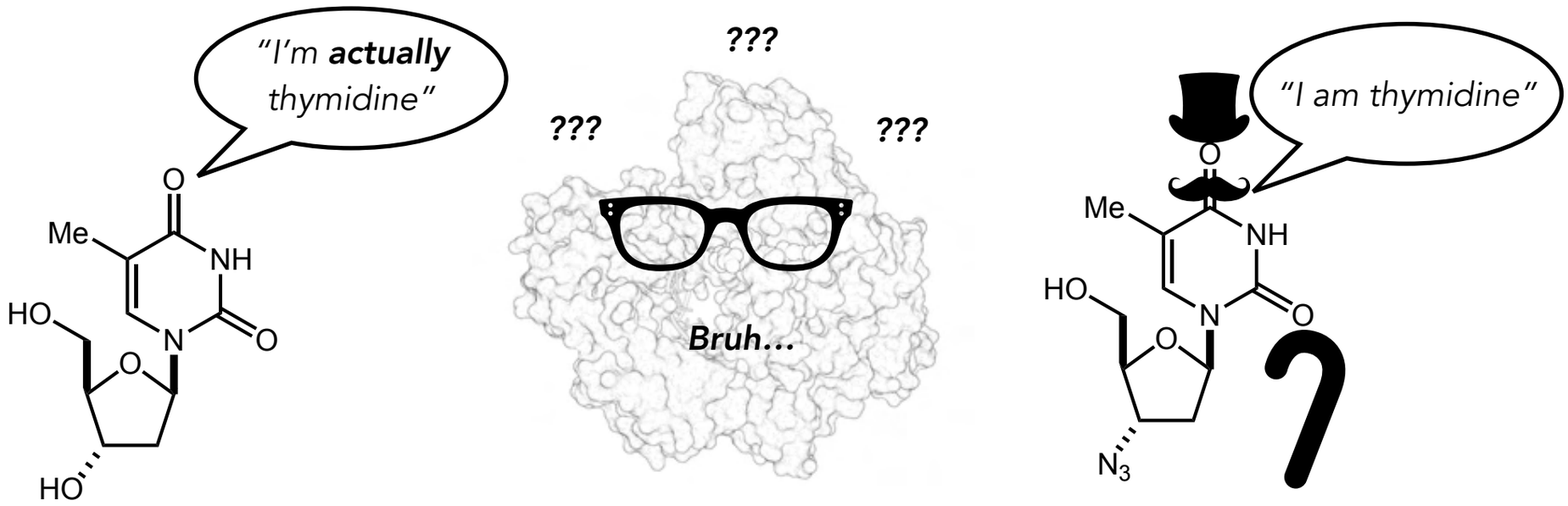
Nucleoside Therapeutics: Mechanism of Action, Development, SAR, and Synthesis



Noah B. Bissonnette
MacMillan Group
Group Meeting
February 6th, 2024

Introduction

Viruses

H1N1 (Swine Flu)
(2009)

MERS
(2015)

Ebola
(2016)

