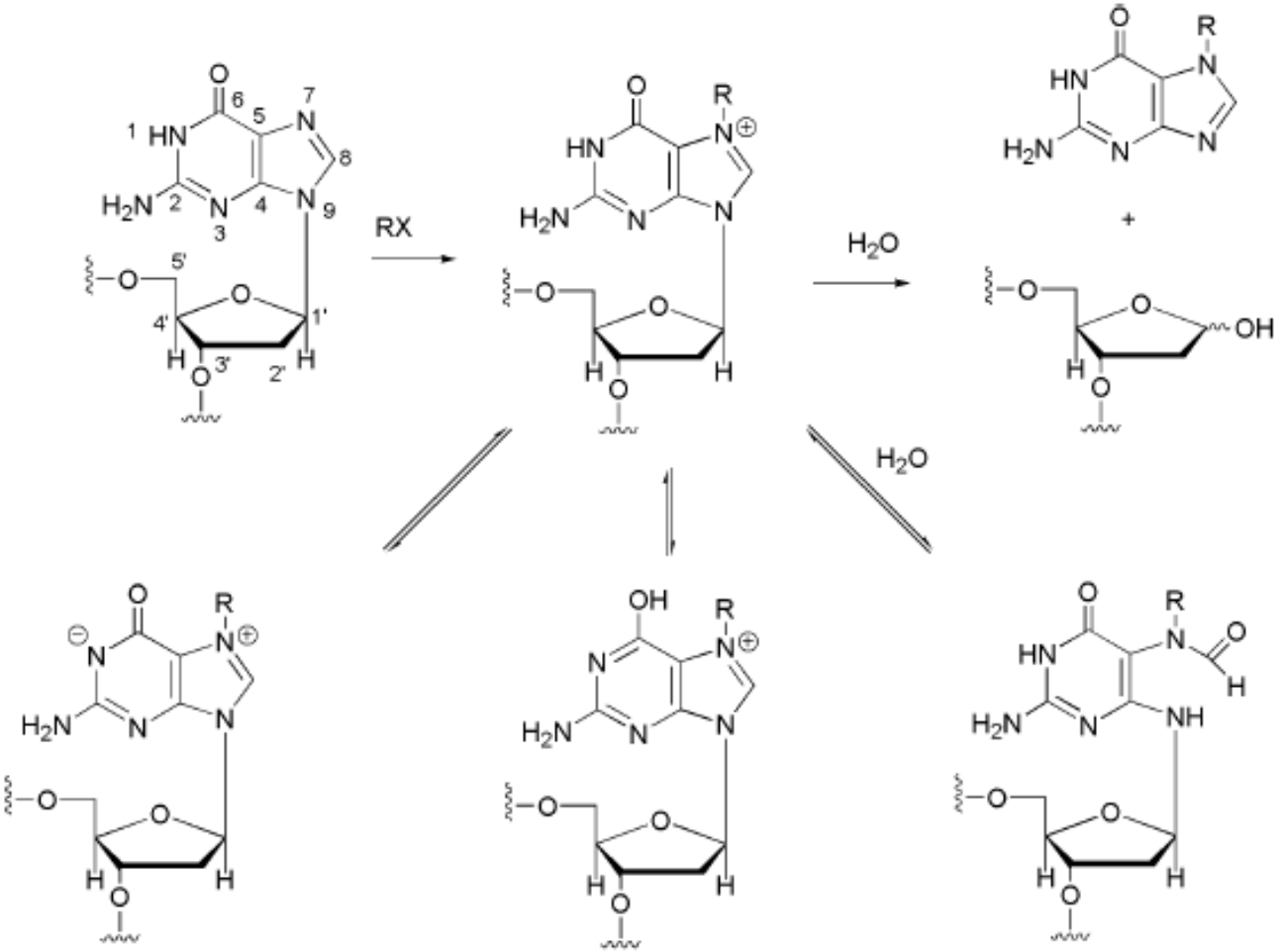
- **Selectively target cancer cells without causing damage to normal cells.**
- **Reduce size of tumors + minimize risks of metastases.**

❖ **unfortunately, most of the available agents are not selective, they also affect rapidly-proliferating normal tissues (bone marrow, gastro intestinal epithelium, hair cells, …), causing serious side-effects (bone marrow suppression, nausea, vomiting, …).**

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)
- 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.

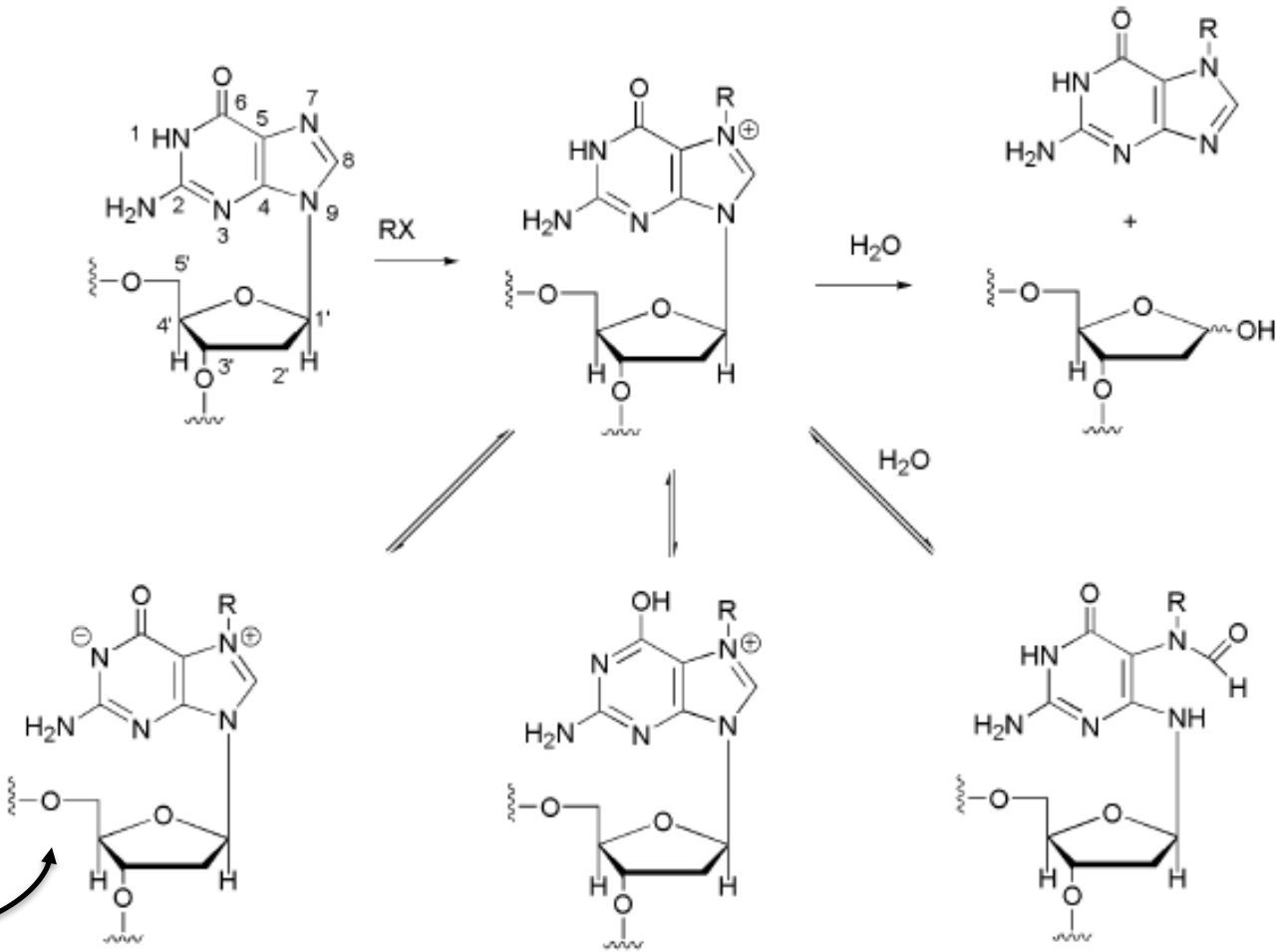
Alkylating Agents



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

Alkylation converts the base to an **effective leaving group** so that attack by water leads to depurination and the loss of genetic information if the resulting depurination is not repaired by the cell

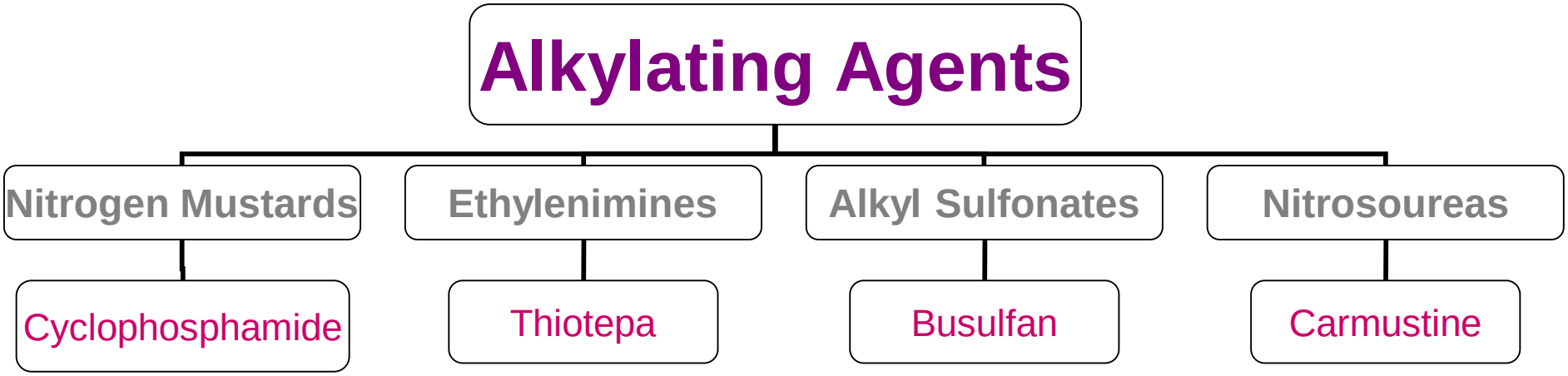
Alkylating Agents



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

Additionally, alkylation has been proposed to result in **altered base pairing** away from the normal G-C: A-T hydrogen bonds because of alterations in tautomerization.
The alkylation also leads to increased acidity of the N-1 nitrogen reducing the pKa from 9 to 7 to 8 giving rise to a zwitterionic form that may also mispair

Classification of Alkylating Agents

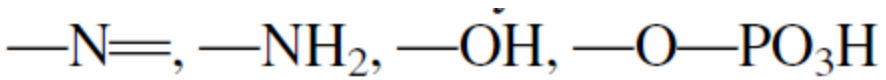


- BisChloroethylAmines Cyclophosphamide,ifosfamide, Chlormethine, Chlorambucil, Sarcolysine
- Nithrosoureas
- Carmustine,Lomustine
- Ethyeneammonium or Aziridines
- Thiotepa, triethylene melamine
- Alkysulfonates:Busulfan

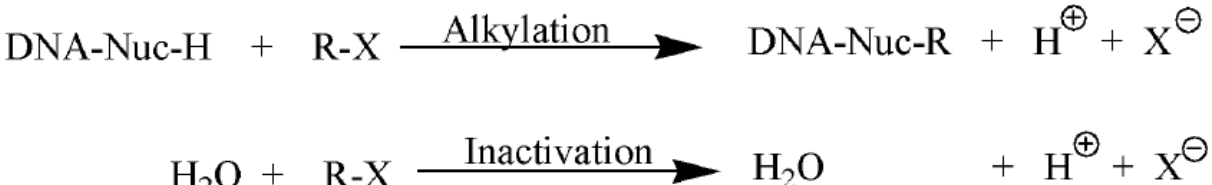
Resistance to alkylating agents

- In many cases, however, 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.
- In this way, cancer cells may become resistant to the effect of alkylating agents.
- Another possibility is that the cancer cells, like normal cells, have various mechanisms by which alkylated DNA bases can be excised.

- The general mechanism for alkylation involves nucleophilic attack by



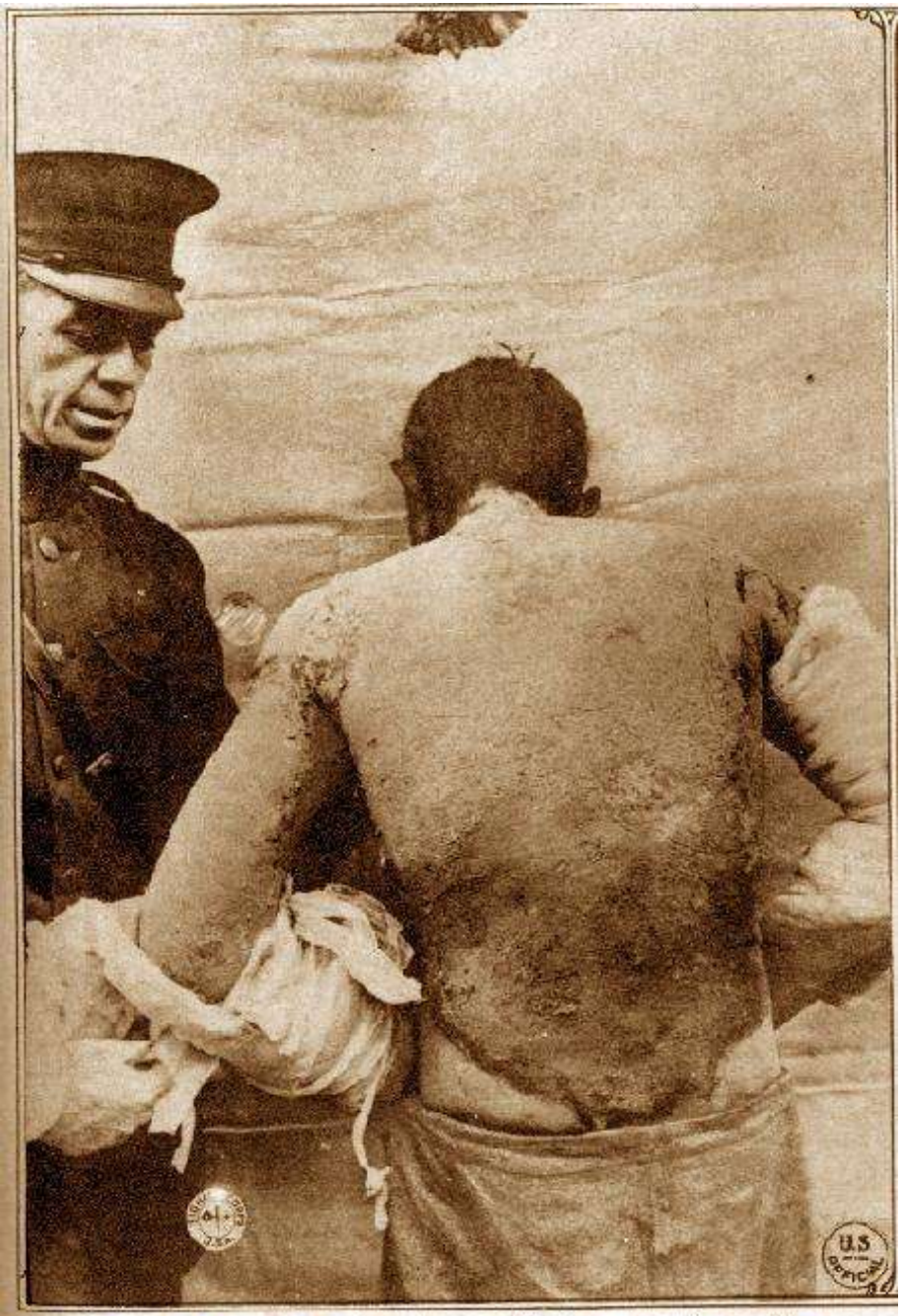
of DNA and RNA



Scheme 10.2 ● General reaction for alkylation and inactivation of alkylating agents.

NITROGEN MUSTARDS

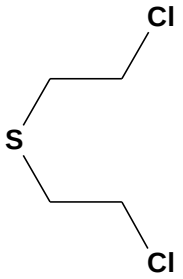
- The nitrogen mustards are compounds that are chemically similar to sulfur mustard or mustard gas developed and used in World War I. The term “mustard” comes from the similarity in the blisters produced by the compound and those seen upon exposure to the oil of black mustard seeds.
- Investigation of sulfur mustard revealed that it possessed antineoplastic properties but because the compound existed as a gas at room temperature, handling and administration of the material were difficult.
- Conversion of the sulfide to a tertiary amine allowed for the formation of salts, which exist as solids at room temperature allowing for easier handling and dosing. The term mustard was then extended to the nitrogen analogs (nitrogen mustards) given their chemical similarity.
- 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 (see figure next page).
- Nucleophilic attack can then occur at the aziridinium carbon to relieve the small ring strain and neutralize the charge on nitrogen.
- This process can then be repeated provided a second leaving group is present



Lieut. Col. William Mook, an army skin expert, treating a soldier burned by mustard gas.

