Nucleic Acids

1869: Miescher- gelatinous material from cell nuceli of white blood cells containing organophosphorous compounds- nuclein (chromatin)- discovery of nucleic acids

1891: Kossel- identified the DNA bases A, T, G and C
identified D-ribose in nucleic acids

1889: Altman- purified DNA

1901: Ascoli- identified U in RNA

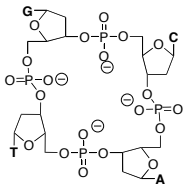
1910: DNA and RNA realized to be separate entities. DNA (thymus) RNA (yeast)

1929: Levene & Jacobs- identified 2'-deoxyribose in DNA

1920's - 1950's: structures of nucleosides and nucleotides. Alexander Todd (Nobel Prize, 1959)

1928: Griffith- first to propose that DNA was genetic material; not widely accepted

1931: Levine & Bass- first proposed structure. Believed to be part of chromosome physiology, but NOT genetic material



1944: Avery, MacLeod & McCarty- Strong evidence that DNA is genetic material

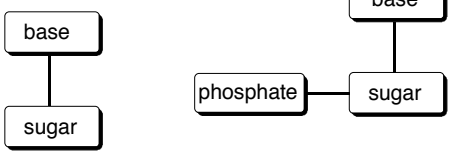
1950: Chargaff- careful analysis of DNA from a wide variety of organisms. Content of A,T, C & G varied widely according to the organism, however: **A=T** and **C=G** (**Chargaff' Rule**)

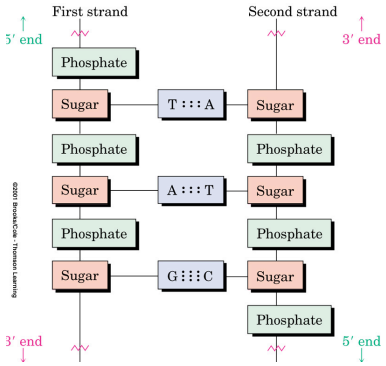
1953: Watson & Crick- structure of DNA (Nobel Prize with M. Wilkens, 1962)

241

Nucleoside, nucleotides and nucleic acids

Blackburn *et al*. Ch. 2

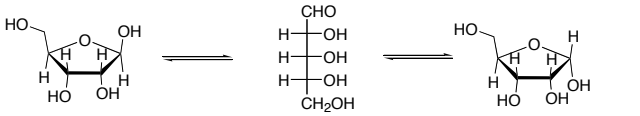




nucleoside nucleotide nucleic acid

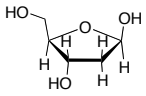
242

Sugar: D-ribose



β-D-ribofuranose

α-D-ribofuranose

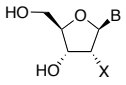


β-D-2-ribofuranose

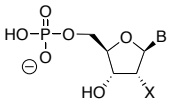
Stereochemistry:
As drawn, the side above the plane of the ring is β
the side below the plane of the ring is α

243

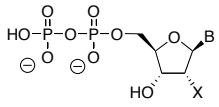
Nucleosides vs Nucleotides



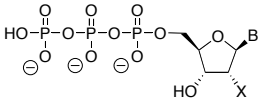
ribonucleoside (X=OH)
deoxyribonucleoside (X=H



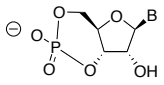
ribonucleotide (X=OH)
deoxyribonucleotide (X=H



nucleotidediphosphate

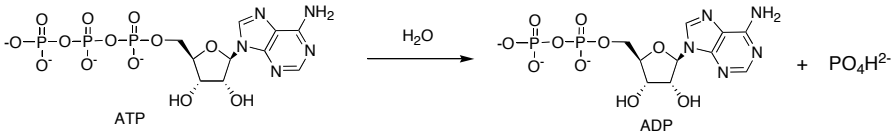


nucleotidetriphosphate



3',5'-cyclic phosphophosphate

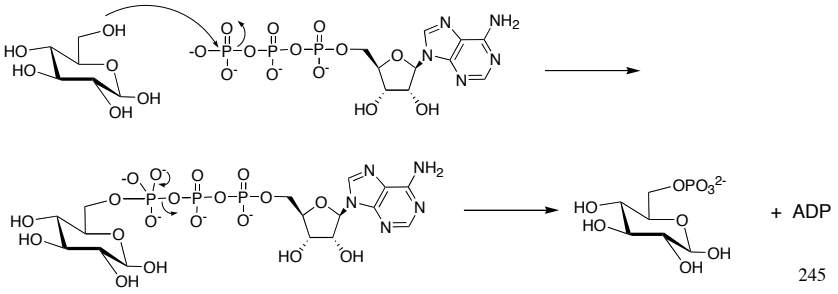
244



+ ~32 KJ/mol (7.3 Kcal/mol)

Hydrolysis of ATP is used to drive may biochemical reactions

Phosphoryl transfer reactions:



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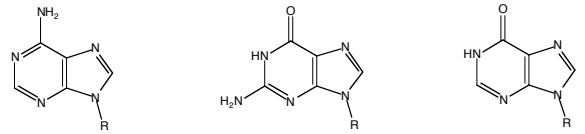
Bases

A. Purines

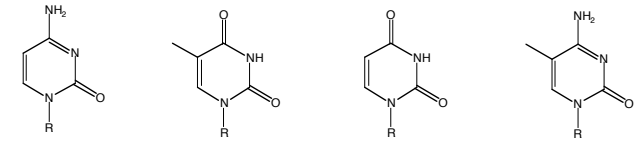


Adenine (R=H)
Adenosine (R= furanose, **A**)

Guanine (R=H)
Guanosine (R= furanose, **G**)

Hypoxanthine (R=H)
Inosine (R=furanose, **I**)

B. Pyrimidines



Cytosine (R=H)
Cytidine (R=furanose, **C**)

Thymine (R=H)
Thymidine (R=furanose, **T**)

Uracil (R=H)
Uridine (R=furnaose, **U**)

5-Methylcytidine
(R=furnaose)

DNA contains A, C, G, T all with 2'-deoxyribose
RNA contains A, C, G, U all with ribose

The stereochemistry of the base is β

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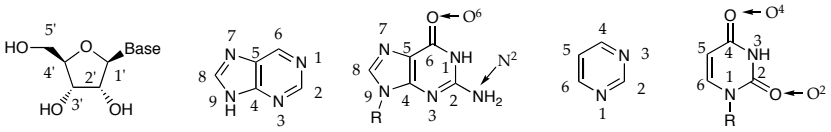
Numbering System



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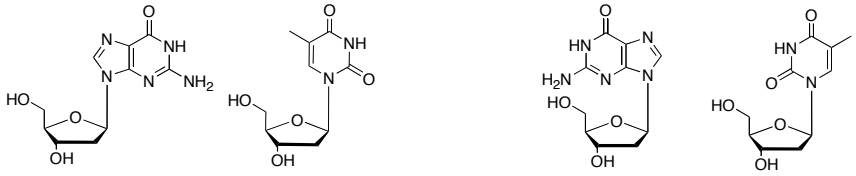
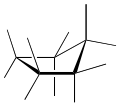
Nucleoside Conformation:

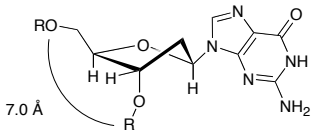


Anti conformation

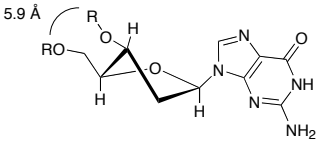
Syn conformation

5-member ring conformation: envelope





C2' - endo conformation found in B-form DNA

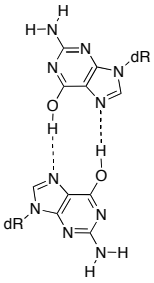


C3' - endo conformation found in A-form DNA

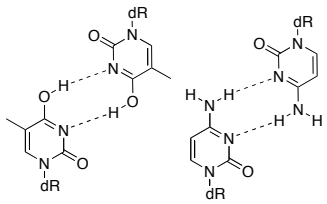
248

Watson & Crick: double helix

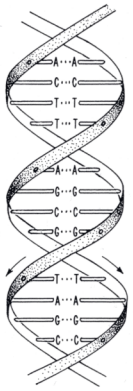
Initial "like-with-like", parallel helix:
Does not fit with with Chargaff's Rule: A = T G = C



purine - purine



pyrimidine - pyrimidine



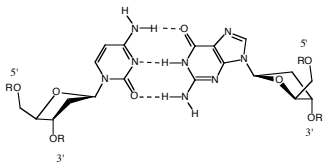
Wrong tautomers !!

