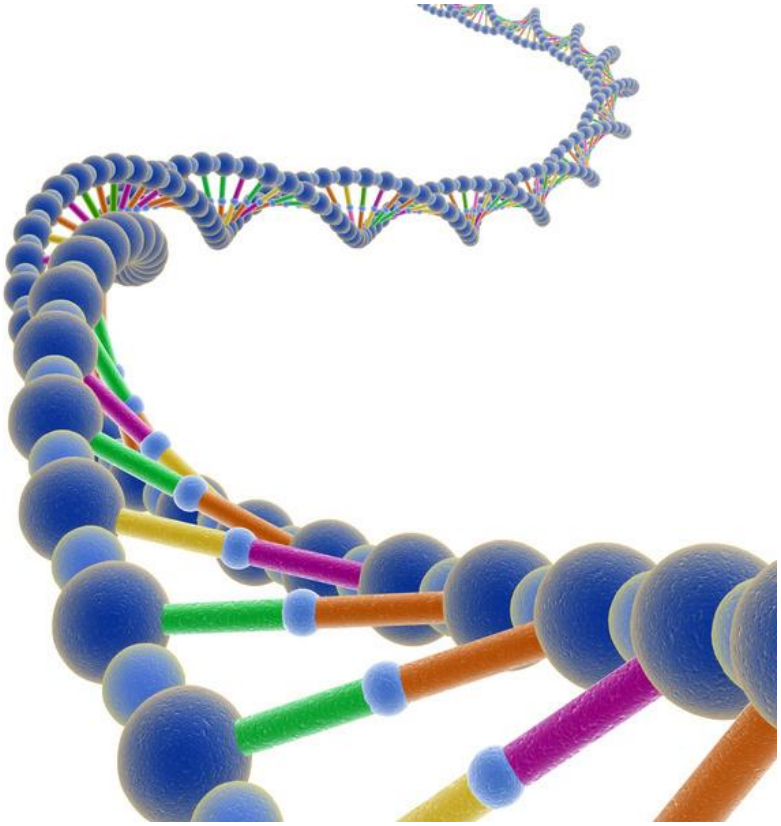
Nucleoside Derivatives as Drug, Their Synthesis & Mode of Action



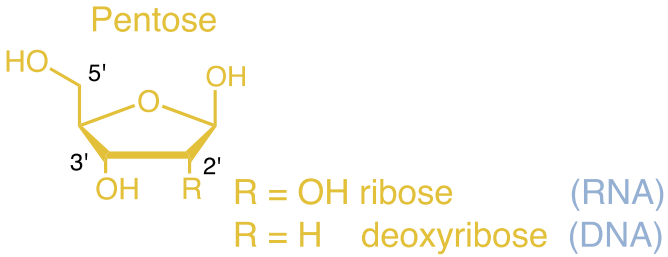
Raphaëlle Berger

MacMillan Group Meeting

Febuary 27, 2013

Introduction: Basics on DNA, RNA Synthesis

- Nucleoside / Nucleotide



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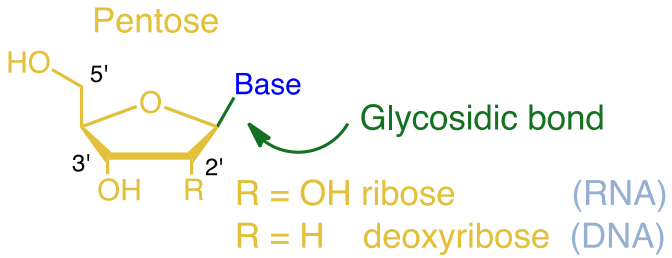
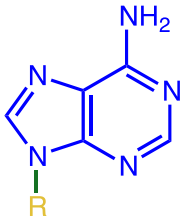
- Nucleoside / Nucleotide



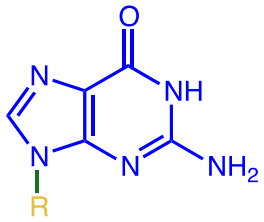
nucleoside

Purines



Adenine

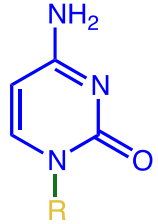
DNA
RNA



Guanine

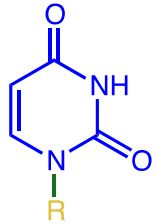
DNA
RNA

Pyrimidines



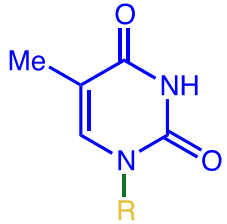
Cytosine

DNA
RNA



Uracil

RNA

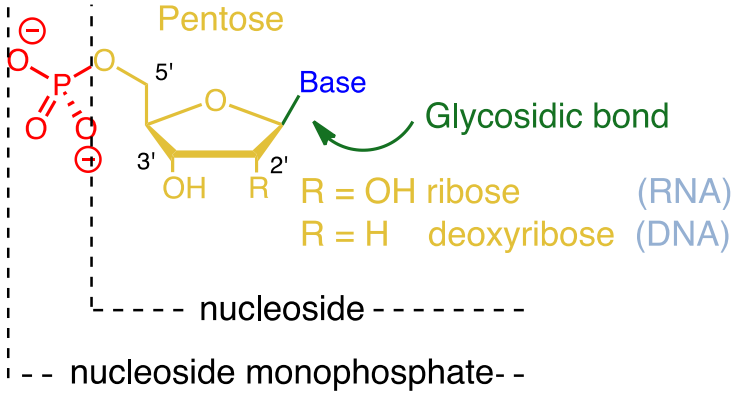


Thymine

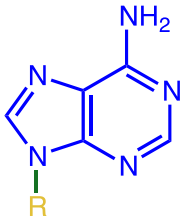
DNA

Introduction: Basics on DNA, RNA Synthesis

- Nucleoside / Nucleotide

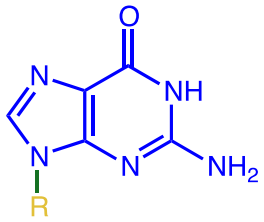


Purines



Adenine

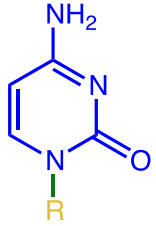
DNA
RNA



Guanine

DNA
RNA

Pyrimidines



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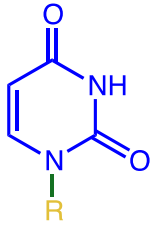
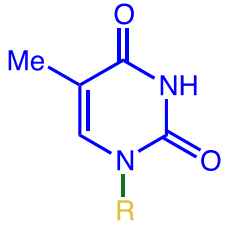
DNA
RNA



Uracil

RNA

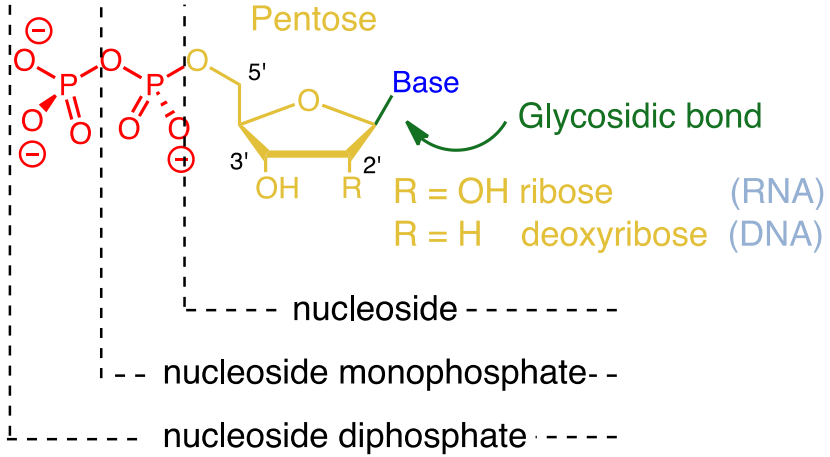


Thymine

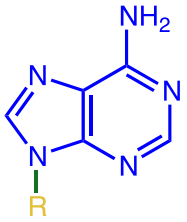
DNA

Introduction: Basics on DNA, RNA Synthesis

Nucleoside / Nucleotide

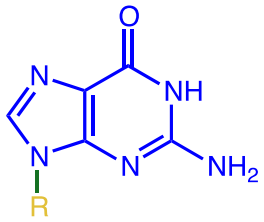


Purines



Adenine

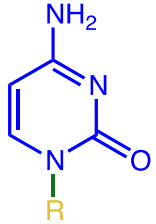
DNA
RNA



Guanine

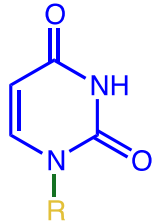
DNA
RNA

Pyrimidines



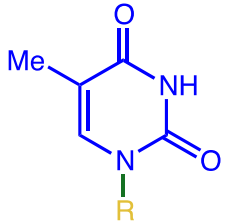
Cytosine

DNA
RNA



Uracil

RNA

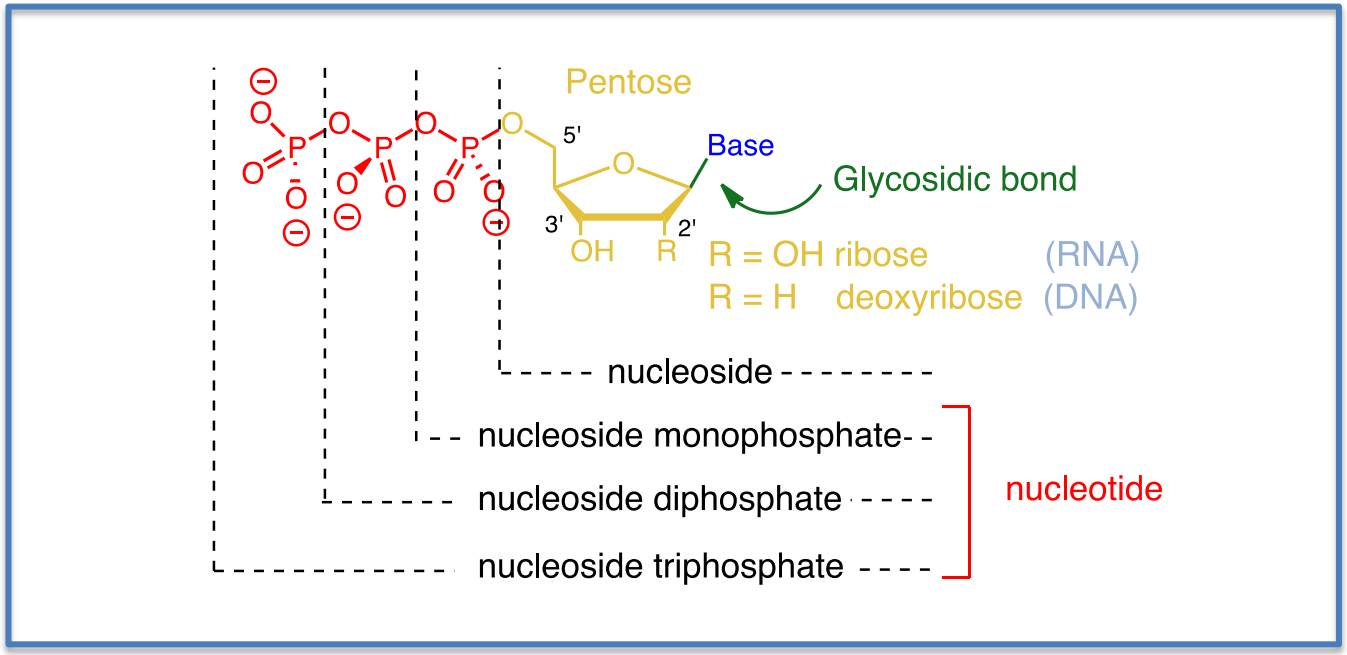


Thymine

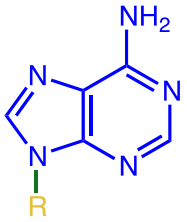
DNA

Introduction: Basics on DNA, RNA Synthesis

Nucleoside / Nucleotide

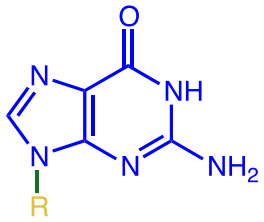


Purines



Adenine

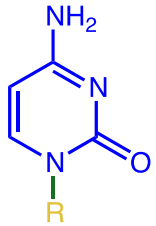
DNA
RNA



Guanine

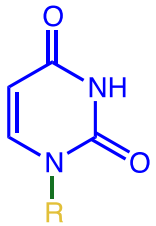
DNA
RNA

Pyrimidines



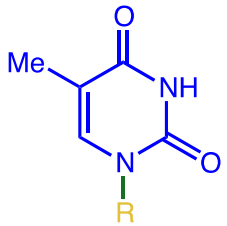
Cytosine

DNA
RNA



Uracil

RNA

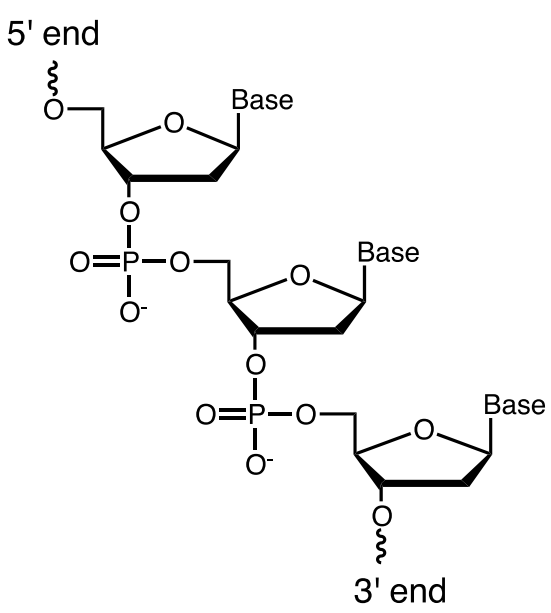


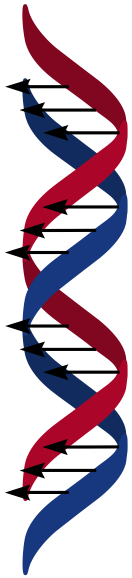
Thymine

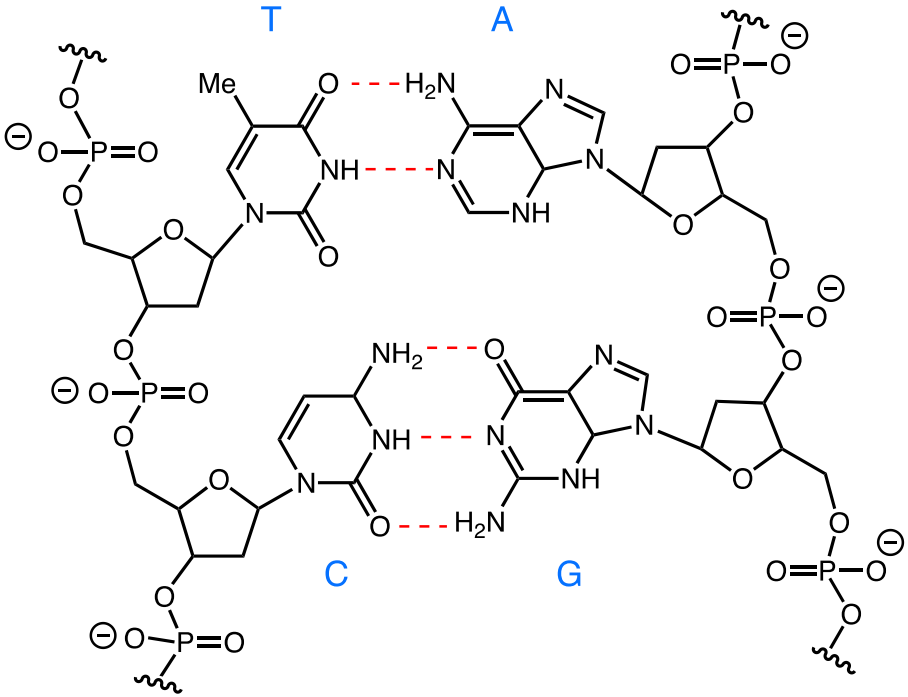
DNA

Introduction: Basics on DNA, RNA Synthesis

- DNA Structure

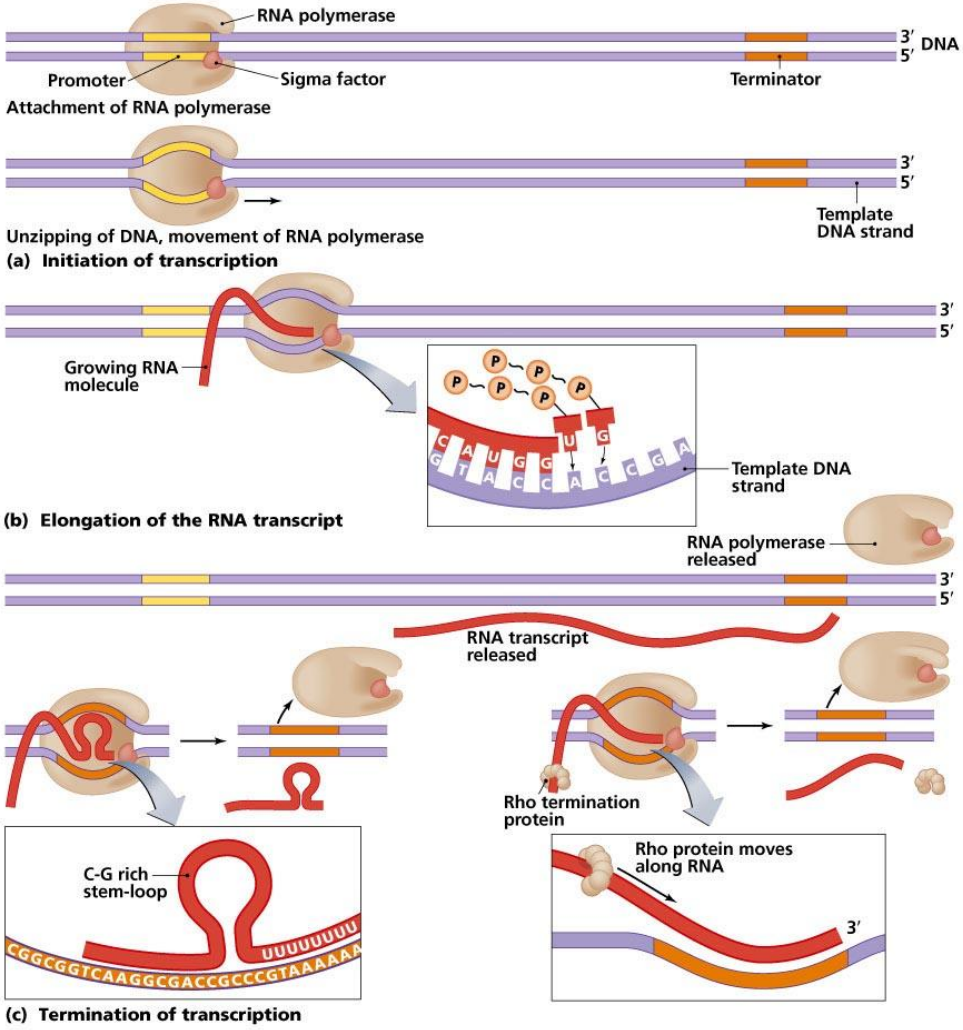






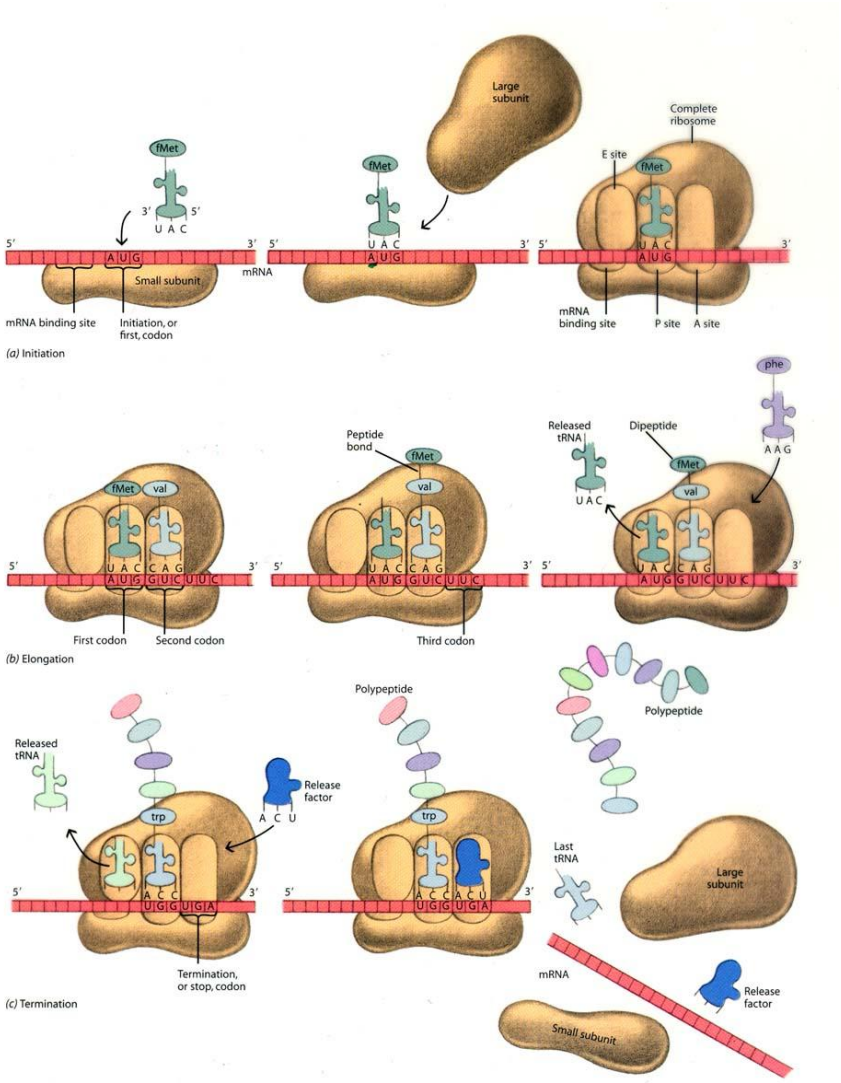
Introduction: Basics on DNA, RNA Synthesis