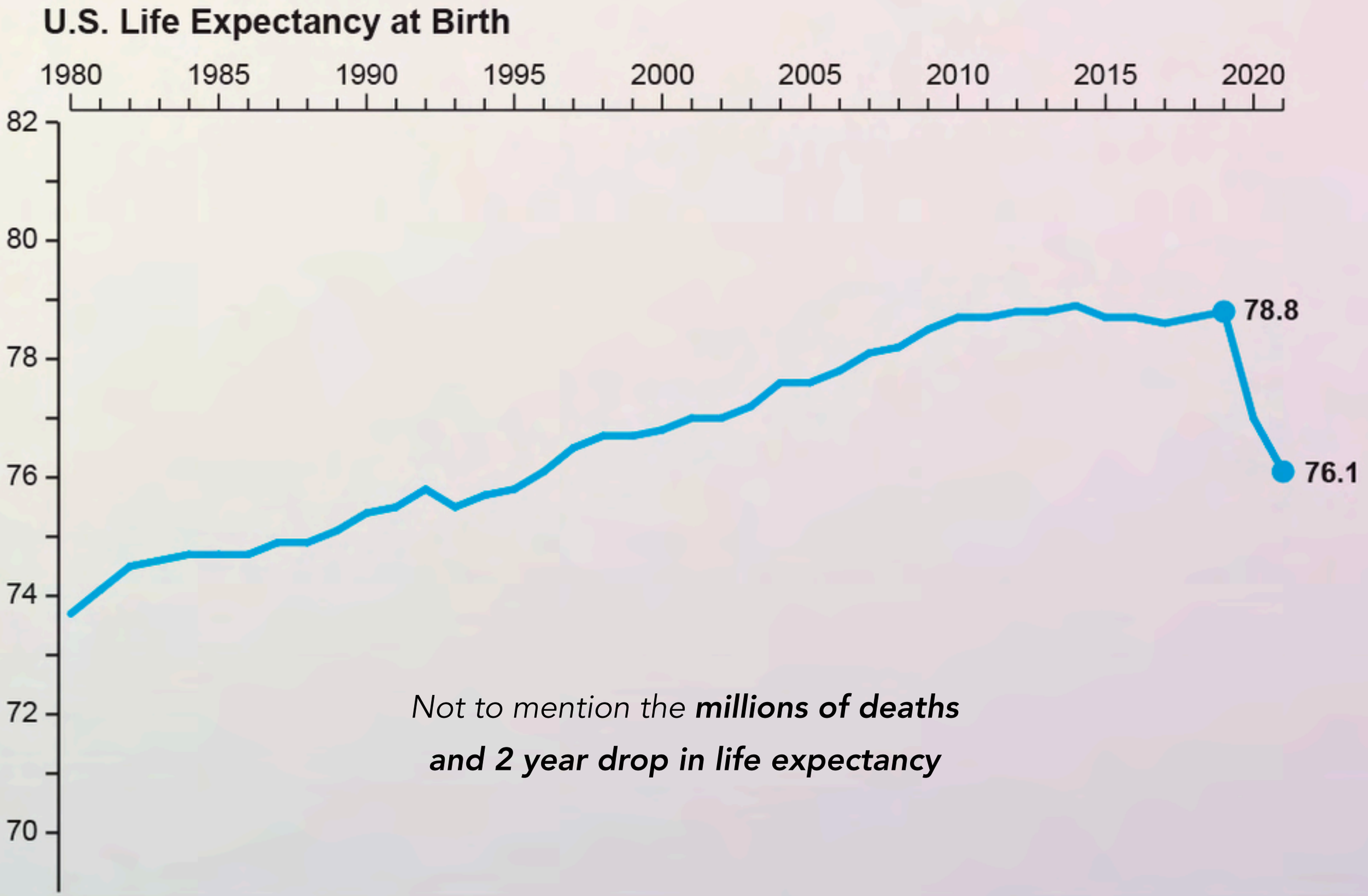
SARS CoV-2
(2019)

*There has been a **multitude of viral outbreaks** within our lifetimes*

Introduction

Viruses

Experts approximate the economic cost
of the SARS CoV-19 pandemic to be
$40 trillion dollars from 2019-23…



