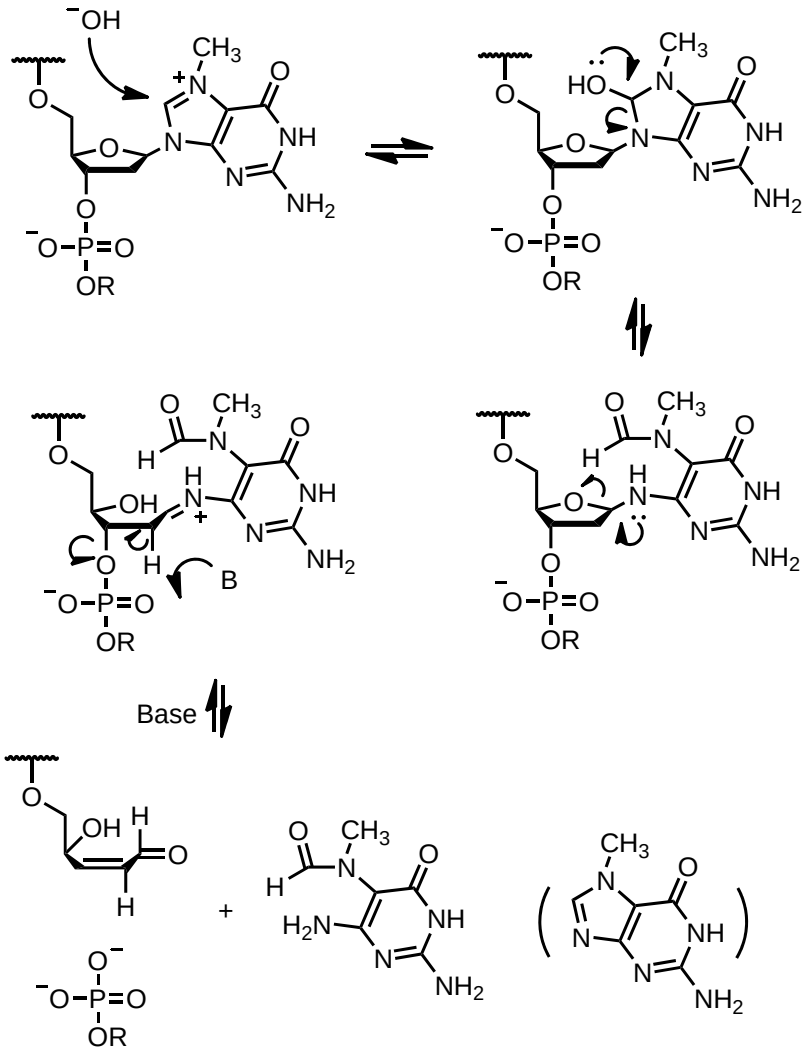
DNA modifications

-Recall from last lecture alkylation by mustards can lead to tautomerization and mispairing

