Biochemistry - II

DNA Synthesis - 1

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Oligonucleotide Synthesis

Why do we need oligonucleotides ??

- **Variety of application in Molecular medicine & biology**
  - **Synthesis of artificial genes**
  - **To be used as anti-sense nucleotides**
  - **Targeted introduction of mutations**

Oligonucleotide Synthesis

What is oligonucleotide synthesis ??

- ***Chemical synthesis of "short fragments" of nucleic acid***
- ***We can define the nucleic acid sequence***
- ***Provides rapid, inexpensive method of custom made oligonucleotides***
- ***Process implemented as "Solid Phase synthesis"***

Solid Phase Synthesis

What is Solid Phase synthesis ??

- ***organic synthesis on solid support***
- ***Solid support held between filters, in columns to enable reagents, solvents to pass freely***
- **building blocks/Reactants are protected at all reactive functional groups**
- **Functional group that need to react are selectively 'de-protected"**
- **Method widely used in peptide , DNA synthesis**

Solid Phase Synthesis

Advantages of Solid Phase synthesis

- ***large excesses of solution-phase reagents can be used to drive reactions quickly to completion***
- ***impurities and excess reagents are washed away and no purification is required after each step***
- ***the process is amenable to automation on computer-controlled solid-phase synthesizers.***

Solid Phase Synthesis

Types of Solid Support

- **Controlled-pore glass (CPG)**
  - Rigid and non-swelling with deep pores in which oligonucleotide synthesis takes place
- **Polystyrene (PS)**
  - Highly cross-linked polystyrene beads

DNA Synthesis

- Naturally occurring nucleotides insufficiently reactive to give oligonucleotides in high yields
- The selectivity and the rate of the formation of internucleosidic linkages is improved by using 3'-*O*-(*N*,*N*-diisopropyl phosphoramidite) derivatives of nucleosides (nucleoside phosphoramidites)
- These act as building blocks

DNA Synthesis

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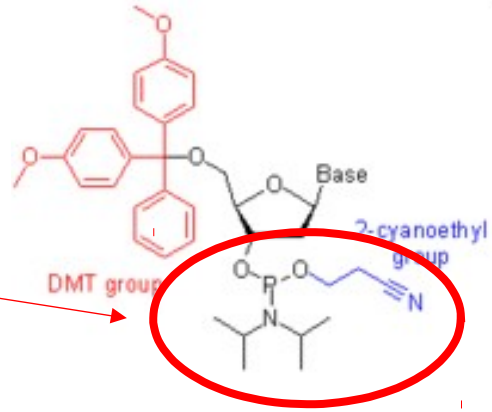
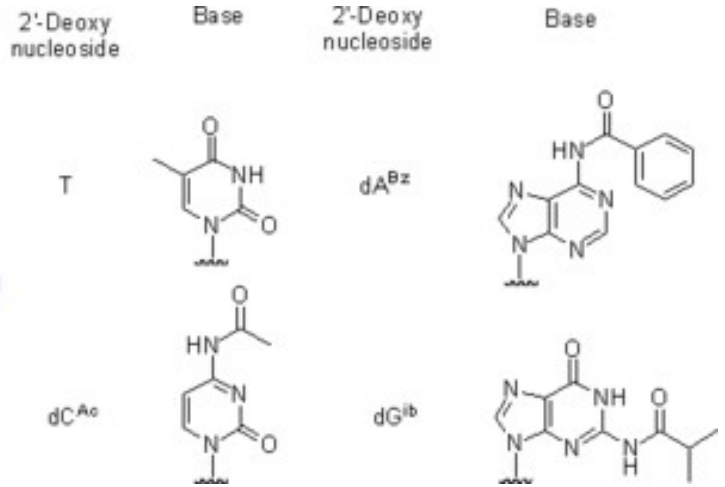
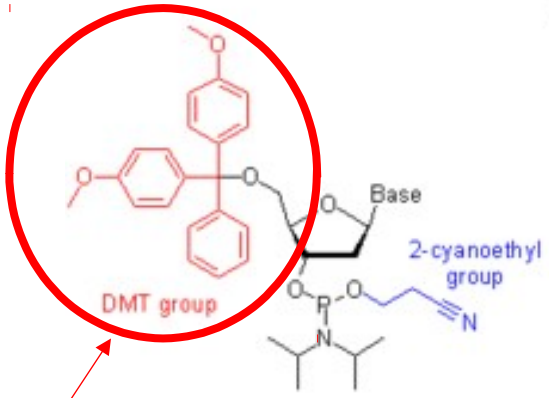
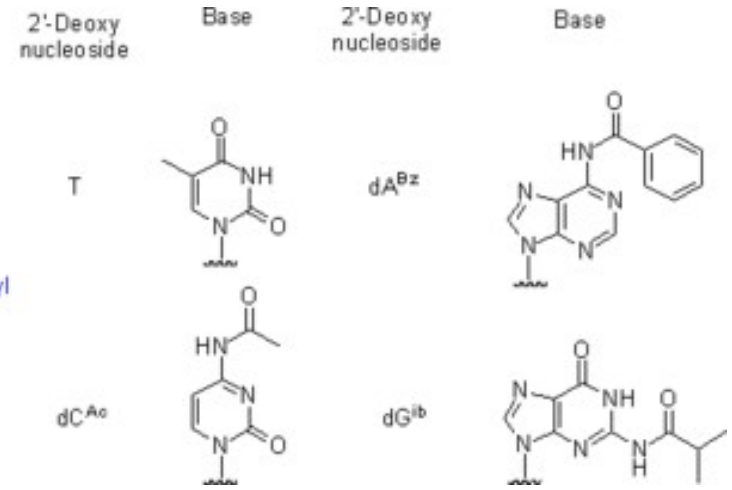