- DNA transcription to RNA



Introduction: Basics on DNA, RNA Synthesis

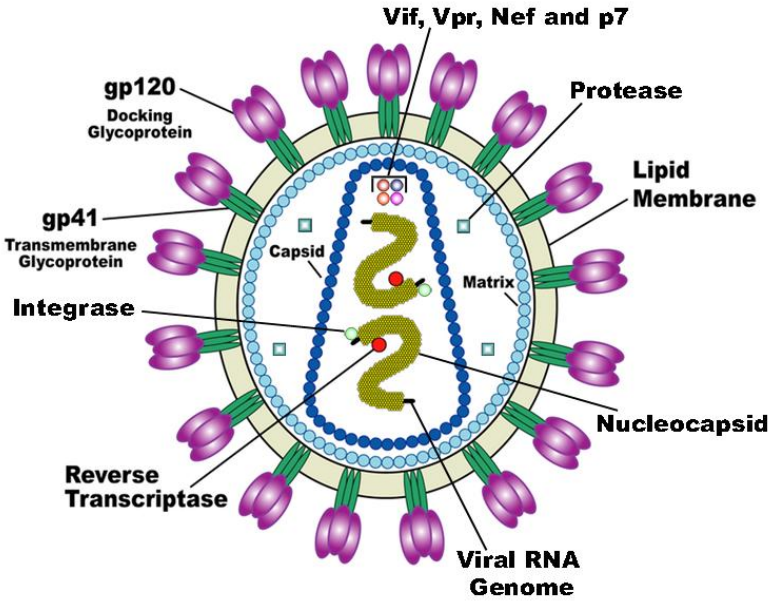
- mRNA translation, protein synthesis



Nucleoside Derivatives as Drug, Their Synthesis & Mode of Action

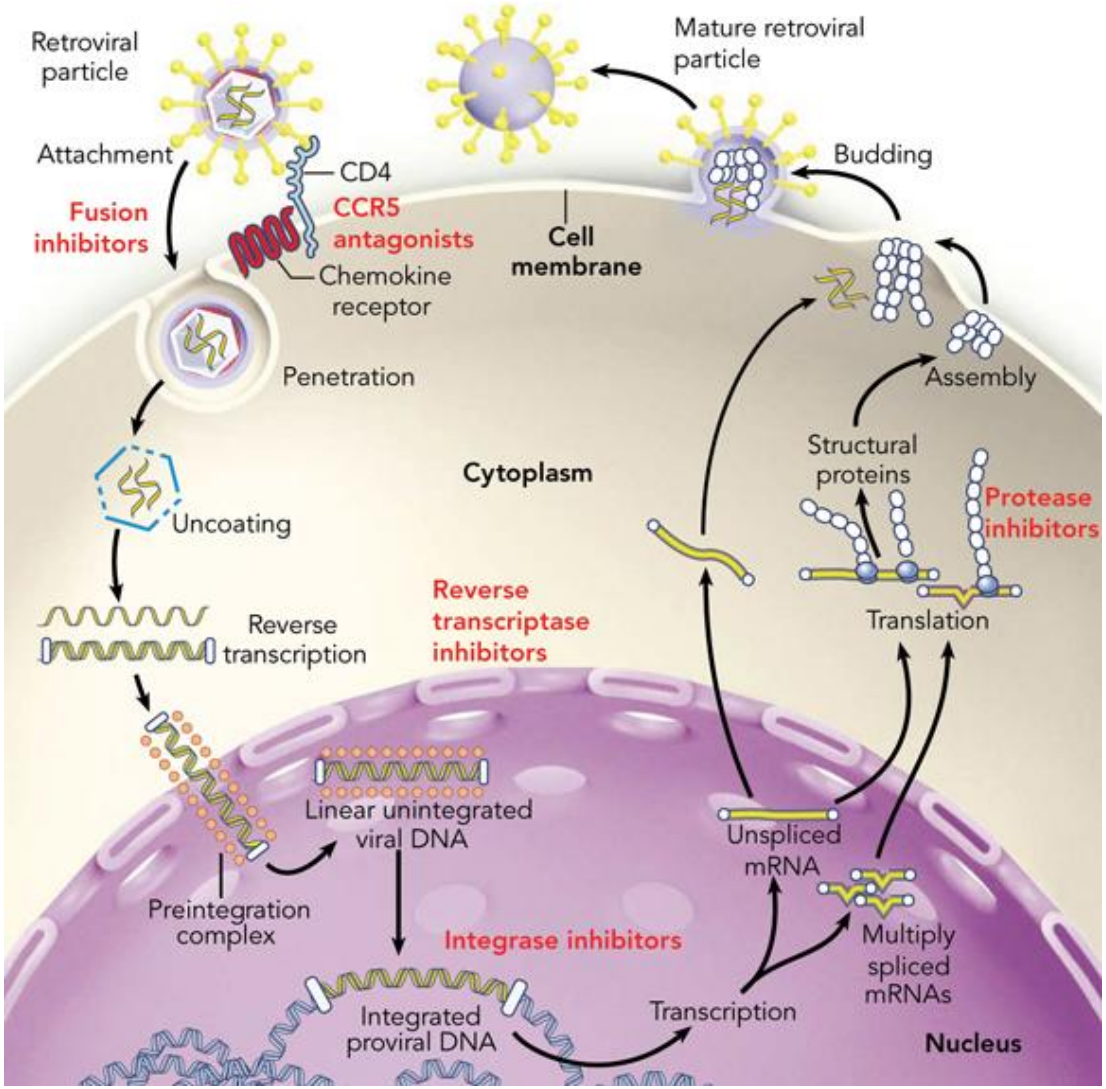
- Use of nucleoside derivatives of the treatment of :
  - HIV (NRTI)
  - Herpes virus (acilovir)
  - Oncology
  - Hepatitis C virus

HIV: 30 years of drug discovery



- AIDS was first clinically observed in 1981 in the United States
- HIV appears to have originated in southern Cameroon through the evolution of a *simian immunodeficiency virus* (SIV) that infects wild chimpanzees
- The names *Human Immunodeficiency Virus* & *Acquired ImmunoDeficiency Syndrome* (HIV / AIDS) were first used in 1983
- Until 1987 no anti-HIV drug was available
- As of 2010 approximately 34 million people have HIV worldwide

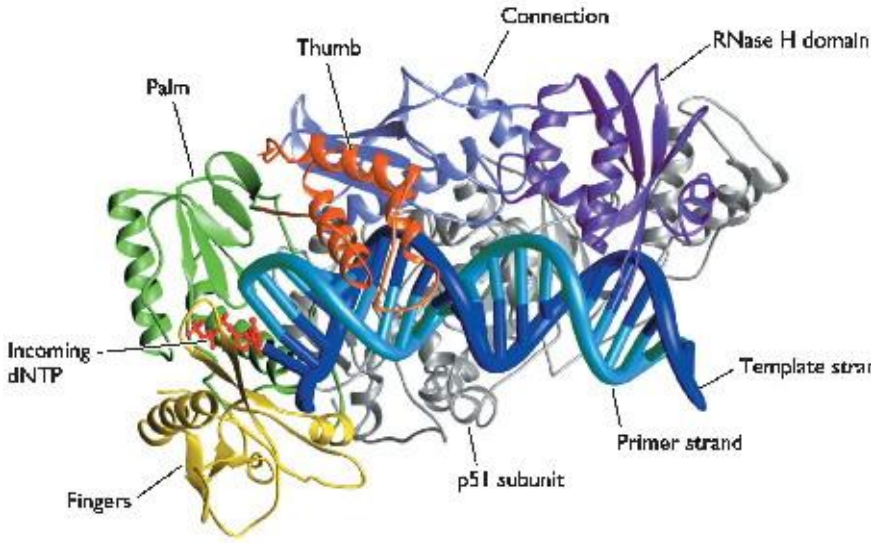
HIV: 30 years of drug discovery

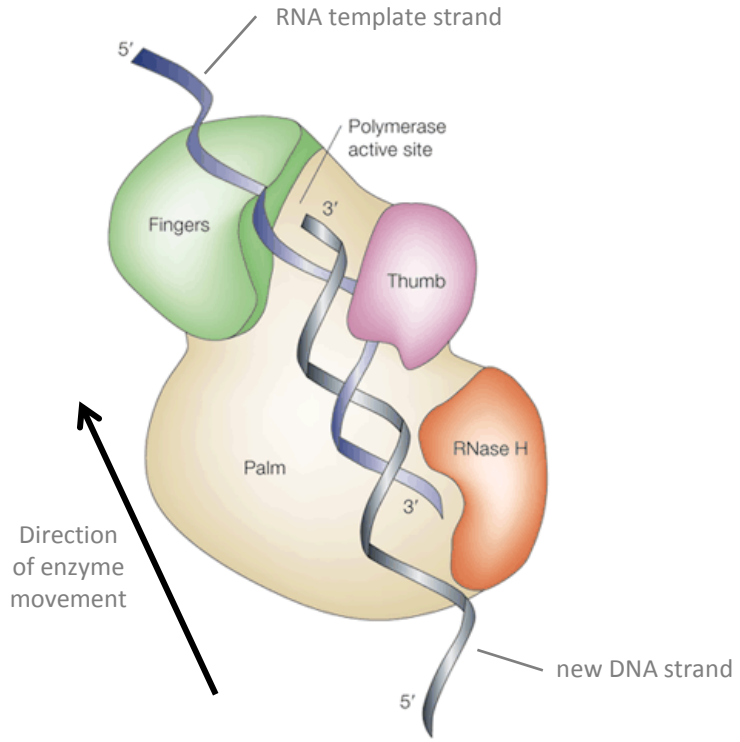


- Understanding of the life cycle of HIV has led to the identification of drug targets
- There are currently 25 FDA approved anti-HIV drugs
- Those drugs include:
  - Viral entry inhibitors
  - Reverse transcription inhibitors
  - Integrase inhibitors
  - Protease inhibitors

HIV: 30 years of drug discovery

- A large class of approved drugs target the reverse transcriptase
  - NRTI & NtRTI Nucleoside & Nucleotide Reverse Transciptase Inhibitors (8 drugs)
  - NNRTI Non-Nucleoside Reverse Transcriptase Inhibitors (5 drugs)

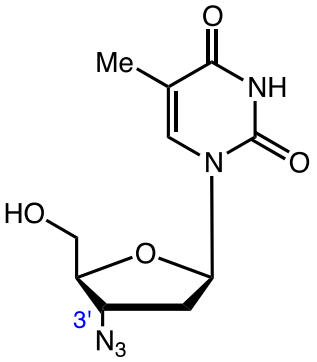




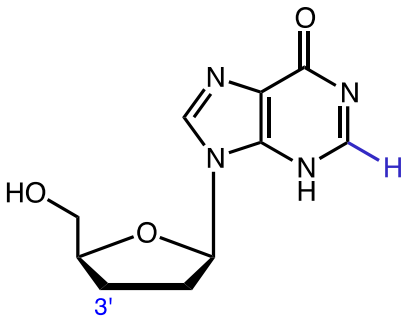
Nature Reviews | Molecular Cell Biology

HIV: 30 years of drug discovery

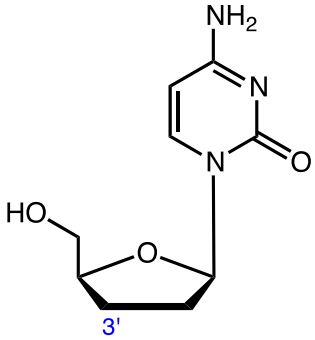
- FDA approved NRTI



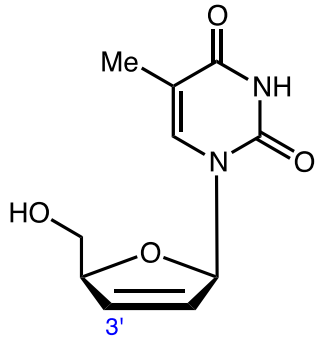
Zidovudine AZT
NIH - GSK 1987



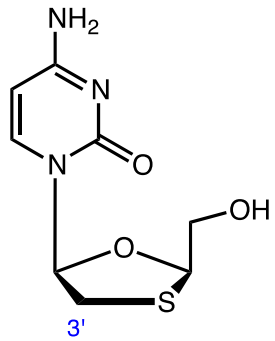
Didanosine ddI
NCI - BMS 1991



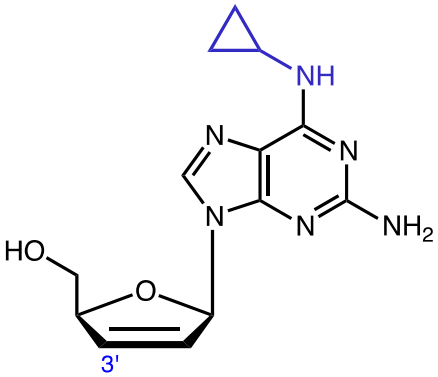
Zalcitabine ddC
NCI - Roche 1992



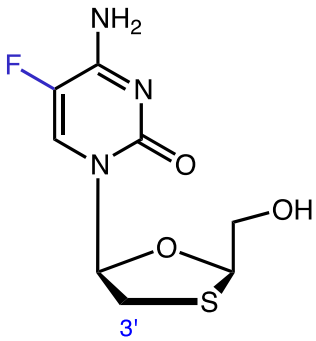
Stavudine d4T
1994



Lamivudine 3TC
GSK 1995



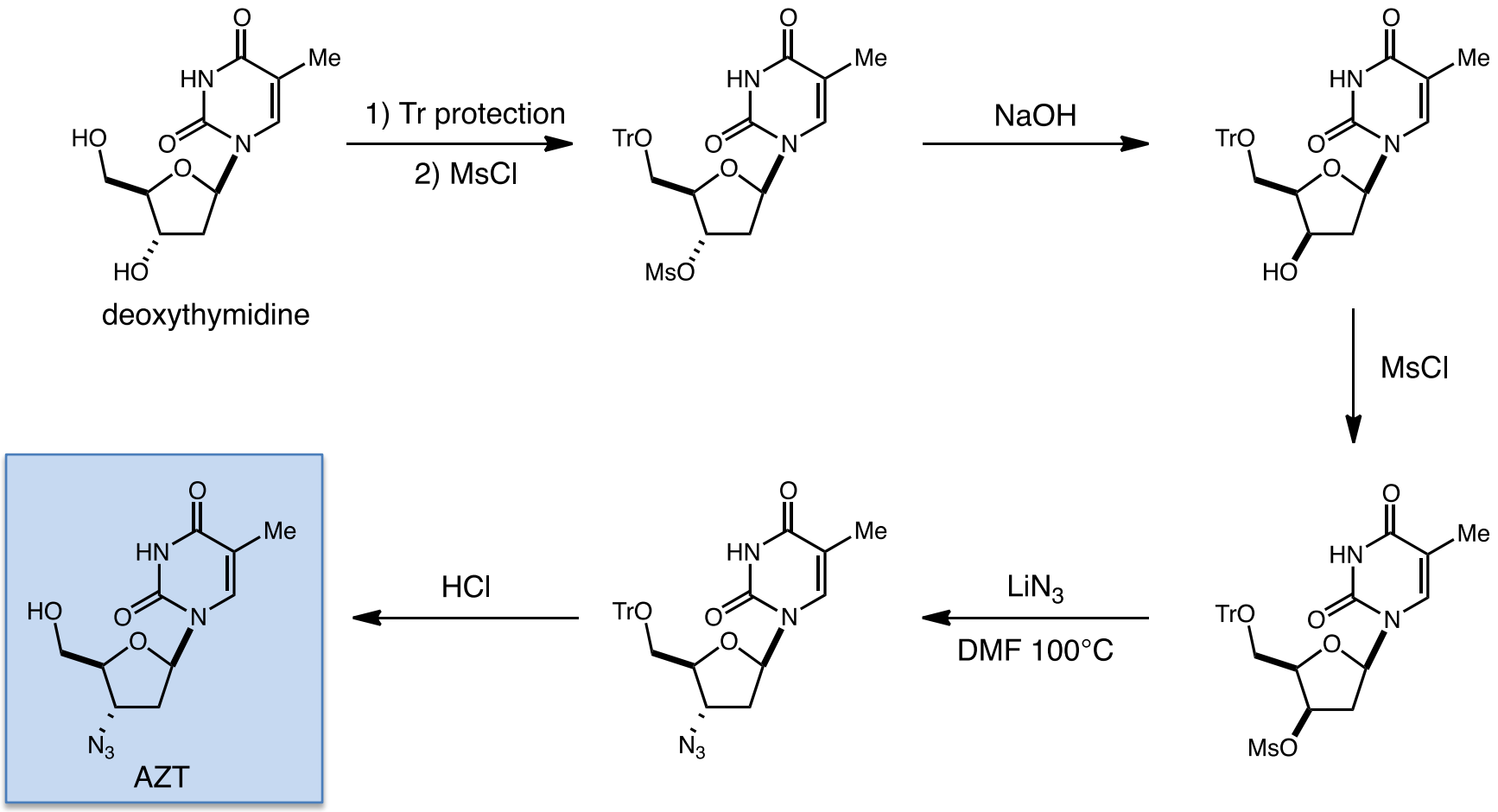
Abacavir ABC
ViiV 1998



Emtricitabine FTC
Gilead - BMS 2006

HIV: 30 years of drug discovery

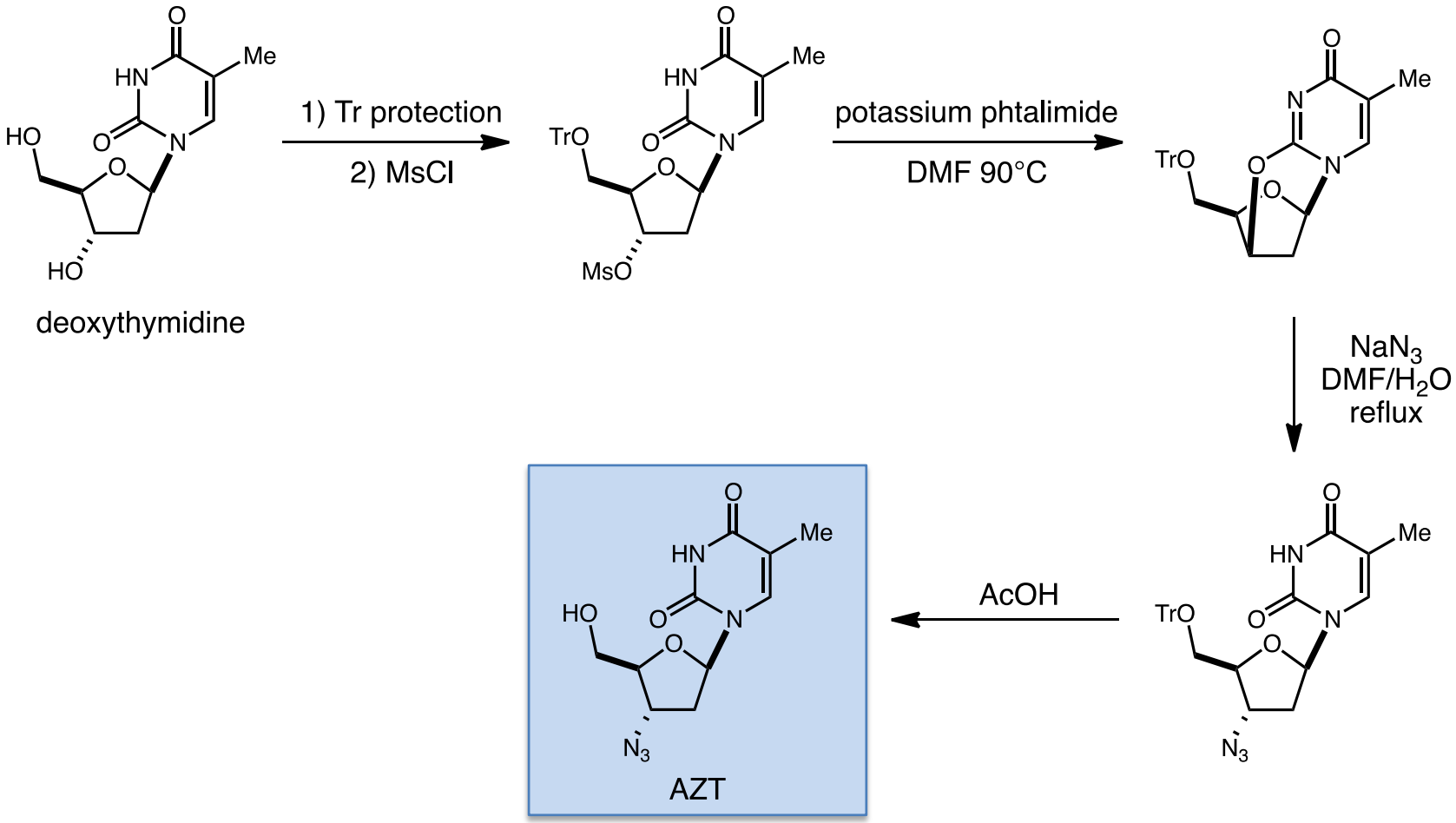
- AZT synthesis: original Horwitz's synthesis



Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076

HIV: 30 years of drug discovery

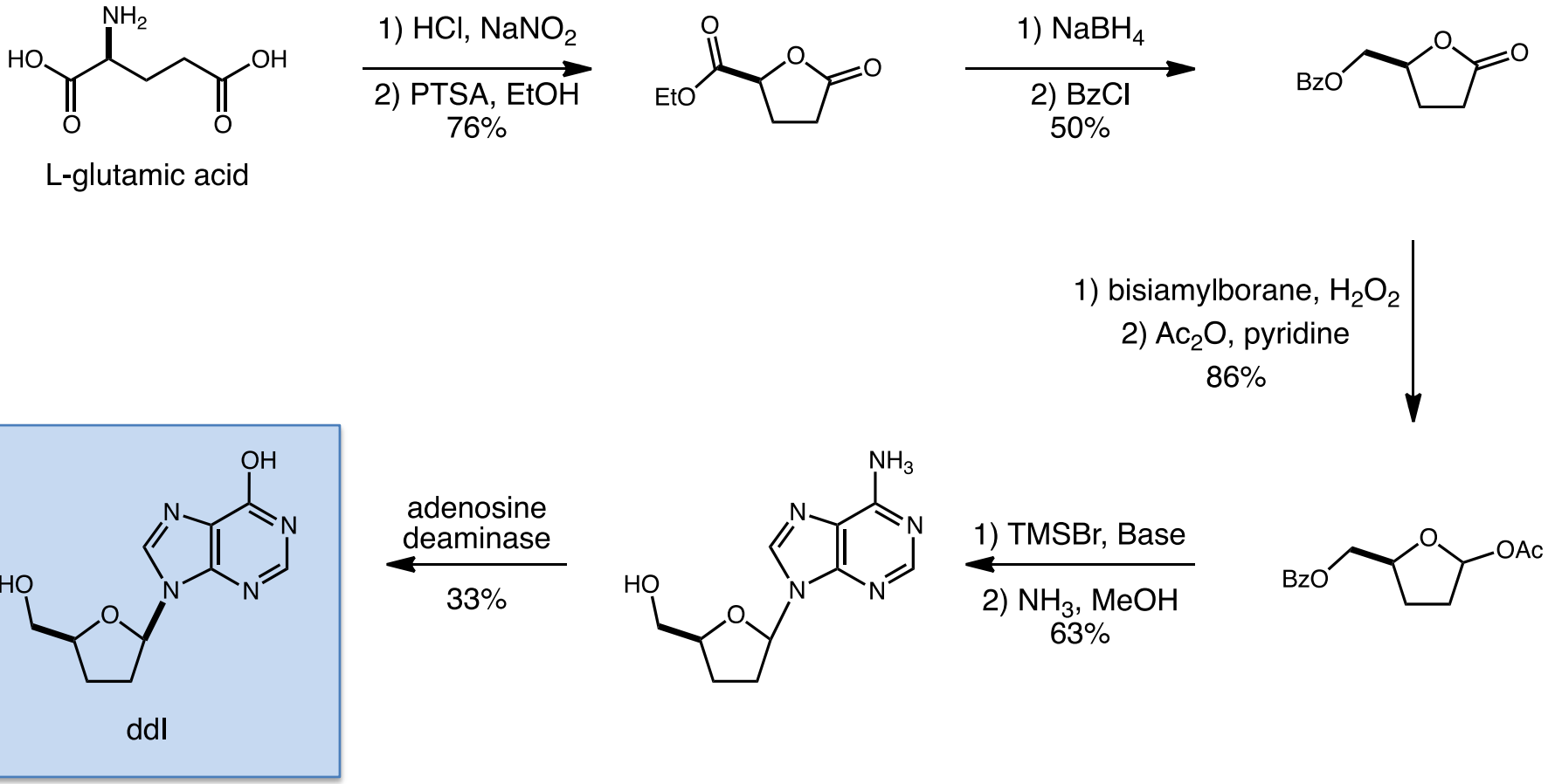
- AZT synthesis: Glinski's synthesis



Glinski, R. P.; Khan, M. S.; Kalamas, R. L.; Sporn, M. B. *J. Org. Chem.* **1973**, *38*, 4299.

HIV: 30 years of drug discovery

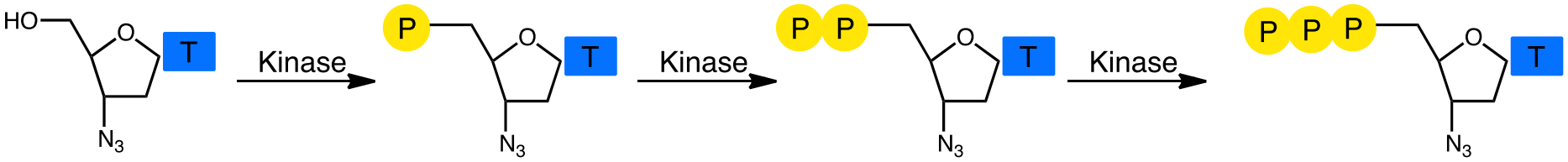
- ddI synthesis by BMS process 1988



Farina, V.; Benigni, D. A. *Tet. Lett.* **1988**, *29*, 1239–1242

HIV: 30 years of drug discovery

- NRTIs are prodrugs that require triphosphorylation to be activated

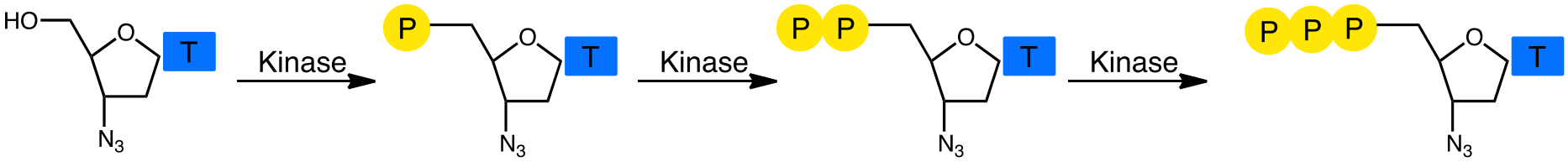


AZT (prodrug)

AZT-tP (active drug)

HIV: 30 years of drug discovery

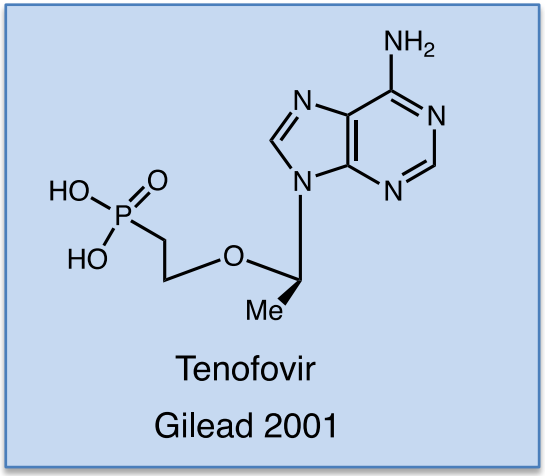
- NRTIs are prodrugs that require triphosphorylation to be activated



AZT (prodrug)

AZT-tP (active drug)

- NtRTIs bypass the difficult first phosphorylation

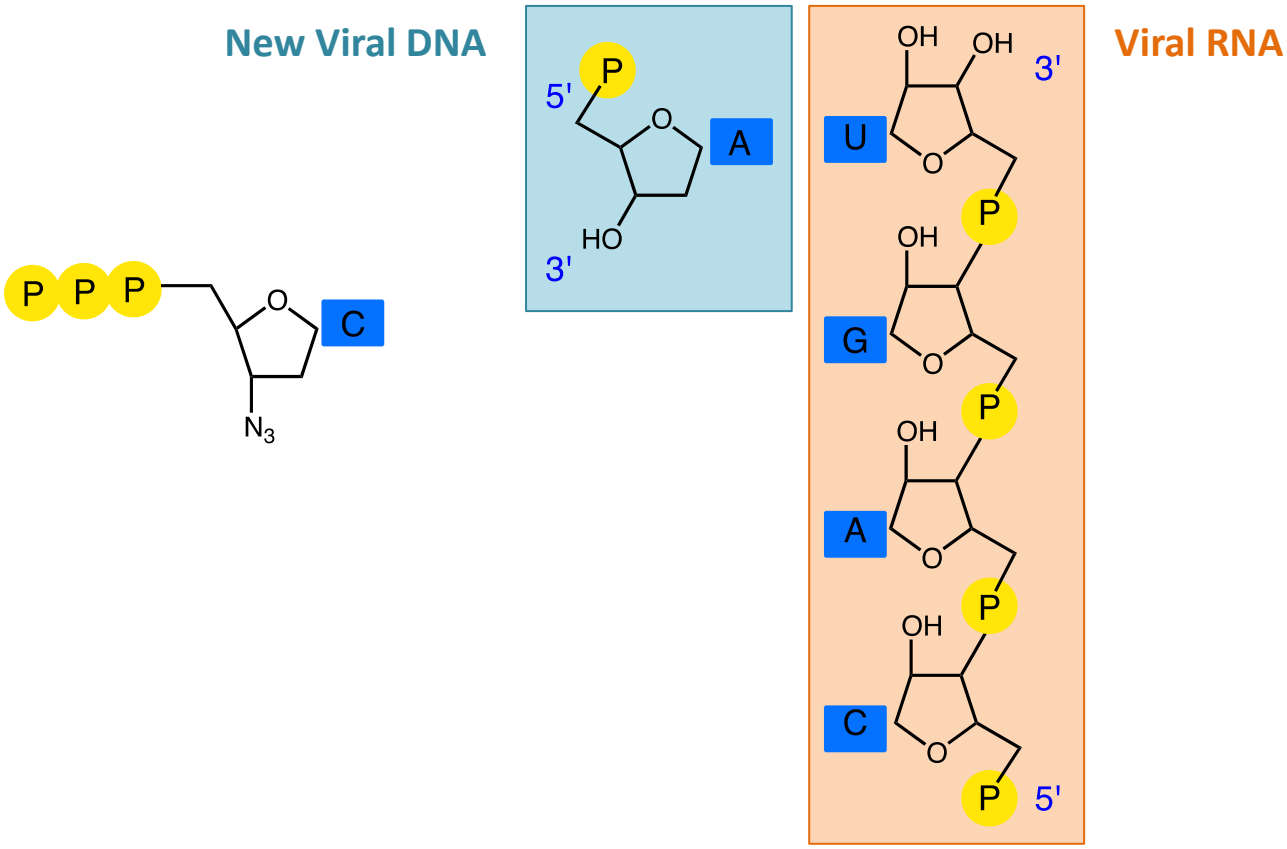


Tenofovir

Gilead 2001

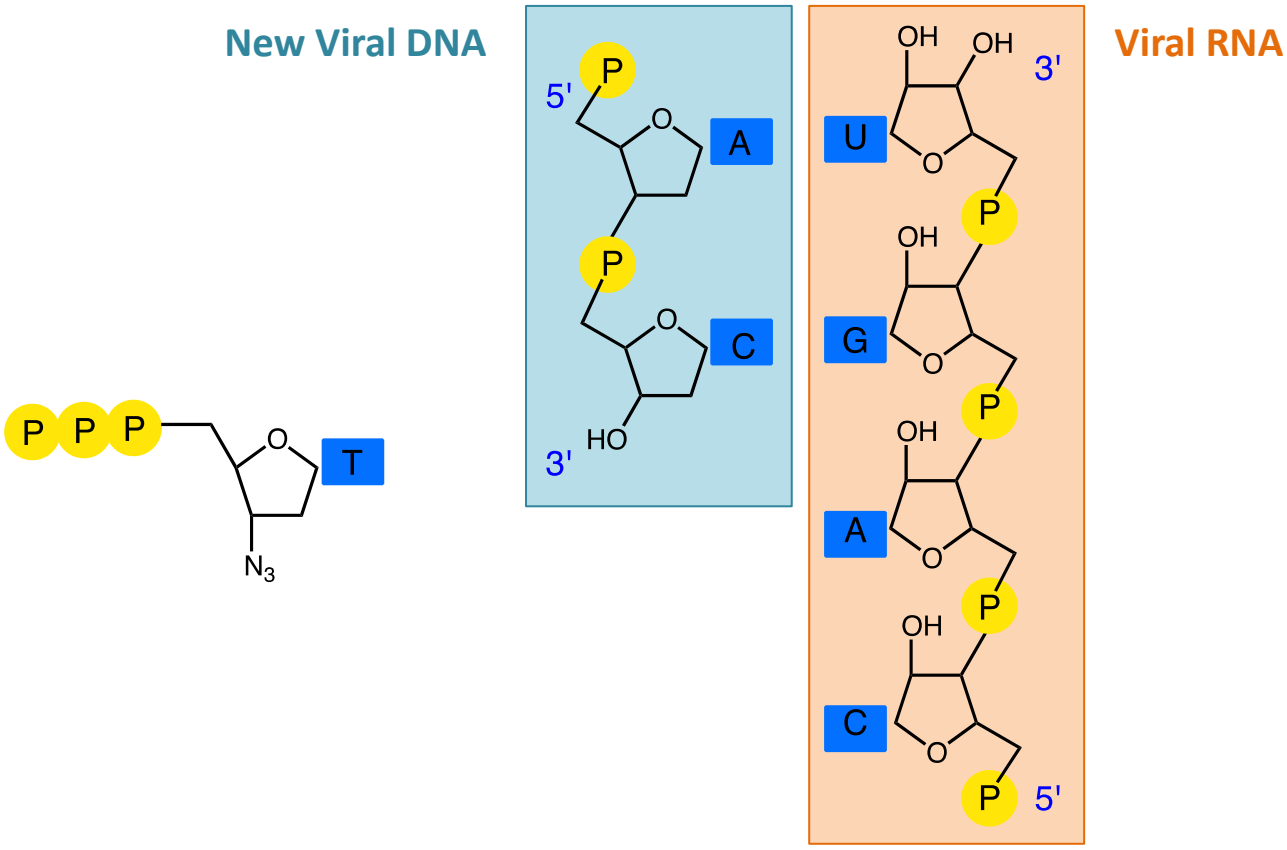
HIV: 30 years of drug discovery

- NRTI mode of action: chain termination

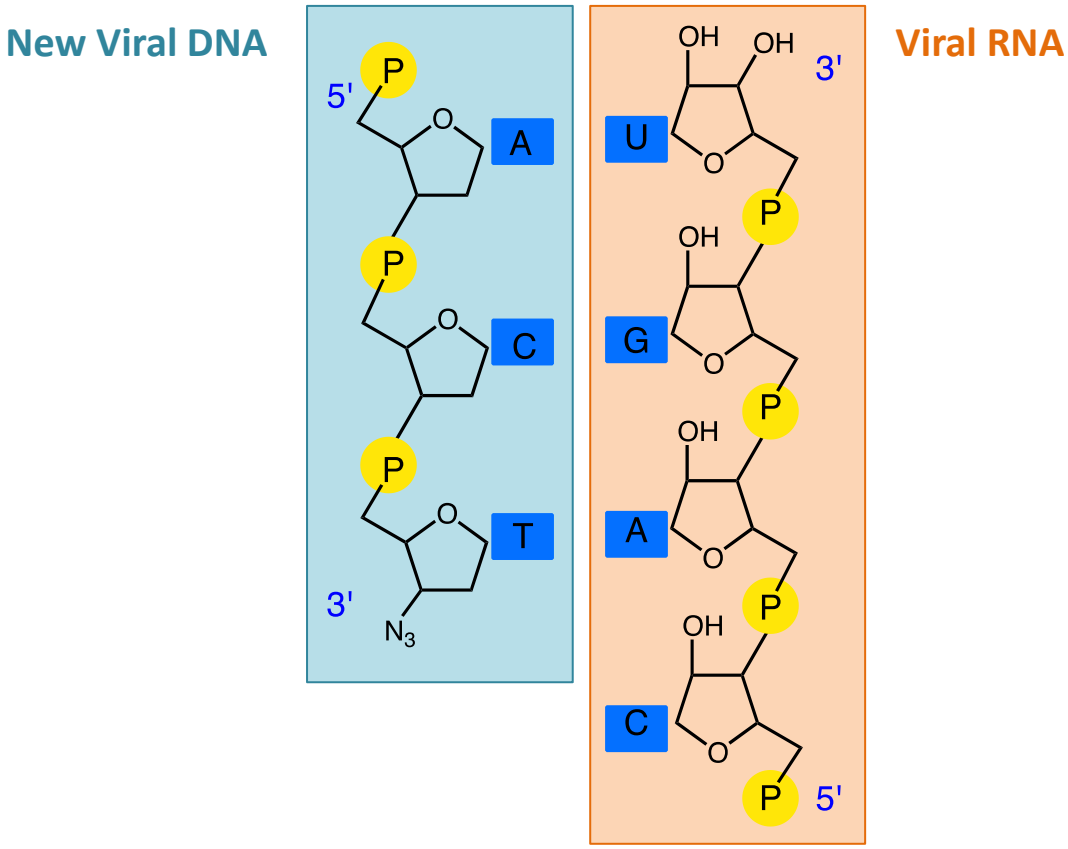


HIV: 30 years of drug discovery

- NRTI mode of action: chain termination



- NRTI mode of action: chain termination



No hydroxy group at the 3' position ⟹ Chain termination

Limitation of the NRTI based treatment

- HIV-1 RT does not have proof-reading activity. This, combined with selective pressure from the drug leads to mutations in reverse transcriptase that make the virus less susceptible to NRTIs and NNRTIs.

Limitation of the NRTI based treatment

- HIV-1 RT does not have proof-reading activity. This, combined with selective pressure from the drug leads to mutations in reverse transcriptase that make the virus less susceptible to NRTIs and NNRTIs.

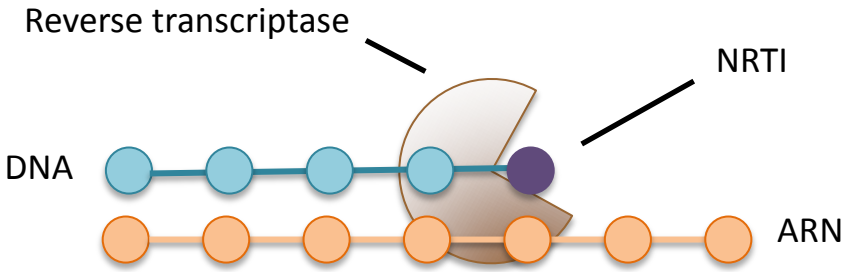
- There are two major mechanisms of NRTI resistance

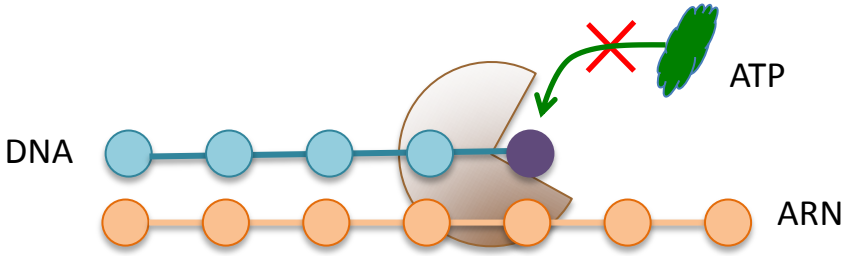
  - Reduced incorporation of the NRTI into DNA over the normal nucleotide. This results from mutations in the N-terminal polymerase domain of the reverse transcriptase that reduce the enzyme's affinity or ability to bind to the drug

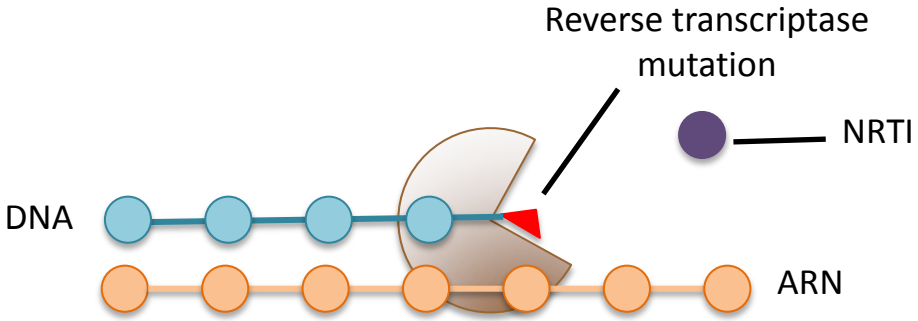
  - Excision or hydrolytic removal of the incorporated drug. This 'unblocks' the DNA chain, allowing it to be extended, and replication to continue.