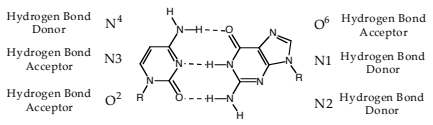
Watson, J. D. *The Double Helix*, 1968

249

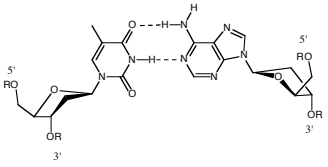
Complimentary Base- Pairing in Nucleic Acids:
antiparallel double helix

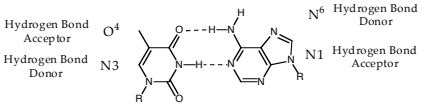
Antiparallel C-G Pair





Antiparallel T-A Pair

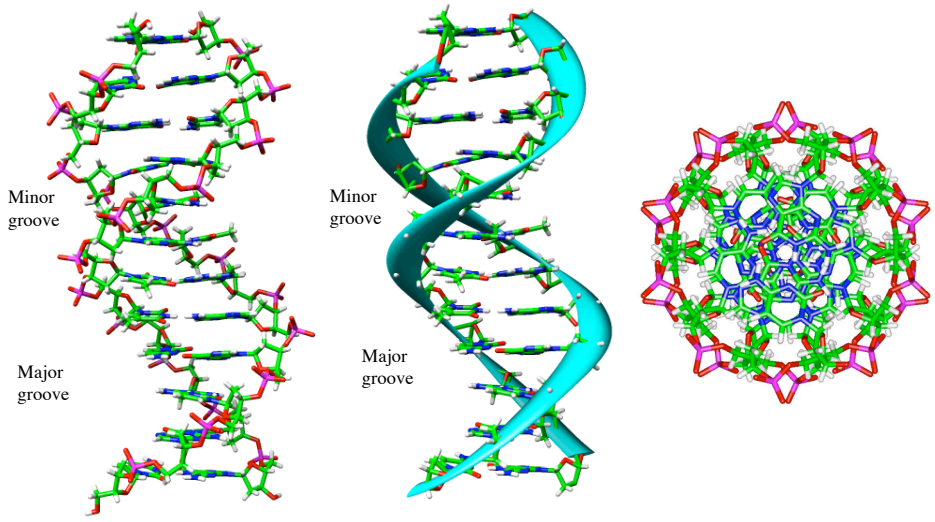




"Molecular Structure of Nucleic Acids" Watson J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737-8

250

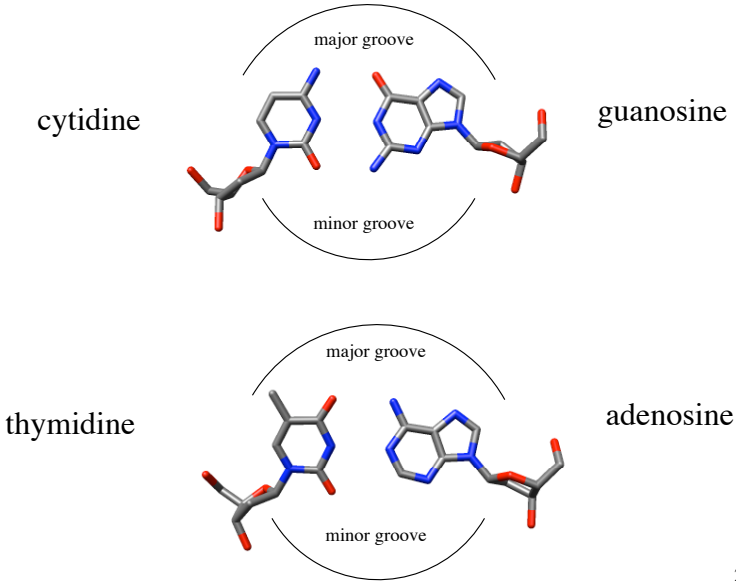
B-DNA



pdb code: 1bna

251

DNA Grooves



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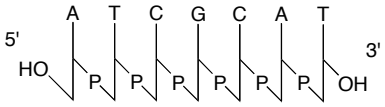
Writing DNA sequences

DNA Sequences are written 5' to 3'

5'-ATCGCAT-3'

5'-d(ATCGCAT)-3'



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Melting Temperature (T_m): a measure a duplex stability

Double stranded $\xrightleftharpoons{\Delta}$ Single strand

Hybridzed: highly ordered structure

Unhybridized: less ordered, random coil

Base stacking causes a deceases in the next UV absorbance when DNA is hybridized (double stranded) versus unhybridized.

Upon thermal denaturation (melting), the UV absorbance increases: hyperchromicity

Blackburn *et al*. Ch. 2.5.1, 11.1.1

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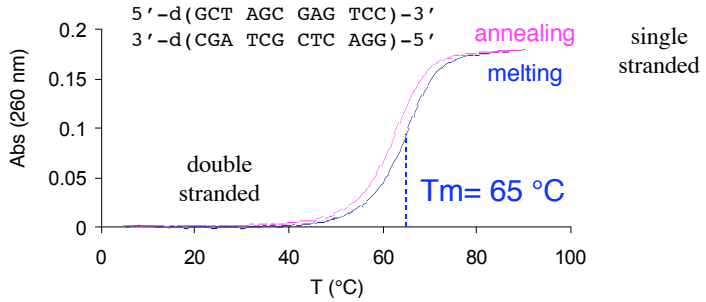
DNA melting curve:



T_m is often taken as a measure of DNA duplex stability

T_m is a measure of ΔG not ΔH

The T_m is dependent upon the length and sequence of the oligonucleotide (CG/AT ratio) and the ionic strength of the medium

255

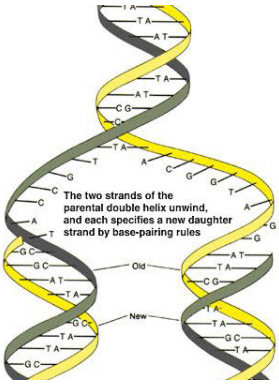
DNA processing enzymes:

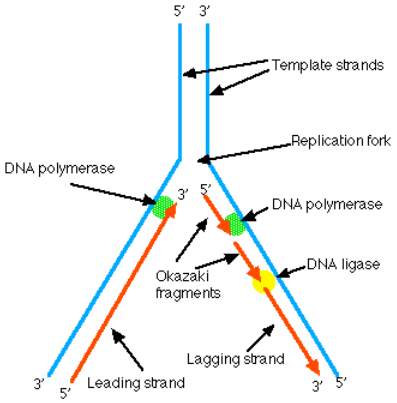
Blackburn *et al*. Ch. 3.6, 5.3, 6.6.4

DNA replication:

Helicase: Unwinds double stranded DNA

DNA polymerase: replicates DNA using each strand as a template for the newly synthesized strand.





"It has not escaped our attention that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
Watson & Crick

256

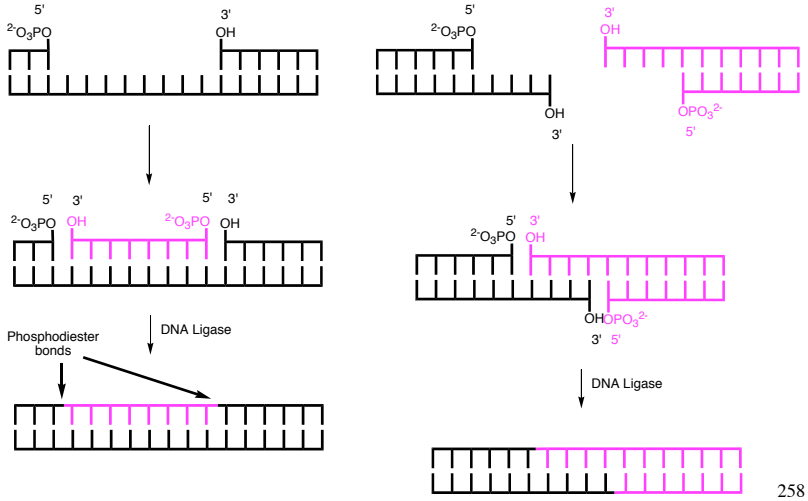
DNA Polymerase: the new strand is replicated from 5' to 3'

DNA polymerase are Mg^{2+} ion dependent

Details regarding the mechanism of recognition of dNTP incorporation for the growing DNA strand are *not* fully understood

257

DNA Ligase: enzyme that will join (ligate) two DNA segment by catalyzing the formation of the phosphodiester bond of a terminal 5'-phosphate of one oligonucleotide and the 3'-hydroxyl group of another oligonucleotide.



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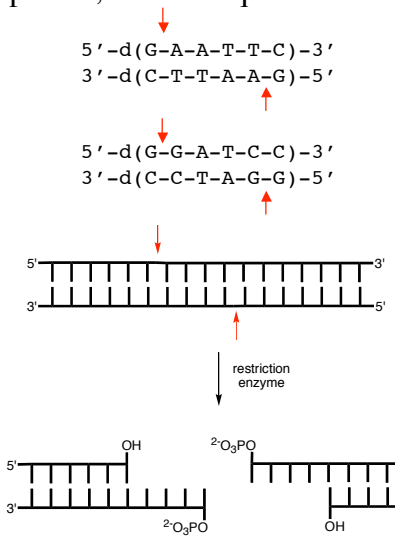
Restriction Enzymes: enzymes that will cleave double stranded DNA at specific, known sequences.



"sticky ends"

Werner Arber, Daniel Nathans and Hamilton Smith
1978 Nobel Prize in Medicine & Physiology

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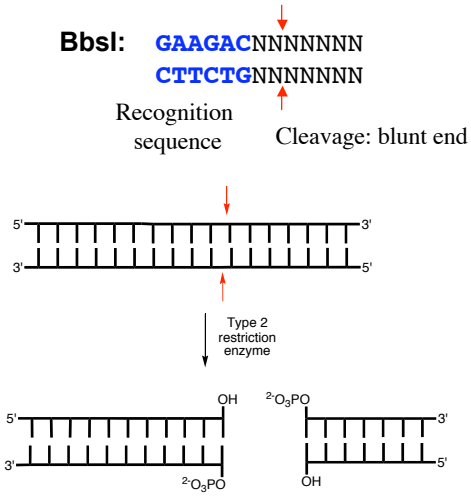
Type I: cleaves at a site very distant from the recognition sequence; Mg^{2+}, SAM and ATP dependent.