DNA Synthesis

- To prevent undesired side reactions, all other functional groups present in nucleosides have to be protected
- Upon the completion of the oligonucleotide chain assembly, <u>all the protecting groups are removed</u> to yield the desired oligonucleotides
- The 5'-hydroxyl group is





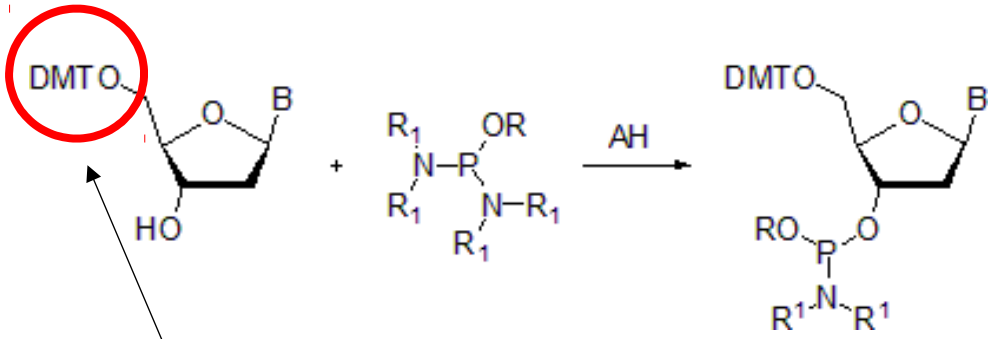
DNA Synthetic Cycle

- Oligonucleotide synthesis is carried out by a stepwise addition of nucleotide residues
- Residues added to 5'-terminus of the growing chain until the desired sequence is assembled

- Each addition is referred to as a synthetic cycle

DNA Synthetic Cycle

- Synthesis of Nucleoside Phophoramidite (building block) :