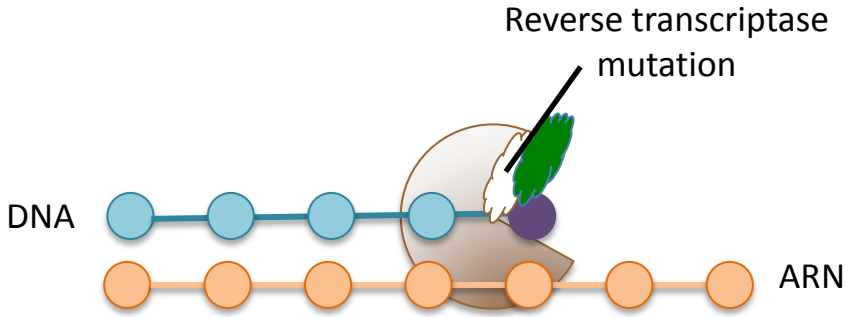
HIV: 30 years of drug discovery

- Some viral mutations result in NRTI inefficacy









Mutation allows reparation of the DNA

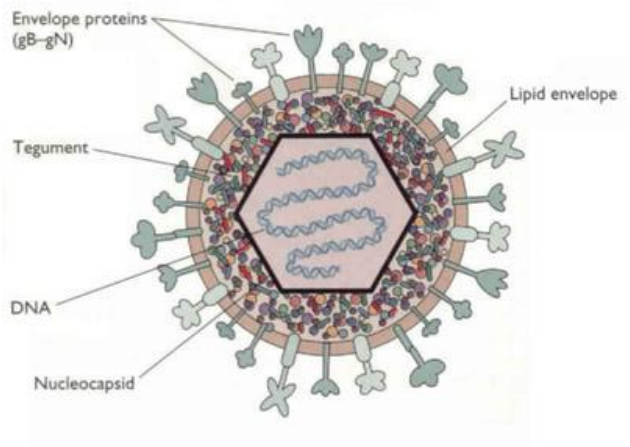
NRTI based treatment limitations

- In order to overcome viral resistance, **HAART** (highly active antiretroviral therapy) has been developed. It uses multiple drugs that act on different viral targets. HAART decreases the patient's total burden of HIV, maintains function of the immune system, and prevents opportunistic infections that often lead to death.

- All NRTI drugs have a lot of side effects including diarrhea, headache, nausea, rash, fever....

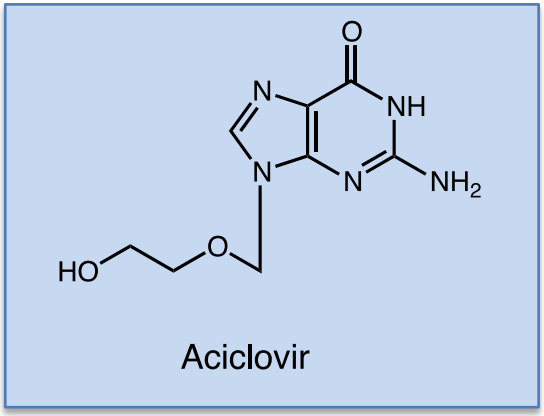
- One of the most important side effect is due to the incorporation on the NRTI during the replication of the DNA. Especially mitochondrial DNA due to the NRTI phosphorilation taking place in the mitochondria. This results in mitochondrial dysfunction and cell energy deprivation.

Herpesviridae



- The Herpesviridae is a large family of DNA viruses also known as herpesvirus
- 5 species of Herpesviridae exist:
  - HSV-1→ facial cold-sores
  - HSV-2 → genital herpes
  - Varicella zoster virus → chicken-pox and shingles
  - Epstein-Barr virus → mononucleosis
  - Cytomegalovirus → not been reported to cause human disease.
- More than 90% of adults have been infected with at least one of these, and a latent form of the virus remains in most people

Treatment of herpes: Aciclovir

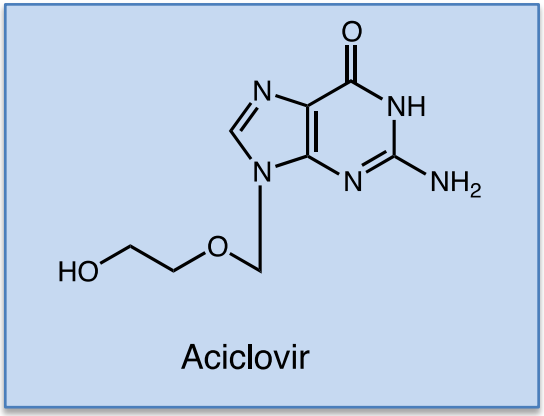


Aciclovir

- Aciclovir was seen as the start of a new era in antiviral therapy as it is extremely selective and low in cytotoxicity

- The synthesis of aciclovir was base on the structure of nucleosides isolated from a Caribbean sponge, *Cryptotethya crypta*

Treatment of herpes: Aciclovir



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- Gertrude Elion received the Nobel prize of Medecine 1988 *"for the discoveries of important principles for drug treatment"* including the synthesis of aciclovir

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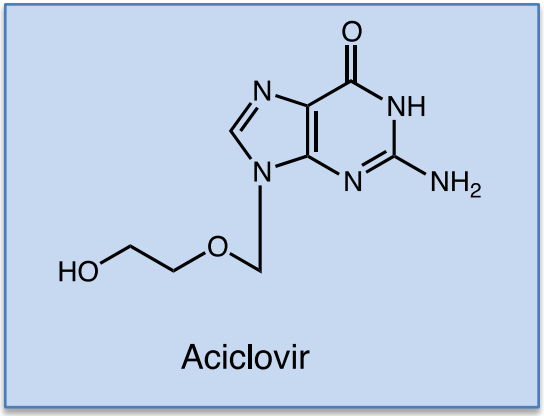

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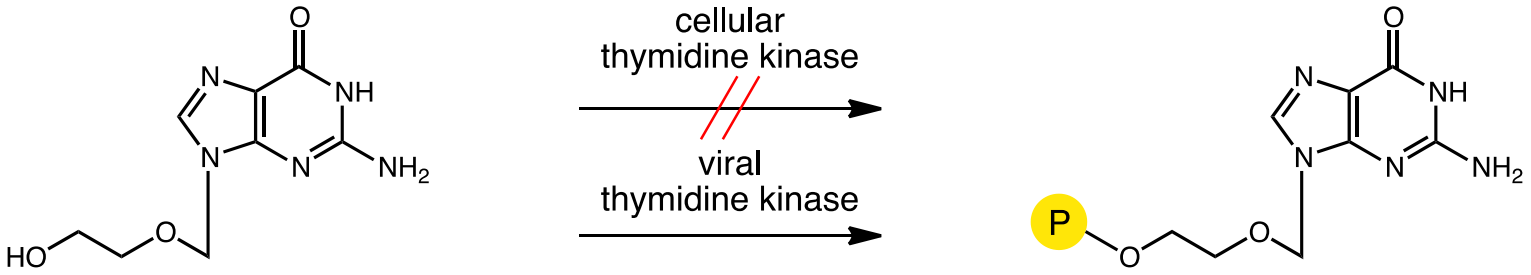
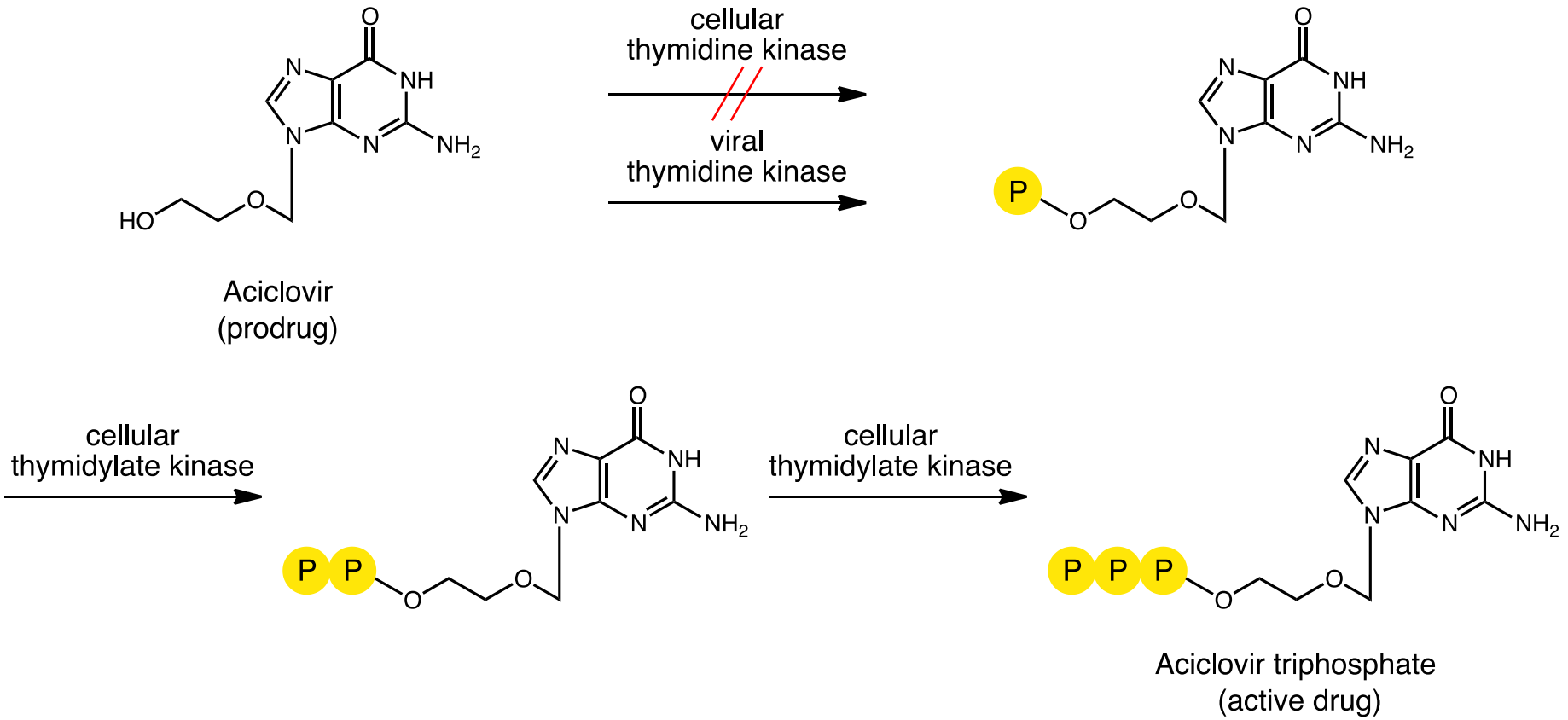
- It was FDA approved in1982 for the treatment of HSV-1, HSV-2 and Varicella zoster virus

Treatment of herpes: Aciclovir



Aciclovir
(prodrug)

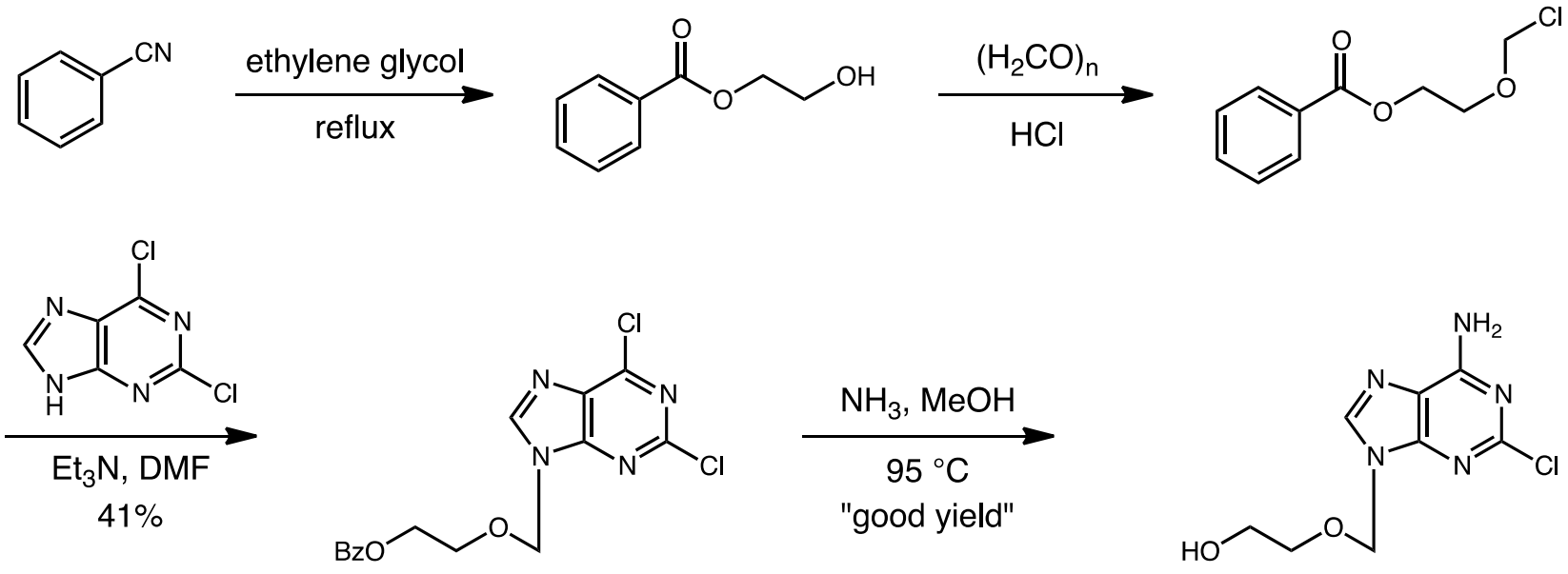
Treatment of herpes: Aciclovir



- Aciclovir is only converted to its active form in infected cells
- Aciclovir triphosphate shows a 50-fold selectivity action against viral DNA polymerases relative to cellular polymerases. DNA incorporation results in premature chain termination of the viral DNA

Treatment of herpes: Aciclovir

- Aciclovir synthesis published in Nature in 1978



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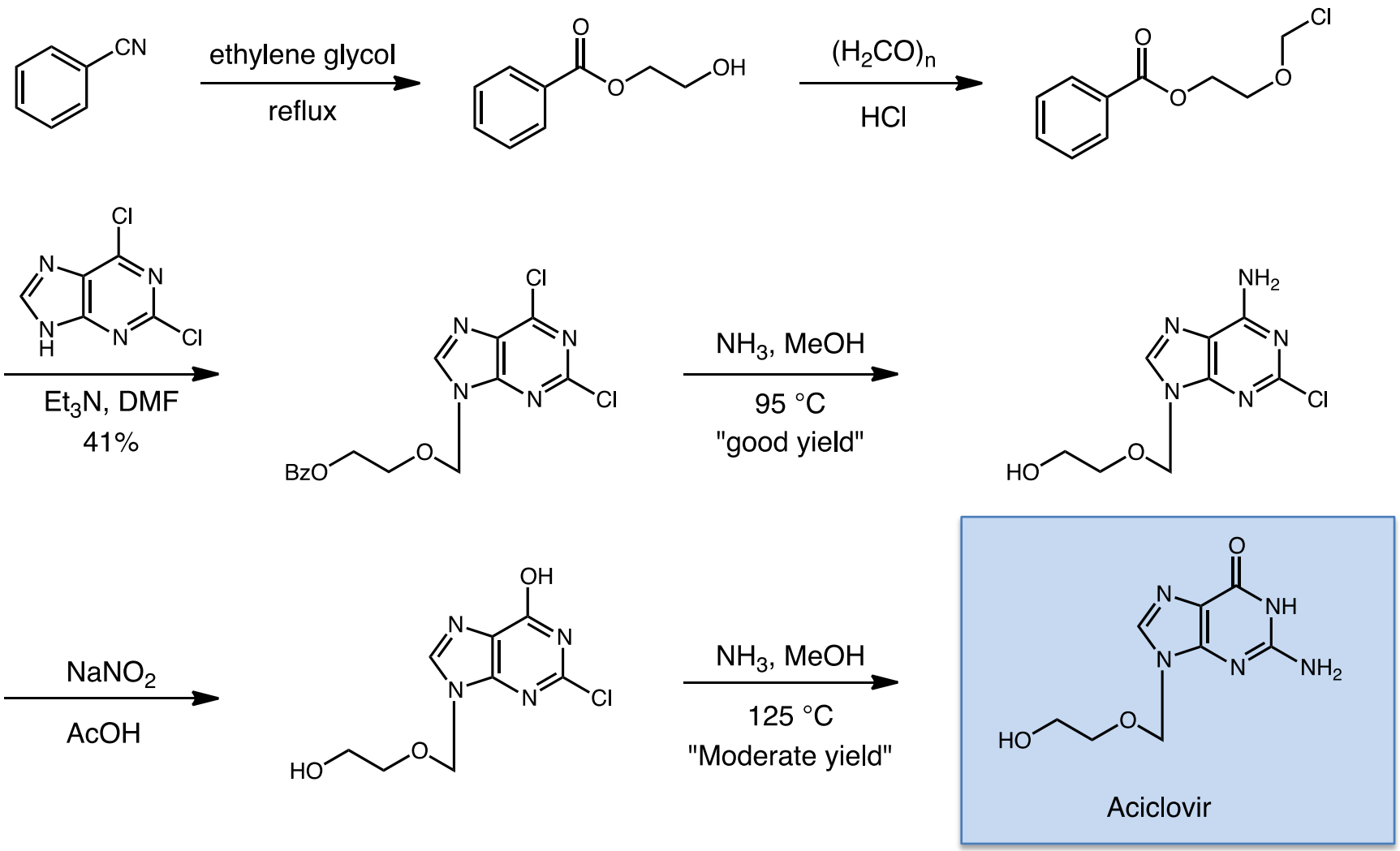

Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature* **1978**, *272*, 583

Nucleoside analogues for the treatment of cancer

Nucleoside analogues for the treatment of cancer

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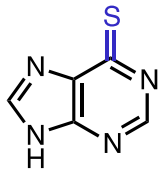
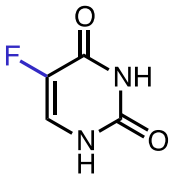
- The basic mechanism of action of purine and pyrimidine antimetabolites is similar. These compounds diffuse into cells and are converted to analogues of cellular nucleotides by enzymes of the purine or pyrimidine metabolic pathway. These metabolites then inhibit one or more enzymes that are critical for DNA synthesis, causing DNA damage and induction of apoptosis

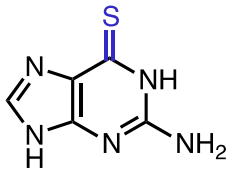
Nucleoside analogues for the treatment of cancer



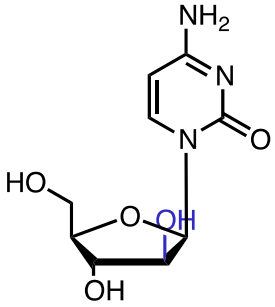
6-mercaptopurine
1953



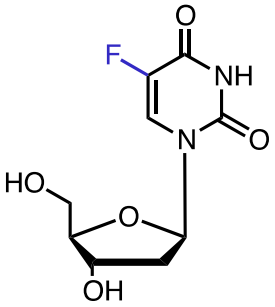
5-fluorouracil
1962



6-thioguanine
1966

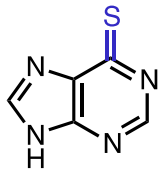


cytarabine
1969



floxuridine
1970

Nucleoside analogues for the treatment of cancer



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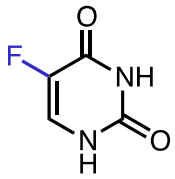

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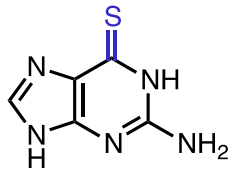

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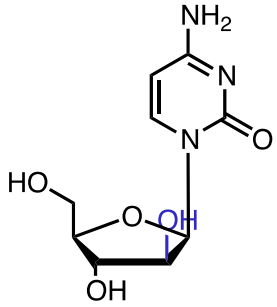

cytarabine
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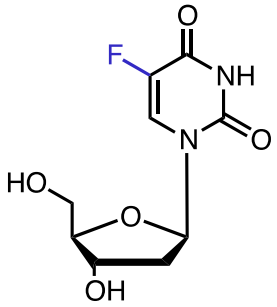