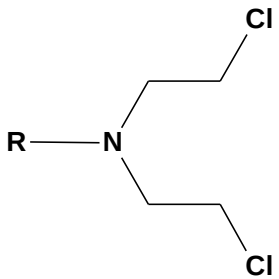
Chemical Warfare – *circa* 1914

- Two most common agents:
  - Chlorine gas
  - Mustard gas



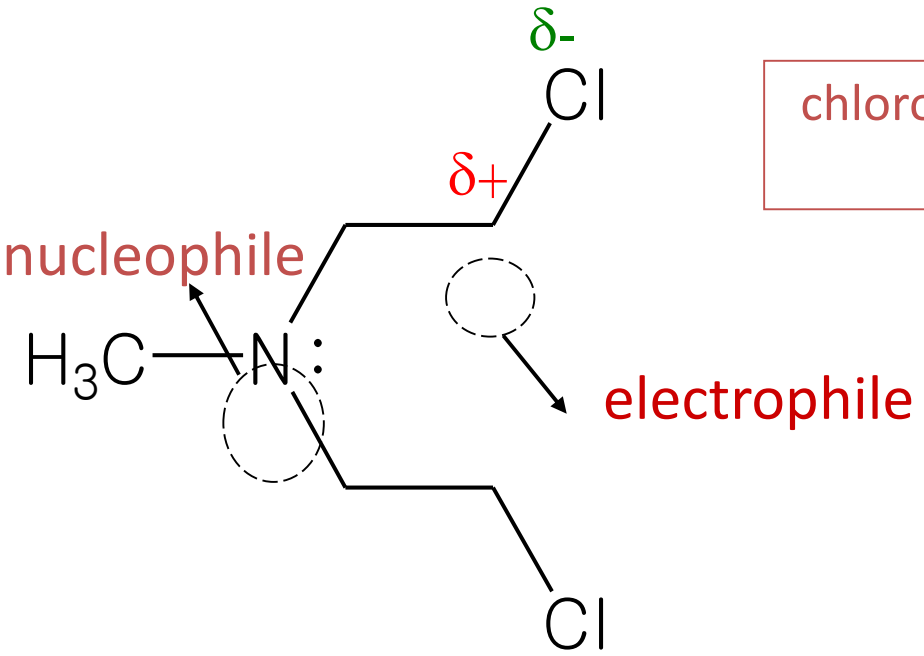
Sulfur Mustard

(chemical weapon) not used clinically

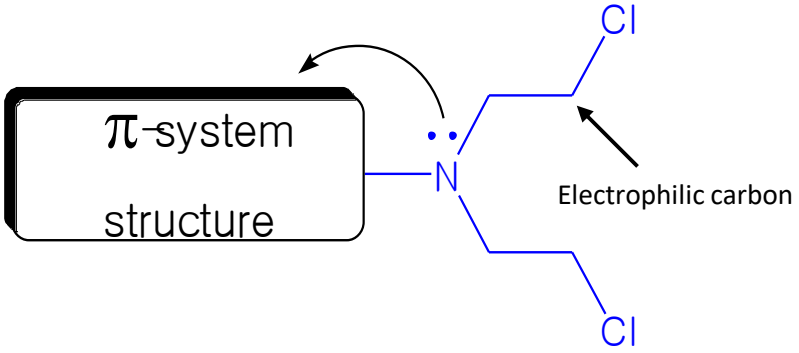


Nitrogen Analog

Nitrogen Mustard



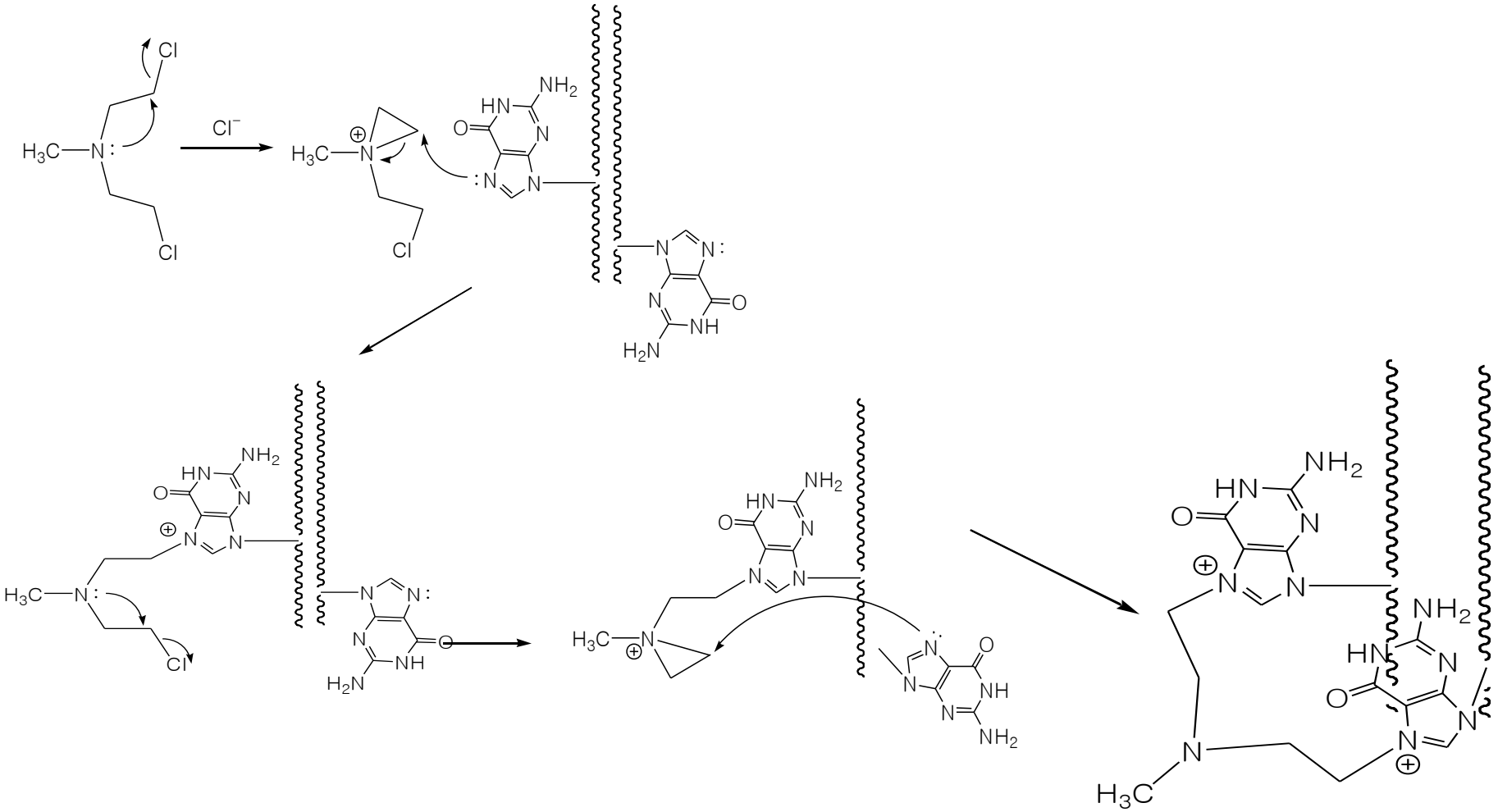
chloroethylamino structure (N-CH_2CH_2-Cl)



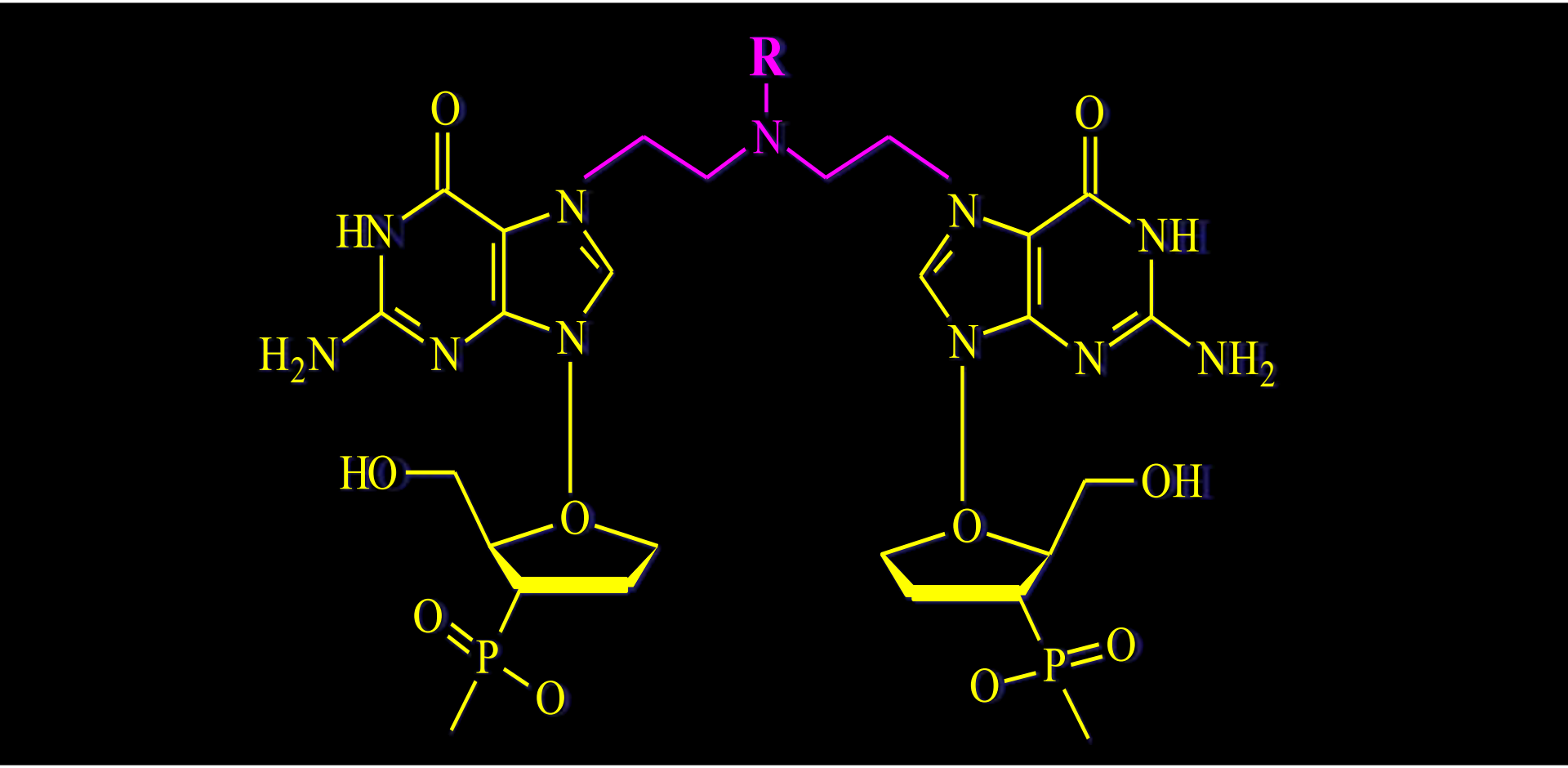
Mechlorethamine (or Chlormethine)

MOA: Nitrogen Mustards

Generation of highly reactive "aziridinium ions" that act as alkylating agents to cross-link DNA producing defective DNA and abnormal cellular function and eventually cell death

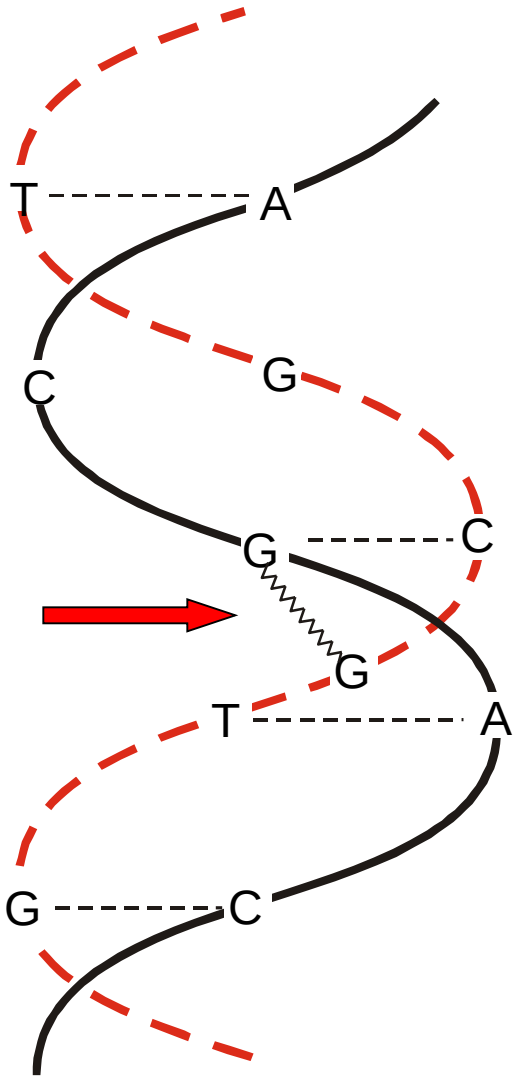


An Example of DNA Crosslinking



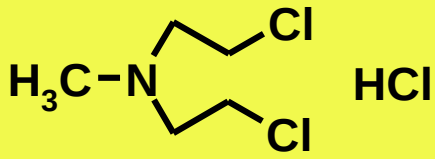
Crosslinking: Joining two or more molecules by a covalent bond. This can either occur in the same strand (intrastrand crosslink) or in the opposite strands of the DNA (interstrand crosslink). Crosslinks also occur between DNA and protein. DNA replication is blocked by crosslinks, which causes replication arrest and cell death if the crosslink is not repaired.

Alkylating Agents (Covalent DNA binding drugs)



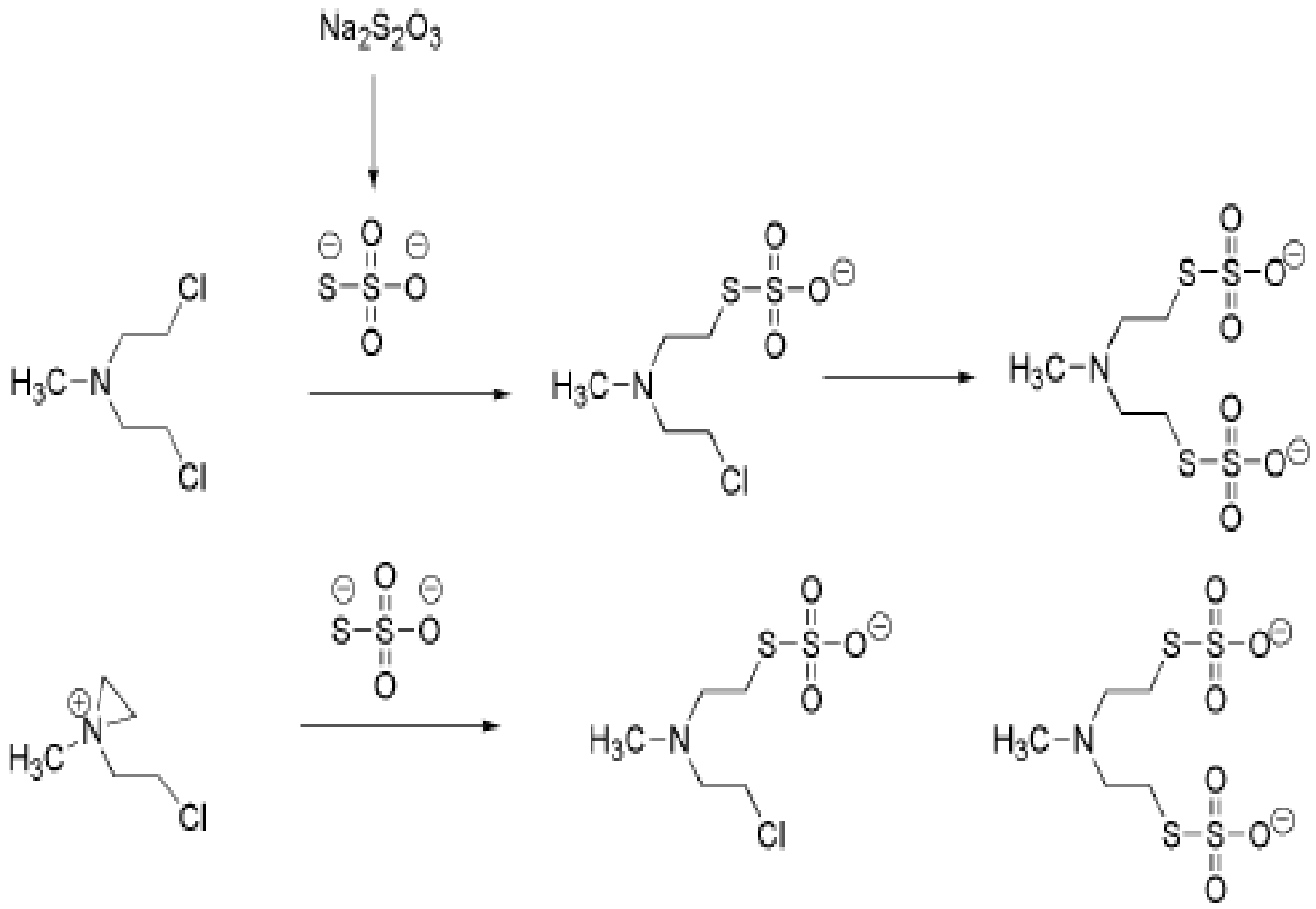
1. The first class of chemotherapy agents used.
2. They stop tumour growth by cross-linking guanine nucleobases in DNA double-helix strands - directly attacking DNA.
3. This makes the strands unable to uncoil and separate.
4. As this is necessary in DNA replication, the cells can no longer divide.
5. Cell-cycle nonspecific effect
6. Alkylating agents are also mutagenic and carcinogenic

Mechlorethamine (or Chlormethine)



Mechlorethamine HCl - Mustargen®

- Mechlorethamine is highly reactive, in fact, too reactive and therefore. In cases of extravasation (drug escapes from the tumor into the underlying tissue), the antidote sodium thiosulfate
- ($Na_2S_2O_3$), a strong nucleophile, may be administered.
- It is capable of reacting with electrophilic sites on the mustard, and once reaction has occurred, the resulting adduct has increased water solubility and may be readily eliminated (Scheme 10.4).
- Cancer patients are at an increased risk of extravasation because of the fragility of their veins resulting from radiation, previous chemotherapy treatments, or malnutrition.



Scheme 10.4 • Thiosulfate Inactivation of mechlorethamine.

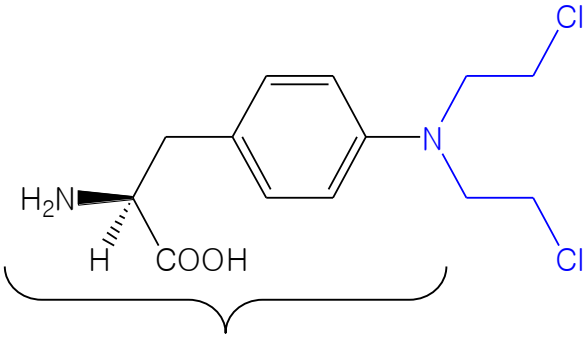
Reducing the reactivity of nitrogen mustard

- 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 (-I).
- This is seen in the case of chlorambucil and melphalan by attachment of nitrogen to a phenyl ring.
- 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.

nitrogen mustard

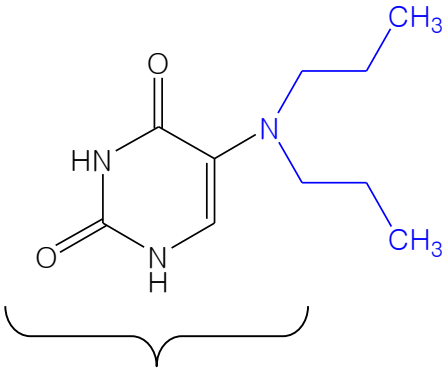
- nitrogen mustard
- amino acid, nucleic base or hormone uptake by carrier protien

Melphalan



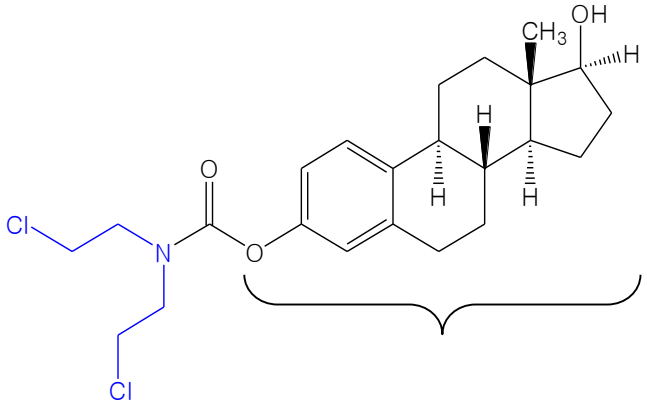
L-phenylalanine
(amino acid)

Uracil Mustard



Uracil
(nucleic base)

Estramustine



Estradiol
(sex hormone)

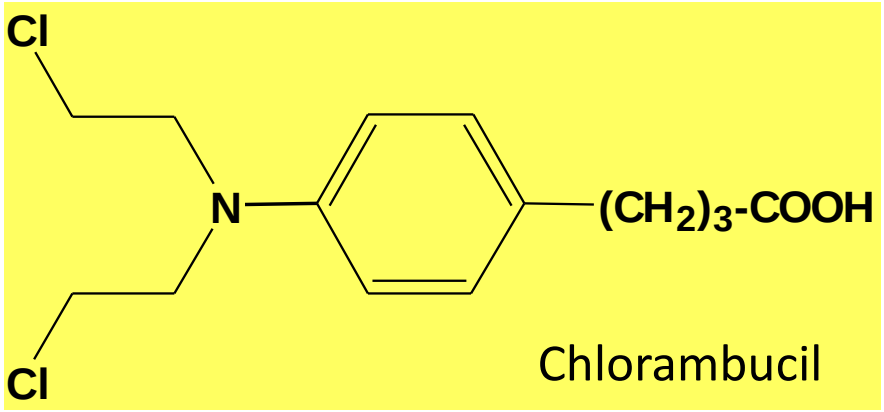
Nitrogen Mustards

Theoretical rationales used to improve nitrogen mustards

-Substituting an aromatic ring for methyl group can be predicted to increase chemical stability and thereby decrease the rate of alkylation because of electron-withdrawing effect.

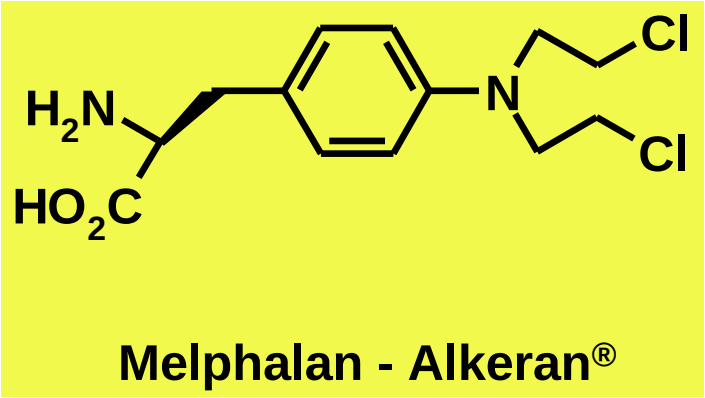
This also, will lead to good oral bioavailability, tissue distribution, before alkylation is widespread.

E.g. Chlorambucil, and melphalan.



4-(*p*-bis(2-Chloroethylamino)phenyl)butyric acid.