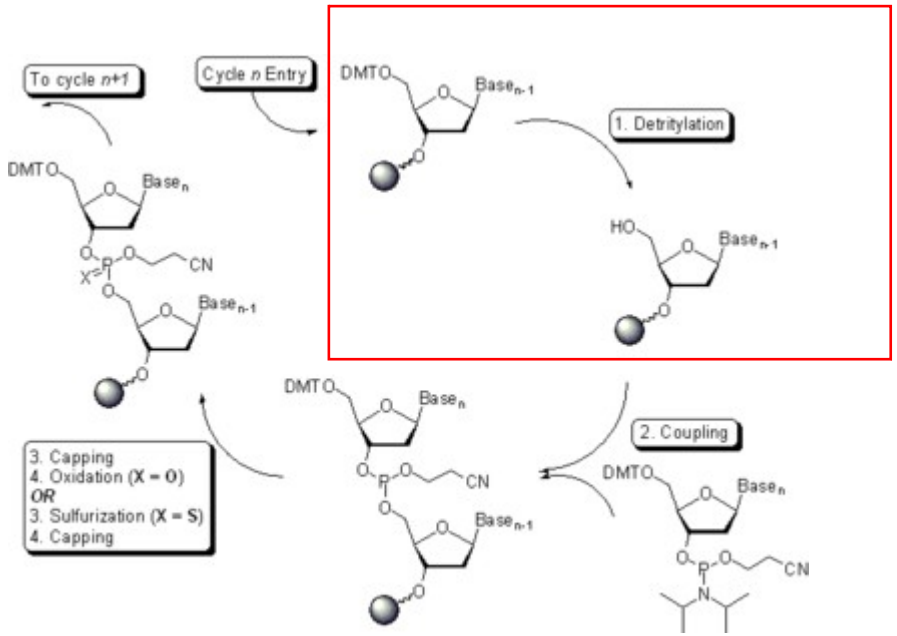
Treatment of a protected nucleoside bearing a single free hydroxy group with phosphorodiamidite under the catalytic action of a weak acid

DNA Synthetic Cycle

- <u>STEP 1 : De-blocking (detritylation)</u>

1. **The DMT group is removed with a solution of an acid, such as 2% trichloroacetic acid (TCA) or 3% dichloroacetic acid (DCA), in an inert solvent (dichloromethane or toluene)**

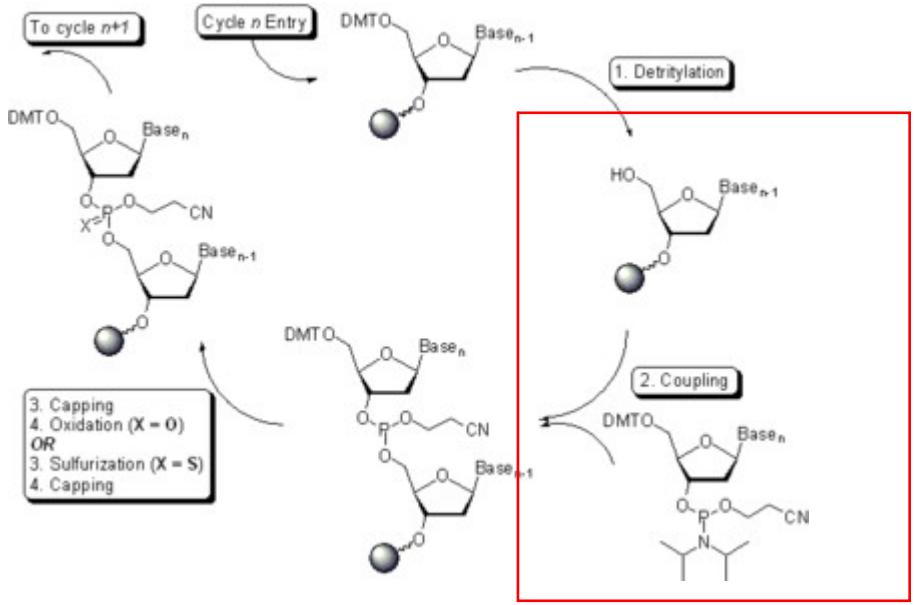
2. **The step results in the solid support-bound oligonucleotide precursor bearing a free 5'-terminal hydroxyl group**