Type II: cleaves DNA at or near the recognition sequence; Mg^{2+} dependent. i.e., EcoR I, BamHI, Bbs I



260

Polymerase Chain Reaction (PCR): method of amplifying DNA using DNA polymerase and cycling temperature

Heat stable DNA Polymerases:

Taq: thermophilic bacteria (hot springs)- no proof reading

Pfu: geothermic vent bacteria- proof reading

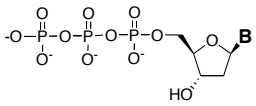
Mg^{2+}

two Primer DNA strands (synthetic, large excess)

one sense primer and one antisense primer

one Template DNA strand

dNTP's



KARY B. MULLIS, 1993 Nobel Prize in Chemistry for his invention of the polymerase chain reaction (PCR) method.

Blackburn *et al*. Ch. 5.2.3, 5.4.2

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A typical PCR temperature cycle

Denaturation:	94 °C	0.5 - 1 min	
Annealing:	55-68 °C	0.5 - 1 min	5 °C below the T_m of the primer
Extension:	72 °C	1 min	+ 1 min per Kb of DNA
# of cycles	25 - 35		
Final extension	72 °C	10 min	

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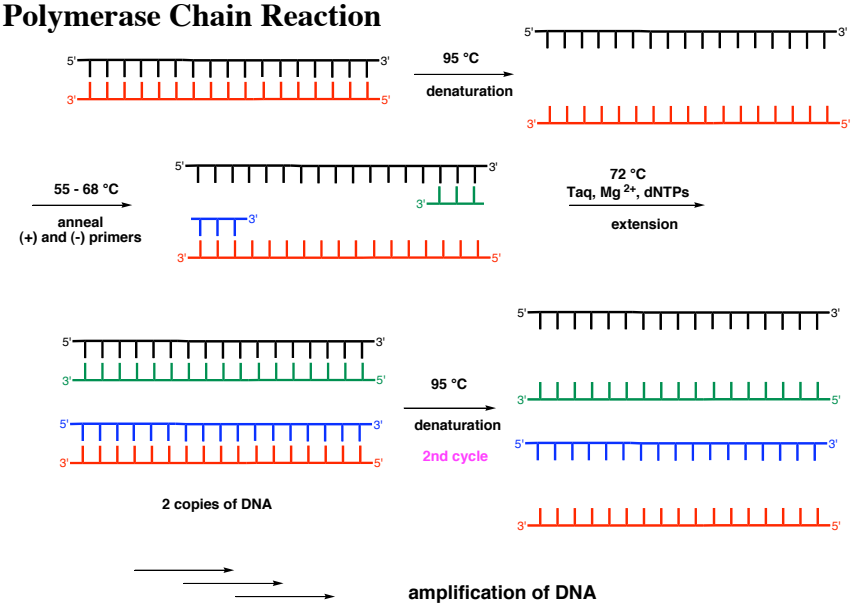
Polymerase Chain Reaction



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For a PCR animation go to: http://www.blc.arizona.edu/INTERACTIVE/recombinant3.dna/pcr.html

$$ The Power of Compounded Interest $$

1 x 2 = 2 x 2 = 4 x 2 = 8 x 2 = 16 x 2 = 32 x 2 = 64 x 2 = 128 x 2 = 256 x 2 = 512 x 2 = 1,024 x 2 = 2,048 x 2 = 4,096 x 2 = 8,192 x 2 = 16,384 x 2 = 32,768 x 2 = 65,536 x 2 = 131,072 x 2 = 262,144 x 2 = 524,288 x 2 = 1,048,576

In principle, over one million copies per original, can be obtained after just twenty cycles

264

Oligonucleotide-Based Site-directed mutagenesis

DNA ⟶ mRNA ⟶ protein

THE STANDARD GENETIC CODE

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

AUG is part of the initiation signal, as well as being the codon for internal methionine.

SYMBOLS FOR AMINO ACIDS

A	Ala	Alanine
B	Asx	Asparagine or aspartic acid
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
Z	Glx	Glutamine or glutamic acid

Blackburn *et al*. Ch. 5.6

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```
3′-GAG ATG ACA CCC AAA-5′
5′-CTC TAC TGT GGG TTT-3′