Not to mention the ***millions of deaths***

and 2 year drop in life expectancy

Centers for Disease Control and Prevention, **2022**

Introduction

Antiviral Therapies

Vaccines and

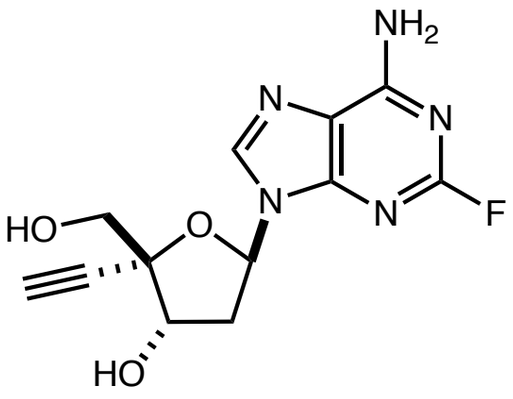
Monoclonal Antibodies

Small Molecule

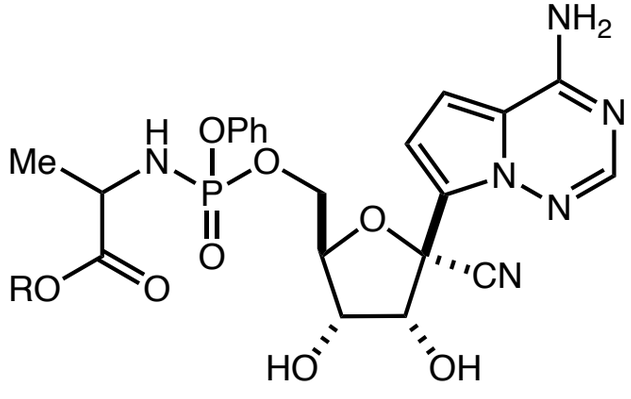
Antivirals

Introduction

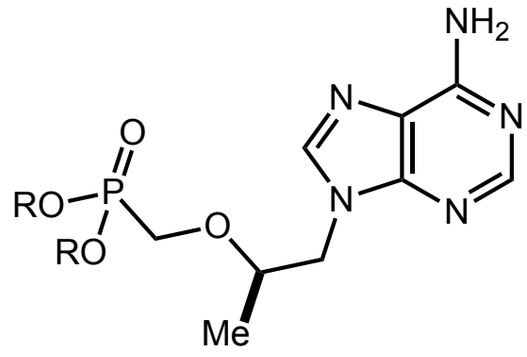
Nuceloside Therapies



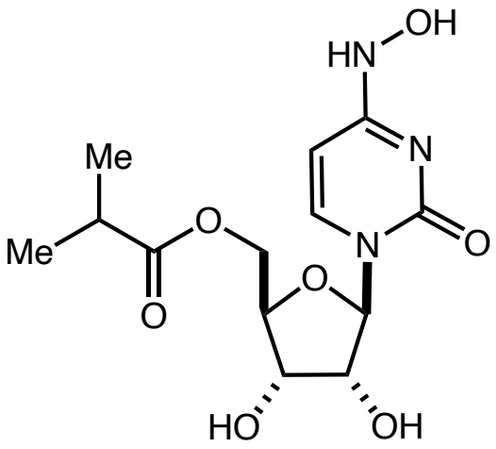
Islatravir
(Merck)



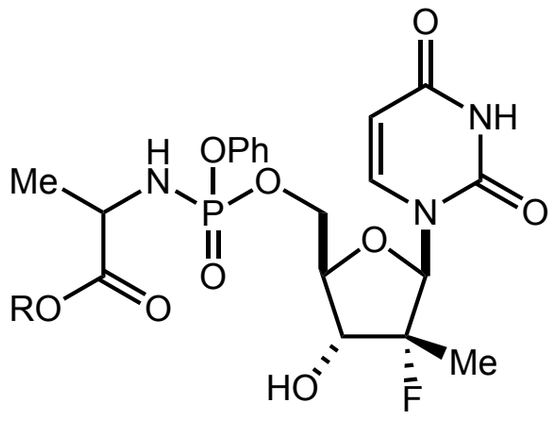
Remdesivir
(Gilead)



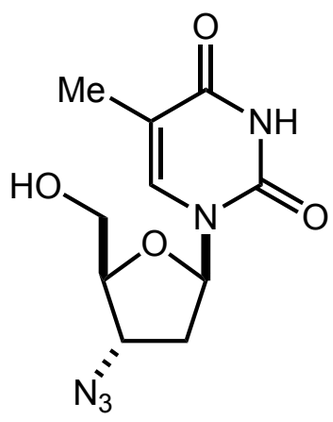
Tenofovir
(Gilead)



Molnupiravir
(Merck)



Sofosbuvir
(Gilead)