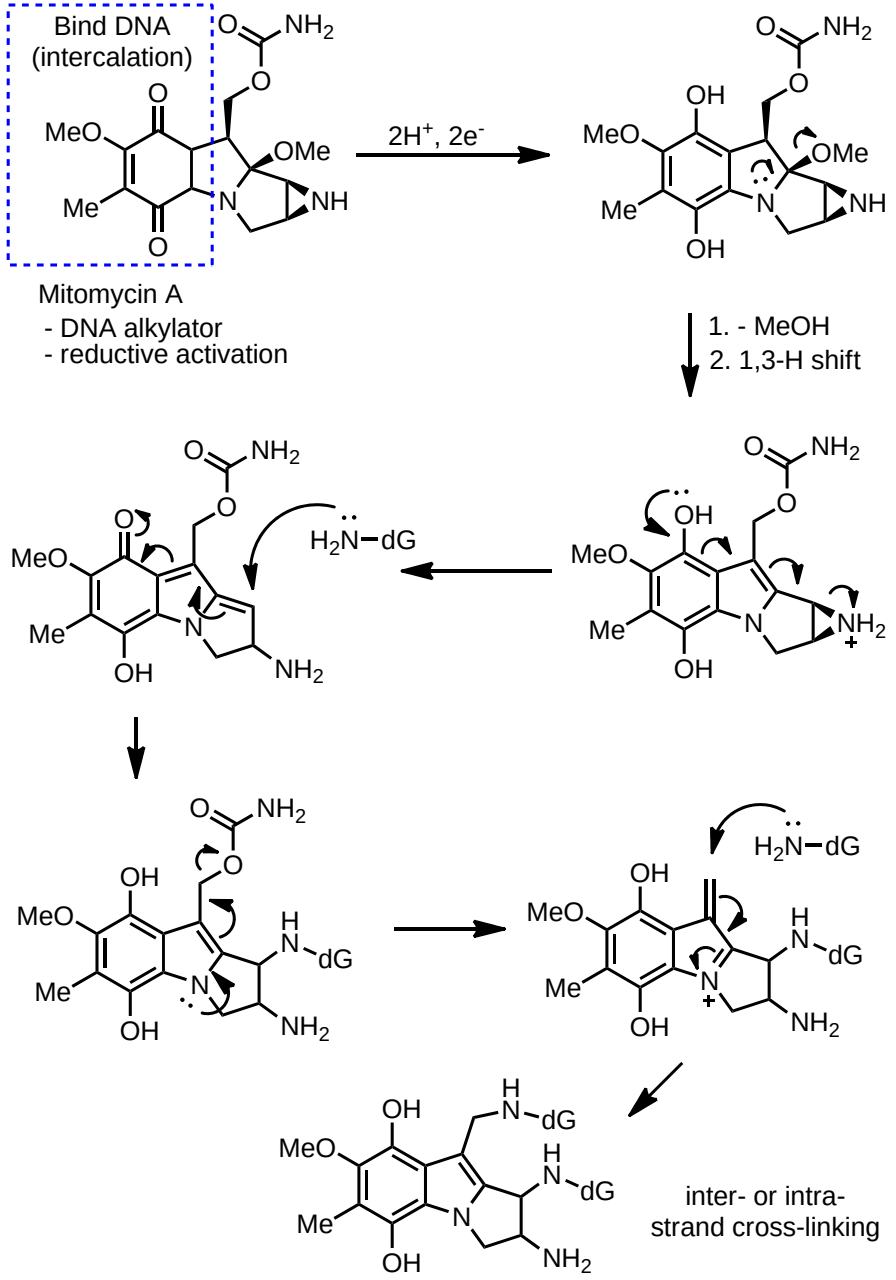
-Alkylation by mustards can also lead to interstrand crosslinking which blocks transcription and replication

-Alkylation at N7 can also lead to backbone cleavage

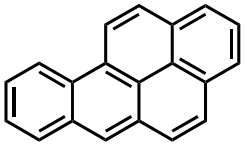


DNA crosslinking molecules

Mitomycin A

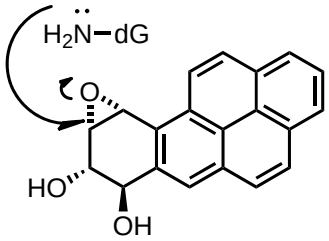


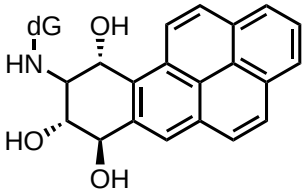
DNA crosslinking molecules



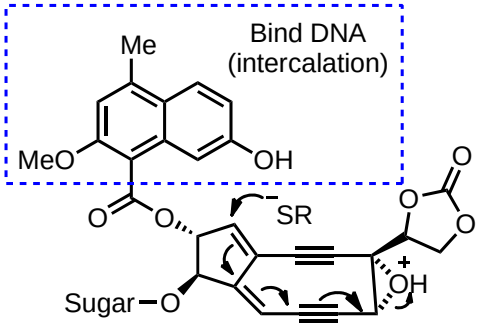
Benzopyrene
- activated by p450 (Cyp1A1)
- from fuel, cigarette smoke, burnt toast
- intercalates into DNA

epoxide hydrolase

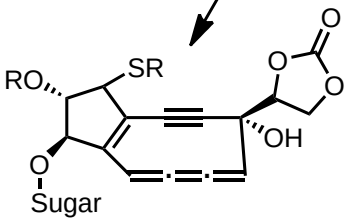




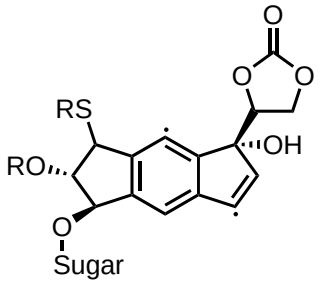
Enediyne (Neocarzinostatin)



Enediyne (Neocarzinostatin)
- extremely toxic
- intercalates into DNA
- bind carrier protein