Nitrogen Mustards

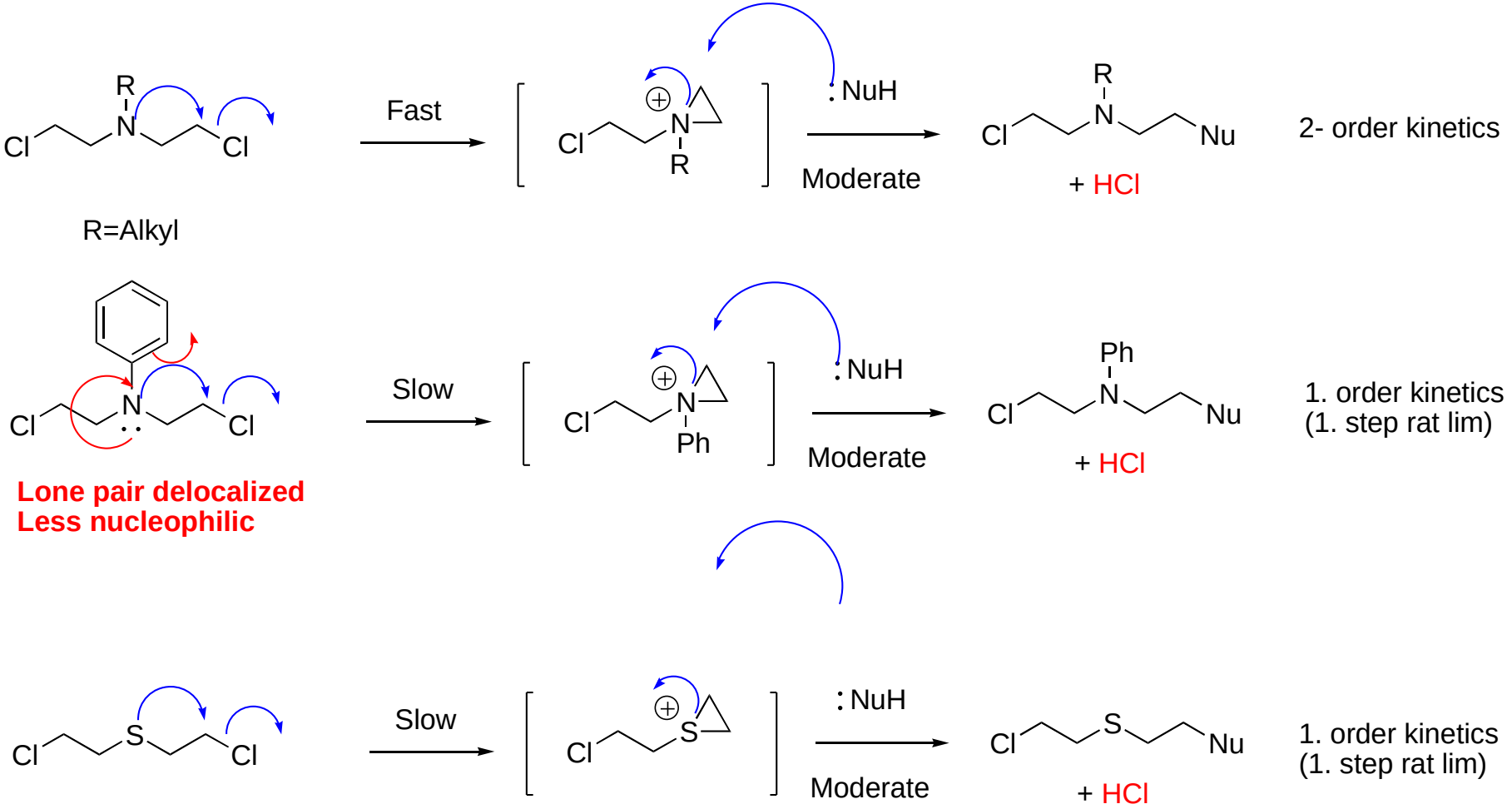


Melphalan - Alkeran®

Oral or IV, severe bone marrow suppression resulting in infection and bleeding

Dosage reduction may be necessary in renal failure as measured by BUN

Known to cause chromosome abnormalities


R
N
Cl
Cl
Fast
Cl
N
R
: NuH
Moderate
R
N
Cl
Nu
+ HCl
2- order kinetics
R=Alkyl
N
Cl
Cl
Slow
Cl
N
Ph
: NuH
Moderate
Ph
N
Cl
Nu
+ HCl
1. order kinetics
(1. step rat lim)
Lone pair delocalized
Less nucleophilic
S
Cl
Cl
Slow
Cl
S
: NuH
Moderate
S
Cl
Nu
+ HCl
1. order kinetics
(1. step rat lim)
less stable than N-aanalog

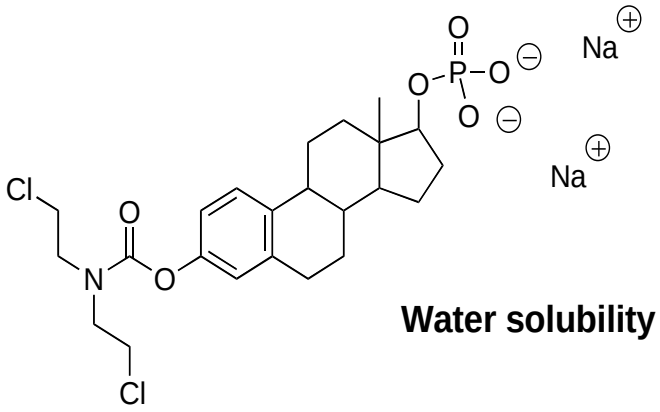
Nitrogen Mustards

To increase selectivity, nitrogen mustards was bonded with natural carrier e.g. **estramustine** which is active against prostate cancer, another examples is the bonding with antimetabolites e.g. uracil mustard.

Estramustine phosphate

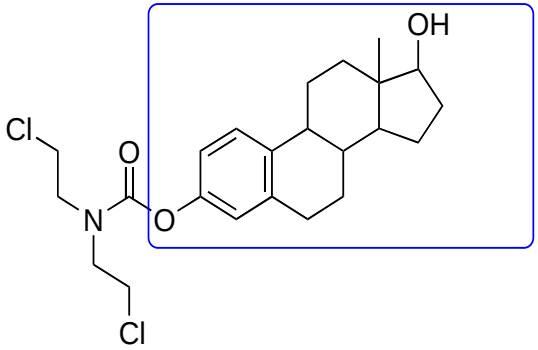
Estracyt®

Pro-drug



Water solubility

1) Oral absorb
2) Fast metabol.



Main comp. plasma

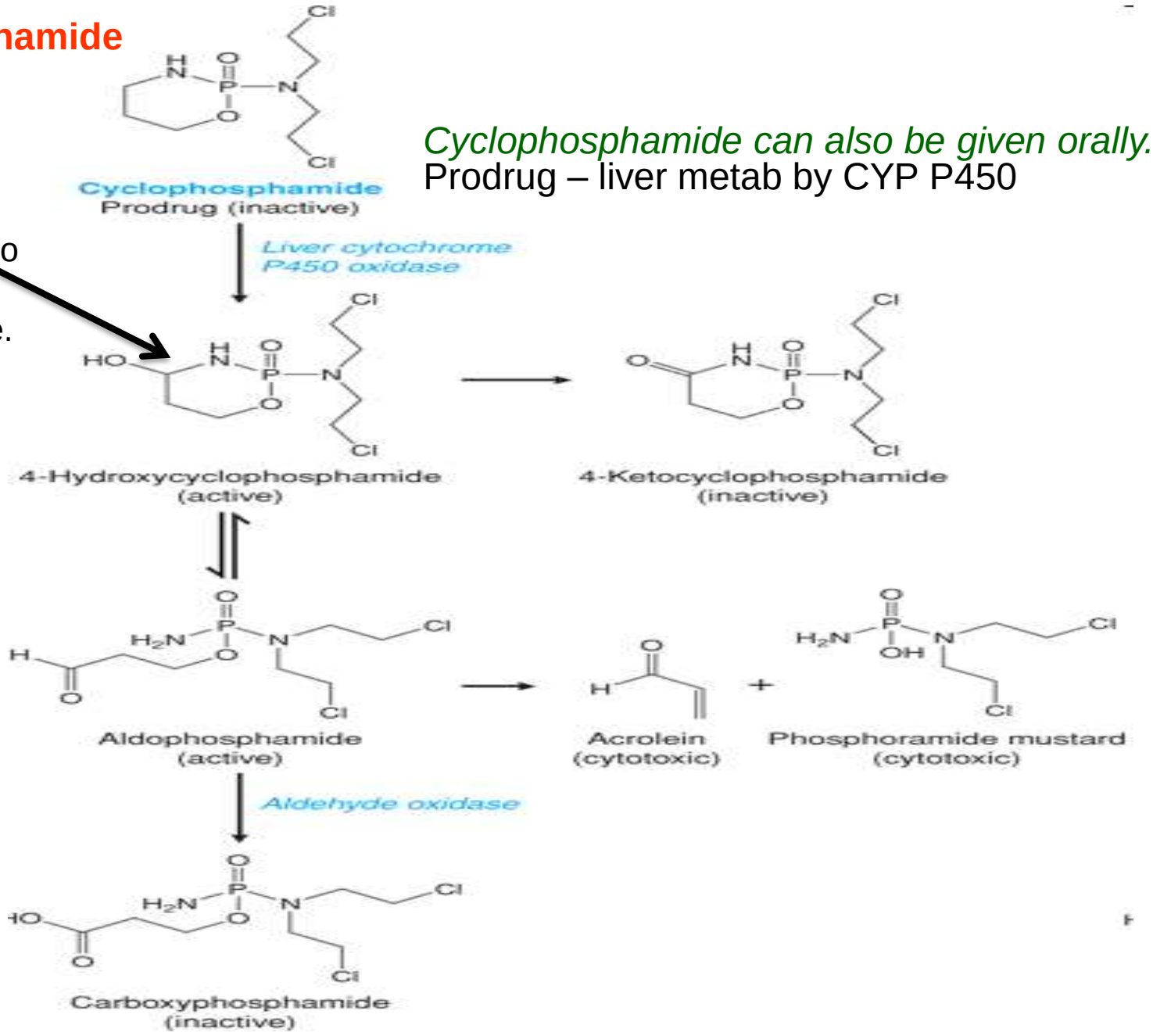
Estradiol
Carry to cells with estrogenic receptors

Estrogenic (Anti-androgenic) effect protate cancer
Cleaved to active alkylating agent?

Cyclophosphamide

Cyclophosphamide can also be given orally.
Prodrug – liver metab by CYP P450

A carbinolamine that could undergo ring opening to give the aldehyde.

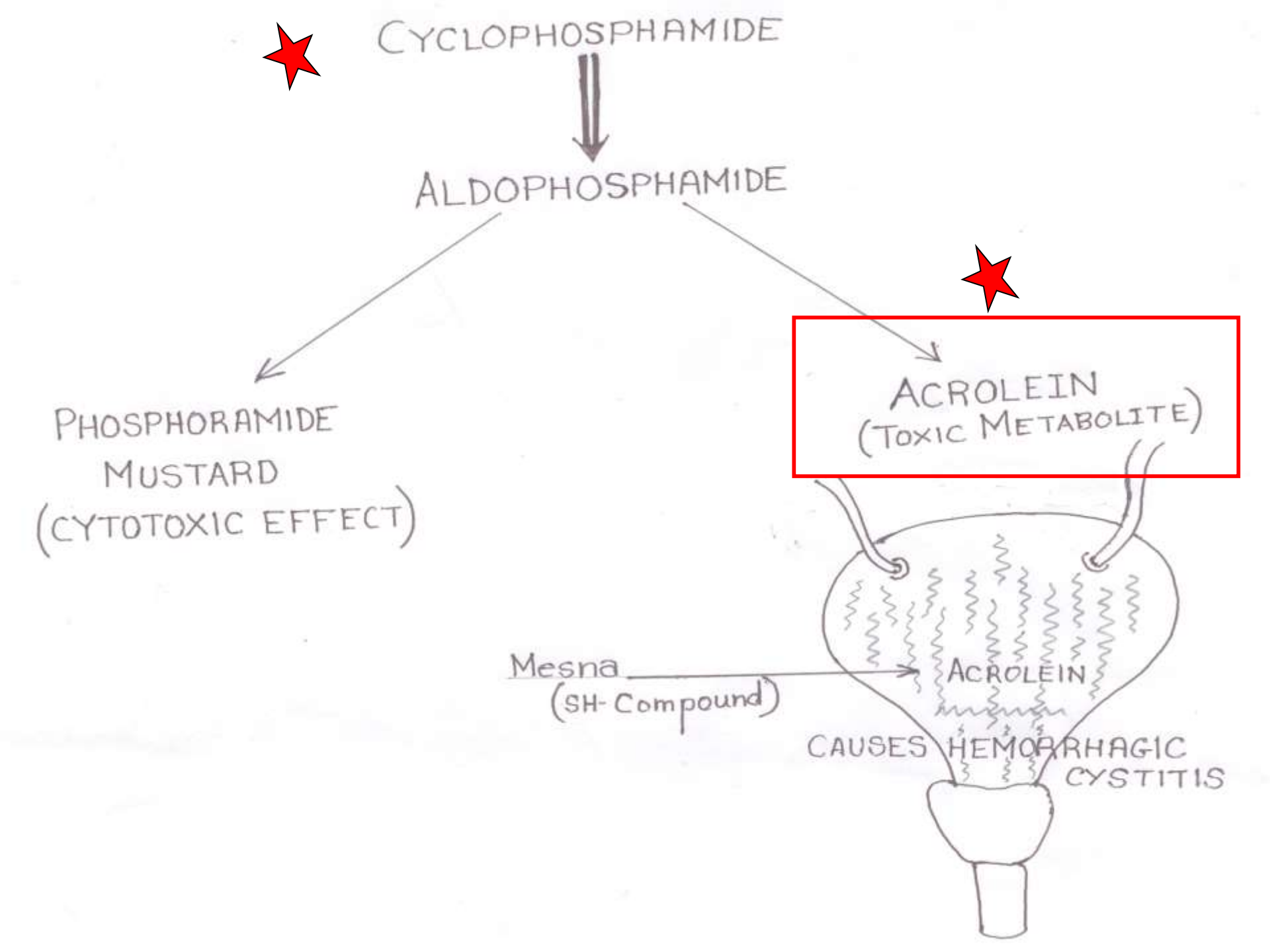


These species pass by passive diffusion into the tumor cell

Metabolism of cyclophosphamide

- the products of cyclophosphamide activation can be converted to the inactive metabolites by the action of alcohol and aldehyde dehydrogenase
- additionally, cyclophosphamide can undergo N-dealkylation

  on either chain
- the product of this reaction is chloroacetaldehyde,which is highly nephrotoxic and neurotoxic
- it can be further oxidized by the action of aldehyde dehydrogenase to chloroacetic acid

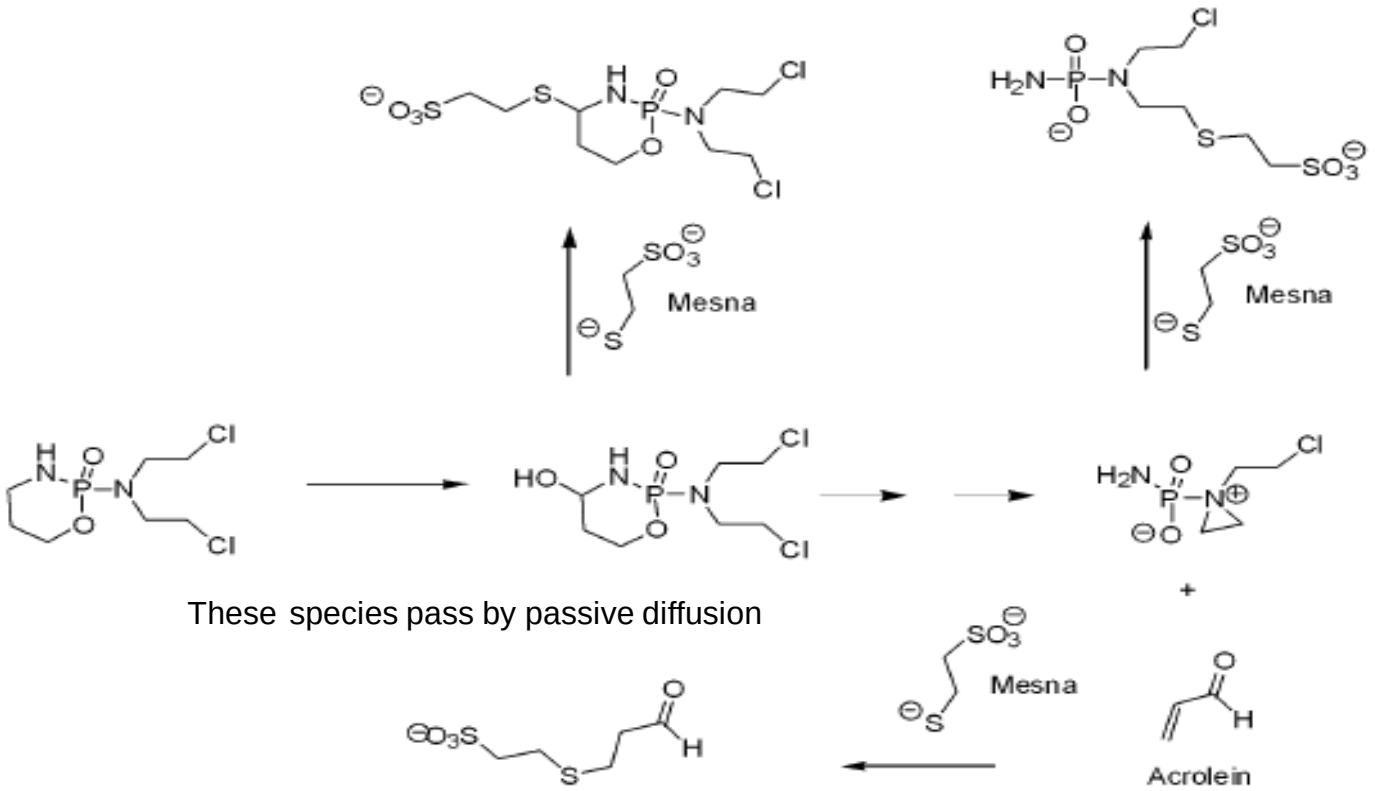

CYCLOPHOSPHAMIDE
ALDOPHOSPHAMIDE
PHOSPHORAMIDE MUSTARD (CYTOTOXIC EFFECT)
ACROLEIN (TOXIC METABOLITE)
Mesna (SH- Compound)
ACROLEIN
CAUSES HEMORRHAGIC CYSTITIS

(HEMORRHAGIC CYSTITIS)

- Arcolein is highly toxic to the bladder and kidney this is caused by reaction with SH containing proteins with the alpha, beta-unsaturated aldehyde (acrolein) to offset this effect, the compound, mesna, can be Administered
- this compound is a thiol sulfonate the thiol of mesna reacts to produce a water soluble sulfonic acid that is readily excreted
- **MESNA** - a mercaptosulfonic acid that concentratesin bladder can be used with cyclophosphamide to decrease toxicity

MESNA is an adjuvant used in cancer chemotherapy involving cyclophosphamide and ifosfamide. It is marketed by Baxter as Uromitexan and Mesnex. MESNA is an acronym for 2-MercaptoEthane Sulfonate sodium. It is a detoxifying agent.

- the nucleophilic sulfhydryl group may react with the carbinolamine, aziridinium cation, the chloro substituents of cyclophosphamide, or via conjugate addition with acrolein (Scheme 10.6).
- This inactivation and detoxification may also be accomplished glutathione. Increased levels of these proteins may occur as cancer cells become resistant to these alkylating agents by other thiol-containing proteins

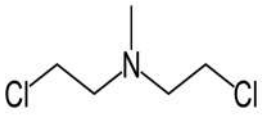


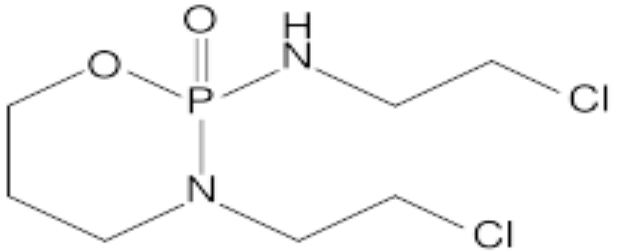
Scheme 10.6 • Detoxification of cyclophosphamide by mesna.