  -Leu Tyr Cys Gly Phe-

              ↓

3′-GAG ATG CGA CCC AAA-5′
5′-CTC TAC GCT GGG TTT-3′

  -Leu Tyr Ala Gly Phe-
```

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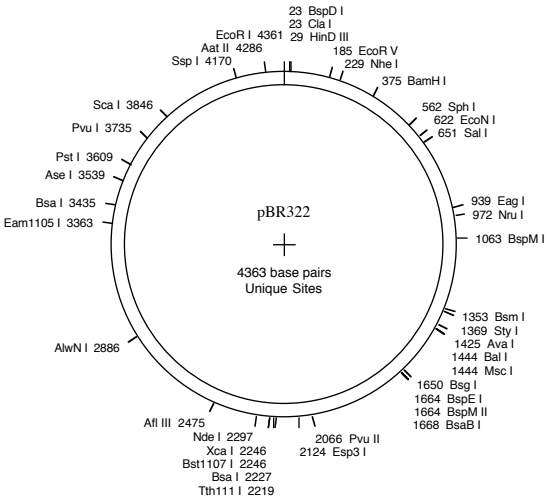
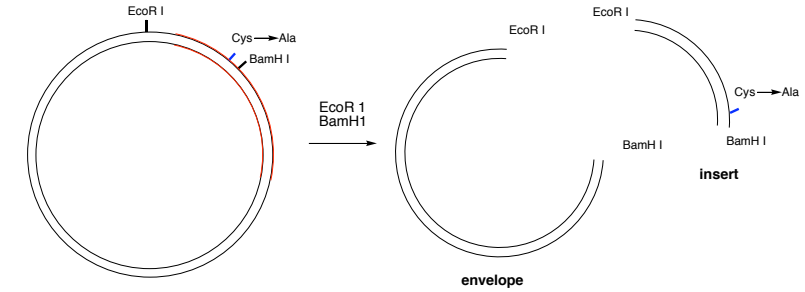
Restriction Map





BAM H1 Cys plasmid (template)

3'-**GAT** **C**CC NNN CTC TAC **ACA** GGG TTT NNN----------5'

5'-**CTA** **G**GG NNN GAG ATG **GCT** CCC AAA NNN-3'

PCR primer with mutation Ala

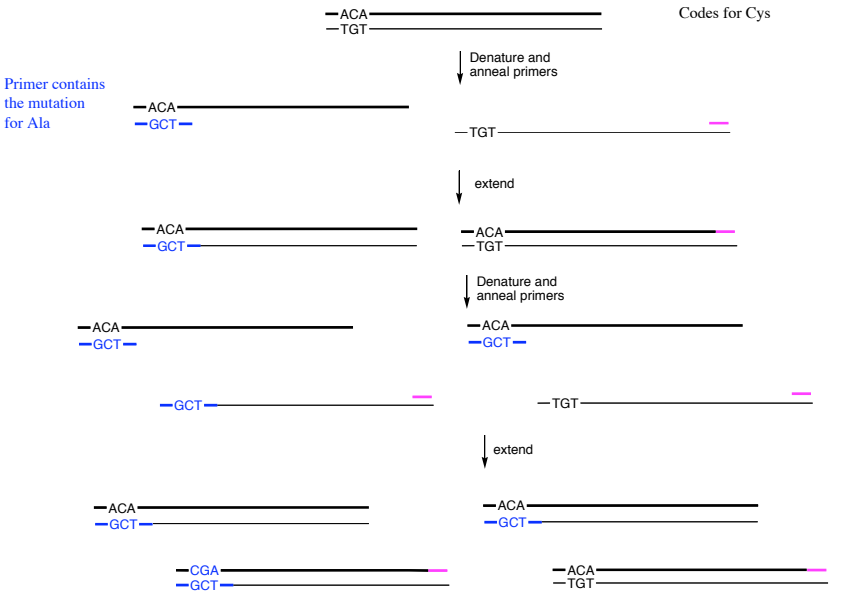
PCR amplifies a new insert with the mutation

Cut the PCR fragments with EcoR1 and BamH1

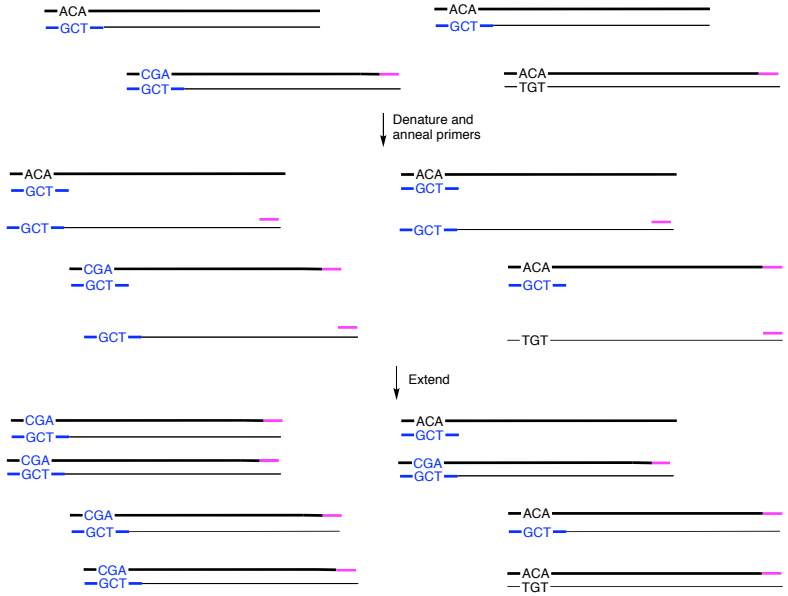
Ligate the new insert (with the desired mutation) back into the envelope

MICHAEL SMITH, 1993 Nobel Prize in chemistry for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies.

Amplification of the mutant sequence by PCR



269



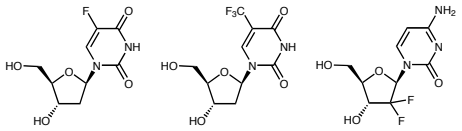
DNA strands with the desired mutation are being amplified much faster than strands without the mutation; after ~25 rounds of PCR, the amount of DNA that does not have the desire mutation become negligible (<< 1 in a million)

270

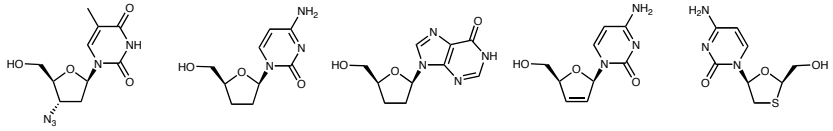
Nucleoside Synthesis: Blackburn *et al*. Ch. 3.1

important class of chemotherapeutic agents
(anticancer and antivirals)
important reagents for biotechnology

Anti-Cancer Nucleosides



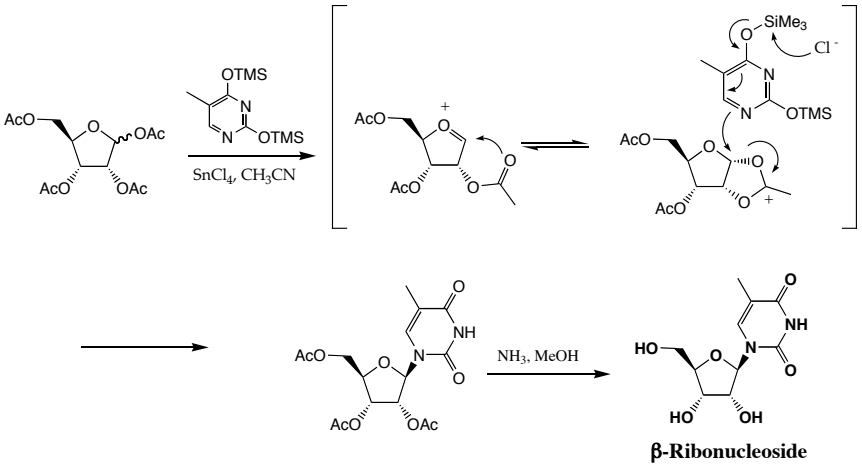
Anti-Viral Nucleosides



AZT ddC ddI d4C (-)-3TC

271

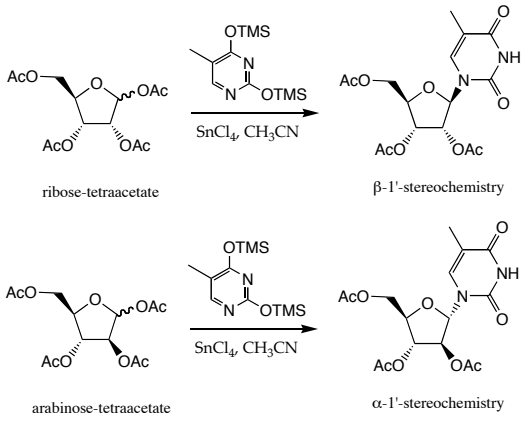
Chemical Synthesis of Ribonucleosides via Vorbrüggen Glycosylation



β-Ribonucleoside

272

The stereochemistry of the C2-ester group of the furanose-tetraester controls the stereochemistry of the glycosylation reaction



ribose-tetraacetate

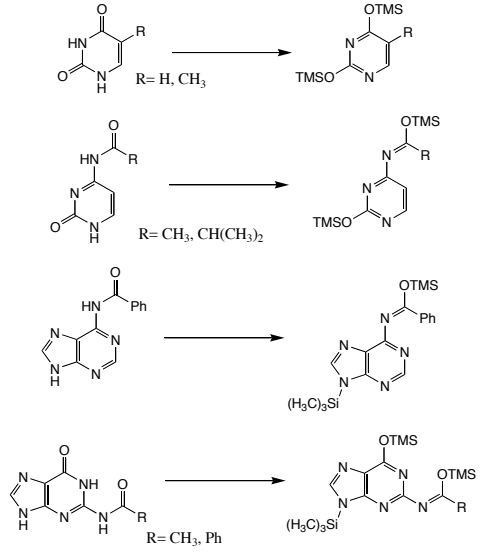
β-1'-stereochemistry

arabinose-tetraacetate

α-1'-stereochemistry

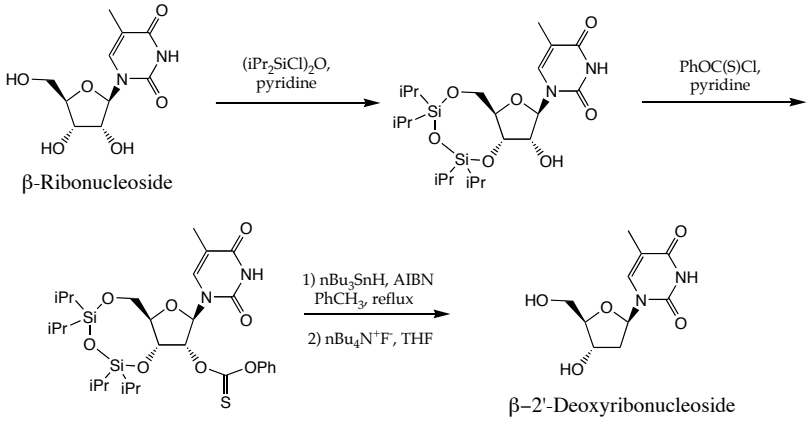
273