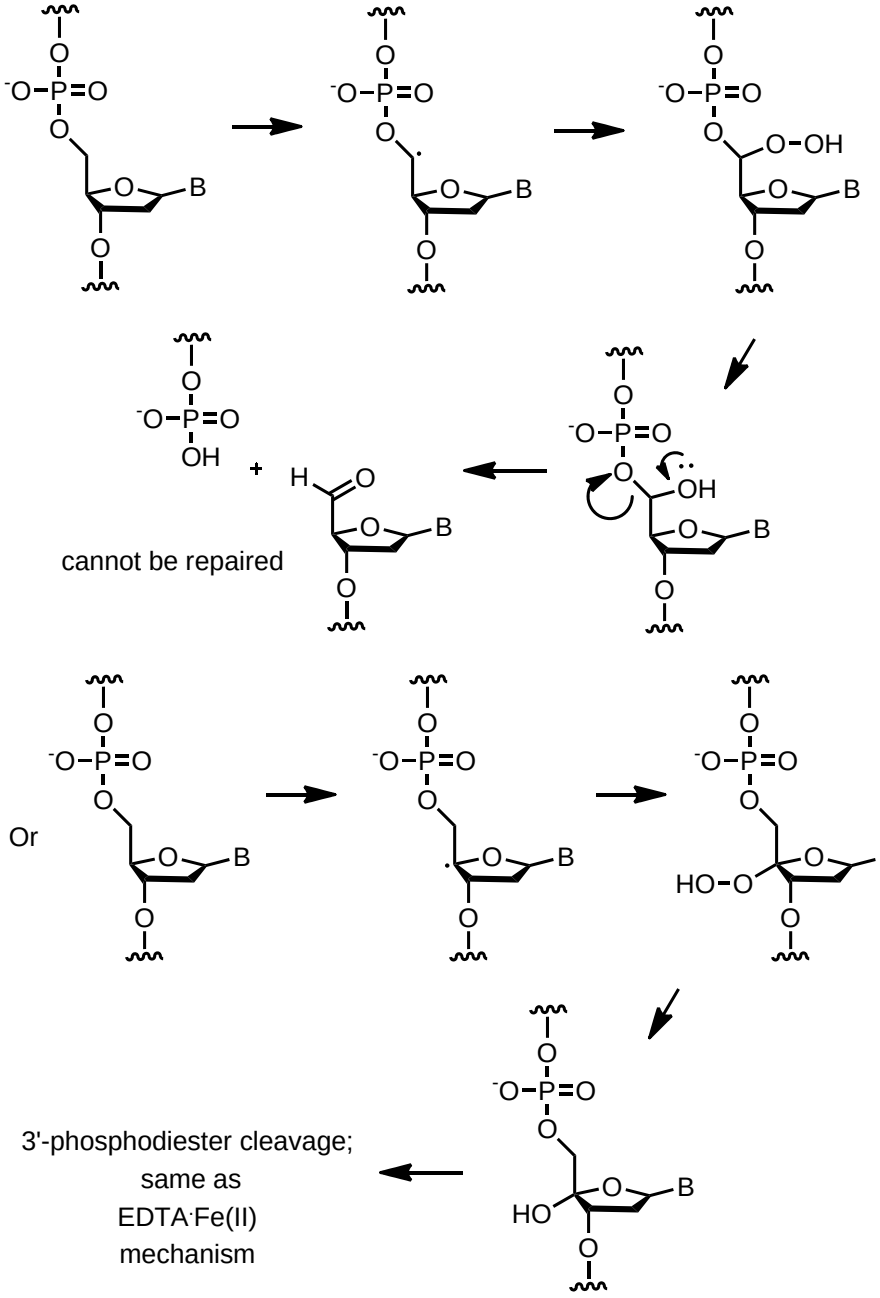


Bergman Cyclization



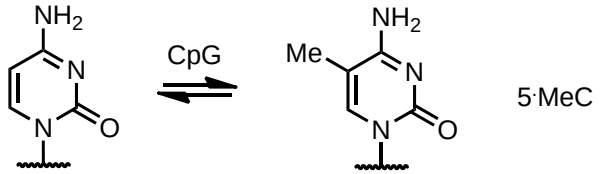
phenyl radical
110 kcal/mol (C-H bond)

Antibiotics (function by modify DNA)