DNA Synthetic Cycle

- <u>STEP 2 : Coupling</u>

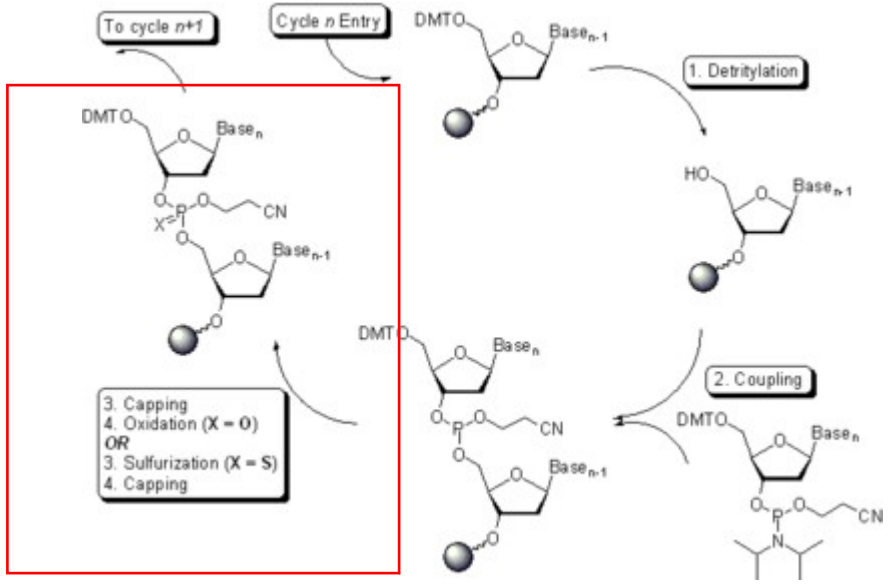
1. **Nucleoside phosphoramidite is activated**
2. **Activated Nucleoside phosphoramidite brought in contact with solid bound oligonucleotide**
3. **Coupling occurs at 5-OH group forming phosphite trimester linkage**



DNA Synthetic Cycle

- <u>STEP 3 : Oxidation</u>

1. **The newly formed tricoordinated phosphite triester linkage is not natural and is of limited stability**
2. **Oxidize the phosphite triester into a tetracoordinated phosphate trimester to make it stable**
3. **Oxidation done by iodine and water in the presence of a weak base**



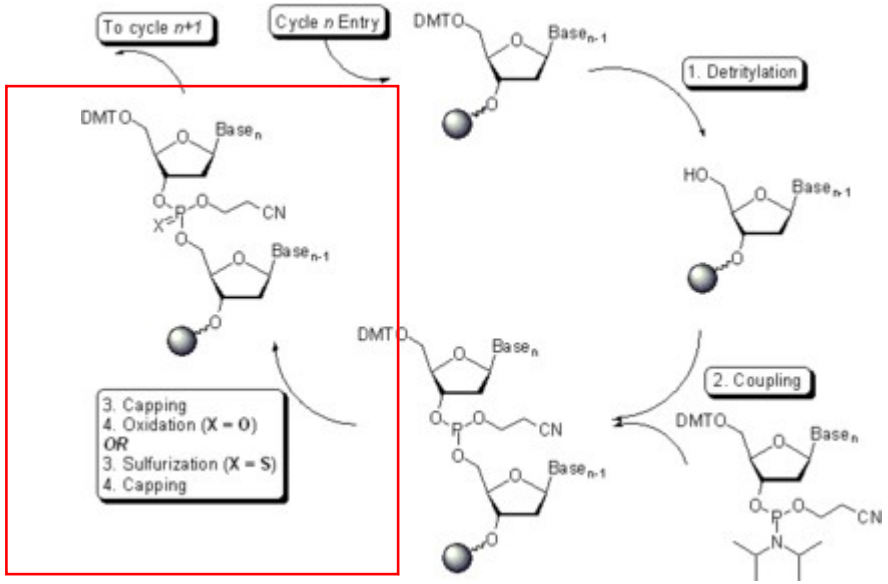
DNA Synthetic Cycle

- <u>STEP 4 : Post Synthetic Processing</u>

1. **After the completion of chain assembly, solid bound oligo-nucleotide needs to be De-protected**

2. **<u>Removal of DMT-</u>**
   Deprotected with trichloroacetic acid in dichloromethane

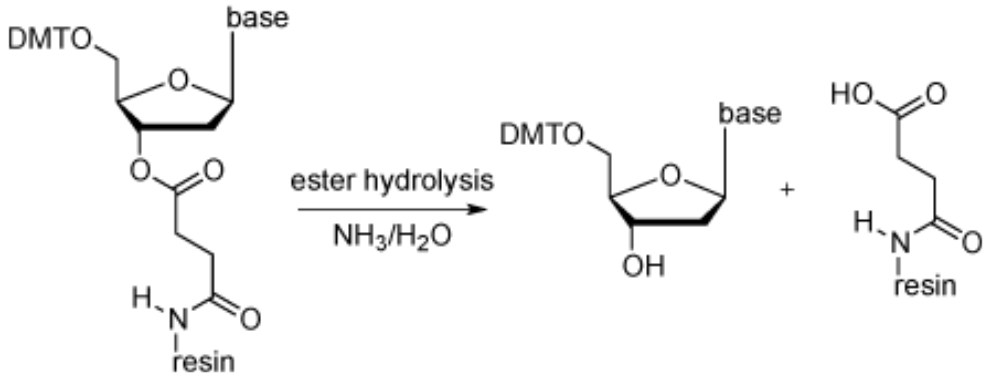
3. **<u>Removal of Phosphate protecting group:</u>**
   Oligonucleotides removed from solid assembly
   deprotected by treatment with *aqueous ammonium hydroxide, aqueous*