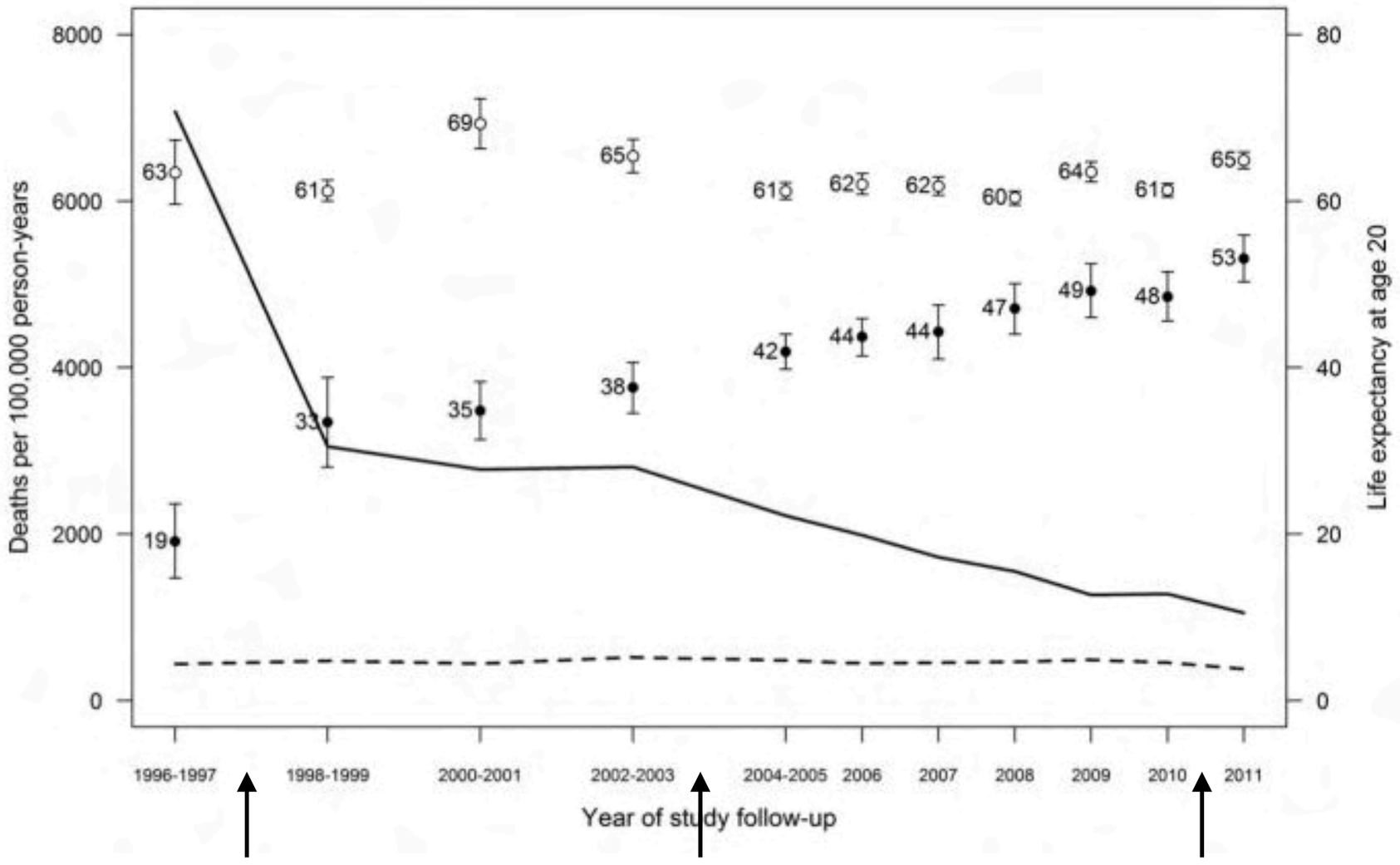
AZT
(Burroughs Wellcome)

*Nucleotides analogs have **drastically impacted patient outcomes***

Introduction

Impact on HIV/AIDS

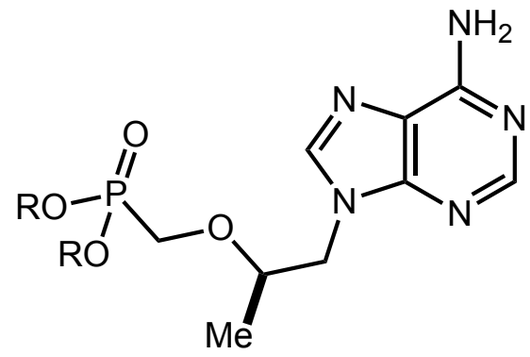
Life expectance and death rate of healthy vs HIV infected individuals



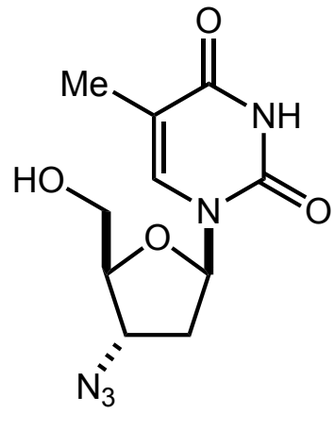
AZT broadly used

Tenofovir approved

Tenofovir combination therapies



Tenofovir
(Gilead)