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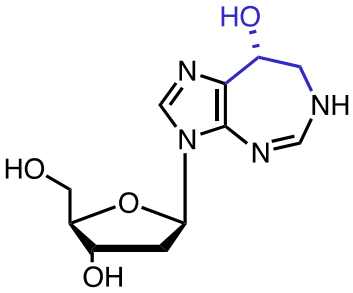
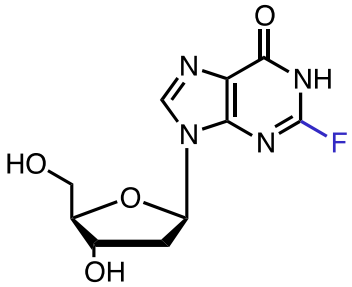
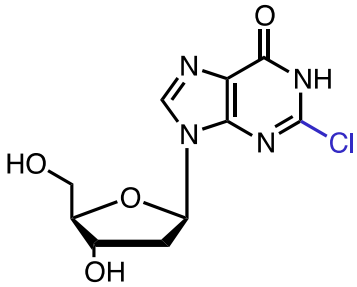


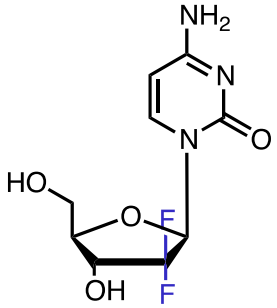
pentostatin
1991



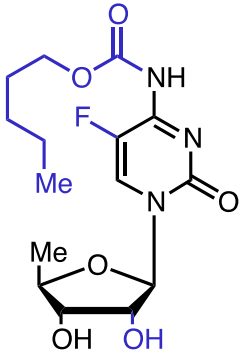
fludarabine
1991



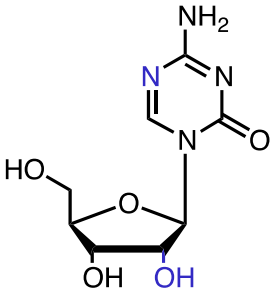
cladribine
1992



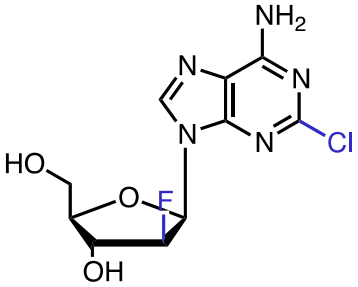
gemcitabine
1996



capecitabine
1998



vidaza
2004



clofarabine
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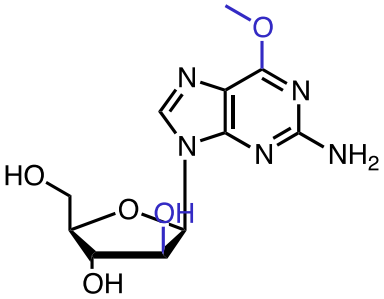
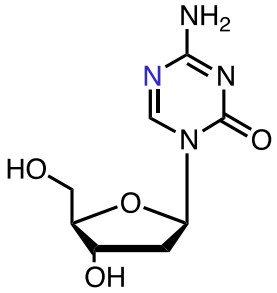
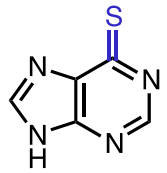


nelarabine
2005



decitabine
2006

Nucleoside analogues for the treatment of cancer



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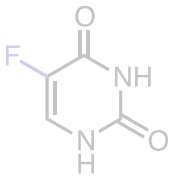

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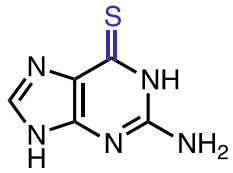

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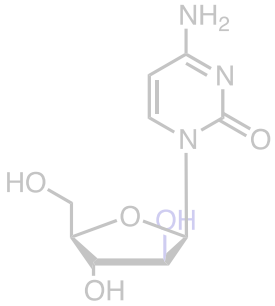
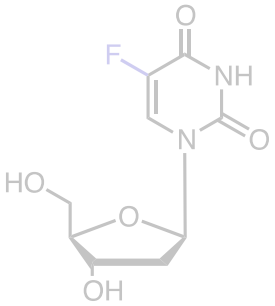
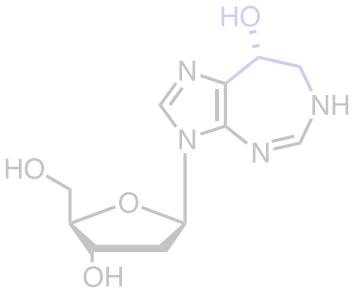


cytarabine
1969



floxuridine
1970



pentostatin
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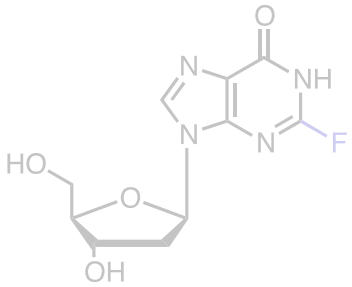
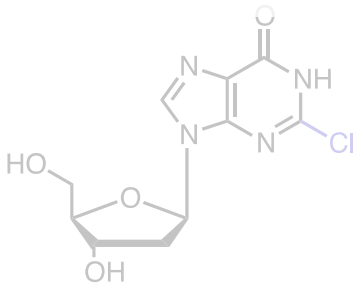
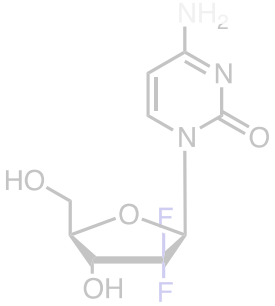


fludarabine
1991



cladribine
1992



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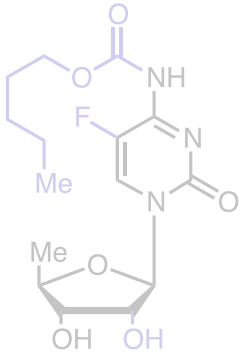

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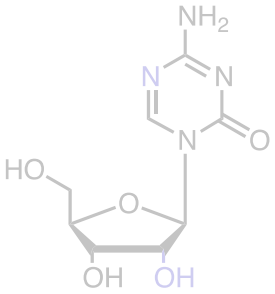

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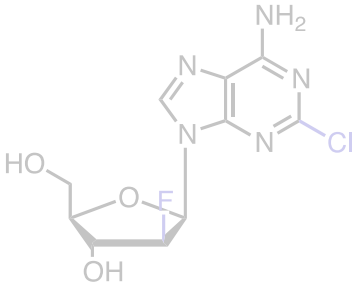

clofarabine
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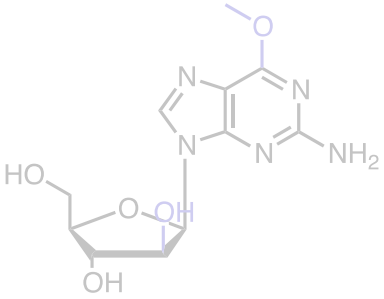
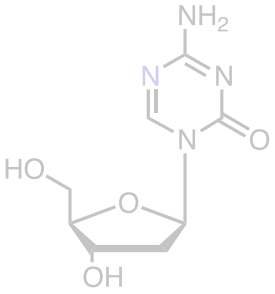


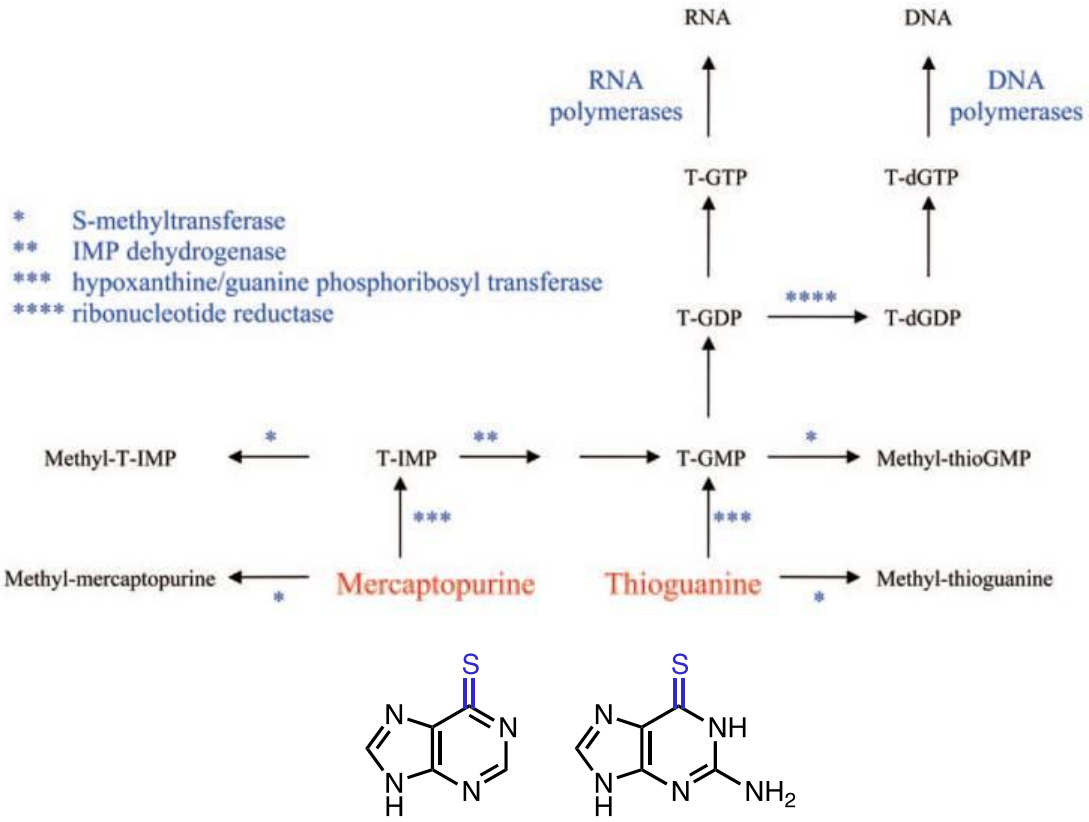
nelarabine
2005



decitabine
2006

Nucleoside analogues for the treatment of cancer

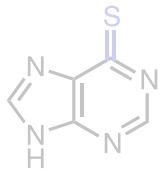
- Thiopurines



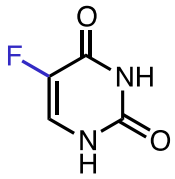
- Treatment of cells with MP does not result in the immediate inhibition of DNA synthesis. But incorporation into DNA results in damage which are recognized by mismatch repair enzymes, which causes a futile cycle of repair that results in lethal DNA damage

Parker, W.B. *Chem. Rev.* **2009**, *109*, 2880

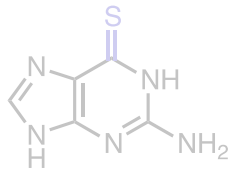
Nucleoside analogues for the treatment of cancer



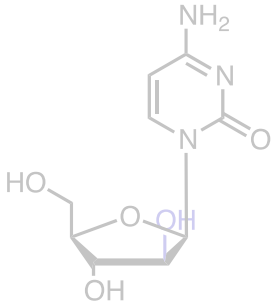
6-mercaptopurine
1953



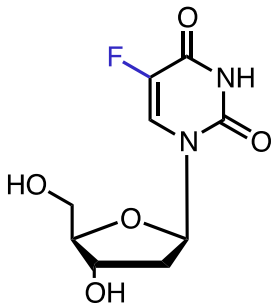
5-fluorouracil
1962



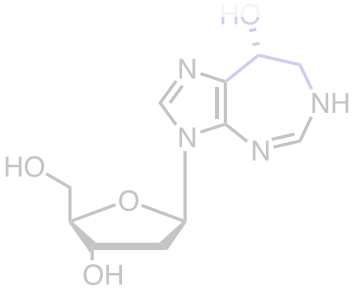
6-thioguanine
1966



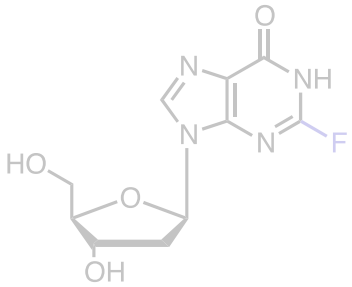
cytarabine
1969



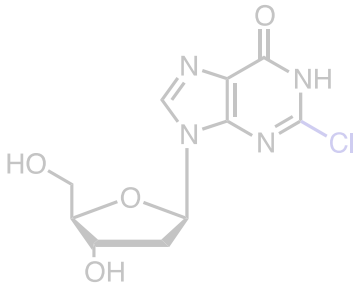
floxuridine
1970



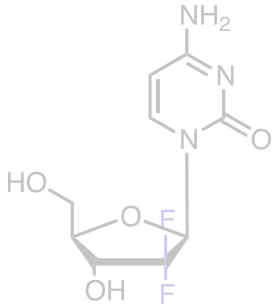
pentostatin
1991



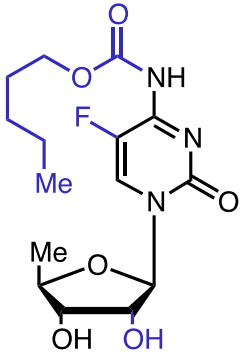
fludarabine
1991



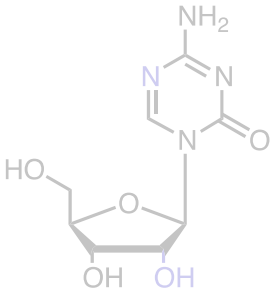
cladribine
1992



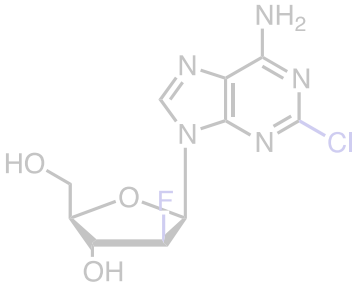
gemcitabine
1996



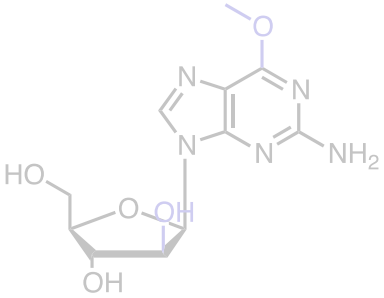
capecitabine
1998



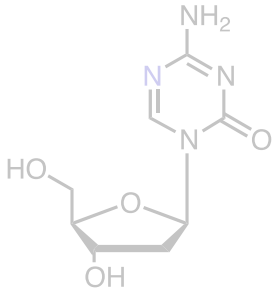
vidaza
2004



clofarabine
2004



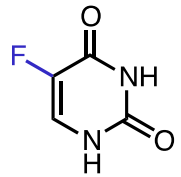
clofarabine
2005



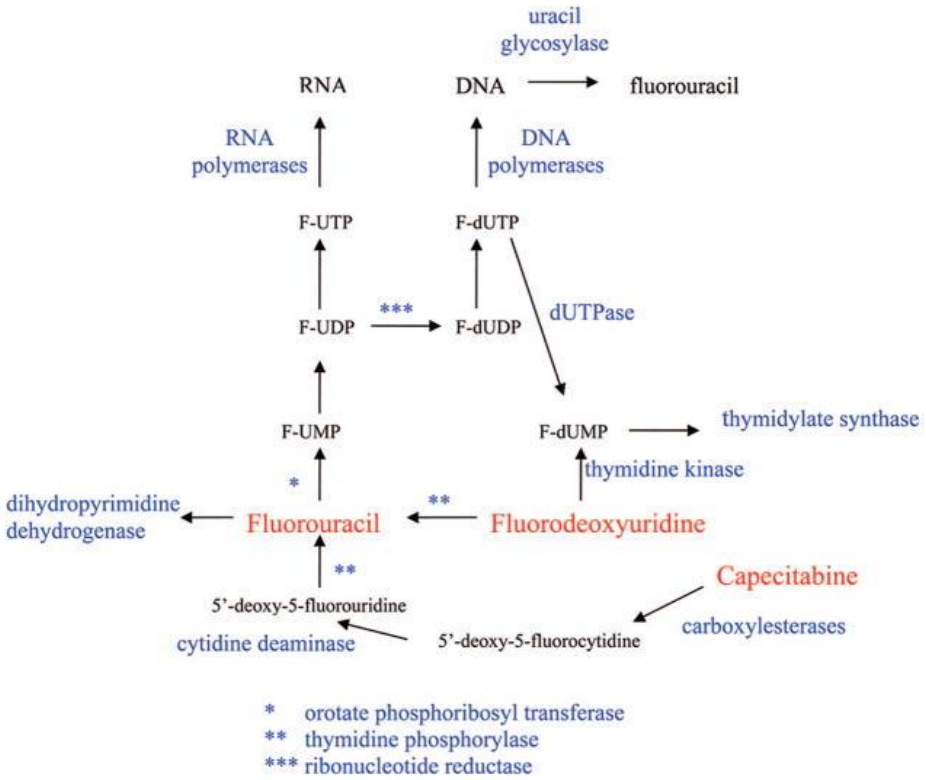
decitabine
2006

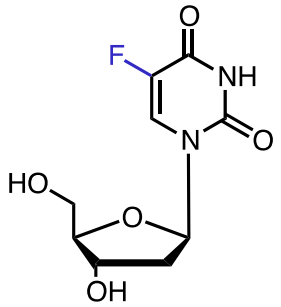
Nucleoside analogues for the treatment of cancer

- Fluoropyrimidines

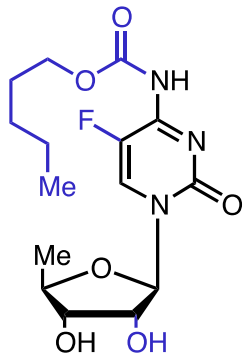


5-fluorouracil





florxuridine



capecitabine

- Treatment of cells with FUra does not result in the immediate DNA synthesis termination. The enzyme responsible for the removal of uracil from DNA, uracil glycosylase recognizes FUra in DNA as a substrate and readily removes it from the DNA causing chain breakage

Parker, W.B. *Chem. Rev.* **2009**, *109*, 2880

Nucleoside analogues for the treatment of cancer

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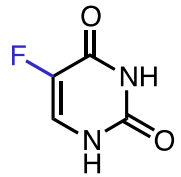
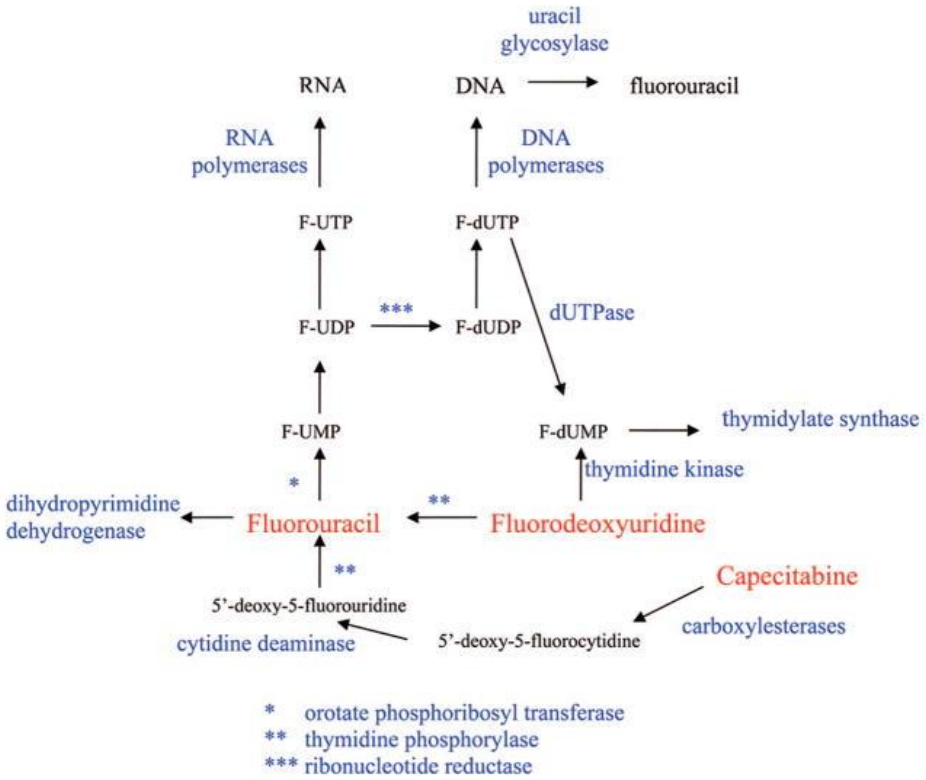
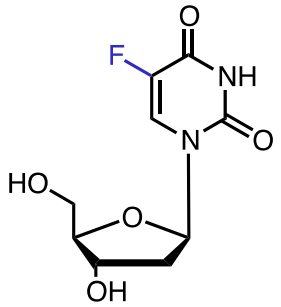
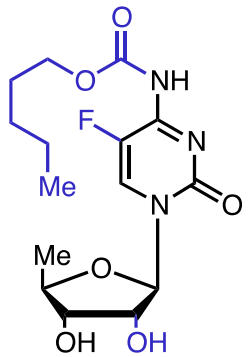