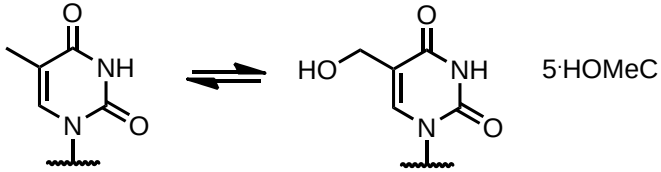


Modification of DNA (Enzyme mediated)

-Epigenetic modifications passed on through multiple rounds of cell divisions; most common is 5-methyl-C



-modifcation of DNA causes silencing/activation of gene expression
-DNA methyl transferases (DNMT) modify hemimethylated DNA
-also 5-hydroxymethylcytosine found in brain



Modification of histone proteins

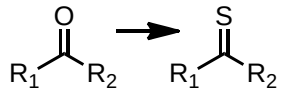
HAT histone acetyl transferase

K14/K9 H3 ⟶ ε-N-Ac-Lys

-neutralize charged lysine (structural effects on chromatin)
-bromodomain of transcription factors binds to ε-N-Ac-Lys (popular drug target) and recruits protein TFs and cofactors

RNA modifcations

-RNA is a subject to many base modifications
-alkylate exocyclic NH_2 of A/G/C
-alkylate ring N of N1A, N7G, N3C
-

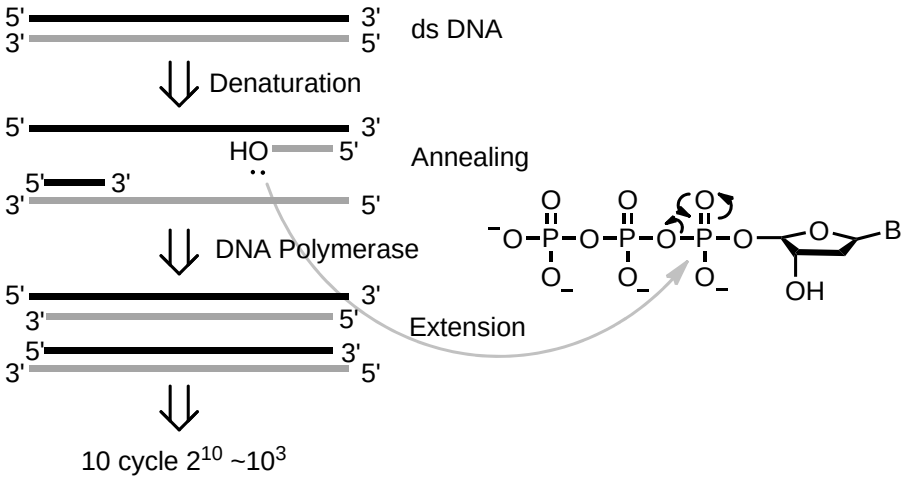


-saturate C(5)-C(4)
-glycosylate (allow phage to identify *E. coli* genome from phage genome.

Tools for DNA

-Synthesize
-Sequence
-Amplify (PCR)

Polymerase Chain Reaction (PCR)

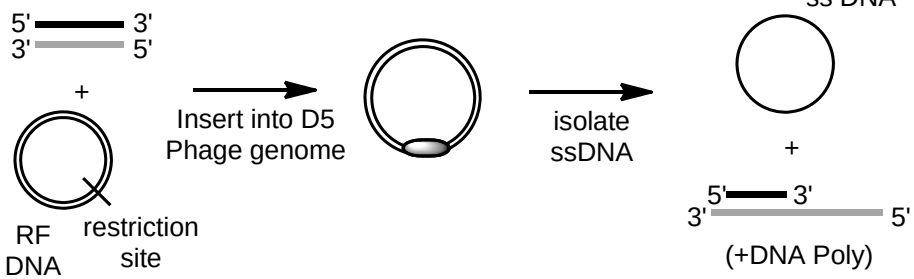


Sequencing of DNA

Chemical sequencing

-A/G/C/T specific reactions enable specify DNA modification to identify A/G/T/C

Sanger sequencing



-M13 phage has ssDNA form
-RF (replicated form) ds DNA
-packaged form ssDNA

Lecture 4- Nucleic Acid Structure

Sequencing of DNA

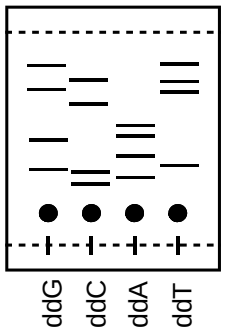
Sanger sequencing uses dideoxy chain terminators

Find G: add dNTPs (A/G/C/T) + PPPO–R (ddGTP)

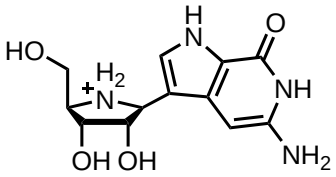
5' C^{dd}G
3' GCATGC 5'

5' CGTAC^{dd}G
3' GCATGC 5'

.....