Exocyclic amino groups of C, A and G require protecting for the Vorbrüggen Glycosylation reaction



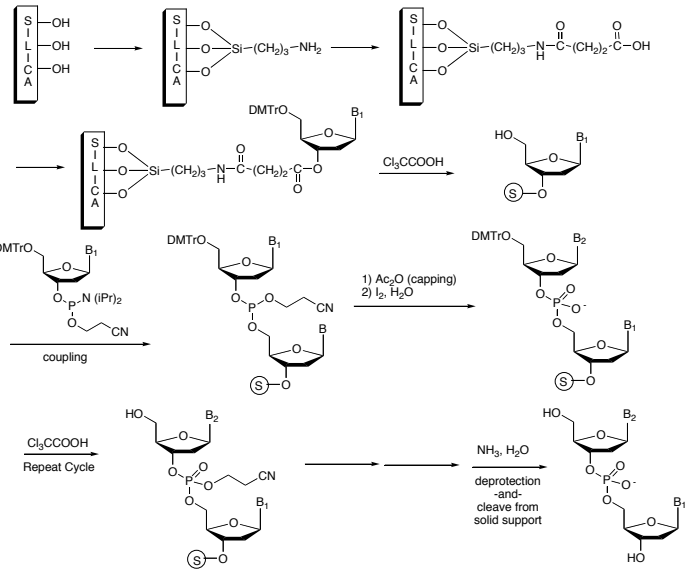
274

Chemical Conversion of Ribonucleoside to 2'-Deoxyribonucleoside: Free radical deoxygenation of the 2'-hydroxyl group



275

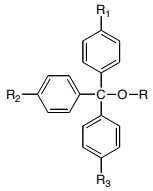
Solid-Phase Oligonucleotide Synthesis



276

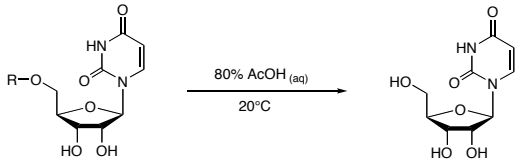
Blackburn *et al*. Ch. 4

The Protecting Groups for Solid Phase DNA Synthesis:
5'- hydroxyl group



Trityl $R_1 = R_2 = R_3 = H$ Tr-OR
(p-Methoxyphenyl)diphenylmethyl ether $R_1 = R_2 = H$, $R_3 = OCH_3$
4'-methoxytrityl MMTr-OR
Di-(p-methoxyphenyl)phenylmethyl ether $R_1 = H$, $R_2 = R_3 = OCH_3$
4',4'-dimethoxytrity DMTr-OR
Tri-(p-methoxyphenyl)methyl ether $R_1 = R_2 = R_3 = OCH_3$
4',4',4'-trimethoxytrityl TMTr-OR

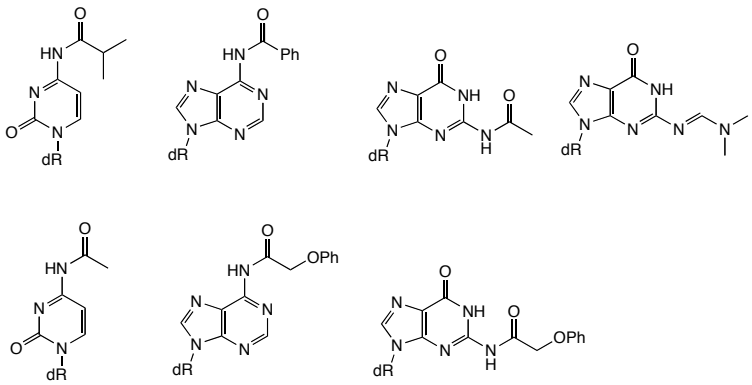
Relative rate of hydrolysis:



R = Tr 48 hr.
R= MMTr 2 hr.
R= DMTr 15 min.
R= TMTr 1 min. (too labile to be useful)

277

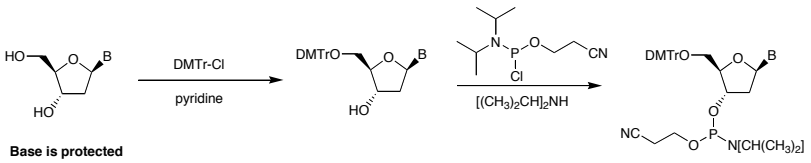
Protecting groups for the exocyclic amino groups



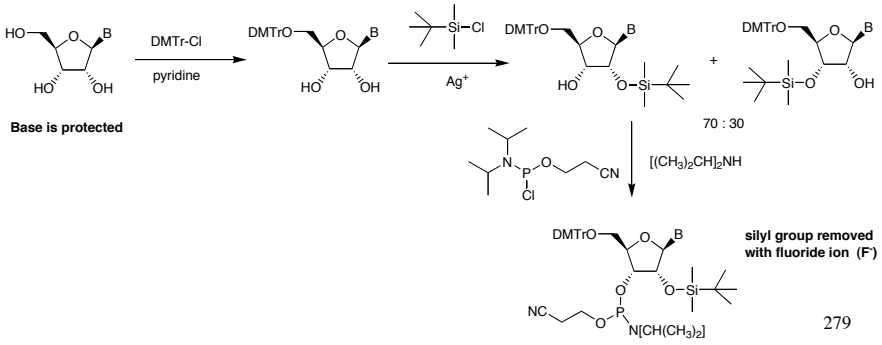
All are removed with NH_3/H_2O at 80° C

278

Phosphoramidite reagents: the monomeric building blocks
for solid-phase DNA synthesis



for solid-phase RNA synthesis



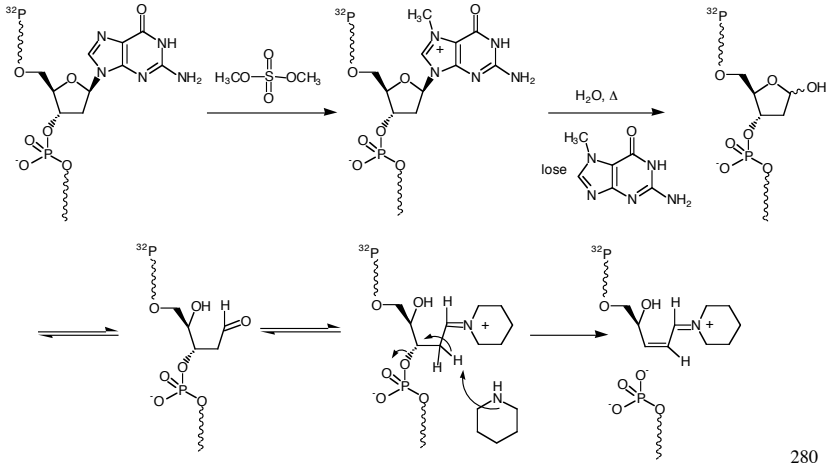
279

DNA Sequencing: Maxam-Gilbert Sequencing Blackburn *et al.* Ch. 5.1

sequence selective chemical cleavage

Detection: 5'-^{32}P-labeling

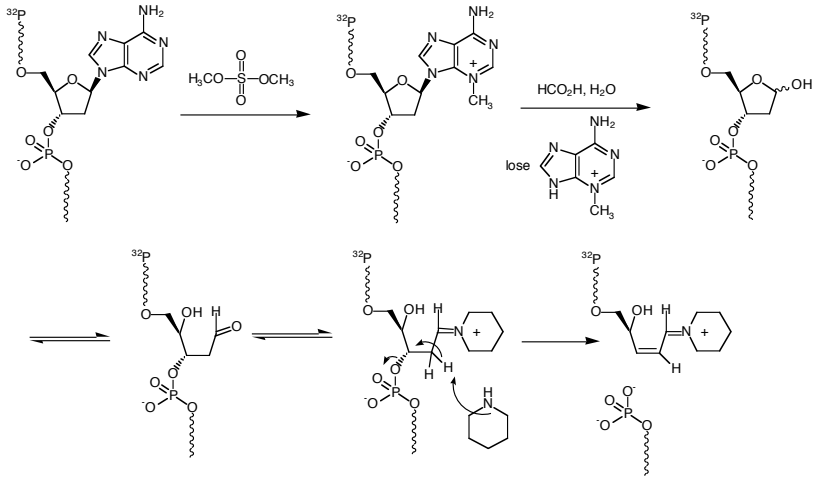
A: Cleavage at the 3'-side of guanosines with a) dimethylsulfate b) Δ, c) piperidine.



280

Maxam-Gilbert Sequencing

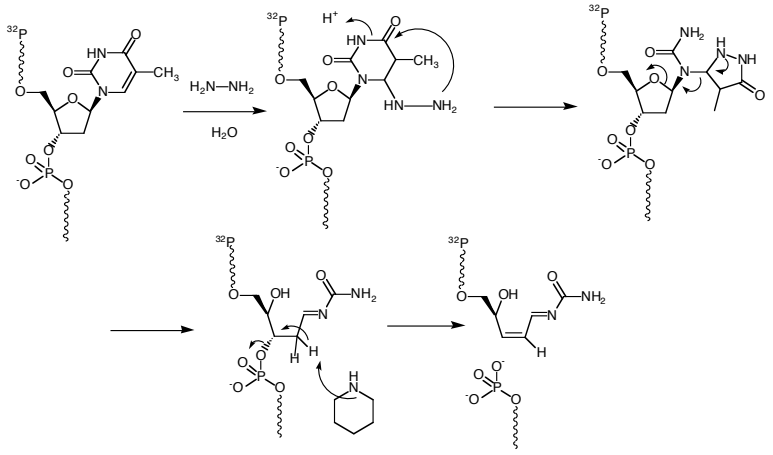
B. Cleavage at the 3'-side of adenosines and guanosines with a) dimethyl sulfate b) HCO_2H, c) piperidine.



281

Maxam-Gilbert Sequencing

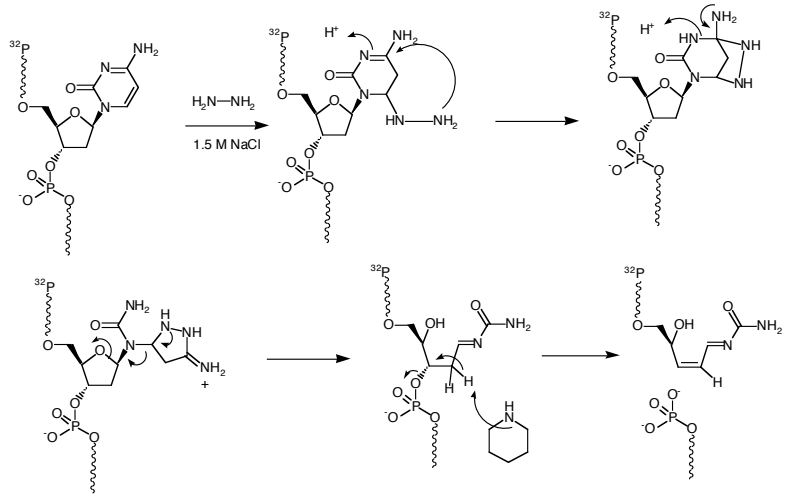