5-fluorouracil





florxuridine



capecitabine

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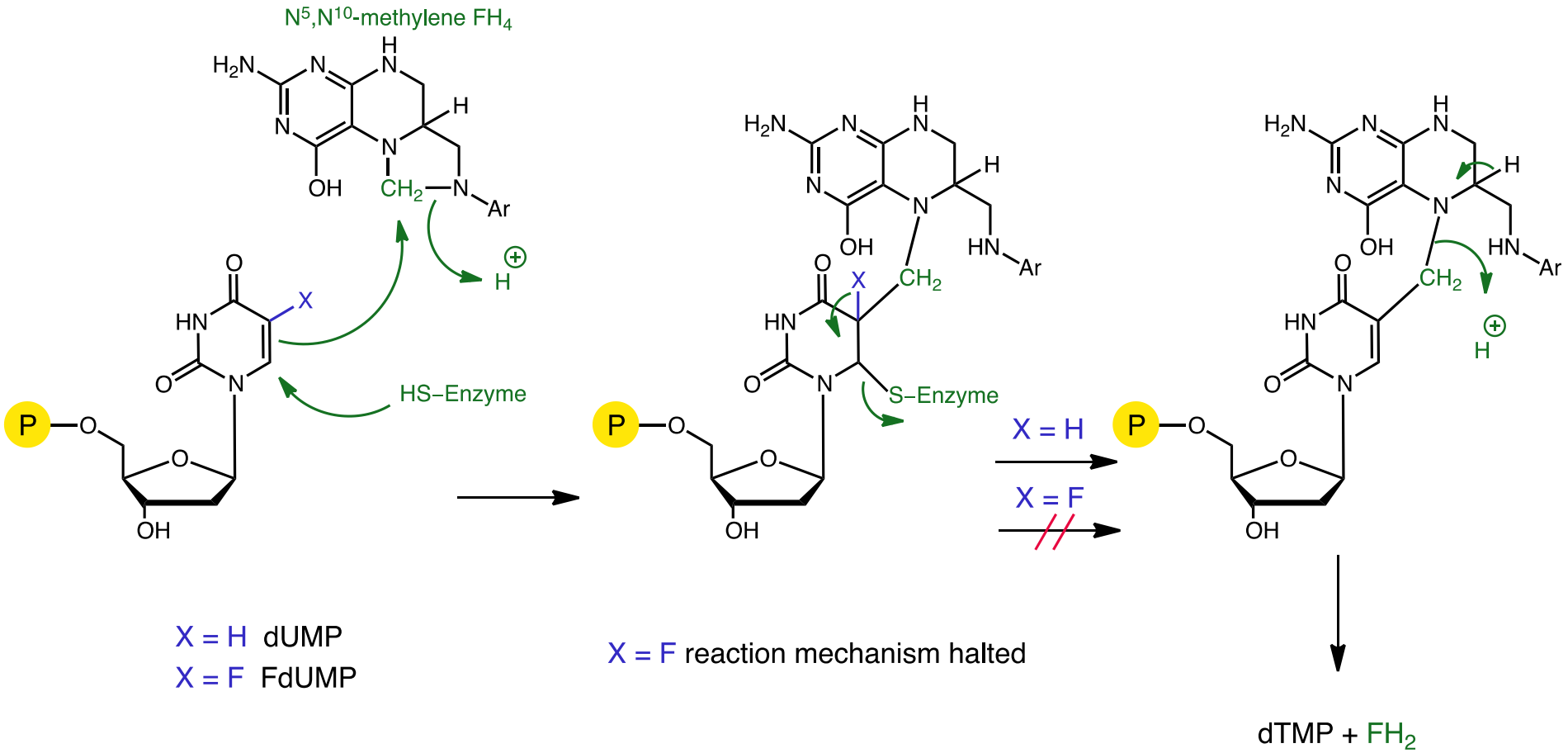
- FUra acts as a suicide substrate for thymidylate synthase blocking the synthesis of thymidine

Parker, W.B. *Chem. Rev.* **2009**, *109*, 2880

Nucleoside analogues for the treatment of cancer

- Fluoropyrimidines form a covalent bond with N^5,N^{10}-methylene FH_4 cofactor needed for the thymidylate synthase

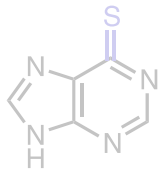


X = H dUMP
X = F FdUMP

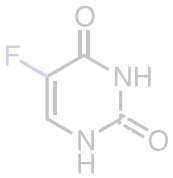
X = F reaction mechanism halted

Patrick, G. L. *An Introduction to Medicinal Chemistry*, Oxford University Press

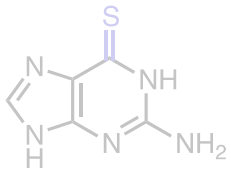
Nucleoside analogues for the treatment of cancer



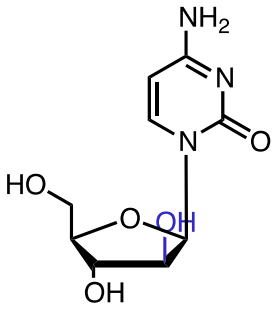
6-mercaptopurine
1953



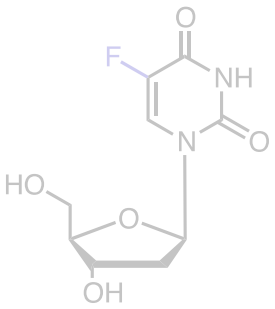
5-fluorouracil
1962



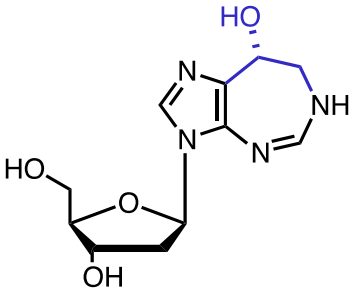
6-thioguanine
1966



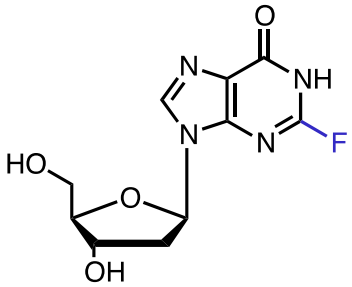
cytarabine
1969



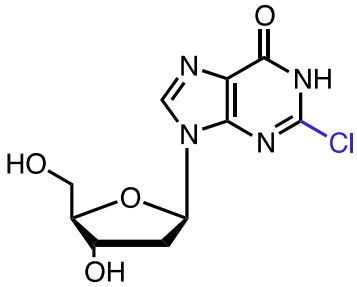
floxuridine
1970



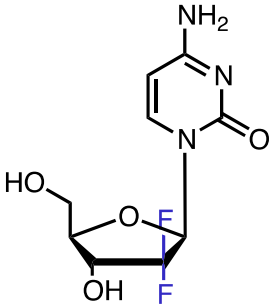
pentostatin
1991



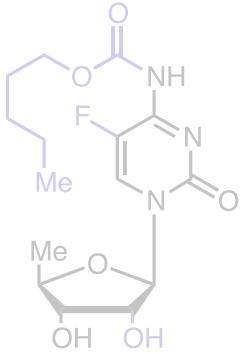
fludarabine
1991



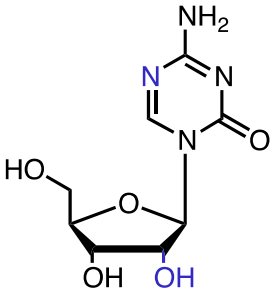
cladribine
1992



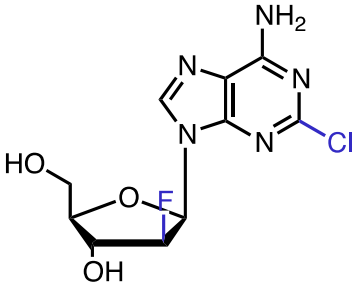
gemcitabine
1996



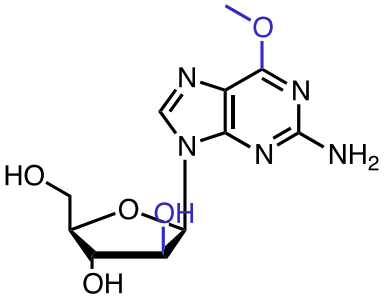
capecitabine
1998



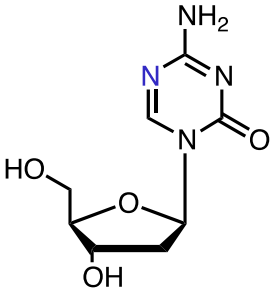
vidaza
2004



clofarabine
2004



clofarabine
2005



decitabine
2006

Nucleoside analogues for the treatment of cancer

- Deoxynucleoside analogues' mechanisms of action are quite similar. They are converted to their respective nucleotide analogues, which inhibit DNA synthesis by inhibition of DNA polymerases and/or ribonucleotide reductase

Nucleoside analogues for the treatment of cancer

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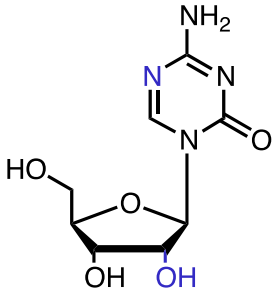
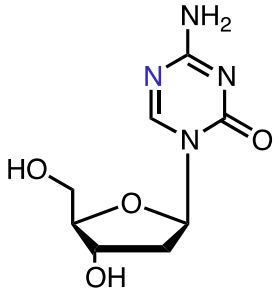
- For example vidaza and decitabine are incorporated into DNA and inhibit DNA methylation. Methylation of the 5 position of cytosine residues in DNA is a major mechanism that is used by human cells to control gene expression. This result in the activation of epigenetically repressed genes



vidaza

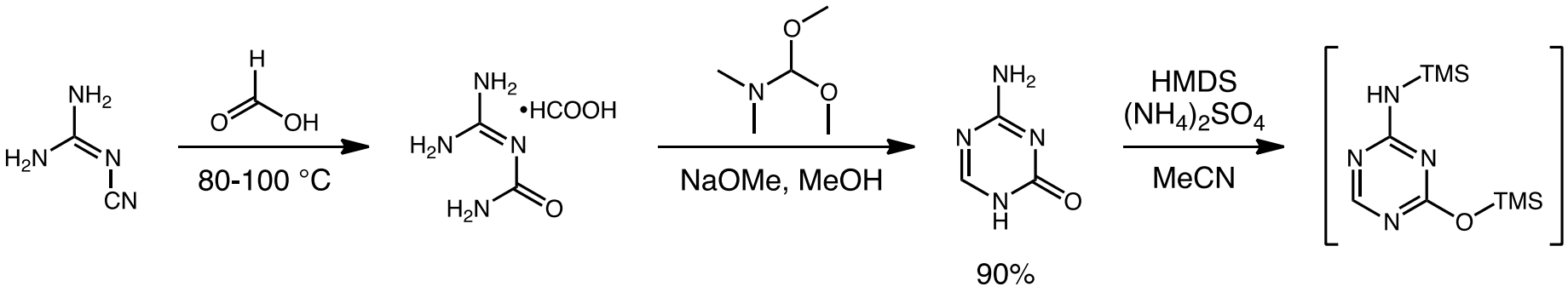


decitabine

Parker, W.B. *Chem. Rev.* **2009**, *109*, 2880

Nucleoside analogues for the treatment of cancer

- Process synthesis of vidaza



Vujjini, S. K. *et al Org. Process Res. Dev.* **2013**, *17*, 303

Nucleoside analogues for the treatment of cancer

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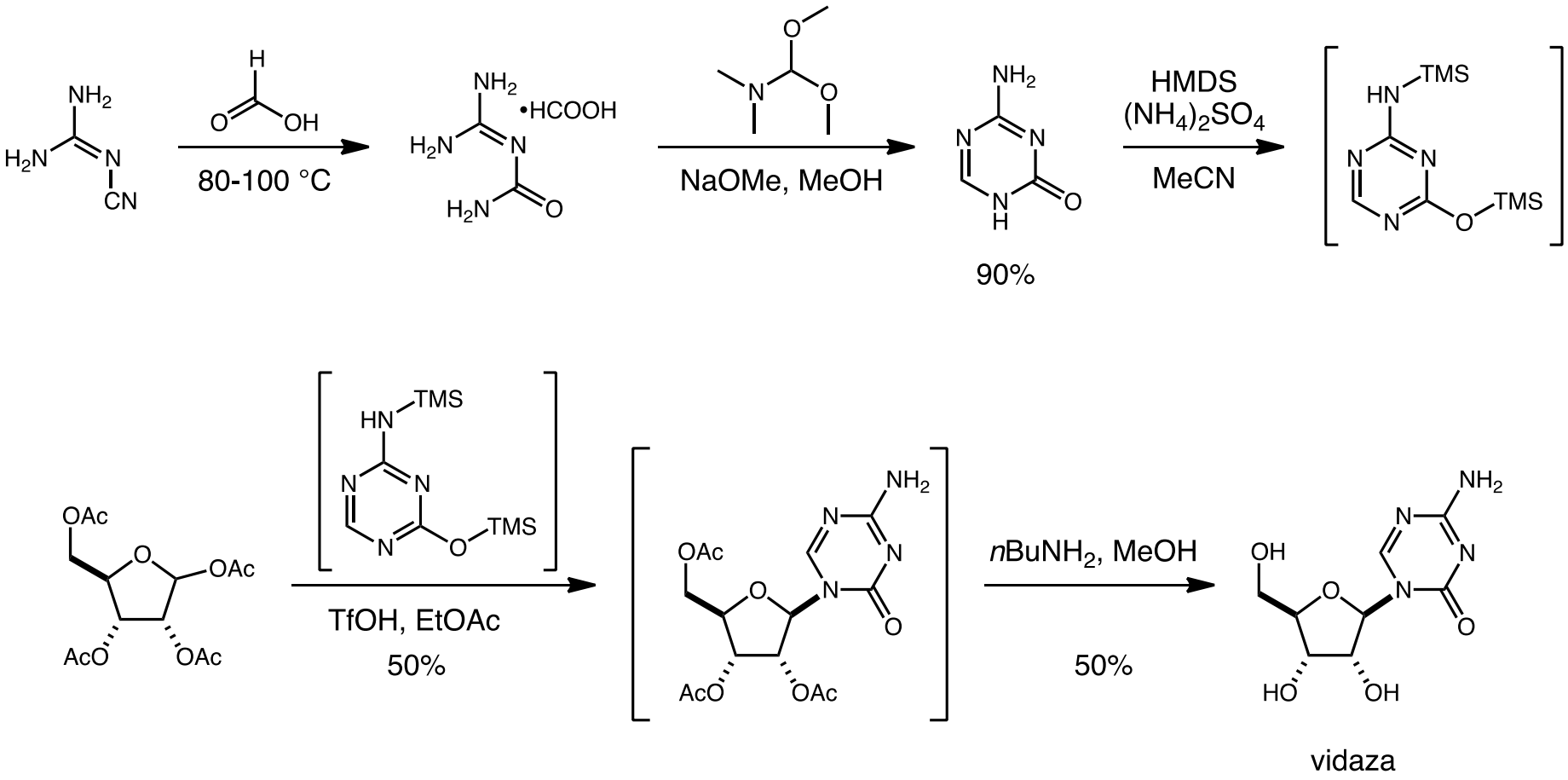
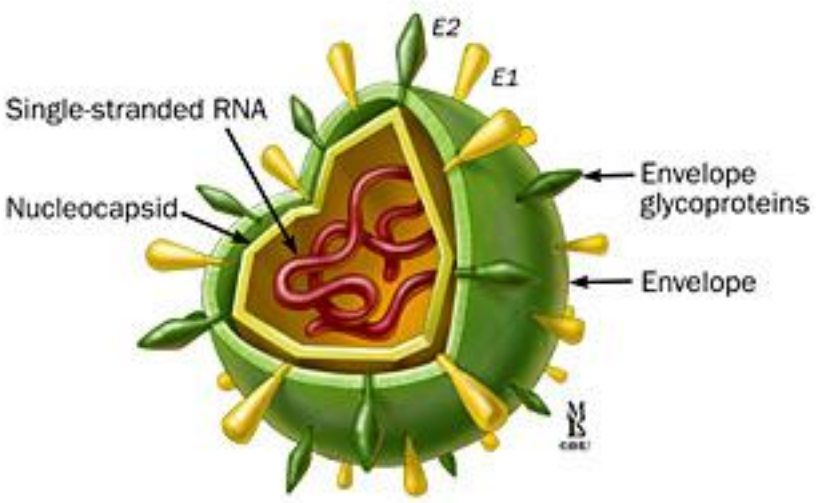


Vujjini, S. K. *et al Org. Process Res. Dev.* **2013**, *17*, 303

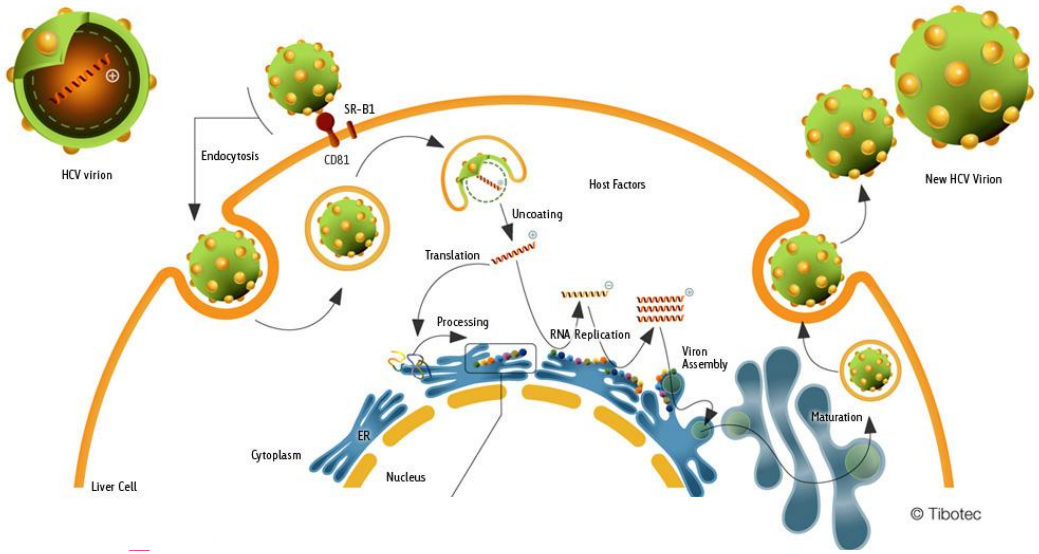
Hepatitis C Virus



- Approximately 180 million people are infected with HCV
- Early stage of HCV are usually asymptomatic and 20% of infected individuals naturally clear the virus
- > 20% progress to develop chronic liver disease such as cirrhosis, hepatocellular carcinoma or liver failure requiring liver transplantation

Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. *J. Med. Chem.* **2012,** 55, 2481

Hepatitis C Virus



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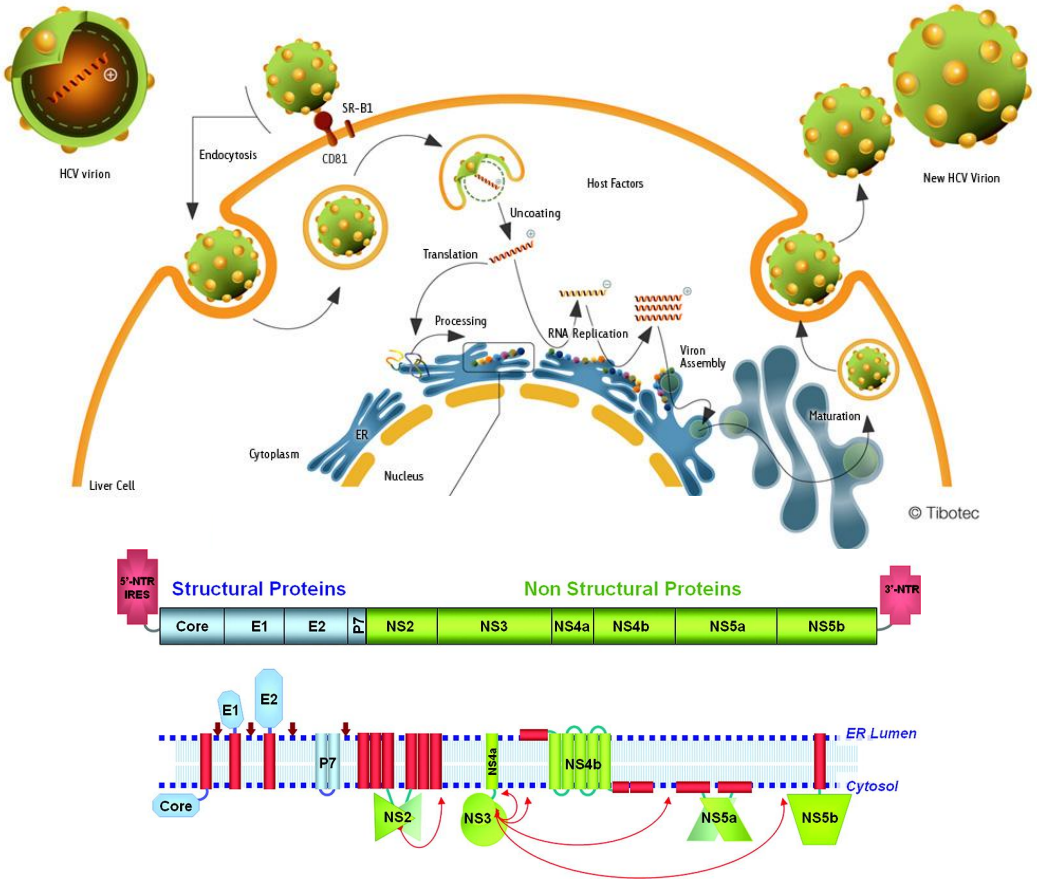
Hepatitis C Virus