Drug for Ebola

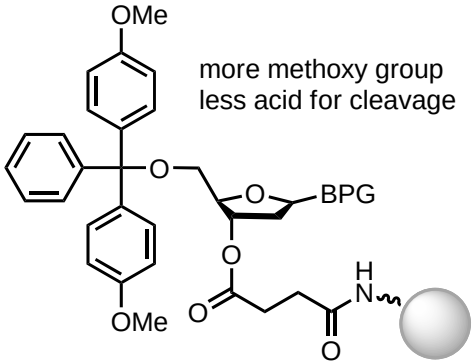


Ebola RNA polymerase will take it as a substrate
Human RNA polymerase will not take it

DNA synthesis

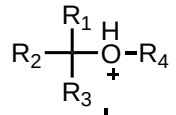
-phosphotriester
-phosphoramidite

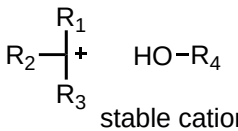
Solid-phase synthesis-rapid way to purify reactants from products



more methoxy group
less acid for cleavage

Acid labile
DMT dimethoxytrityl



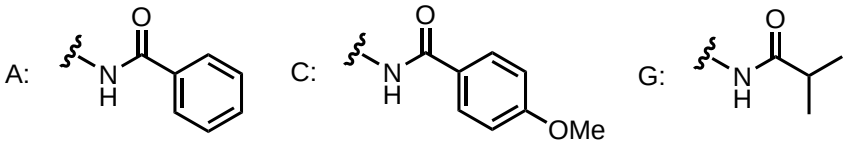


stable cation

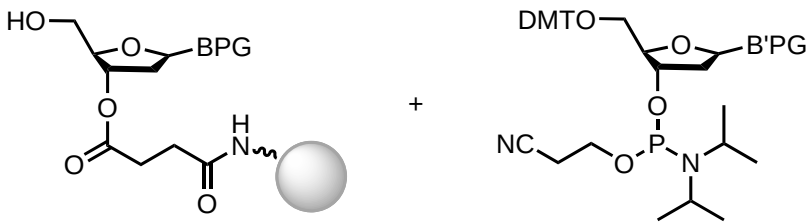
DNA synthesis (continue)

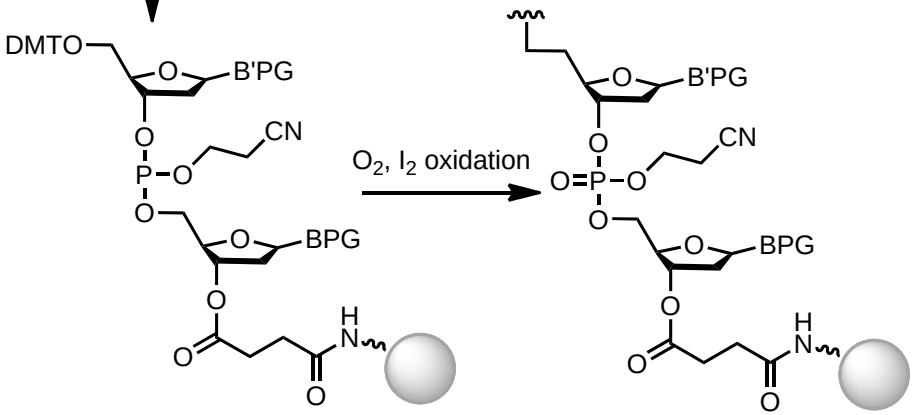
Base labile

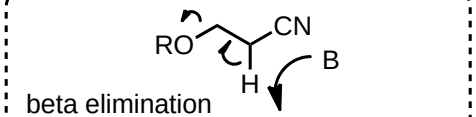
A: C: G:



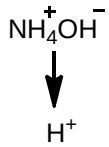
Acid Label/Base Label-orthogonal protecting groups







beta elimination
to avoid phosphodiester bond cleavage



1. H+ deprotection
2. coupling
3. solid phase purification