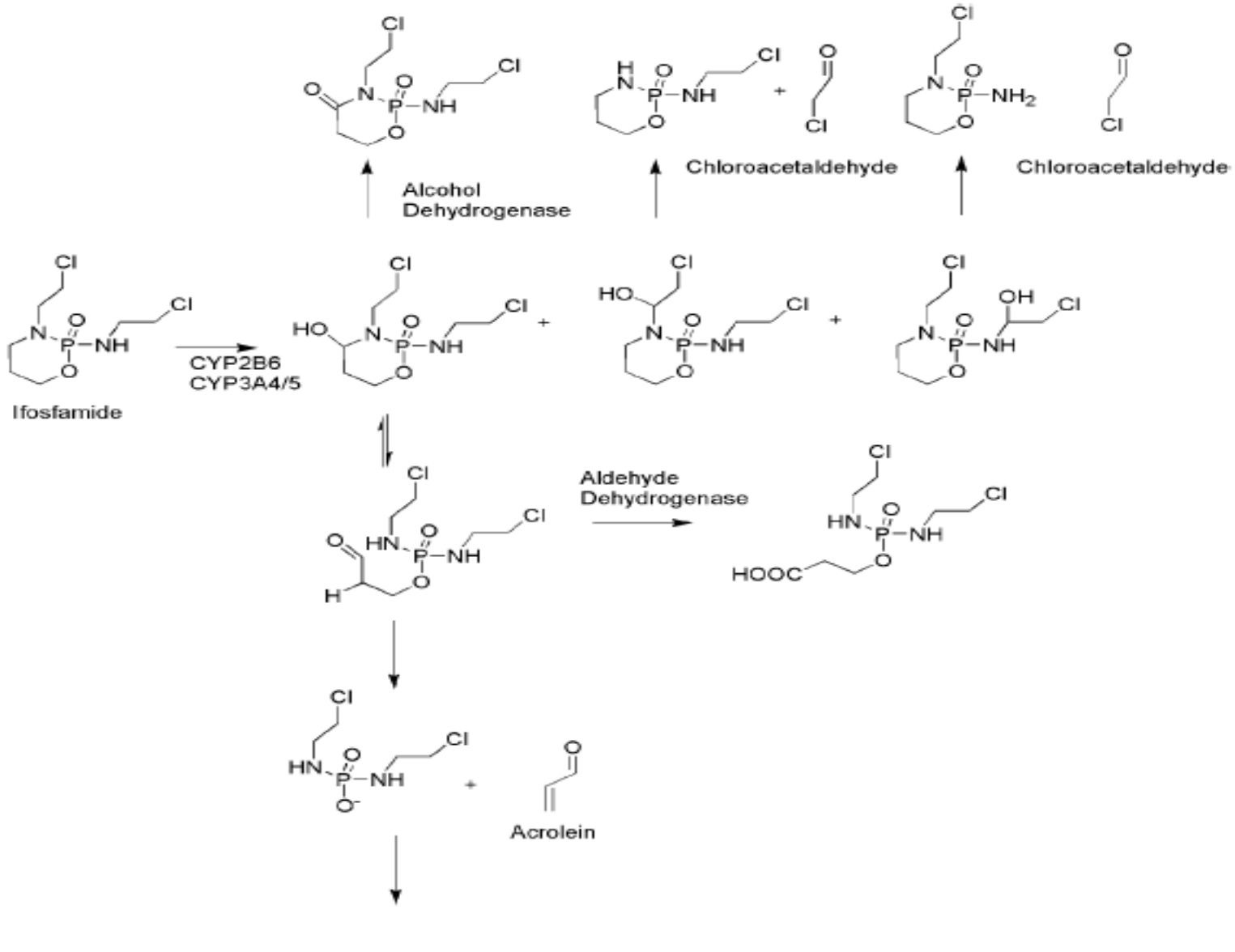
Ifosfamide

- Analogue to cyclophosphamide.
- Look at the 2 chloroethyl arms , they are not attached to the same nitrogen.
- Metabolic activation is required.
- usd as third-line therapy

- the toxicity of ifosfamide is greater than cyclophosphamide due to greater N-dealkylation to produce chloroacetaldehyde (45% as compared to 10%)
- mesna can be administered to react with the acrolein that is generated in the bioactivation of ifosfamide

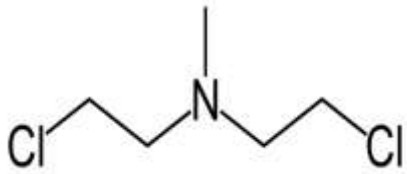




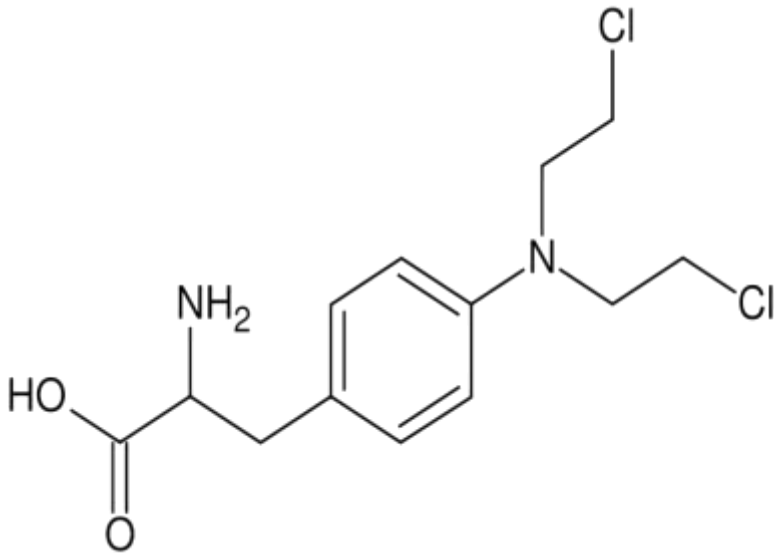


Scheme 10.7 • Metabolic and chemical activation of Ifosfamide.

Melphalan



- L-phenylalanine mustard or L-PAM, a nitrogen mustard that is linked to aminoacids.
- is transported by L-amino acid active transporters
- D-isomers require very high doses to reach the same cytotoxicity levels as L- isomers levels



Alkylating Agents—Nitrosoureas

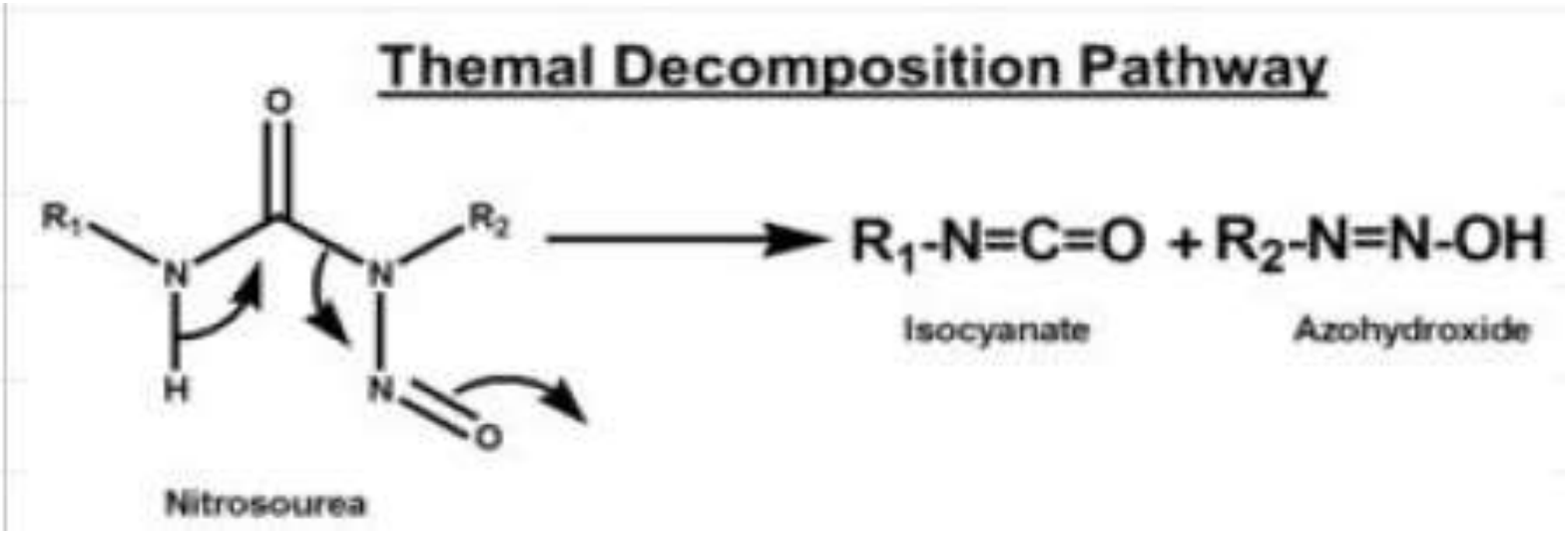
- the nitrosoureas are another type of alkylating agent that must undergo bioactivation
- nitrosoureas typically contain a 2-chloroethyl or methyl group on the nitrogen bearing the nitrosogroup

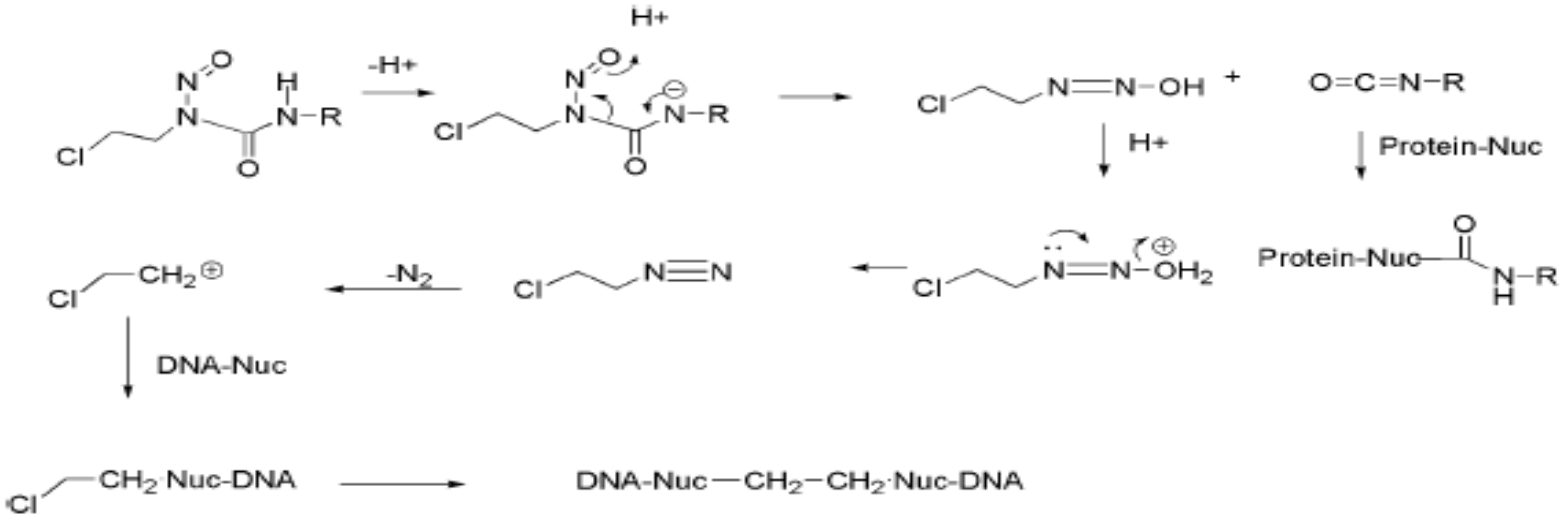
Alkylating Agents——Nitrosoureas

- ***Carmustine, Lomustine, Semustine:*** Also activated in vivo
- Alkylate DNA BUT alk'n proteins → toxicity
- Pharmacokinetics:
- Nitrosoureas are highly lipophilic and reach cerebrospinal fluid concentrations that are about 30% of plasma concentrations.
- Indications:
- Because of their excellent CNS penetration, carmustine and lomustine have been used to treat brain tumors.

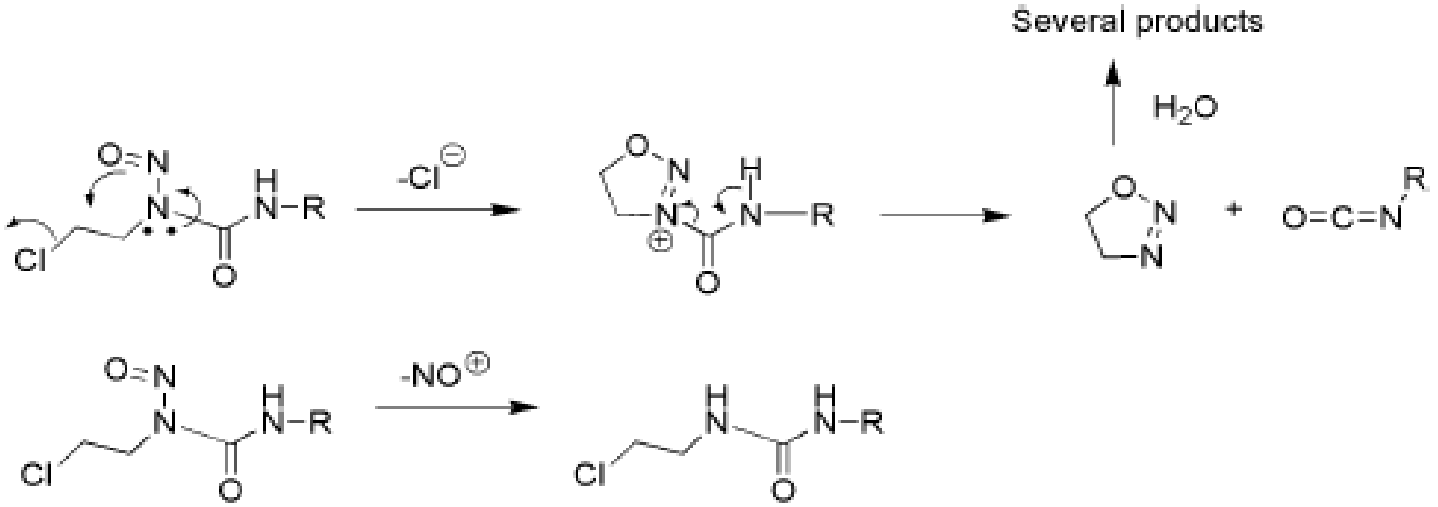
Thermal decomposition pathway:

- the thermal decomposition of nitrosoureas generates an isocyanate and an azohydroxide





Scheme 10.11 • Mechanism of DNA alkylation by nitrosoureas



Scheme 10.12 • Metabolic and chemical inactivation of nitrosoureas.

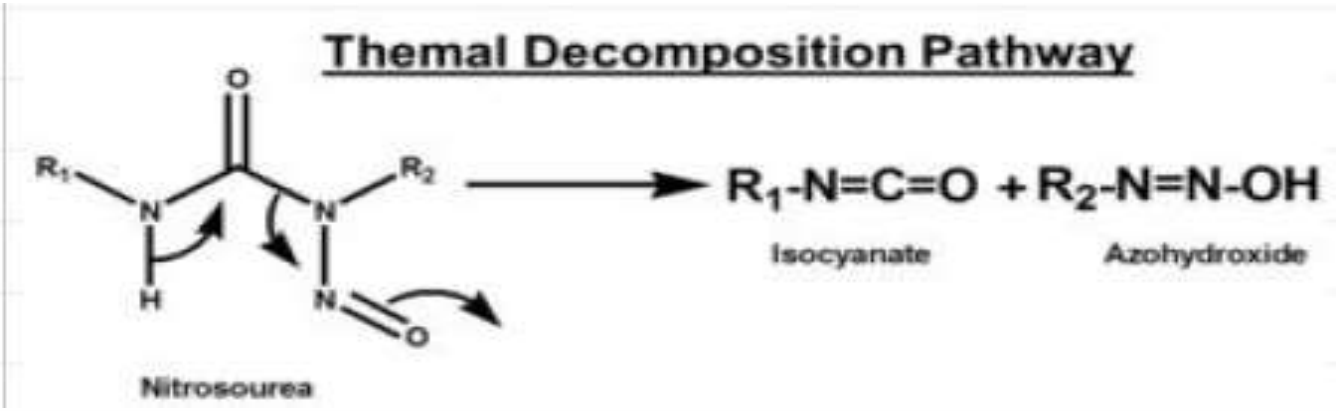
Fate of decomposition products of nitrosoureas

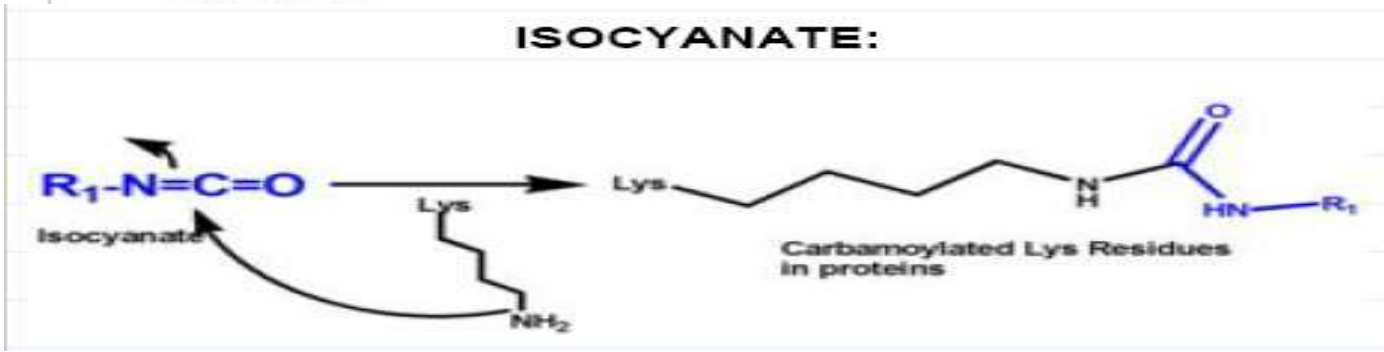
- THE AZOHYDROXIDE that is generated by thermal decomposition of a nitrosourea can decompose to nitrogen and a chloroethyl cation
- this cation can serve as a biological alkylating agent (bis-alkylating agent) or undergo reactionwith water (to form an alcohol) or Cl- (to form dichloroethane)
- it can also rearrange to react with water to produce acetaldehyde

ISOCYANATE:

- isocyanates that are generated by decomposition of nitrosoureas can react with amino groups on Lys side chains of proteins
- the amino groups are said to be carbamoylated which alters protein structure may lead to inhibition of several types of DNA repair enzymes
- the isocyanate that is generated by decomposition of nitrosoureas, is probably responsible for adverse effects by carbamoylating amino acid side chains that are contained in proteins