C. Cleavage at the 3'-side of thymidines and cytidines with a) hydrazine, b) Δ, c) piperidine.



282

Maxam-Gilbert Sequencing

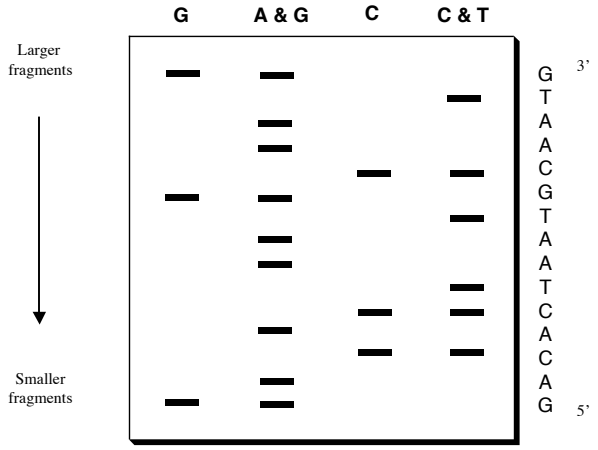
D. Cleavage at the 3'-side of cytidines with a) hydrazine, 1.5 M NaCl, b) Δ, c) piperidine.



283

Maxam-Gilbert Sequencing

Separation and detection of ^{32}P-labeled DNA fragments by polyacrylamide gel electrophosesis (PAGE). DNA fragments separated based upon charge, which is proportional to the length of the DNA fragment.

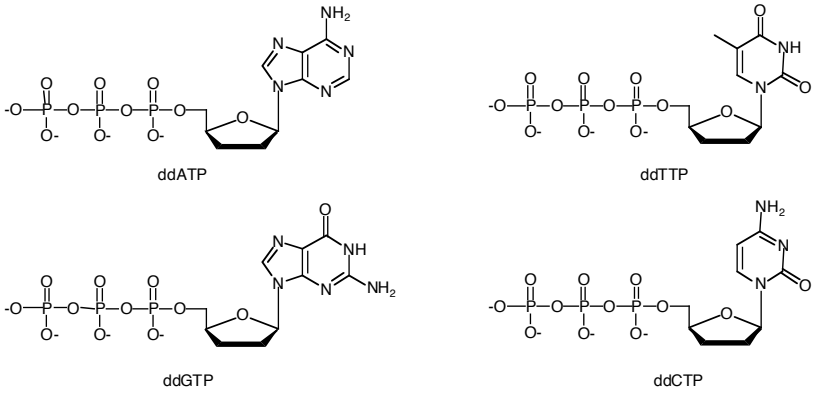


284

Sanger Sequencing

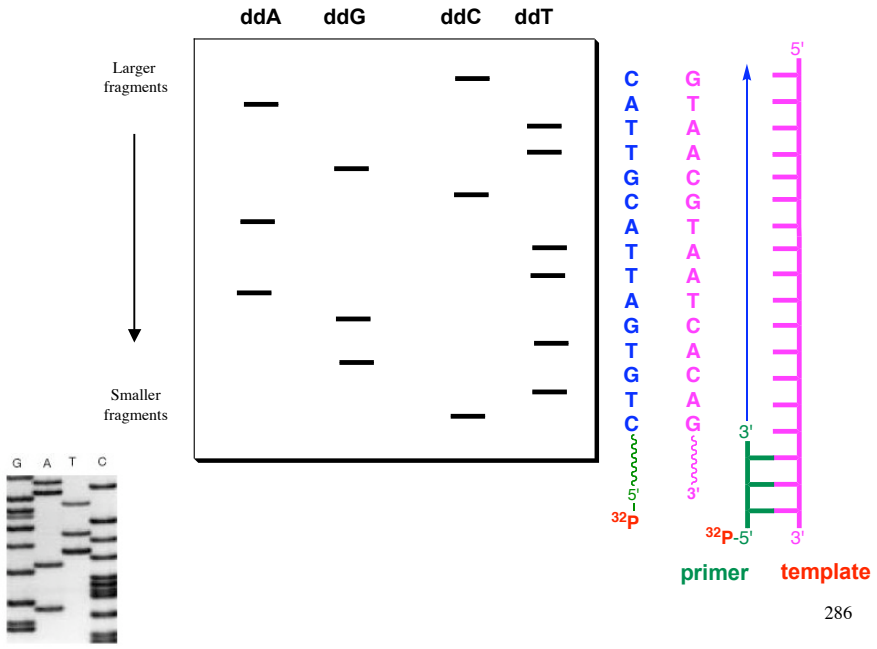
Enzymatic replication of the DNA fragment to be sequnced with DNA polymerase, Mg^{+2}, ddNTP's and ^{32}P-labeled primers.

ddNTP's



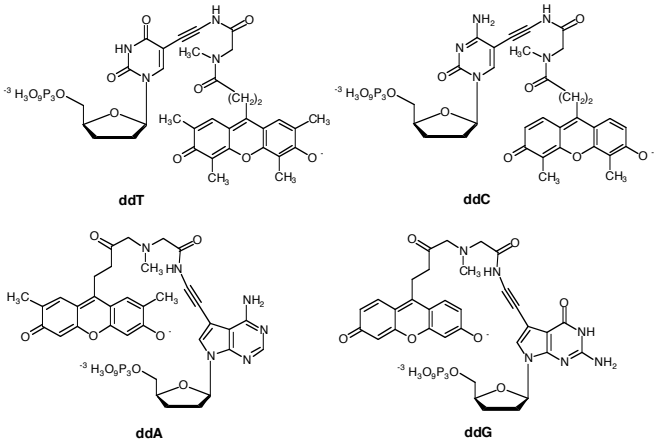
285

Sanger Sequencing



286

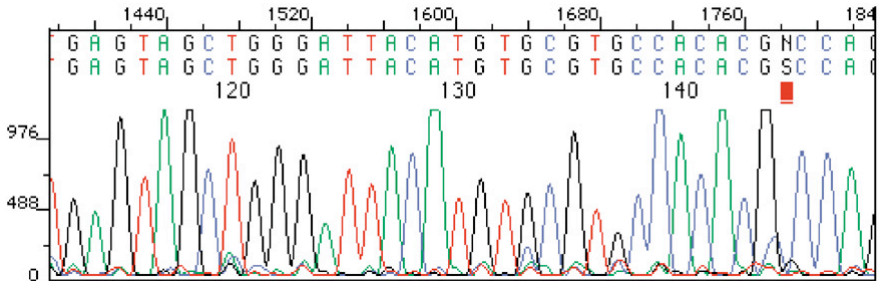
Sanger sequencing with flourescent ddNTP's



Excitation: ~ 490 nM, Emission: ddT= 526 nm. ddC= 519 nm, ddA= 512 nm, ddG= 505 nm

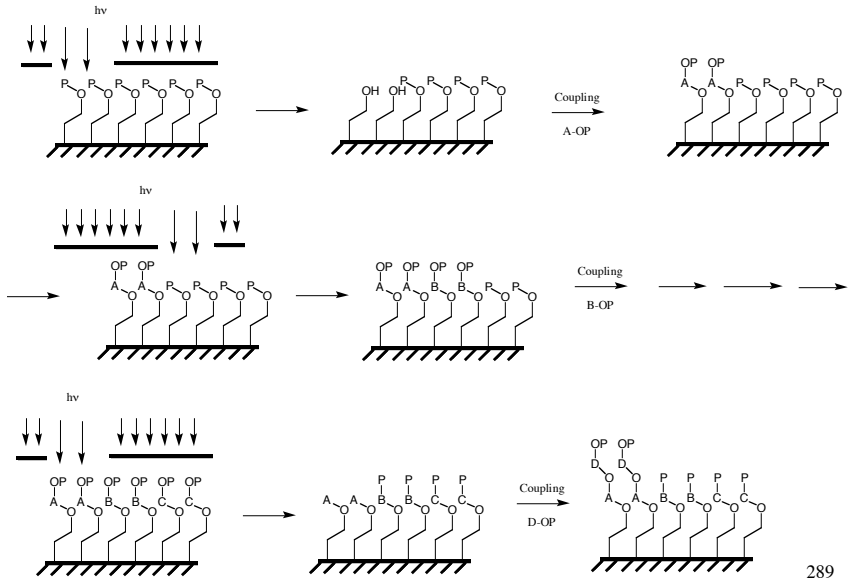
287

Sanger sequencing using flourescent ddNTP's
terminated DNA strands are separated by capillary electrophoresis



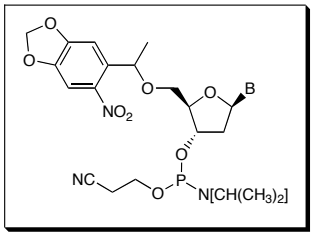
288

Light-Directed, Spatially Addressable Parallel Synthesis

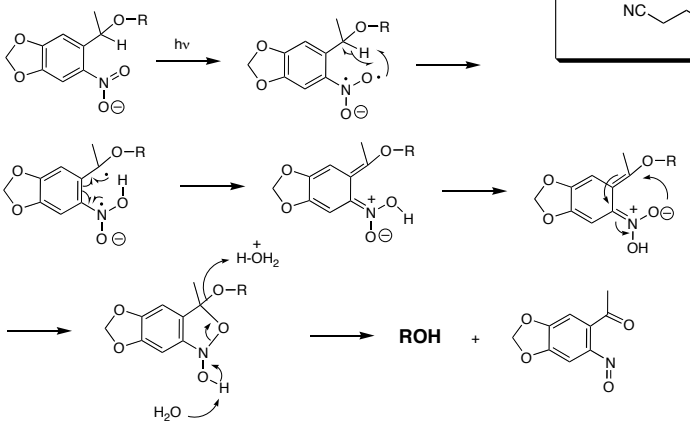


289

Photoremovable protecting group: o-nitrobenzyl ether derivatives



Mechanism: hν (λ ~ 350 nm)

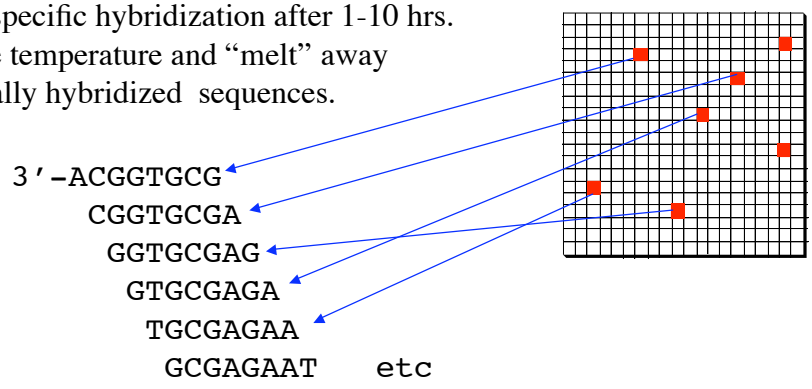


290

Spatially addressable: 8-mer chip: 65, 536 different sequences
12-mer chip: 1,677,216 different sequences

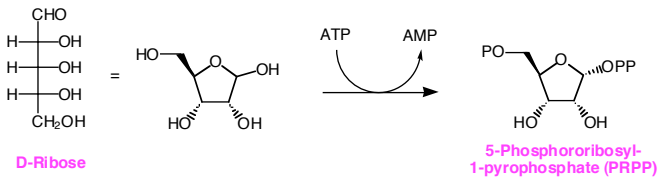
DNA fragment ⟶ DNA–fluorophore

Place DNA on the chip, then wash away non-specific hybridization after 1-10 hrs. Raise temperature and "melt" away partially hybridized sequences.



3′-ACGGTGCGAGAAT---5′ (from the chip)
5′-TGCCACGCTCTTA---3′

Nucleotide Biosynthesis

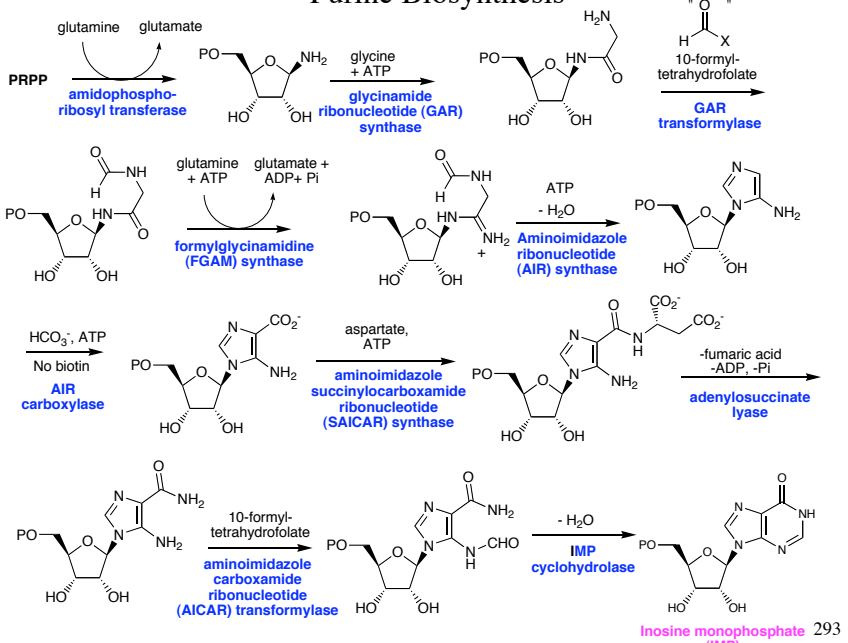


D-Ribose

5-Phosphororibosyl-1-pyrophosphate (PRPP)