DNA Synthetic Cycle

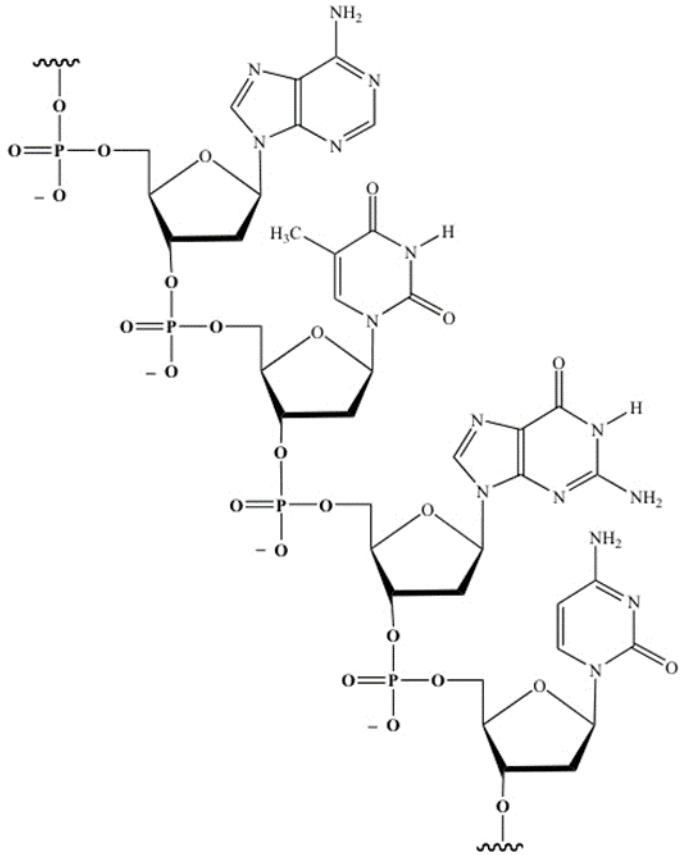
- <u>STEP 4 : Post Synthetic Processing</u>

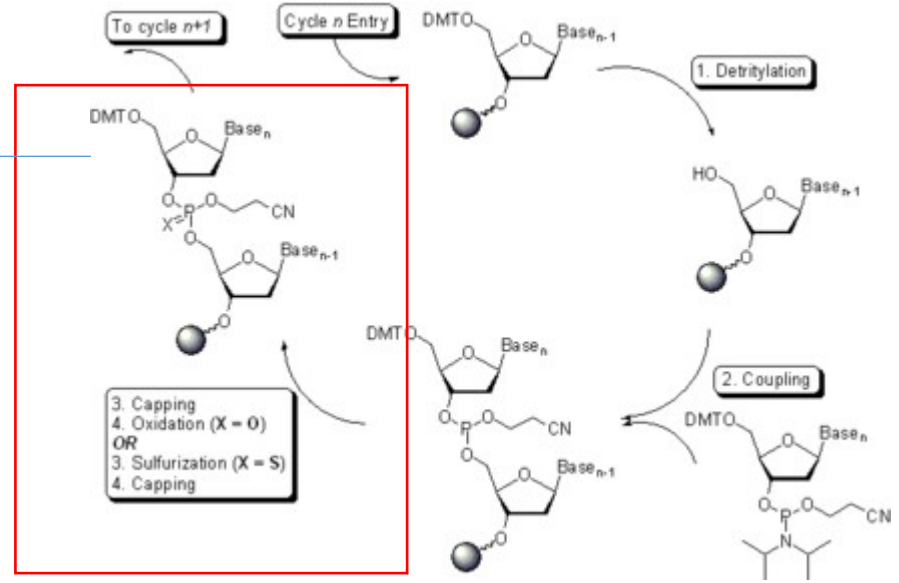
1. **After the completion of chain assembly, solid bound oligo-nucleotide needs to be removed from solid support**
2. **The linker is the chemical entity that attaches the 3′-end of the oligonucleotide to the solid support**
3. **The linker used most frequently in oligonucleotide synthesis is the succinyl linker**