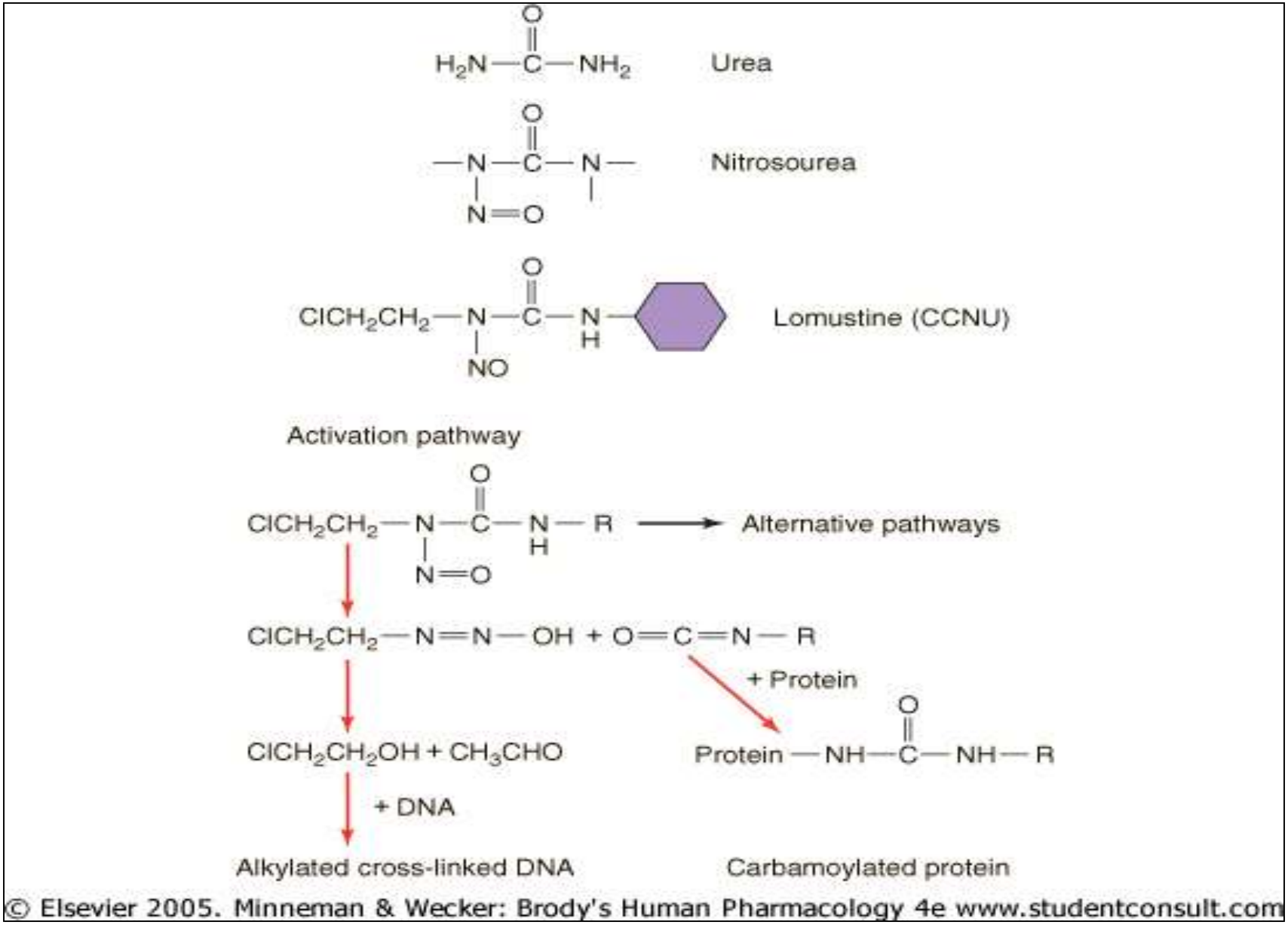
- the isocyanate carbon is electropositive (due to the electron withdrawing effects of the oxygen and nitrogen), thus it is readily attacked by biological nucleophiles (e.g. the amino groups of Lys)
- note that this produces a urea function on the side chain nitrogen. the protein structure is therefore oltered by carbamoylation





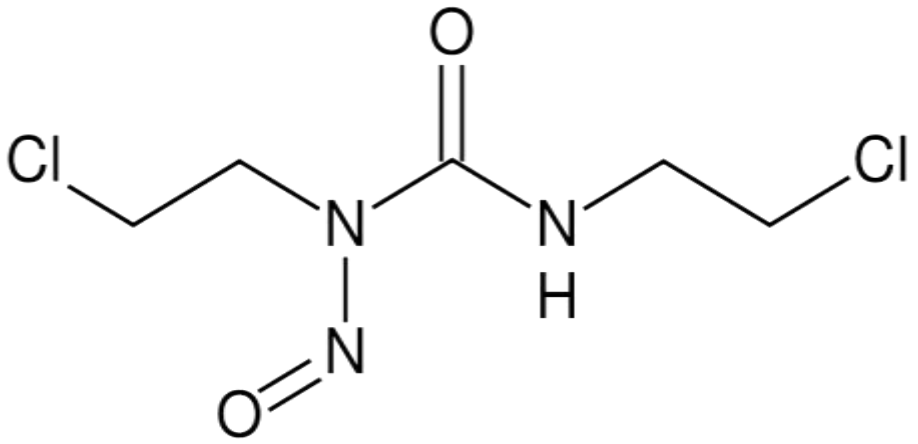
- an alternative activation of a nitrosourea can exist in its tautomeric enol tautomer
- the tautomer can be attacked by water that gives a species that breaks down to yield a carbamic acid and an azohydroxide
- carbamic acids are unstable and eliminate carbon dioxide to yield amines
- the other product of this decomposition is an Azohydroxide as mentioned earlier, the azohydroxide breaks down to yield nitrogen and a cation
- this cation can then serve as an alkylating agent cross-linked DNA by the 2-chloroethyl cation
- the cation can react with the N7 position of guanine and the Cl group is readily displaced by the N3 nucleophilic nitrogen of adenine
- the carbon to which the Cl is attached is electropositive due to the electron withdrawing effect of the Cl. thus, it is readily displaced by a nucleophile

Nitrosoureas

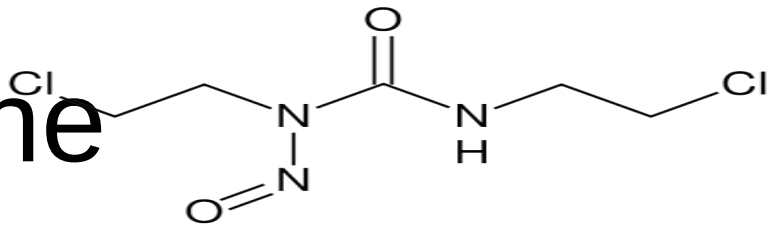


Carmustine

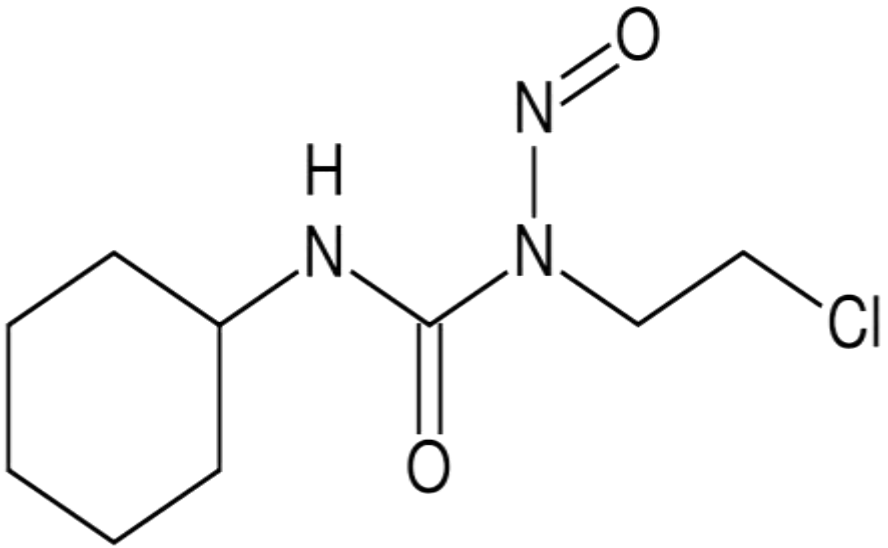
- BCNU(1,3-bis(chloroethyl)-1-nitrosourea.
- Neutral, lipophilic & poorly soluble in water: cross BBB providing higher CSF to plasma ratio of drug compared to other alkylating agents.



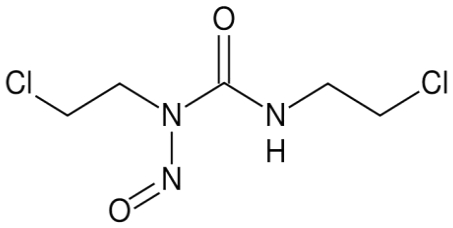
Lomustine



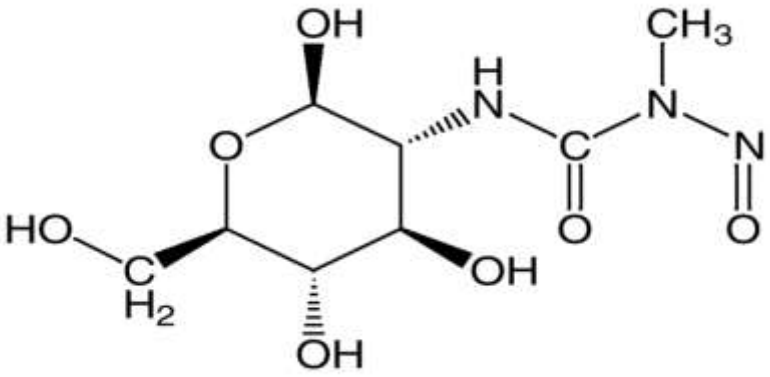
- CCNU(1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-urea).
- Similar to carmustine.



Streptozocin



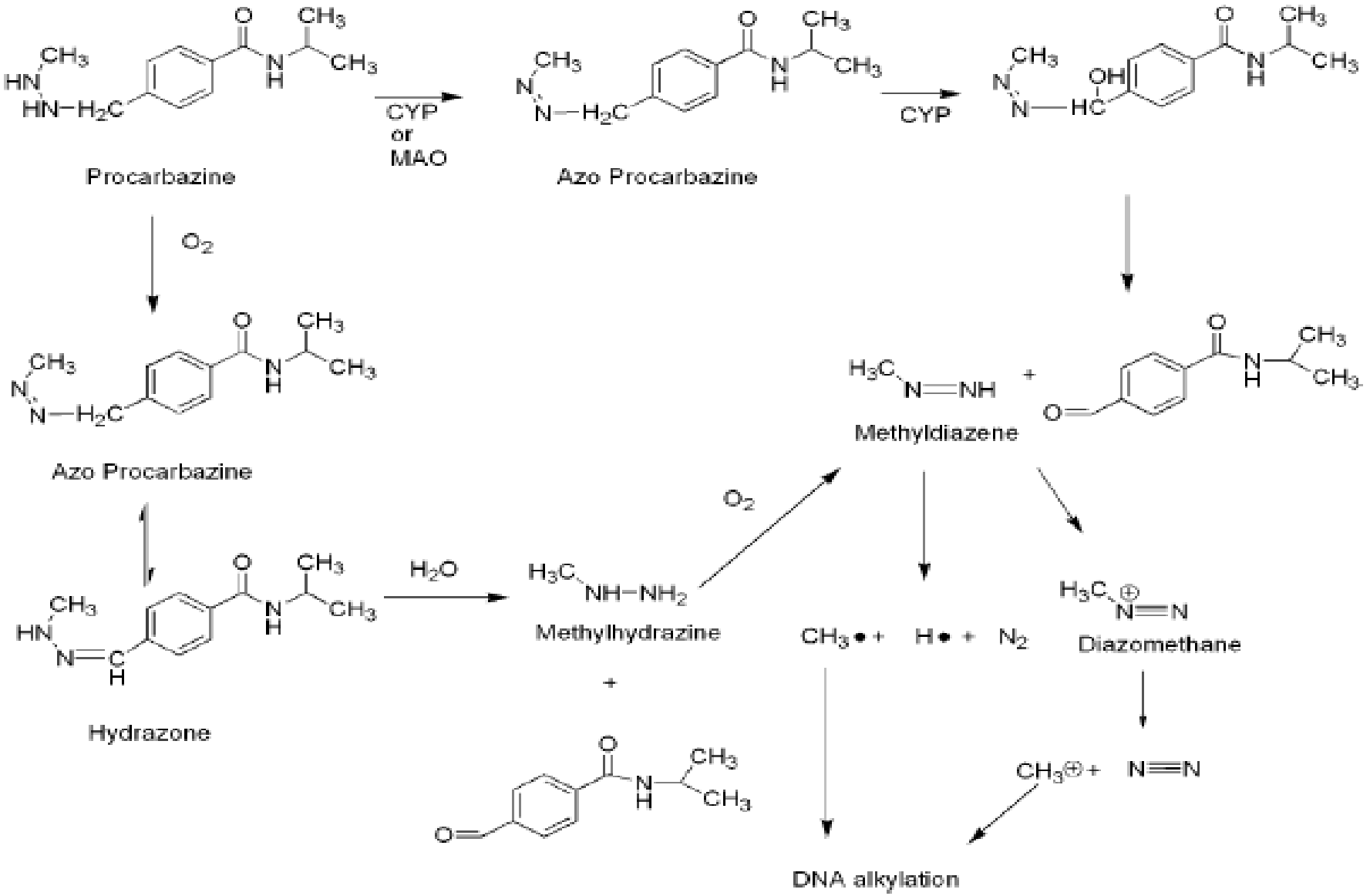
- Streptozotocin (Streptozocin, STZ, Zanosar) a chemical that is particularly toxic to the insulin-producing beta-cells of the pancreas in mammals.
- Composed of a combination of glucopyranose amino sugar & nitrosourea.
- The sugar imparts good water solubility as compared to other lipophilic analouges.
- Used in cancers of the islet of Langerhans.
- Long been known to produce diabetes-like syndrome in animals.



Methyl hydrazines: Procarbazine

- methyl hydrazines can also act as alkylating agents (again by bioactivation)
- formation of methyl carbonium ion (methylating species) from procarbazine:
- procarbazine is actually a prodrug for the highly reactive methylcarbonium ion
- the compound is a hydrazine that undergoes N-hydroxylation by P450
- the resulting N-OH compound undergoes loss of water to form a diazo CH3N=N-R intermediate
- the diazo intermediate undergoes benzylic carbon oxidation by P450
- remember - a benzylic carbon is the carbon alpha (adjacent) to the phenyl ring .the resulting carbinol-diazo species loses methyl diazene
- this species loses a proton to form methyldiazonium which in turn loses nitrogen and forms a highly reactive carbonium ion
- the methylcarbonium ion can alkylate the N7 position of guanine bases in DNA

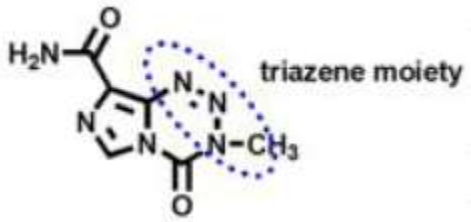
ultimately, there is a mis-pairing of bases in DNA, leading to altered amino acids and a disruption of protein function



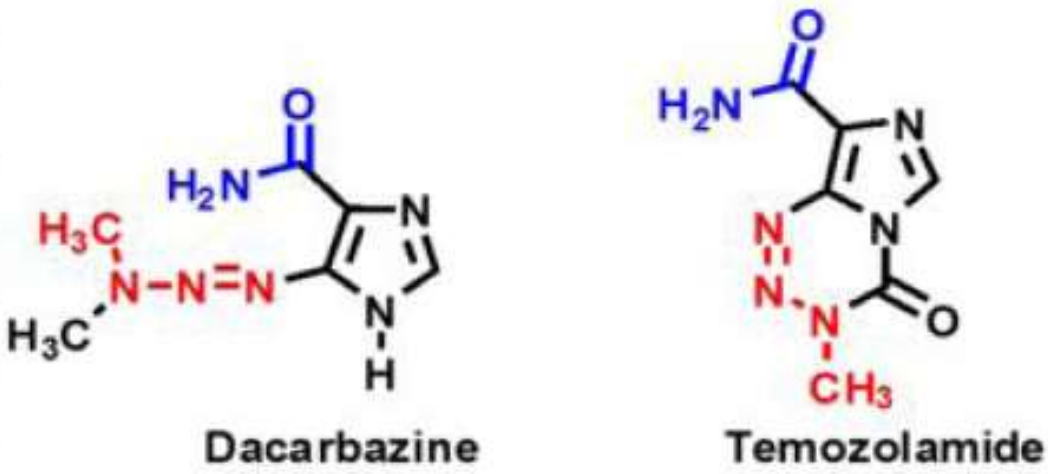
Scheme 10.13 • Metabolic and chemical activation of procarbazine.

Triazines: Dacarbazine & Temozolamide

1. are able to yield methyl diazonium (a highly reactive species and can generate a methyl carbonium ion) hydrazines are similar to amines, but with 2 adjacent nitrogens
2. like amines hydrazines are basic functional groups
3. dacarbazine is a DNA methylating agent it is a triazene (note 3 adjacent nitrogens in which there is unsaturation (double bond))
4. it is activated by P450 to yield methyl diazonium which is highly reactive (note the positive charge on nitrogen)
5. it can be attacked by a nucleophile to displace nitrogen gas or it can break down to yield a methyl carbonium ion in either case, alkylation occurs. in this case the methyl group is attached to a nucleophilic site on a prurine or pyrimidine base
6. the triazene structure is incorporated in the ring structure in temozolamide, thus, it is more stable to UV light

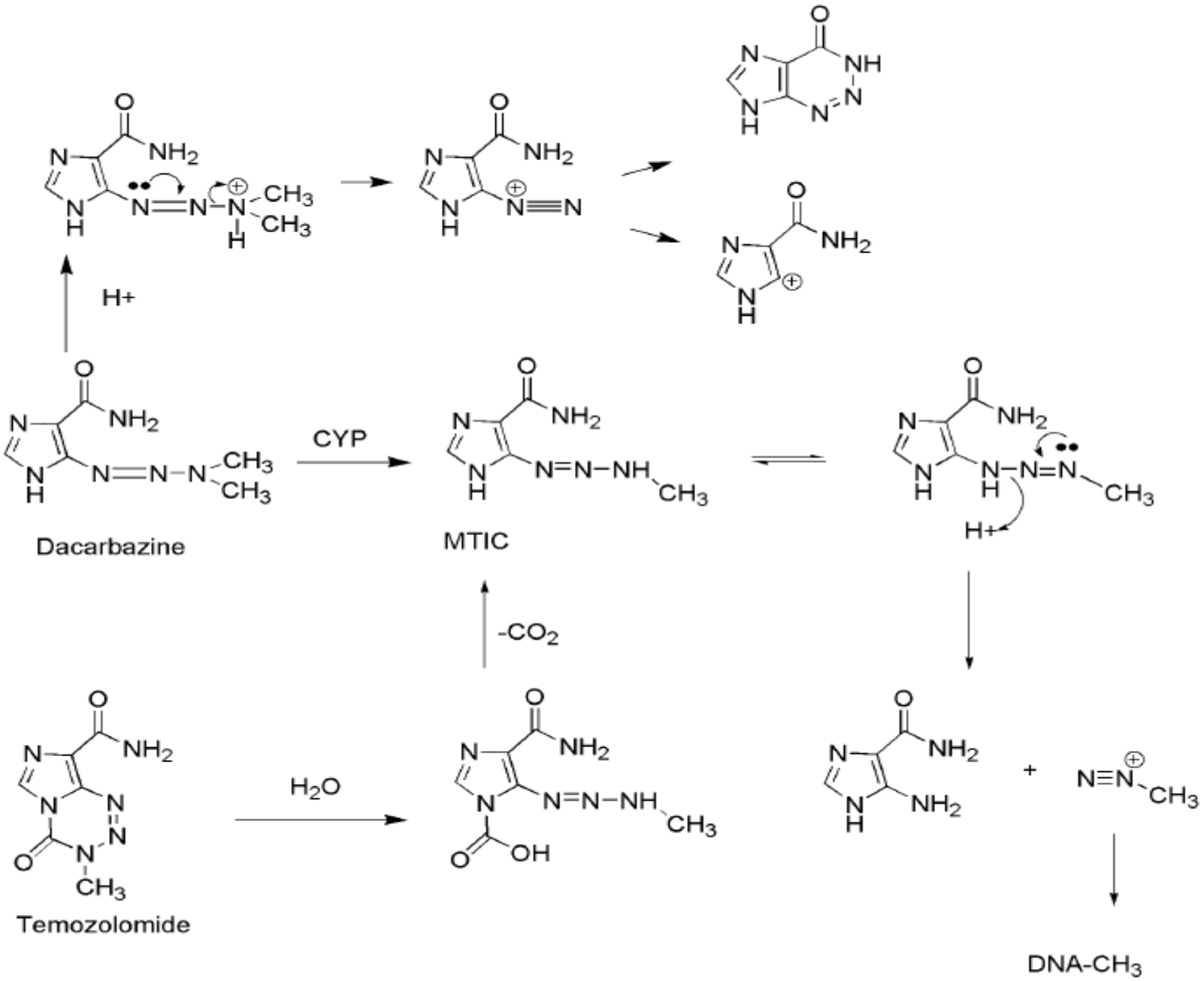


structural comparison between dacarbazine and temozolamide:



temozolamide is a prodrug of dacarbazine

the triazene structure is incorporated in the ring structure in temozolamide, thus, it is more stable to UV light



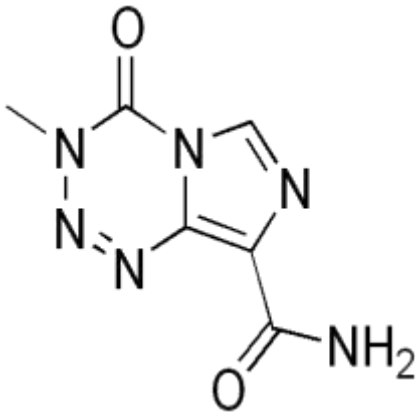
Scheme 10.14 • Metabolic and chemical activation of dacarbazine and temozolomide.