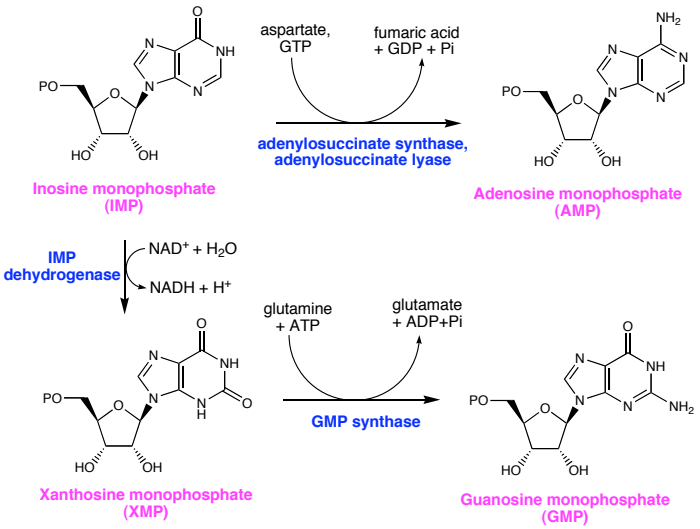
Blackburn *et al*. Ch. 3.4, 3.5

Purine Biosynthesis



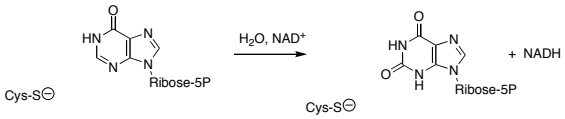
293

Purine Biosynthesis (con't)



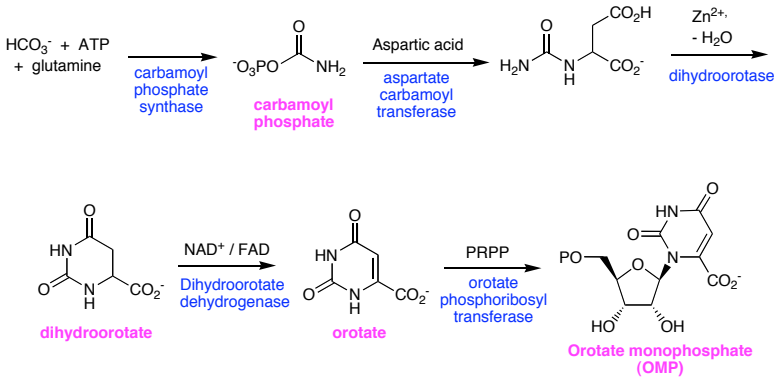
294

IMP Dehydrogenase

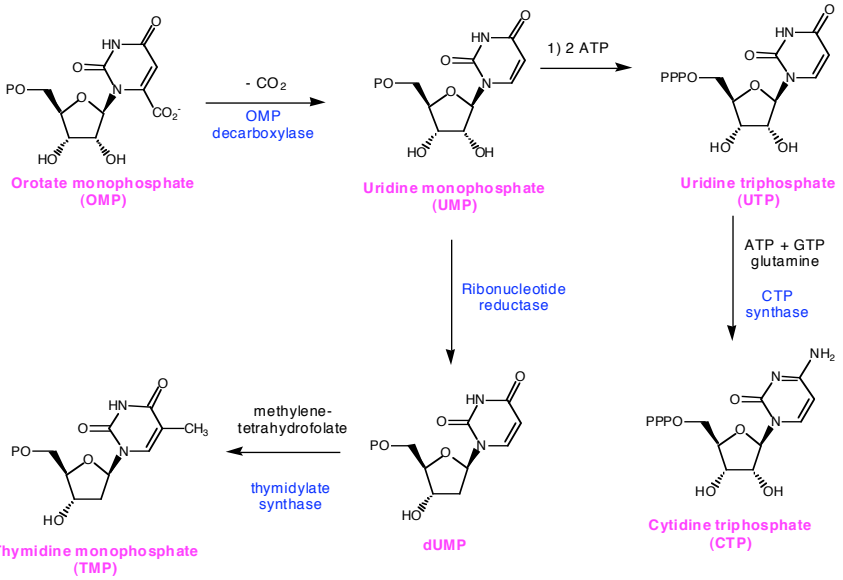


295

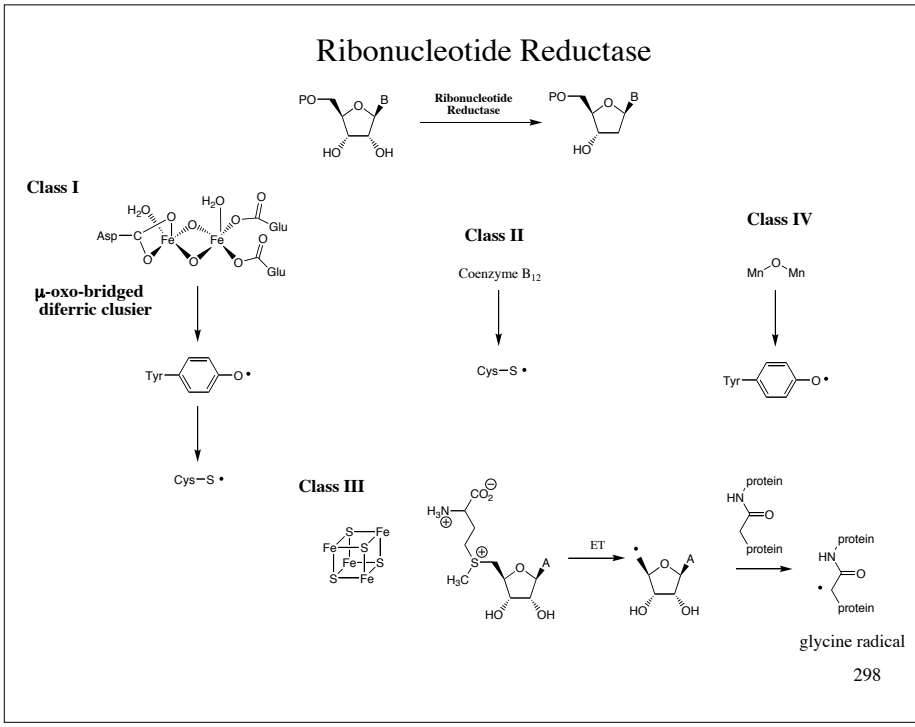
Pyrimidine Biosynthesis



296

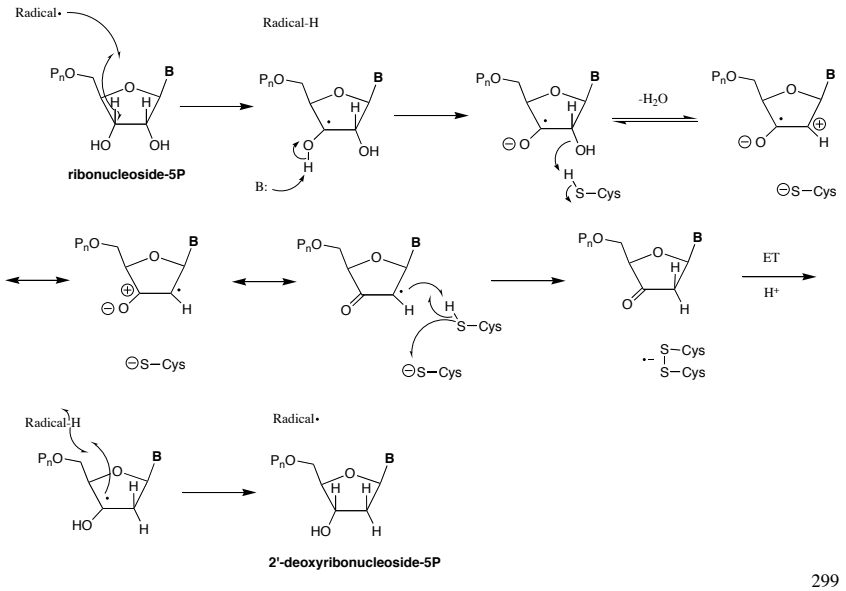

- CO2
OMP
decarboxylase
1) 2 ATP
Orotate monophosphate
(OMP)
Uridine monophosphate
(UMP)
Uridine triphosphate
(UTP)
ATP + GTP
glutamine
CTP
synthase
Ribonucleotide
reductase
methylene-
tetrahydrofolate
thymidylate
synthase
Thymidine monophosphate
(TMP)
dUMP
Cytidine triphosphate
(CTP)

297


Ribonucleotide Reductase
Ribonucleotide
Reductase
Class I
μ-oxo-bridged
diferric clusier
Class II
Coenzyme B12
Class IV
Class III
ET
protein
glycine radical

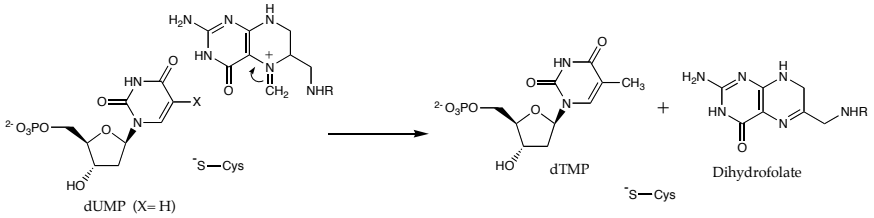
298

Mechanism of Ribonucleoside Reductase



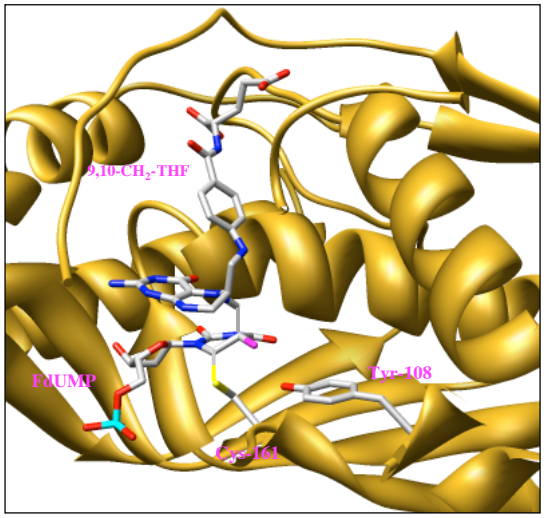
299

Mechanism and Inhibition of Thymidylate Synthase



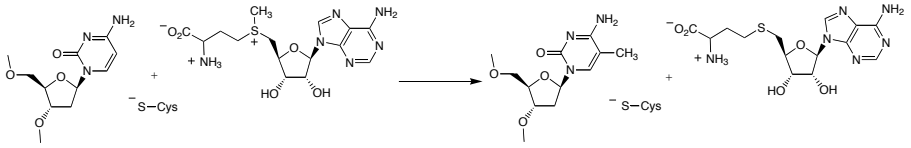
300

Ternary complex of thymidylate synthase with 5'-fluoro-dUMP and 5,10-dihydrotetrahydrofolate

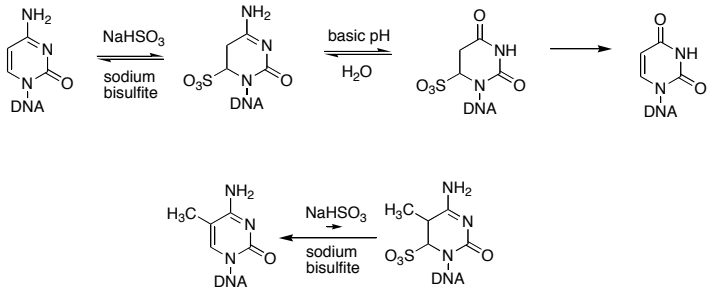


pdb code: 1B02

DNA Methylation: 5-methyl-2'-deoxycytidine



5-Methyl-C (Epigenetic) sequencing



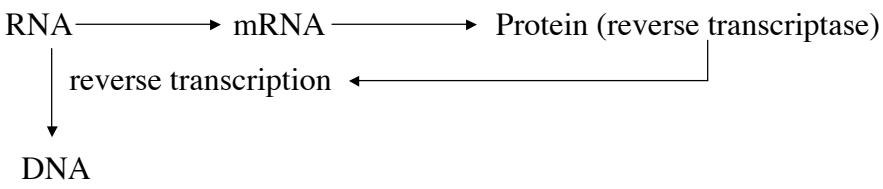
Sodium bisulfite converst C's to U's, but has no effect on 5-methyl-C

Antiviral nucleosides:

2,3-dideoxynucleosides as reverse transcriptase inhibitors

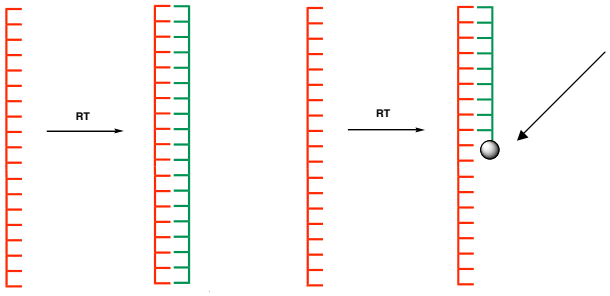
Protein Biosynthesis

Retrovirus (hepatitis B, HIV)



Blackburn *et al*. Ch. 3.7

Mechanism of action of 2,3-dideoxynucleosides:
recall Sanger sequencing- termination of a growing DNA chain by enzymatic incorporation of a 2,3-dideoxynucleosides



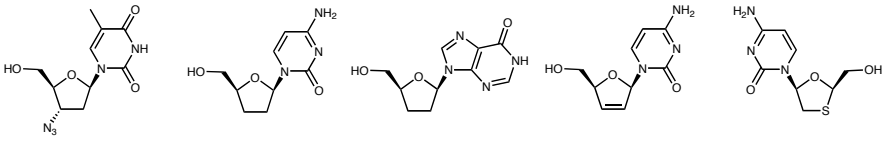
Incorporation of a ddNTP

Termination of elongation