- The HCV genome consists of a single open reading frame that is 9600 nucleotide bases long
- This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins

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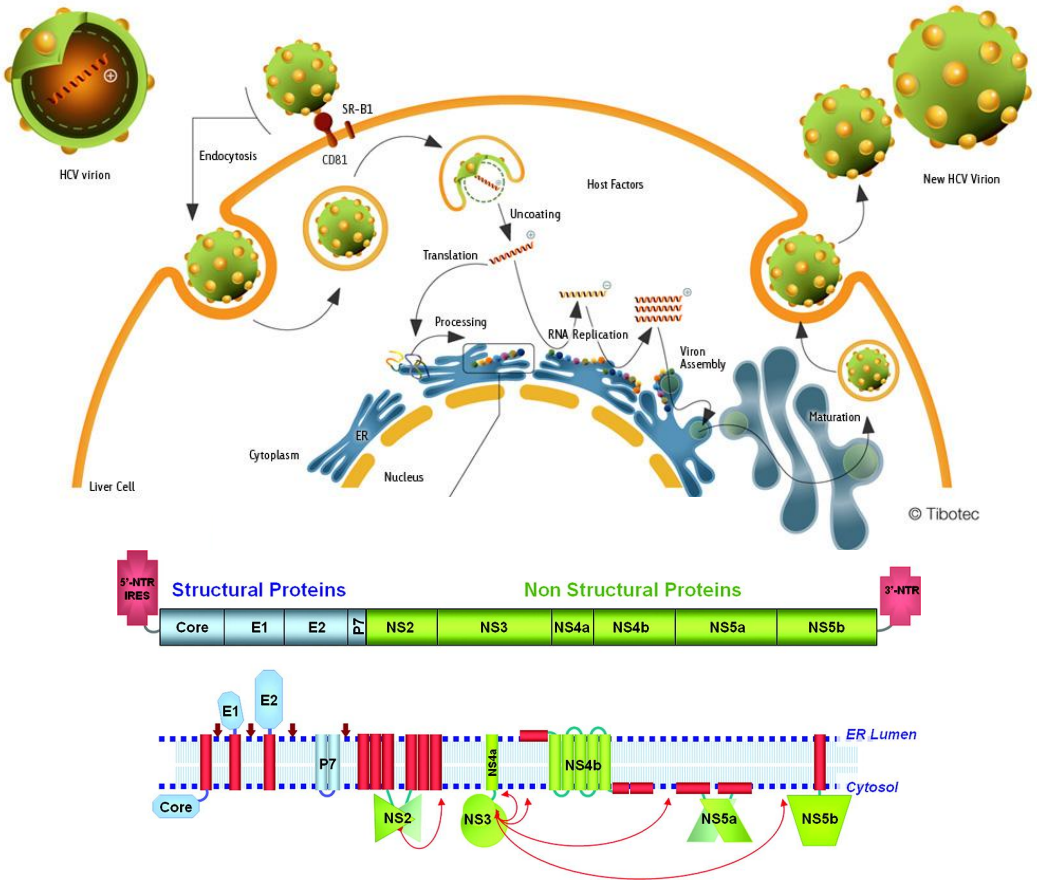
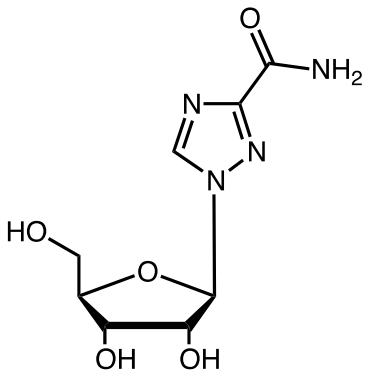


- The HCV genome consists of a single open reading frame that is 9600 nucleotide bases long
- This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins
- In contrast to HIV, HCV does not incorporate its genome into the host cell, so the therapeutic goal is to obtain total clearance of the virus from infected cell

Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. *J. Med. Chem.* **2012,** 55, 2481

Hepatitis C Virus

- Until 2011 the standard of care consists of the use of ribavirin with a weekly injection of pegalated α-interferon. This functions by boosting the host immune system and does not act directly on the virus



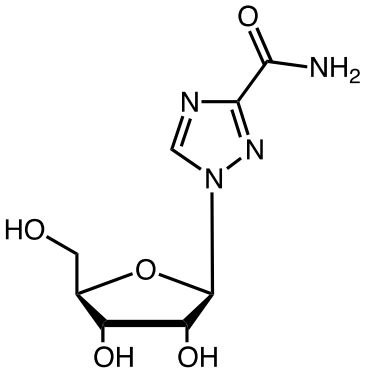
ribavirin

Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. *J. Med. Chem.* **2012,** 55, 2481

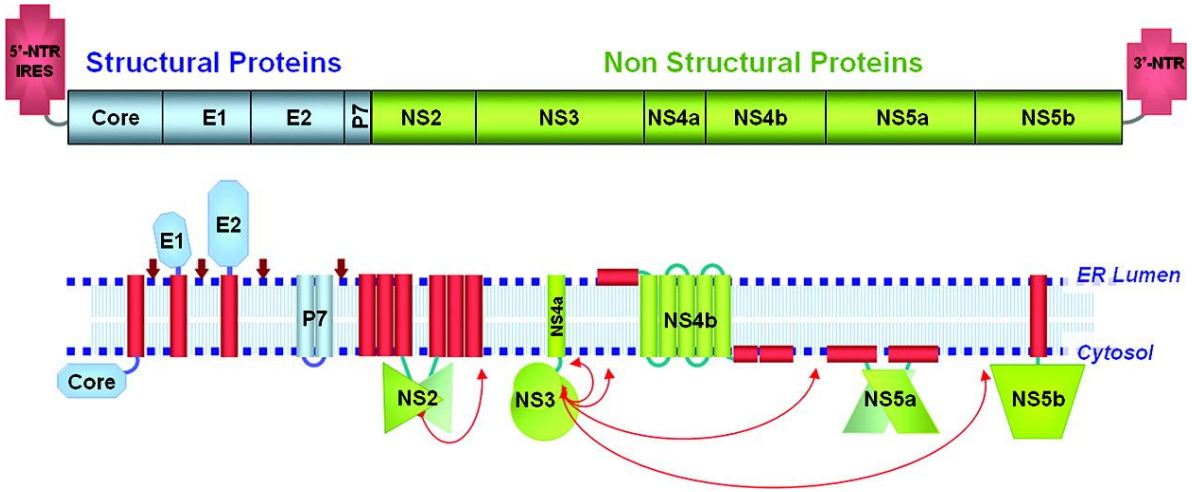
Hepatitis C Virus

- Until 2011 the standard of care consists of the use of ribavirin with a weekly injection of pegalated α-interferon. This functions by boosting the host immune system and does not act directly on the virus

- Two major drug targets have been identified: the NS3/4A serine protease and NS5B RNA-dependent RNA polymerase (RdRp)



ribavirin



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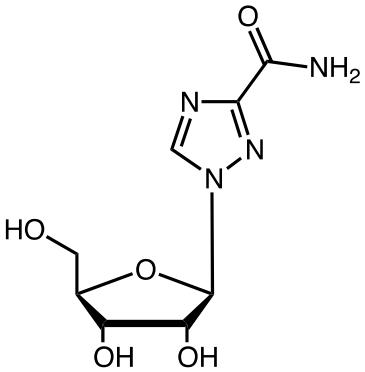
- Two major drug targets have been identified: the NS3/4A serine protease and NS5B RNA-dependent RNA polymerase (RdRp)

- In 2011 two peptidomimetics Boceprevir (Merck) and Telaprevir (Vertex - J&J) targeting NS3/4A were approved by the FDA

- Nucleoside analogue inhibitors are the main category of RdRp inhibitors with multiple drug candidates in phase II and III



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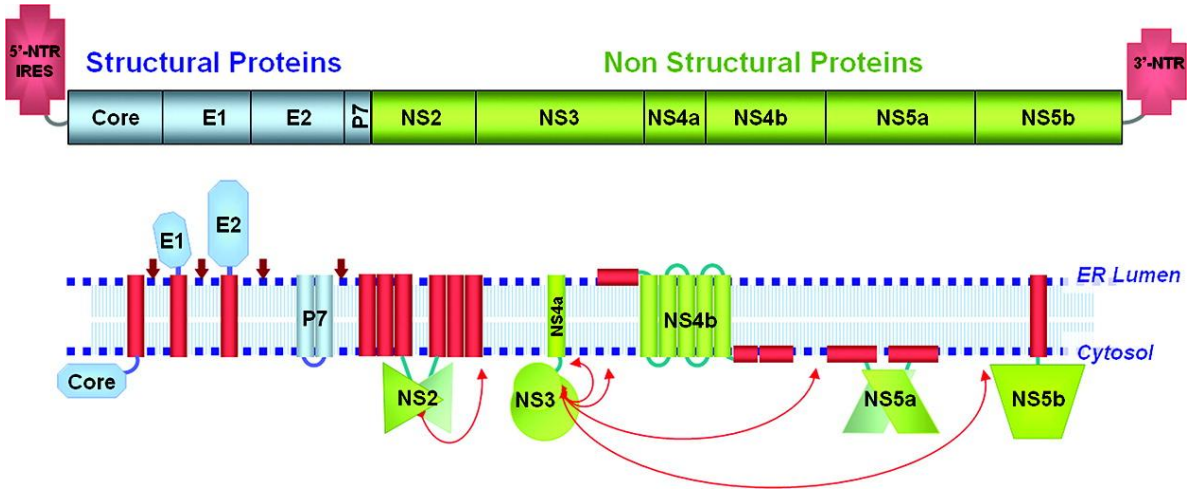

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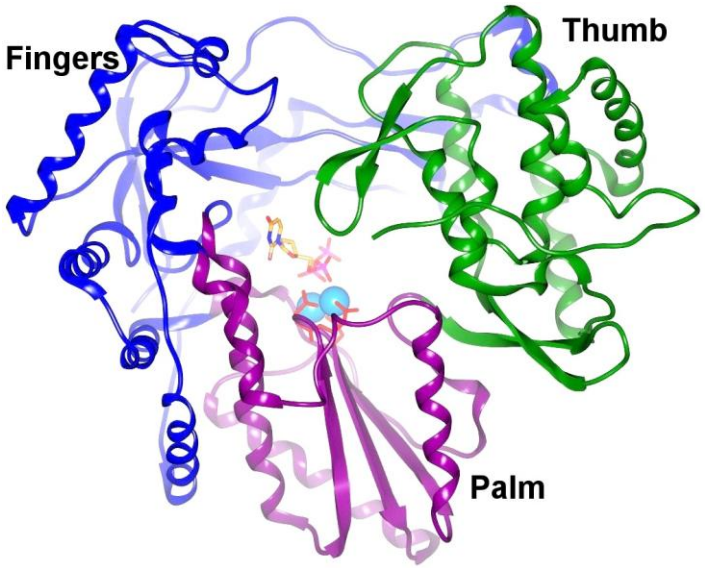
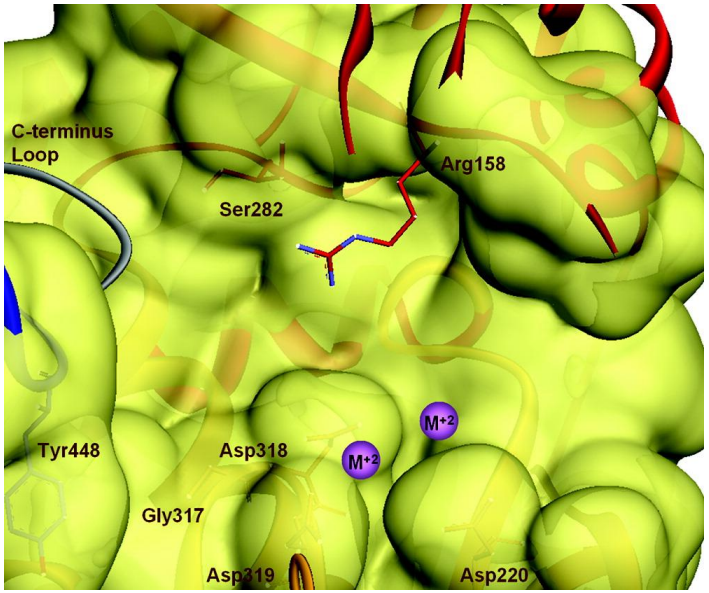
Nucleoside Inhibitors of the HCV NS5B Polymerase

- HCV NS5B polymerase has a hand shape with the catalytic site located in the palm

- At this site a ribonucleoside 5'-triphosphate binds through the coordination of a divalent metal (Mg^{2+} or Mn^{2+}) and is subsequently added to the 3'-end of the growing RNA chain.





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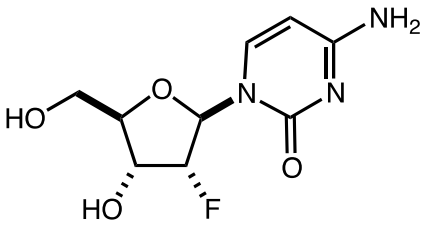
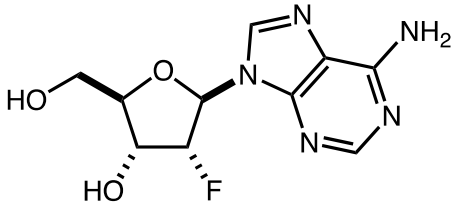


EC_{50} >50 μM



EC_{50} = 1.2 μM

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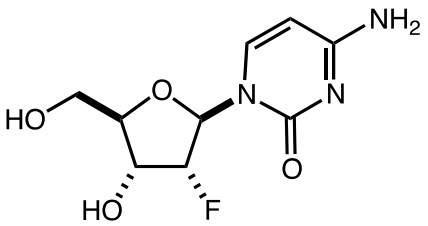
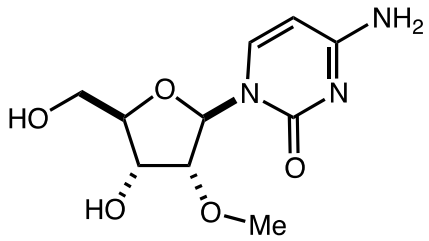
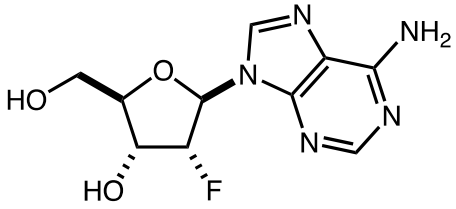


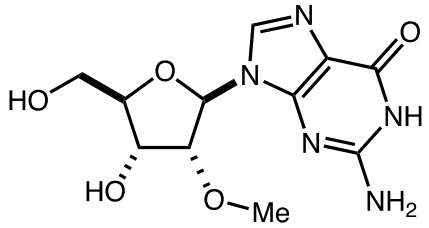
EC_{50} >50 μM



EC_{50} = 11 μM



EC_{50} = 1.2 μM



EC_{50} >50 μM

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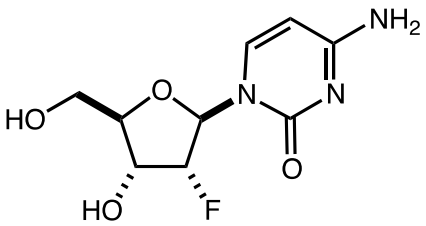
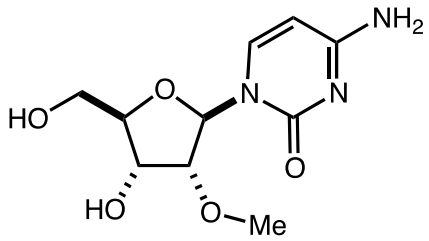
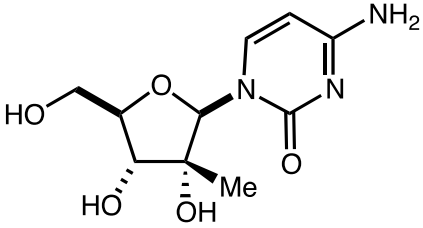


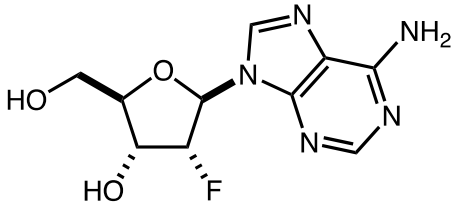
EC_{50} >50 μM



EC_{50} = 11 μM



EC_{50} = 1.23 μM



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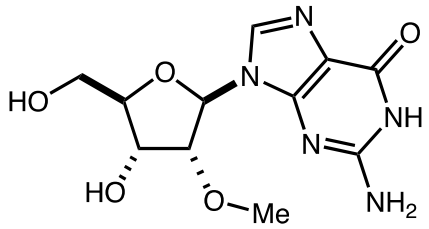

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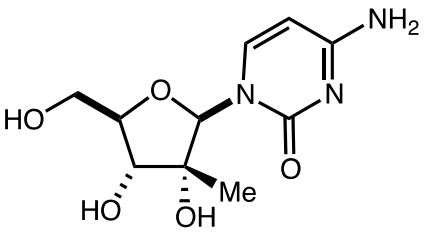
Nucleoside Inhibitors of the HCV NS5B Polymerase

- The poor oral bioavailability led to the development of a ester prodrug for clinical studies



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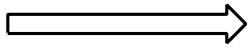

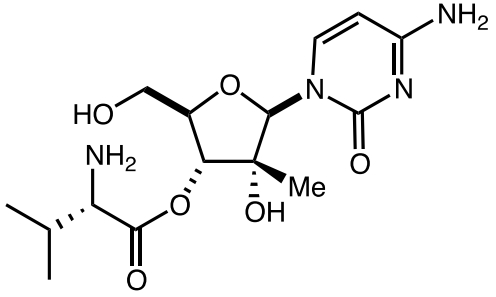

increased oral bioavailability

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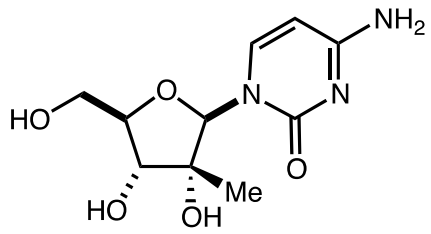

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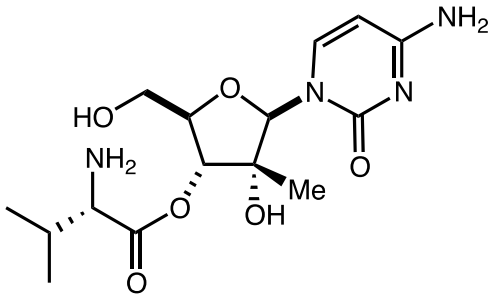
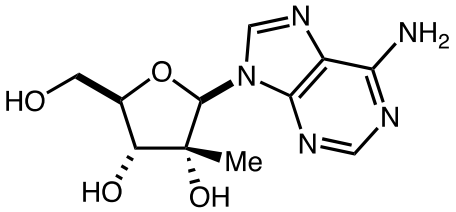
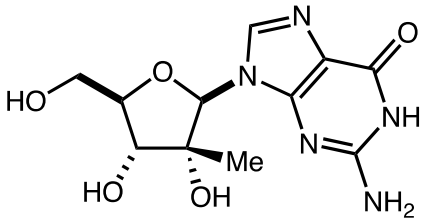


increased oral bioavailability

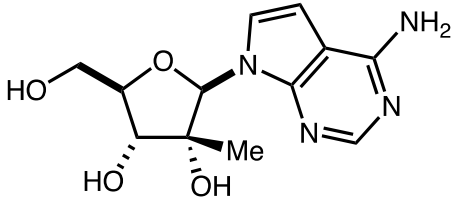
- Purine bases improved activity



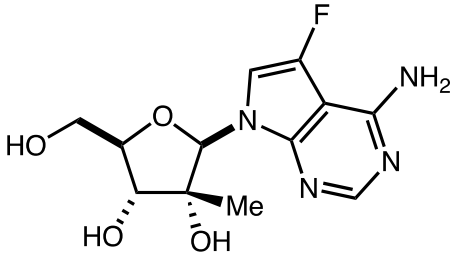
EC_{50} = 0.25 μM



EC_{50} = 3.5 μM



EC_{50} = 0.25 μM



EC_{50} = 0.07 μM

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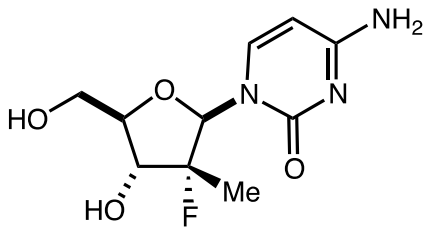
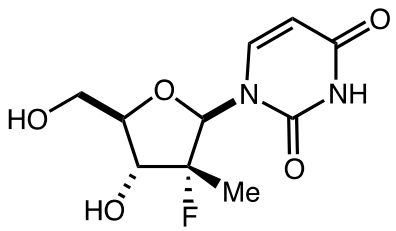
Nucleoside Inhibitors of the HCV NS5B Polymerase