Disadvantages of dacarbazine

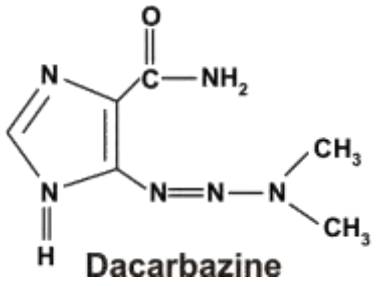
- poor water solubility (IV administration necessary)
- photosensitivity (protect IV bags from light)
- dacarbazine is broken by UV light to liberatedimethyl amine and a dizaonium species
- the diazonium species reacts with itself
- thus, it no longer functions as an alkylating agent

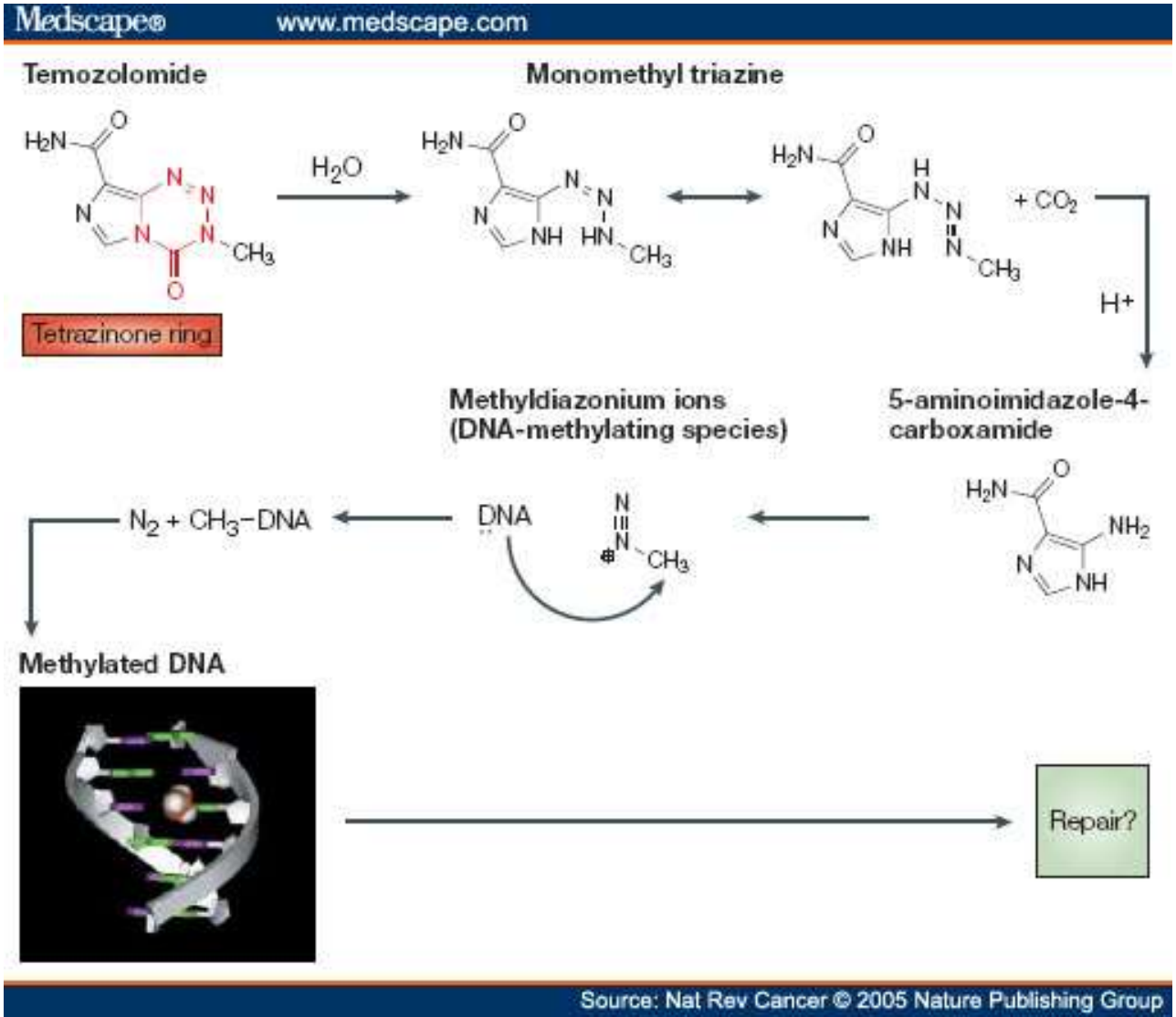
Temozolomide

- Prodrug, which is non-enzymaticaly converted to MTIC (monomethyl triazeno imidazole carboxamide)which then alkylates DNA like DTIC
- FDA approved for the use in treatment of brain tumors.
- Major advantage ,this drug can be given orally.



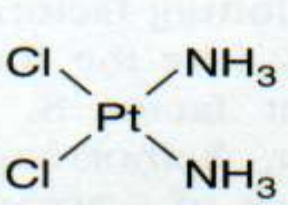
- Methylates G, A → improper G-T base pairing



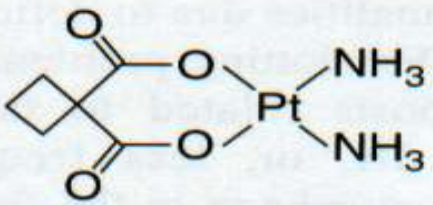


Platinum Coordination Complexes :

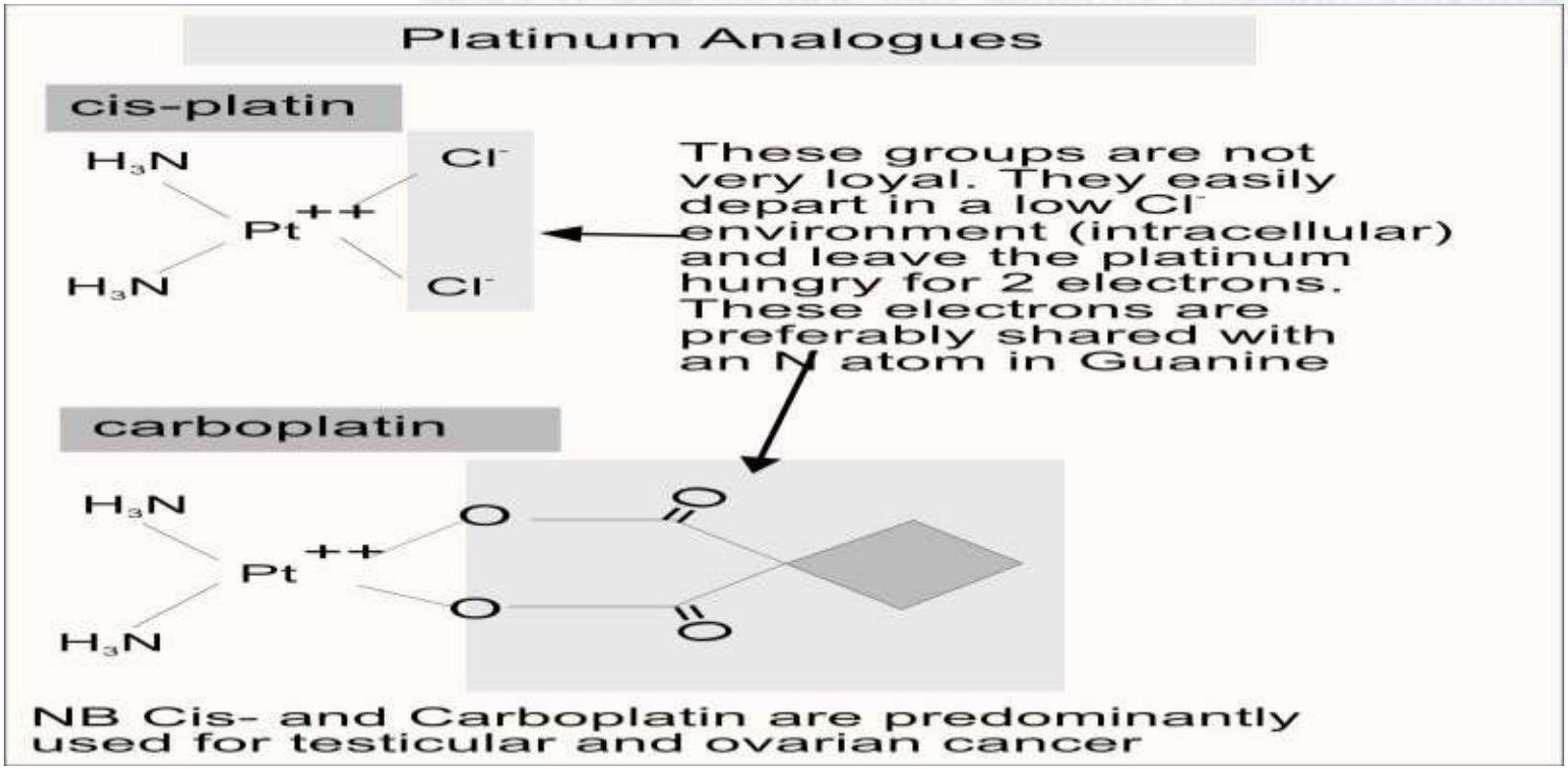
- Cisplatin,
- Carboplatin,
- Oxaliplatin,&



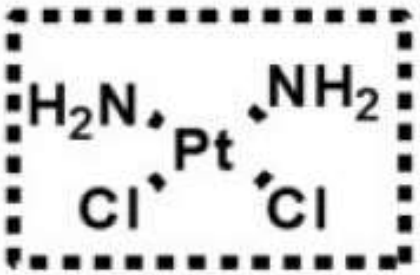
CISPLATIN



CARBOPLATIN



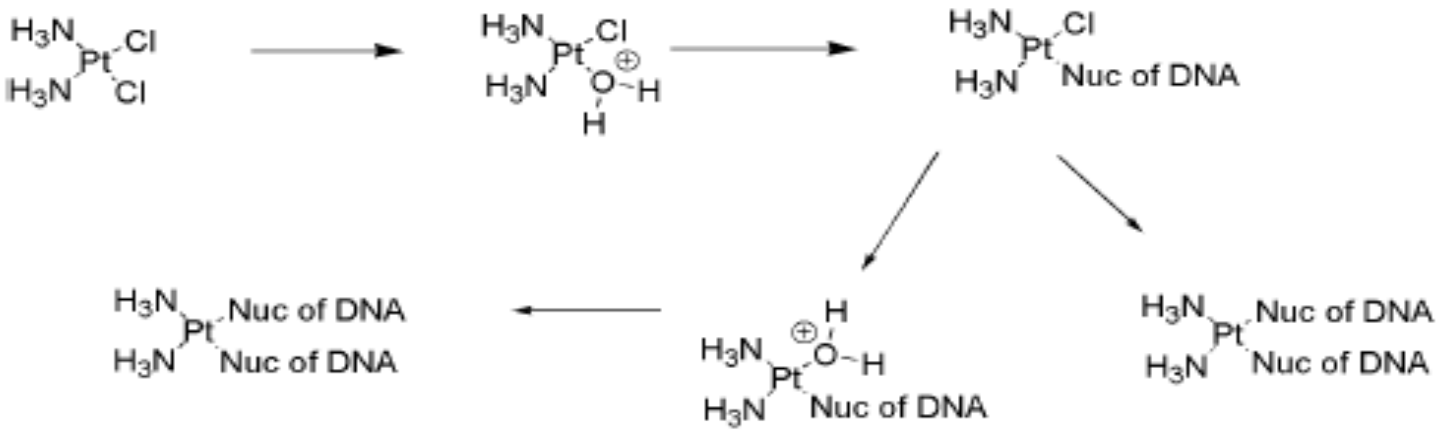
- These related drugs covalently bind to DNA with preferential binding to the N-7 position of guanine and adenine.
- They are able to bind to two different sites on DNA producing cross-links, either intrastrand (within the same DNA molecule; >90%) or interstrand (bewteen two different DNA molecules; <5%).
- The modification of the DNA (formation of DNA adducts) results in inhibition of DNA synthesis and transcription. Binding of the drugs to nuclear and cytoplasmic proteins may also result in cytotoxic effects.



Square Geometry

- electron deficient, but a net charge of zero on (pt) due to the electron releasing effects of the cl groups
- platinum compounds have a zero net charge and a square geometry
- they contain 2 groups (usually amino groups) that facilitate the binding of the compounds to the pohsphate backbone of DNA and 2 leaving groups (Cl or some type of oxygen species)
- platinum compounds must be bioactivated through water generation of the di-aquo species:

in this case, the activation of cisplatin is shown it passes into the cell and then is converted to its diaquo form by water. note that the oxygen derived from water has a positive charge



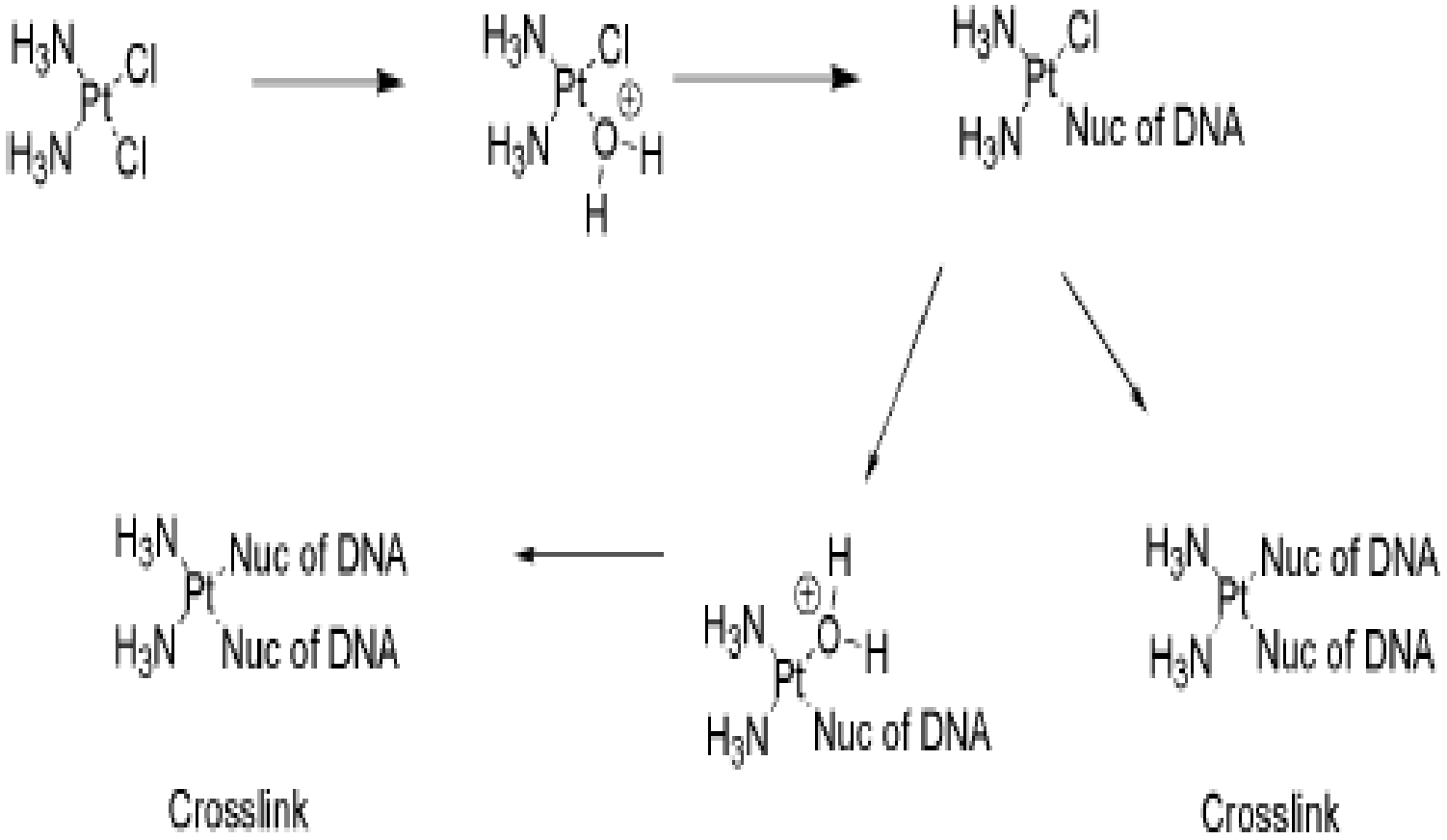
Scheme 10.10 • Mechanism of cisplatin activation and formation of DNA adducts.

Activation and fate of cisplatin:

- cisplatin can enter the cell via the copper transporter (CTR1) or possibly by passive diffusion in the cytoplasm
- Once inside the tumor cell, the drug encounters a lower chloride concentration and one chloro group is substituted by a water molecule in a process known as *aquation*.
- This serves to “trap” the molecule in the cell as a result of

ionization.

- cisplatin undergoes hydrolysis to form the activated di-aquo form



Scheme 10.10 • Mechanism of cisplatin activation and formation of DNA adducts.