Truncated DNA also inhibits RT

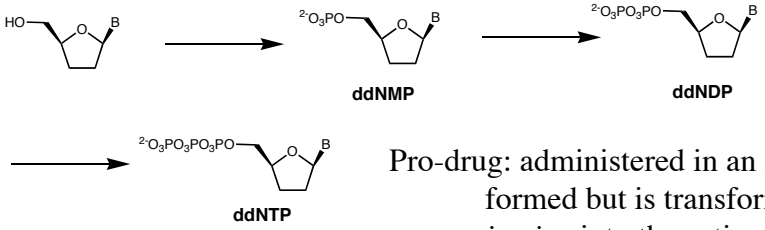
305

Nucleoside-based reverse transcriptase inhibitors



AZT ddC ddI d4C (-)-3TC

2,3-dideoxynucleoside is enzymatically phosphorylated at the 5-hydroxyl to the ddNTP, which is the active RT inhibitor



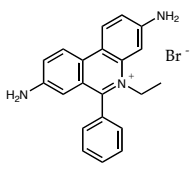
Pro-drug: administered in an inactive formed but is transformed *in vivo* into the active drug

306

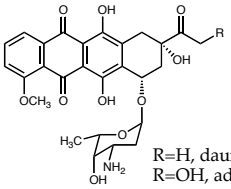
DNA as a target for therapeutic agents:

Intercalation:

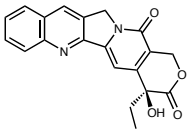
- planar aromatic molecules "slide" between stacked bases
- intercalators span the minor and major grooves of DNA
- driven by hydrophobic interactions
- intercalators stabilize DNA structure
- causes an "unwinding" and elongation of DNA in the area of the intercalation



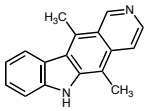
Ethidium Bromide



R=H, daunomycin
R=OH, adriamycin



Campthothecin



Ellipticine

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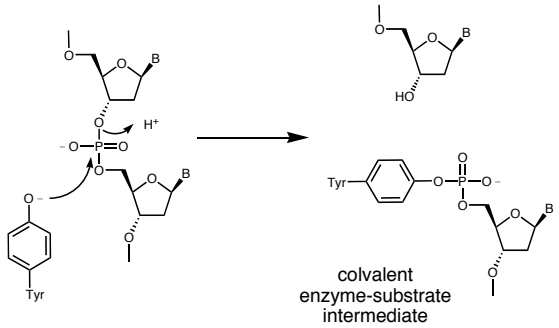
Topological relationship of DNA: supercoiling, tangling, knotting for replication (transcription), the supercoiling of DNA must first be "relaxed"

Topoisomerase (mammalian) DNA gyrase (bacteria)

Mechanism of the DNA cleavage step



Topo II inhibitors stabilize the covalent DNA•Topo II complex (protein bound double strand cleaved DNA), which causes chromosomal abnormalities and leads to *apoptosis*

Corbett, A.H.; Osheroff, N. *Chem. Res. Toxicol.* **1993**, 6, 585-597

Topo II movies: http://berger.berkeley.edu/Pages/Teaching.html

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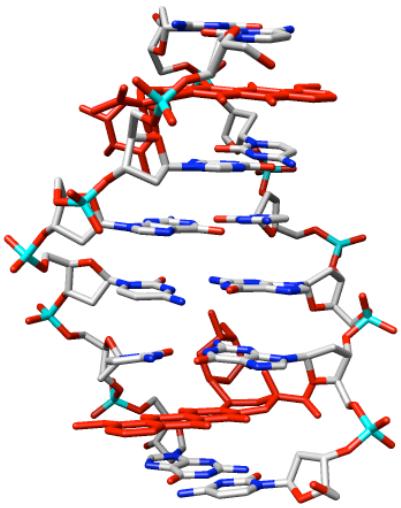
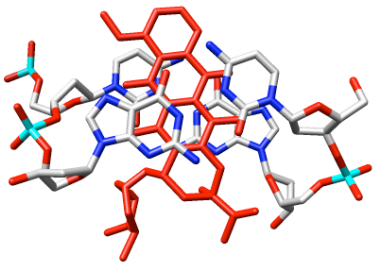
Intercalation

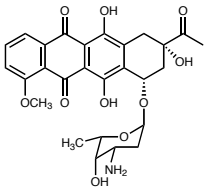


pdb code: 110D

major
groove



minor
groove



daunomycin

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