DNA Synthetic Cycle

- STEP 4 : Post Synthetic Processing

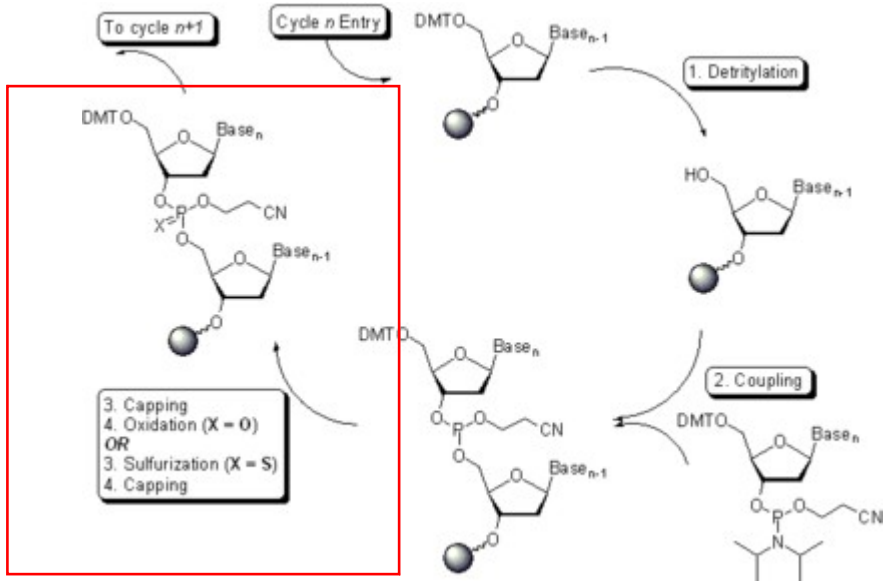




DNA Synthetic Cycle

- <u>STEP 4 : Post Synthetic Processing</u>

1. **The fully deprotected product is used as is, or the desired oligonucleotide can be purified by a number of methods.**



Drugs modulating DNA Replication Pathway

DNA Polymerase Inhibitors

Drugs	Mechanism of Action	Therapeutic Implications
Acyclovir (Acy)	Guanine nucleoside (lacking a cyclic sugar) that is selectively phosphorylated by viral thymidine kinases and selectively incorporated into DNA by viral DNA polymerases. This terminates further extension of the DNA strand. Competitive inhibition of viral DNA polymerase	Anti-viral Drug Herpes Simplex Virus 1 and 2 infections; Chicken pox, Shingles
Gemcitabine (dFdC)	nucleoside analog that is incorporated into replicating DNA, resulting in partial chain termination and stalling of replication forks.	Anti-Cancer Drug Pancreatic, lung and bladder Cancers

Drugs modulating DNA Replication Pathway

DNA Topoisomerase Inhibitors

Drugs	Mechanism of Action	Therapeutic Implications
Camptothecin (CPT)	five-membered ring alkaloid that traps topoisomerase I-DNA complexes. This causes protein-linked DNA single-strand breaks and replication double-strand breaks	Colon, lung and ovarian carcinomas; Leukemia and gastric cancer
Doxorubicin (Dox) Daunorubicin	Anthracycline that intercalates into DNA and traps topoisomerase II-DNA complexes. Inhibits Topoisomerase enzyme.	Breast cancer, Lymphomas Leukemia

Drugs modulating DNA Replication Pathway

DNA Telomerase Inhibitors

Drugs	Mechanism of Action	Therapeutic Implications
Camptothecin (CPT)	five-membered ring alkaloid that traps topoisomerase I-DNA complexes. This causes protein-linked DNA single-strand breaks and replication double-strand breaks	Colon, lung and ovarian carcinomas; Leukemia and gastric cancer
Doxorubicin (Dox) Daunorubicin	Anthracycline that intercalates into DNA and traps topoisomerase II-DNA complexes. Inhibits Topoisomerase enzyme.	Breast cancer, Lymphomas Leukemia