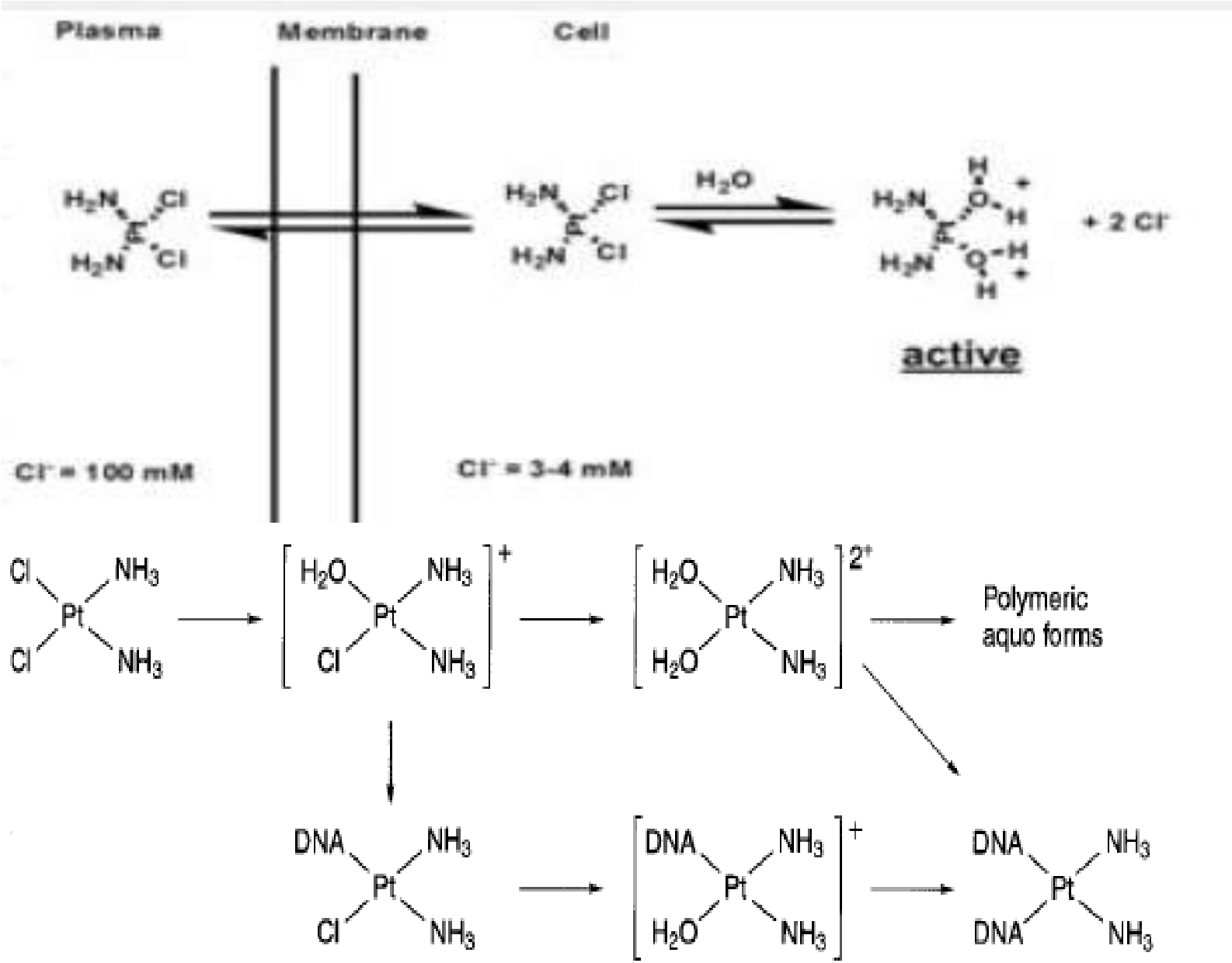
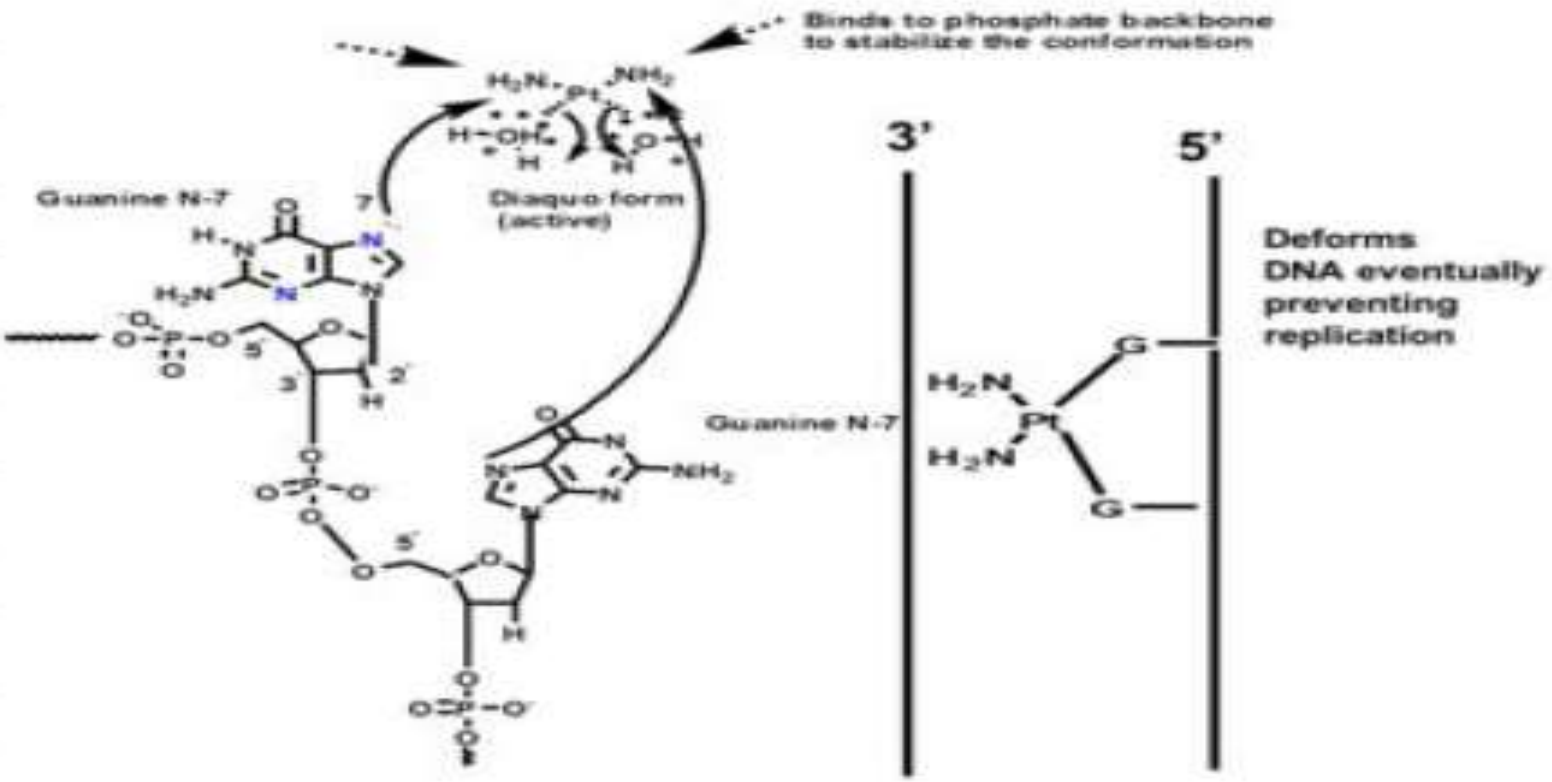


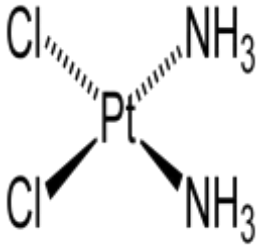
Figure 2.3. Reaction of platinum species with DNA.

intrastrand cross linking by Pt complexes

- platinum compounds produce DNA intrastrand cross links
- the key here is displace the positively charge water species from platinum by the purine base on DNA. Reaction with DNA occurs preferentially at the N-7 of guanine of two adjacent guanine residues resulting in primarily (95%) intrastrand cross-links



Cisplatin

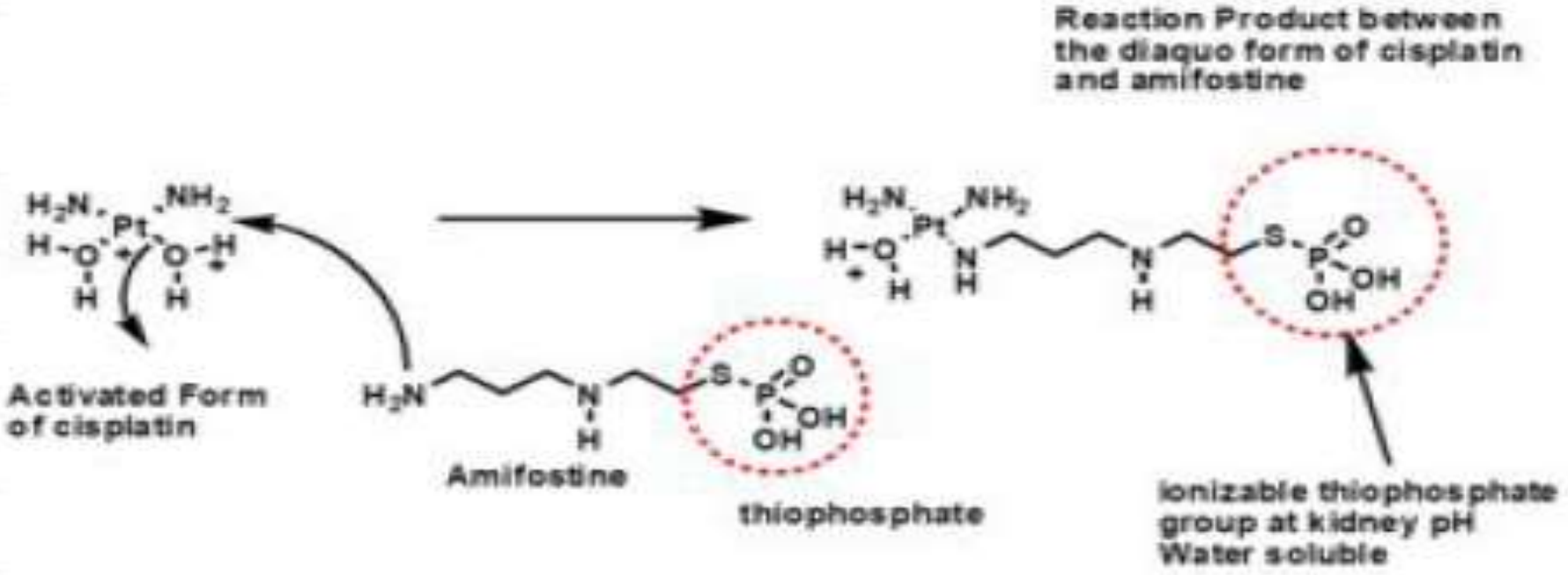


- Pt-based drug used to treat various types of cancers, including sarcomas, some carcinomas & Lymphomas and germ cell tumors.
- Contains 2 ammonia molecules & 2 chloride atoms in a cis configuration.
- Nominal solubility of cisplatin in water.
- Cisplatin is highly protein-bound.
- IN I.V avoid any thing with AL- highlyn reacting.

ADR

- Nephrotoxicity (prevented by Amifostine)
- Ototoxicity
- Peripheral neuritis
- Severe nausea and vomiting

- **t**he compound amifostine can be used to offset this Toxicity
- in the case of amifostine it reacts with platinum to form a water soluble salt that is ionized at the pH of the kidne**y**



cisplatin is highly nephrotoxic

Resistance does occur to cisplatin and may be attributed to a number of factors:

1) cisplatin requires a transporter to enter the cell and low levels of the transporter can cause resistance

2) the reactive intermediate reacts with thiols such as glutathione. thus, resistance can occur if glutathione levels are high

3) the reactive form of cisplatin is used up (reaction with biological nucleophiles) prior to its ability to react with DNA

4) an efflux transporter can pump the activated form outside the cell

5) increased DNA repair. However, a deficiency in a type of DNA repair known as mismatch repair (MMR) has also been implicated in resistance

to cisplatin and carboplatin.

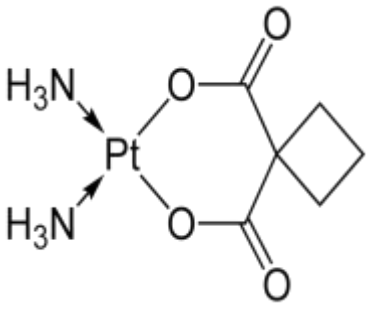
- The process of MMR involves several enzymes that are responsible for maintaining the integrity of the genome, and interest has focused on the interaction of these enzymes with repeating units found throughout DNA known as microsatellites. When MMR processes are not operating, these microsatellites may become longer or shorter and this is known as microsatellite instability.
- This can result in frame shift errors such that tumor suppressor genes may become less effective, and the tumor cells therefore fail to undergo apoptosis even if alkylation has occurred
- When there is a deficiency in the MMR enzymes, cells may be resistant to cisplatin and carboplatin because both of these agents produce the same DNA adduct

Binding of cisplatin- and carboplatin- DNA adducts by the MMR enzymes results in increased cytotoxicity of these agents.

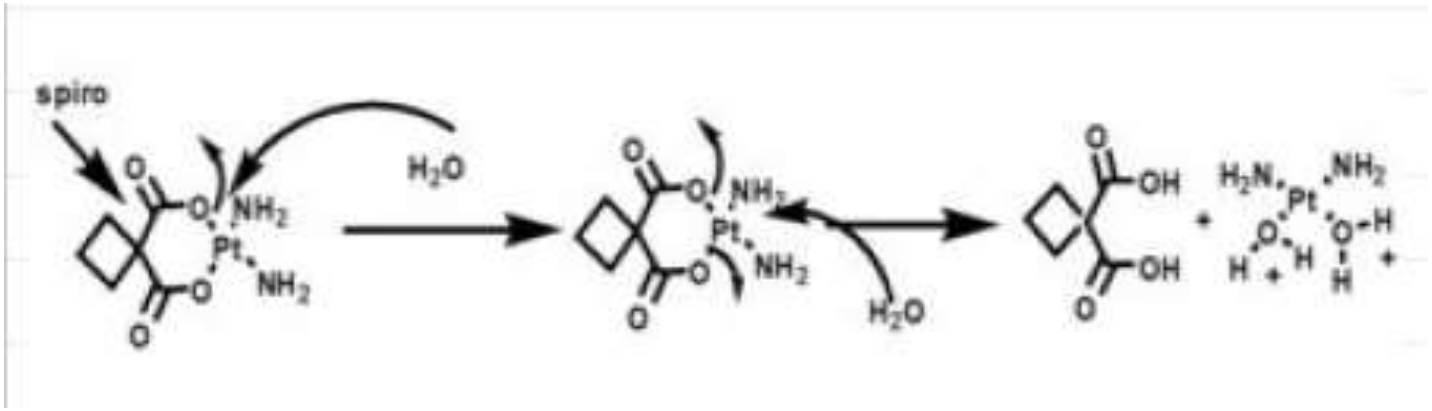
Several rationales have been put forward as to why this occurs, including the involvement of MMR enzymes in downstream signaling that activates apoptosis.

A second rationale involves the ability of MMR enzymes to remove replication errors that occur past the point of adduct formation, and in the process of removing these errors, gaps in the DNA are created, which lead to cell death.

Carboplatin

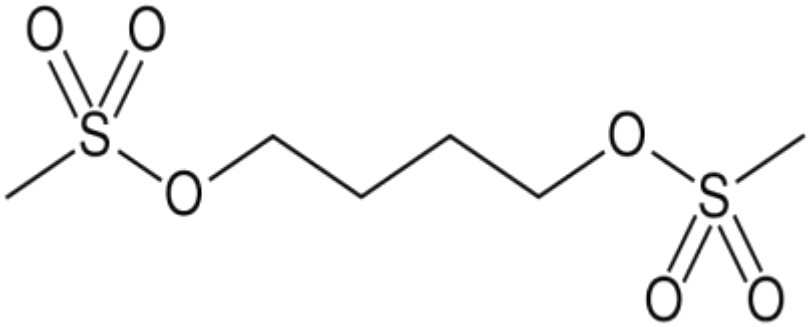


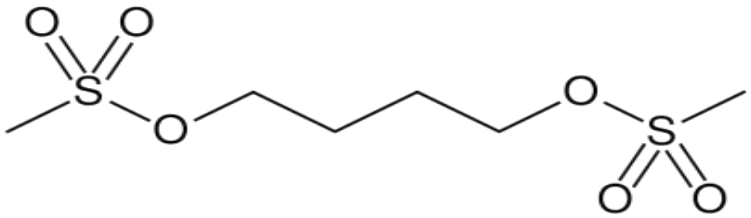
- Similar to cispaltin, cis-diamine groups & Pt II.
- Mainly for ovarian carcinoma, lung, head and neck cancers.
- It was introduced in the late 1980s and has since gained popularity in clinical treatment due to its vastly reduced side-effects compared to its parent compound cisplatin.
- less extensively bound to plasma proteins than cisplatin
- less side effects compared to cisplatin the leaving group is the oxygen atom contained on the diester function



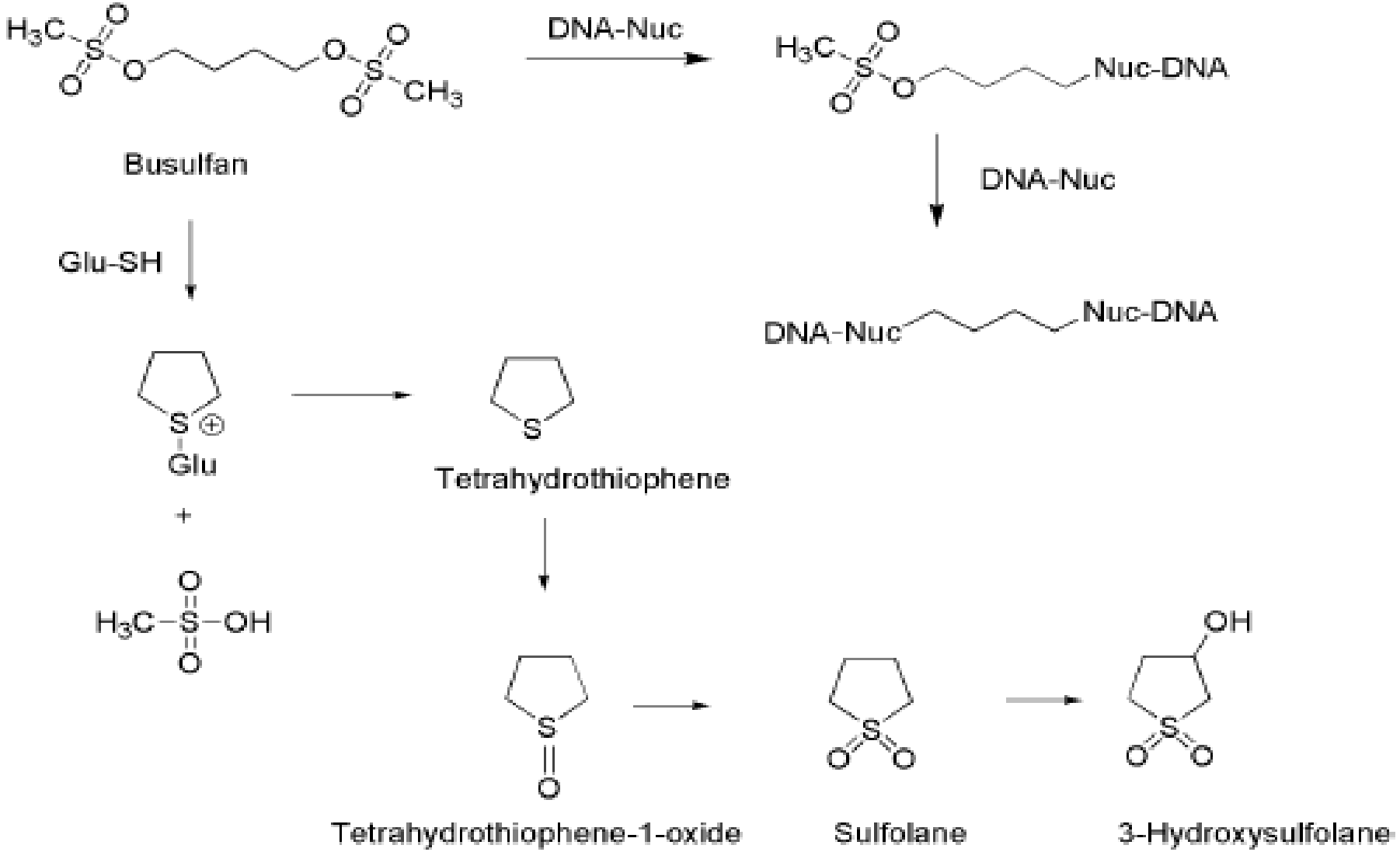
alkyl sulfonates **:Busulfan**

- A nucleophilic center (N or O)
- A cell cycle non-specific alkylating agent.
- More specifically it belongs to a subclass of alkylating agents known as alkyl sulfonates.
- It is marketed in the U.S. by GlaxoSmithKline under the brand name Myleran, and has been in clinical use since 1959. Busulfan is also available in an IV formulation



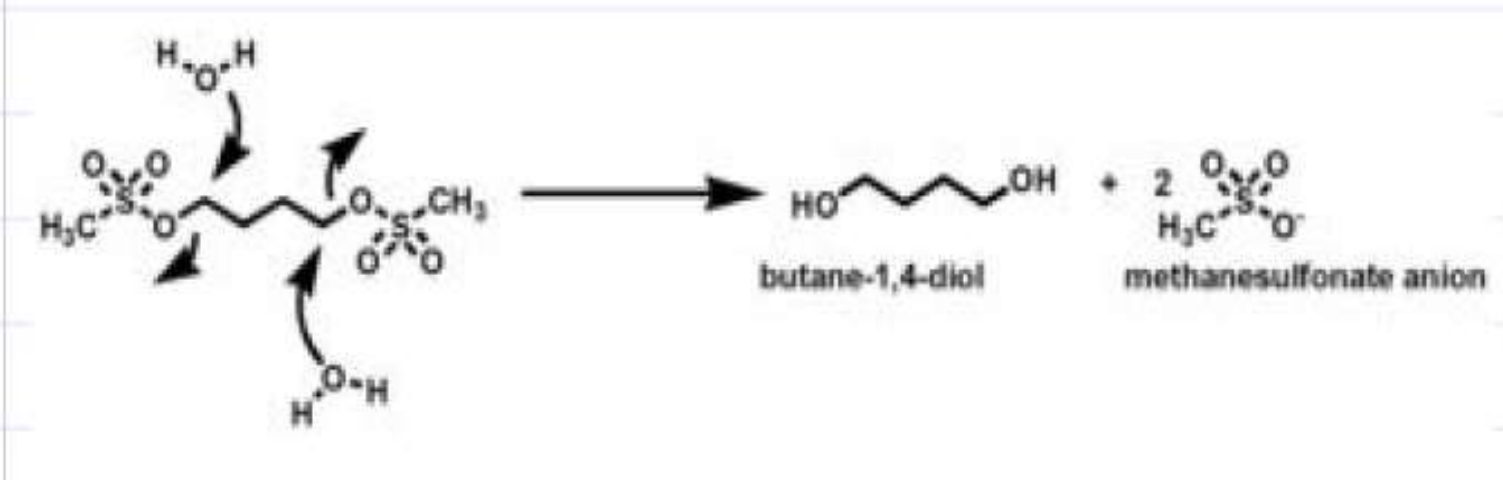


- It is a sulfonic acid ester that is an electrophile with methane sulfonic acid acting as a good leaving group, although no unchanged drug is found in the urine of patients , but most of the labeled sulfur-35 radiolabeled Busulfan was recovered in the urine as labeled methanesulfonic acid, hydrolysis or alkylation (major metabolic pathways).
- Both monoalkylation & di-alkylation of N-7 guanine of DNA.
- **Busulfan is a neutral molecule with poor water solubility .**



Scheme 10.9 • Metabolic and chemical reactions of busulfan.