- The 2'-OH was then replace by Fluorine



PSI-6130

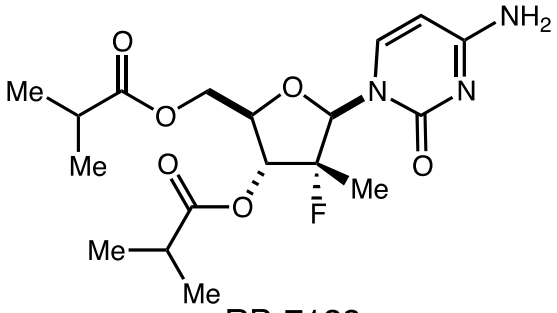
EC_{50} = 4.50 μM



PSI-6206

EC_{50} = >100 μM

- Ester prodrug RB-7128 is in phase 3 clinical trial



RB-7128

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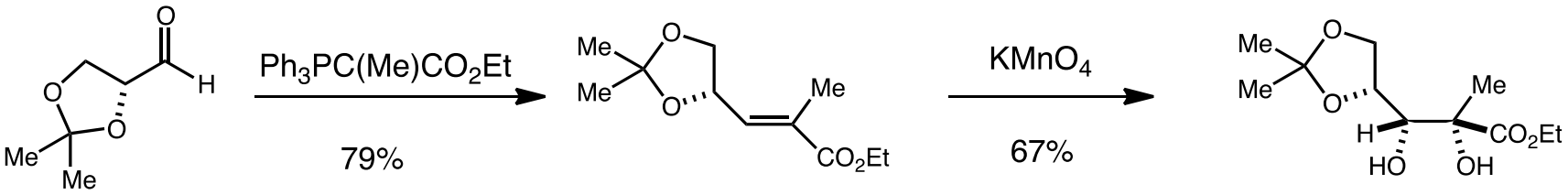
Nucleotide Inhibitors of the HCV NS5B Polymerase

- Synthesis of PSI-6130



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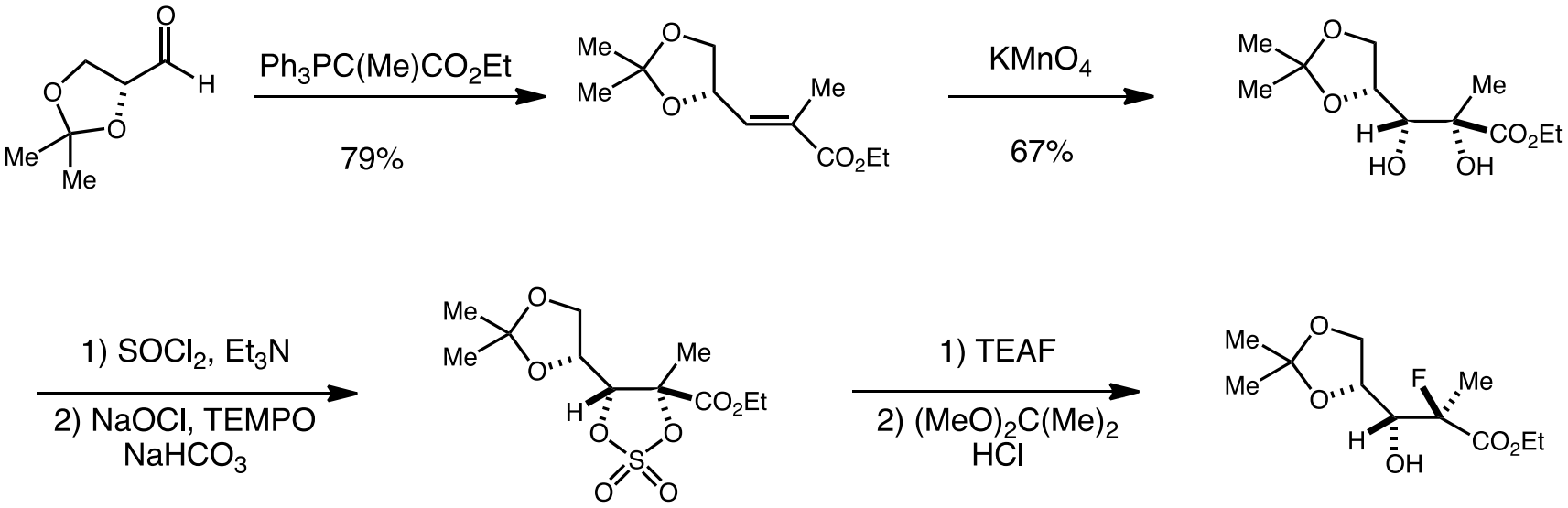

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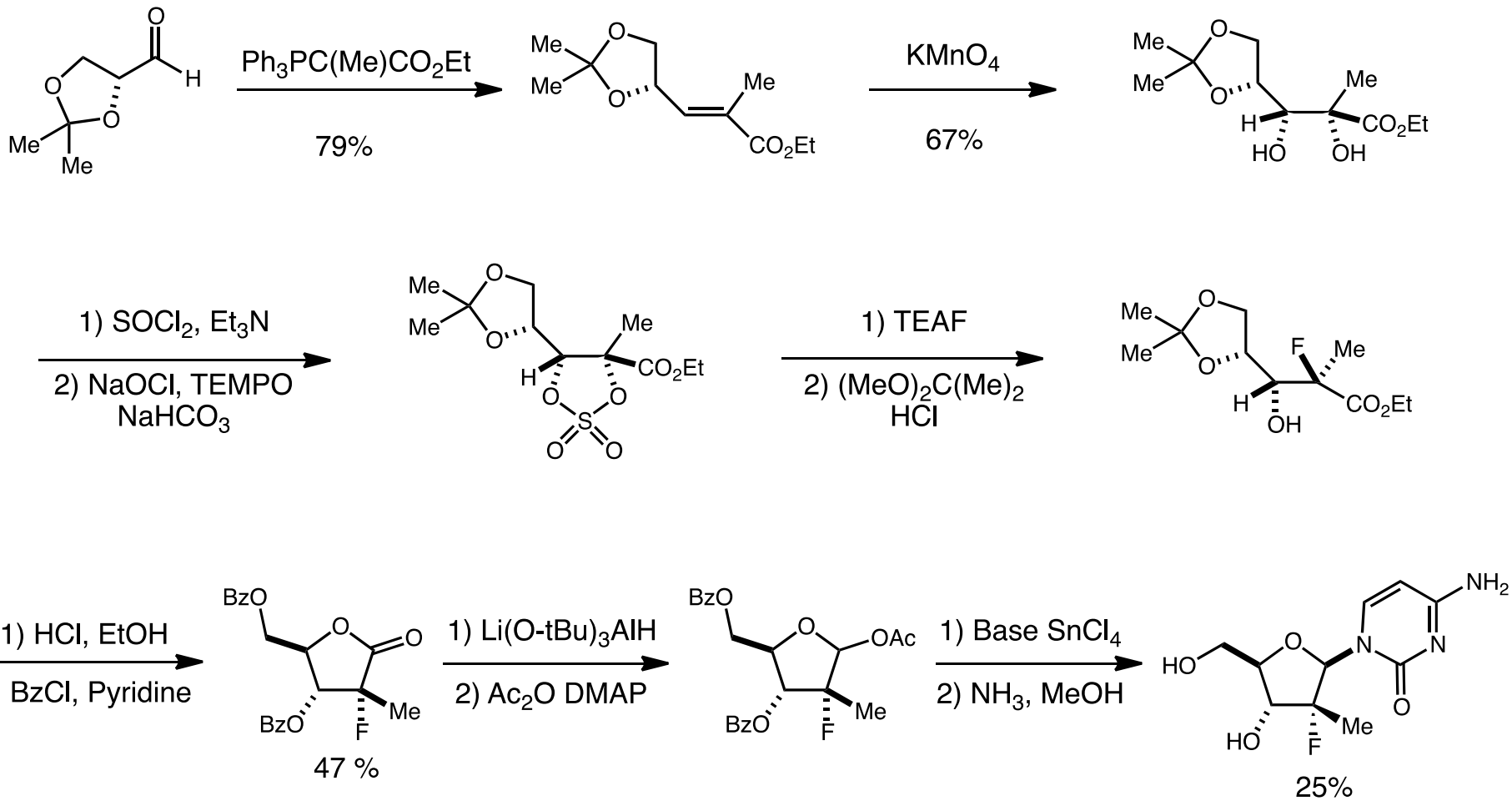

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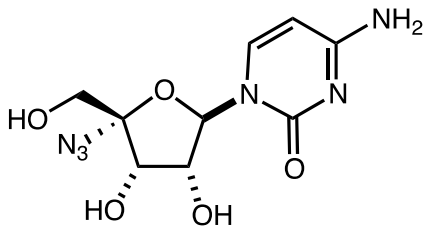
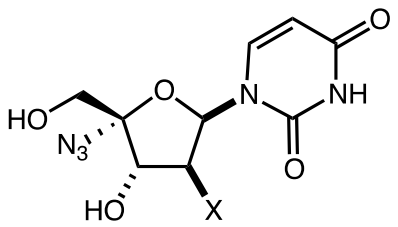
Nucleoside Inhibitors of the HCV NS5B Polymerase

- 4' azido-nucleoside proved to be potent polymerase inhibitors



EC_{50} = 1.28 μM



H = OH EC_{50} = 0.17 μM

H = F EC_{50} = 0.024 μM

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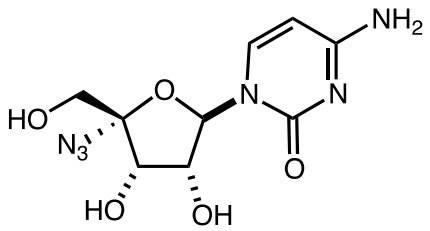

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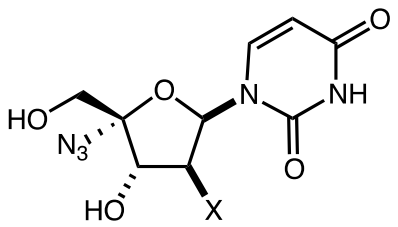

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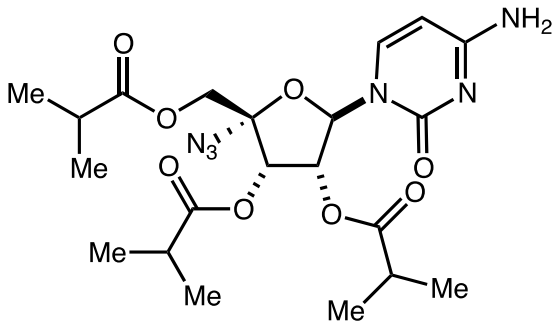
- Ester prodrug R1626 was discontinued after significant hematological adverse events were observed in a phase 2b study



R1626

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Nucleotide Inhibitors of the HCV NS5B Polymerase

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- In most cases the problematic step is the formation of the monophosphate by the first kinase as it is the most discriminating of the three kinase enzymes involved

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  - Chemically stable for oral administration and towards gastrointestinal track

  - Good absorption and be able to reach the liver intact

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- To target the liver selectively, hepatic enzymes should be involved with unmasking the 5'-monophosphate group

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Nucleotide Inhibitors of the HCV NS5B Polymerase

- Three main strategies have be employed:

ProTides

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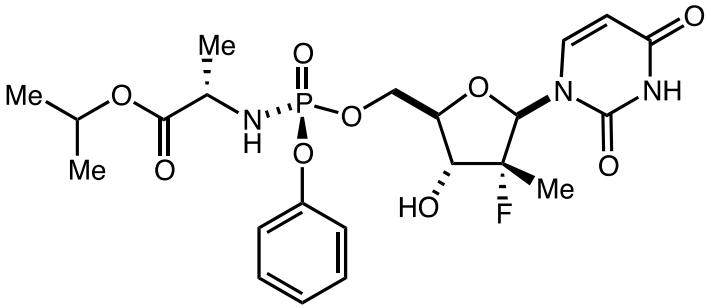

PSI-7977

EC_{50} = 0.42 μM

Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. *J. Med. Chem.* **2012,** 55, 2481

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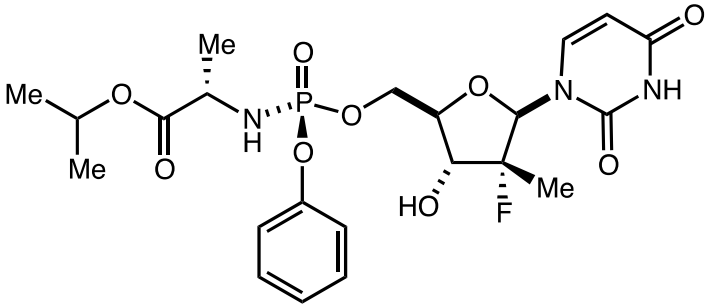

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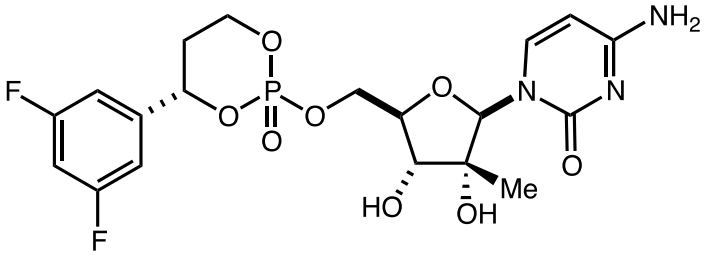

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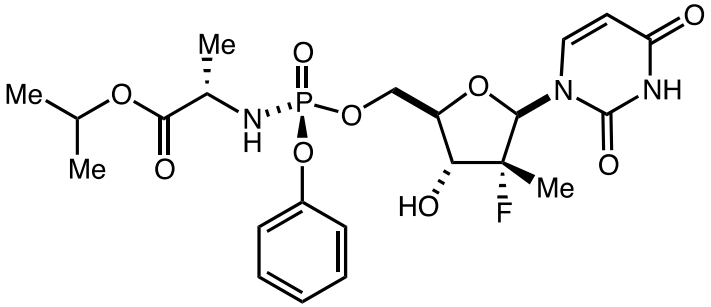

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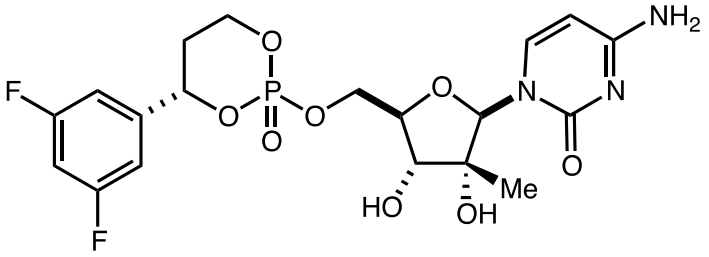
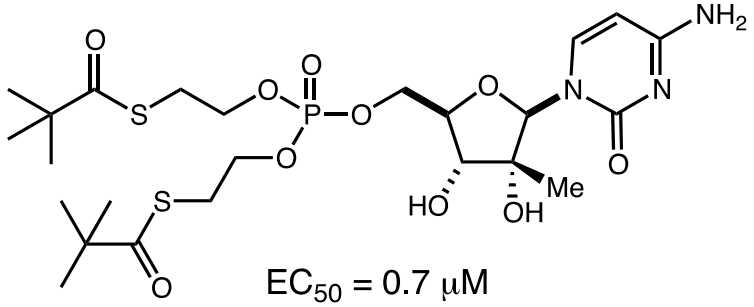


SATE

bis(acyl-2-thioethyl) phosphate esters)



EC_{50} = 0.7 μM

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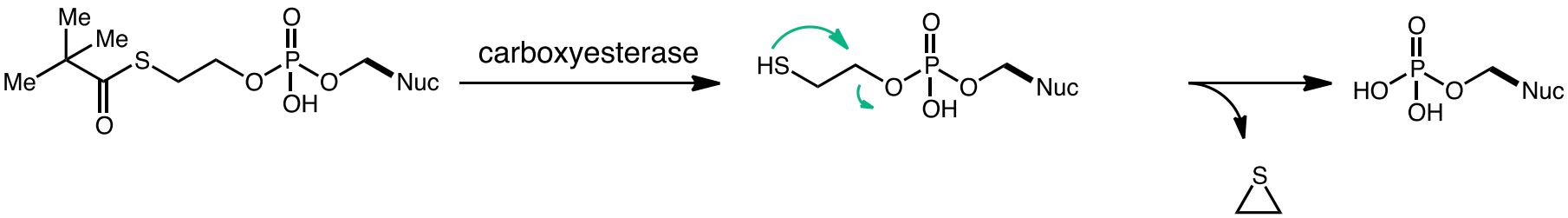
Nucleotide Inhibitors of the HCV NS5B Polymerase

- Metabolic activation of SATE monophosphate prodrugs



Bobeck, D. R.; Schinazi, R. F.; Coats S. J. *Antiviral Therapy* **2010**, 15, 935

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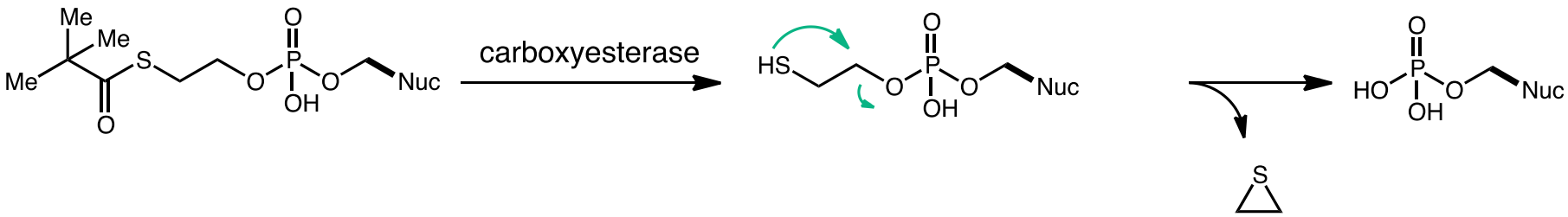
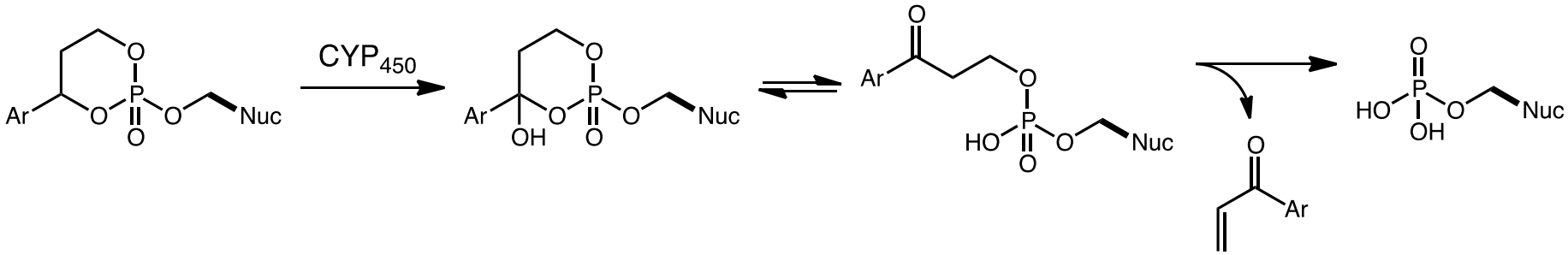


- Metabolic activation of HepDirect monophosphate prodrugs



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Nucleoside Derivatives as Drug, Their Synthesis & Mode of Action

- Due to the importance of nucleoside in the cell cycle of life, nucleoside analogue have been used in a broad range of therapeutic area

- It is interesting to note that even though the structures of these analogues are similar they display a large array of mode of action

- The development of new mono-phosphate analogues will enable the development of more active and selective drug