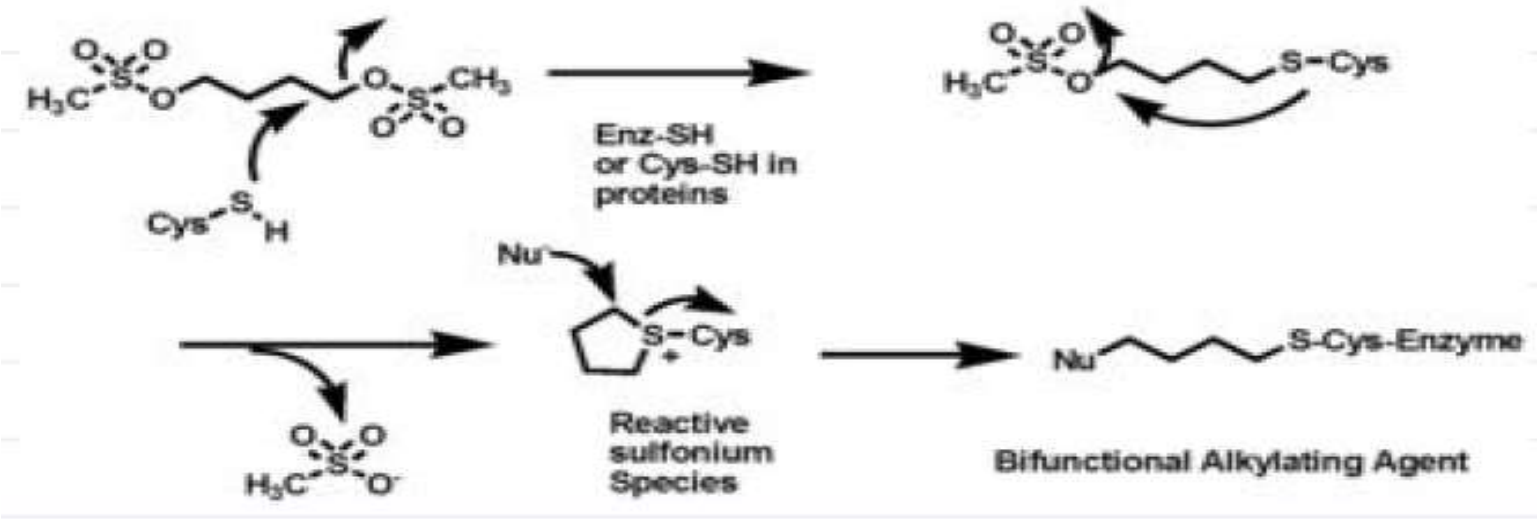
- in addition to alkylation of DNA, busulfan can undergo slow hydrolysis with water to give a diol (2 OH) which is biologically inactive
- much of busulfan is subject to sulfur stripping in that the compounds can react with endogenous SH compounds such as Cys-SH groups and glutathione:



- busulfan can undergo a process known as sulfur stripping

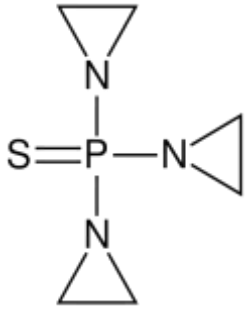
in this case, certain thiol-containing proteins displace one of the fulfonate groups

- the sulfur from thiol containing proteins then can displace the other sulfonate group
- this creates a cyclic sulfonium species (not the positive charge on sulfur) his charge can be eliminated by reaction with biological nucleophiles



aziridine-Thiotepa

- Thiotepa containing the thiophosphoramide functionality
- Thiotepa incorporates a less reactive aziridine ring compared with that formed in mechlorethamine.
- The adjacent thiophosphoryl is electron withdrawing and, therefore, reduces the reactivity of the aziridine ring system.
- Although thiotepa is less reactive than many other alkylating agents, it has been shown to form cross-links
- At acidic pH, the aziridine group is protonated to give the aziridinium ion that is known to alkylate DNA.

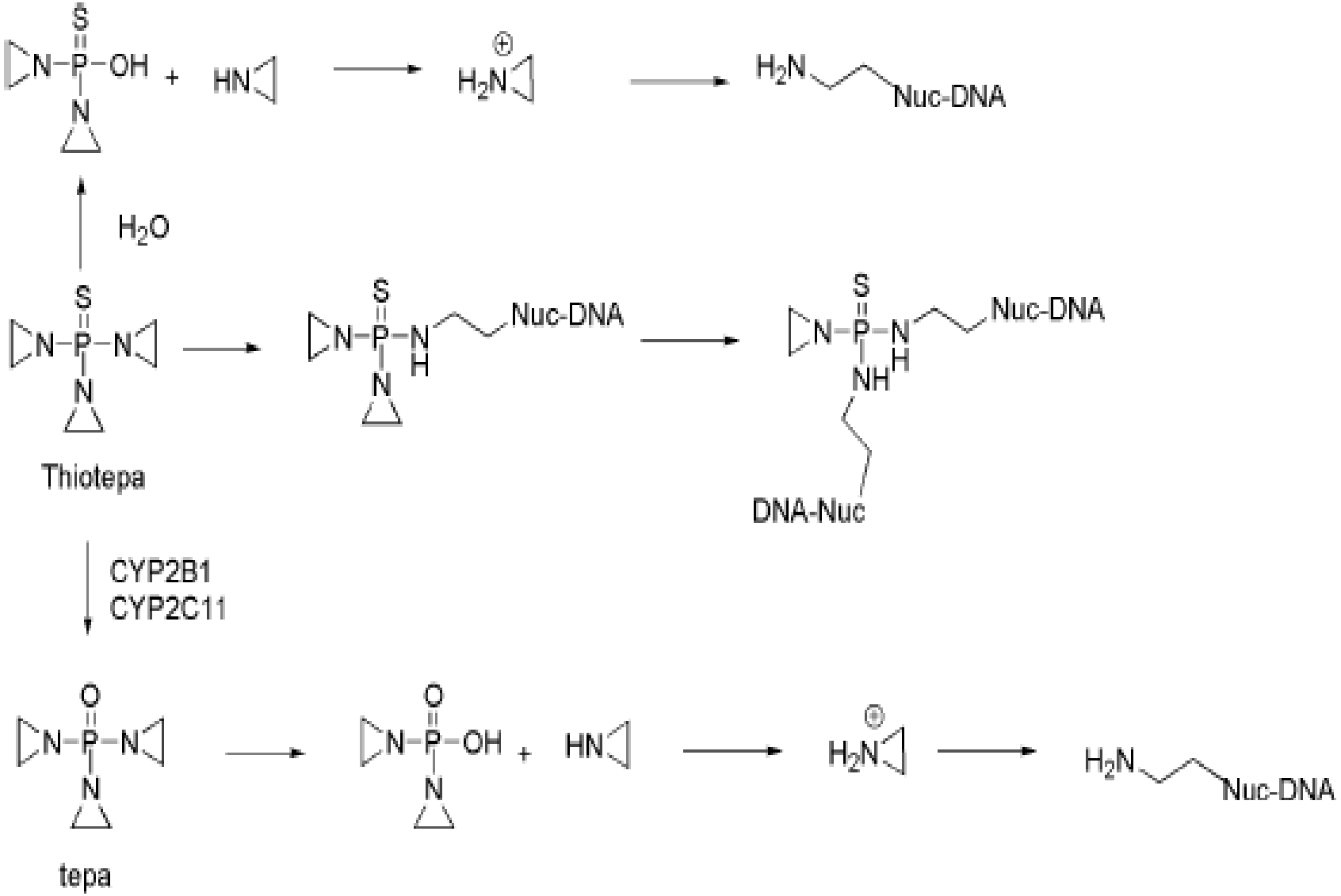


- This is believed to occur by sequential reactions of thiotepa itself with DNA (Scheme 10.8).
- Monoalkylation is also possible as a result of aziridine formation via hydrolysis of thiotepa.
- Thiotepa is also metabolized by oxidative desulfurization mediated by CYP2B1 and CYP2C11.
- The decreased stability of the resulting TEPA undergoes hydrolysis to give aziridine, which may function to monoalkylate DNA

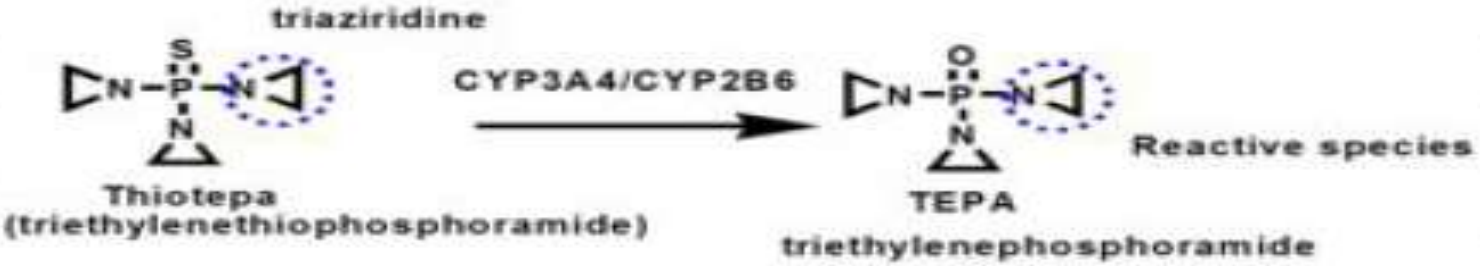
- The conclusion that aziridine is the active alkylating agent

once thiotepa has been converted to TEPA is based on the fact that when TEPA is incubated with DNA, no crosslinks are formed and only monoadducts are generated.

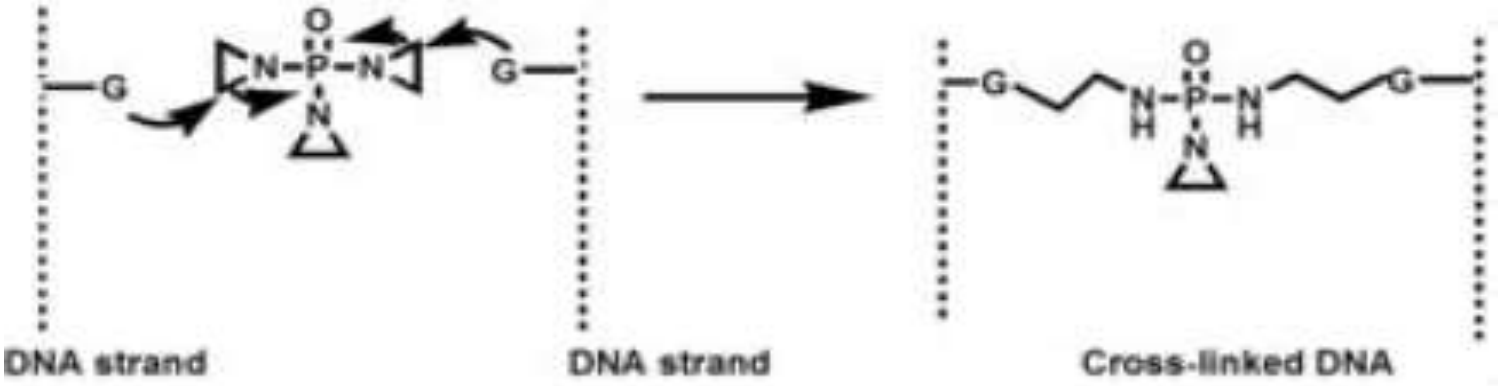
- The reactivity of aziridine generated by either route may be somewhat enhanced within cancer cells, where the pH is normally reduced 0.2 to 0.4 pH units resulting in an increase in reactivity toward nucleophilic attack.



Scheme 10.8 • Metabolic and chemical interactions of thiotepa with DNA.



MUST BE ACTIVATED BY OXIDATIVE DESULFURATION



Toxicity

- Bone marrow depression, with leukopenia
- *Cyclophosphamide/Ifosfamide* - hemorrhagic cystitis
  - Reduced by coadministration with MESNA
- *Cisplatin/Carboplatin* - ototoxic and nephrotoxic
  - Nephrotoxicity reduced by chloride diuresis and hydration

Major Clinically Useful Alkylating Agents

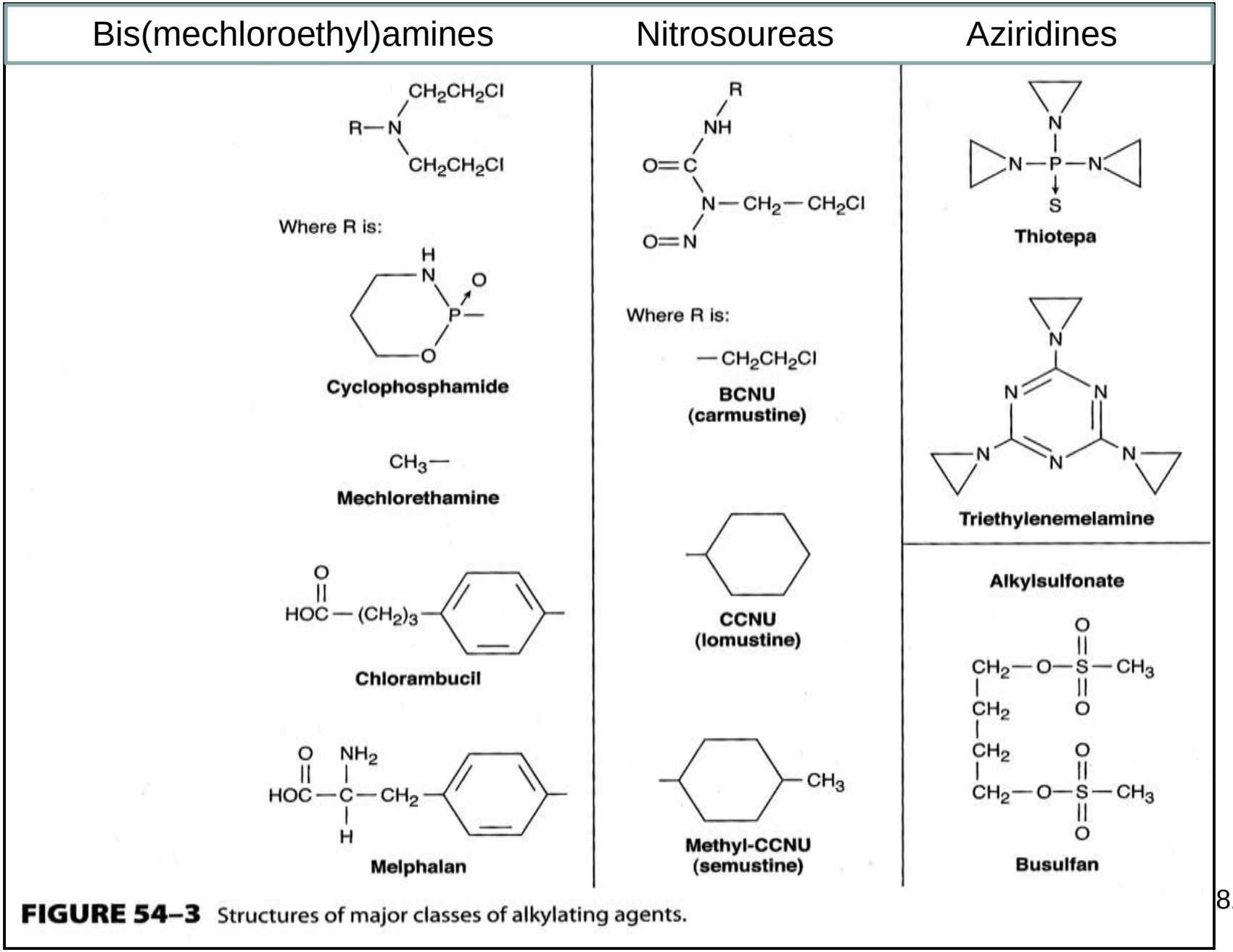


FIGURE 54–3 Structures of major classes of alkylating agents.

A. Alkylating agents

	1. Mechanism of Action	2. Clinical application	3. Route	4. Side effects
a. Nitrogen Mustards				
A. Mechlorethamine	DNA cross-links, resulting in inhibition of DNA synthesis and function	Hodgkin's and non-Hodgkin's lymphoma	Must be given Orally	Nausea and vomiting, decrease in PBL count, BM depression, bleeding, alopecia, skin pigmentation, pulmonary fibrosis
B. Cyclophosphamide	Same as above	Breast, ovarian, CLL, soft tissue sarcoma, WT, neuroblastoma	Orally and I.V.	Same as above
C. Chlorambucil	Same as above	Chronic lymphocytic leukemia	Orally effective	Same as above
D. Melphalan	Same as above	Multiple myeloma, breast, ovarian	Orally effective	Same as above
E. Ifosfamide	Same as above	Germ cell cancer, cervical carcinoma, lung, Hodgkins and non-Hodgkins lymphoma, sarcomas	Orally effective	Same as above

A. Alkylating agents

	1. Mechanism of Action	2. Clinical application	3. Route	4. Side effects
b. Alkyl Sulfonates				
A. Busulfan	Atypical alkylating agent.	Chronic granulocytic leukemia	Orally effective	Bone marrow depression, pulmonary fibrosis, and hyperuricemia

c. Nitrosoureas	**1. Mechanism of Action**	**2. Clinical application**	**3. Route**	**4. Side effects**
A. Carmustine	DNA damage, it can cross blood-brain barrier	Hodgkins and non-Hodgkins lymphoma, brain tumors, G.I. carcinoma	Given I.V. must be given slowly.	Bone marrow depression, CNS depression, renal toxicity
B. Lomustine	Lomustine alkylates and crosslinks DNA, thereby inhibiting DNA and RNA synthesis. Also carbamoylates DNA and proteins, resulting in inhibition of DNA and RNA synthesis and disruption of RNA processing. Lomustine is lipophilic and crosses the blood-brain barrier	Hodgkins and non-Hodgkins lymphoma, malignant melanoma and epidermoid carcinoma of lung	Orally effective	Nausea and vomiting, Nephrotoxicity, nerve dysfunction
C. Streptozotocin	DNA damage	pancreatic cancer	Given I.V.	Nausea and vomiting, nephrotoxicity, liver toxicity

A. Alkylating agents

d. Ethylenimines	**1. Mechanism of Action**	**2. Clinical application**	**3. Route**	**4. Side effects**
A. Triethylene thiophosphoramide (Thio-TEPA)	DNA damage, Cytochrome P450	Bladder cancer	Given I.V.	Nausea and vomiting, fatigue
B. Hexamethylmelamine (HMM)	DNA damage	Advanced ovarian tumor	Given orally after food	Nausea and vomiting, low blood counts, diarrhea

d. Triazenes	**1. Mechanism of Action**	**2. Clinical application**	**3. Route**	**4. Side effects**
A. Dacarbazine (DTIC)	Blocks, DNA, RNA and protein synthesis	Malignant Melanoma, Hodgkins and non-Hodgkins lymphoma	Given I.V.	Bone marrow depression, hepatotoxicity, neurotoxicity, bleeding, bruising, blood clots, sore mouths.

Summary

A. Alkylating agents

1. Interfere with cell division in all rapidly proliferating tissues

2. Most susceptible tissues are hematopoietic and GI epithelium

3. Mechanism of action: react with body fluid to form carbonium ions which bind guanine residue of DNA; such binding could result in:

   a. Miscoding of DNA
   b. Imidazole ring cleavage
   c. Excision of guanine residue producing DNA chain scission
   d. Cross linkages between DNA strands; this effect thought to be primary mode of cytotoxic action

4. Development of resistance possibly due to

   a. Decreased cellular permeability
   b. Production of substances which compete with DNA for alkylation
   c. Increased rate of DNA repair

5. Toxic side effects

   a. Bone marrow depression
   b. Nausea and vomiting especially when given I.V.
   c. Can get additive effects when used with ionizing radiation and antimetabolites