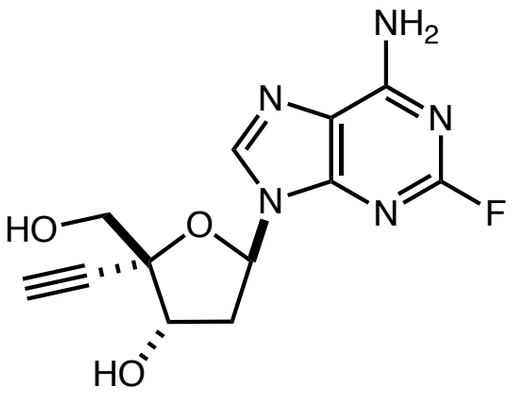
AZT
(Burroughs Wellcome)

*Today the life expectancy of a person diagnosed with HIV is **nearly the same** as a healthy individual*

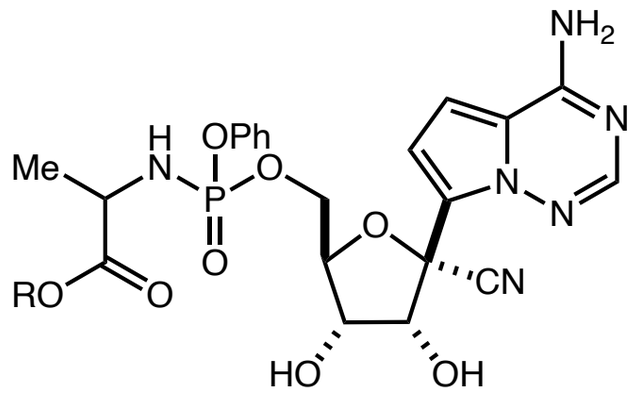
Marcus, J. L; *et. al.*; *J. Acquir Immune Defic Syndr.* **2016**. *73*, 39-46.

Introduction

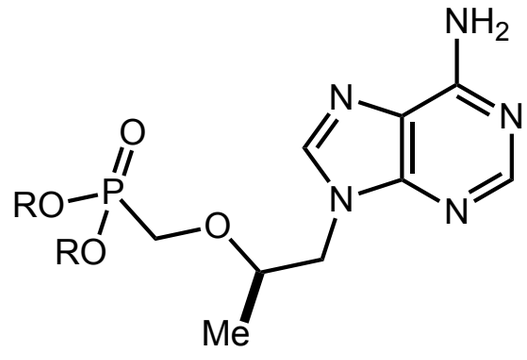
Nuceloside Therapies



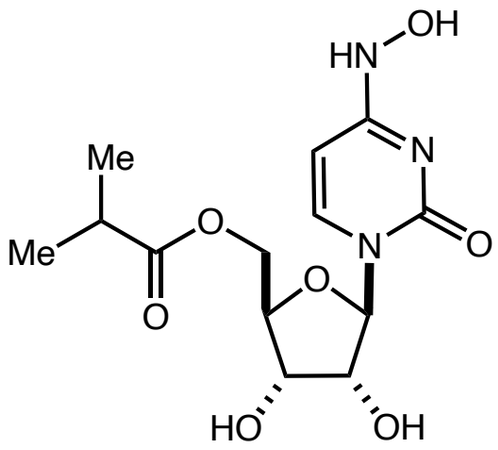
Islatravir
(Merck)



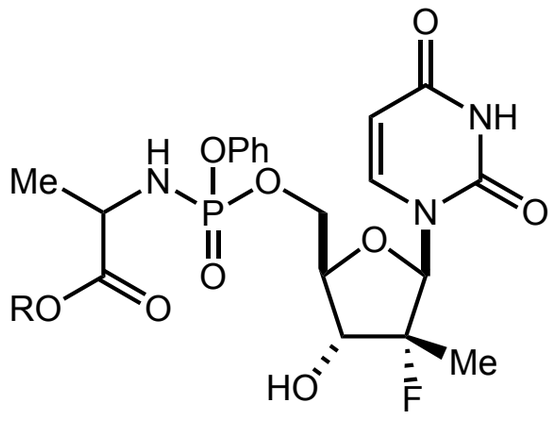
Remdesivir
(Gilead)



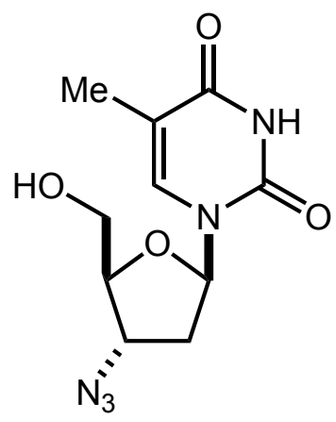
Tenofovir
(Gilead)



Molnupiravir
(Merck)



Sofosbuvir
(Gilead)

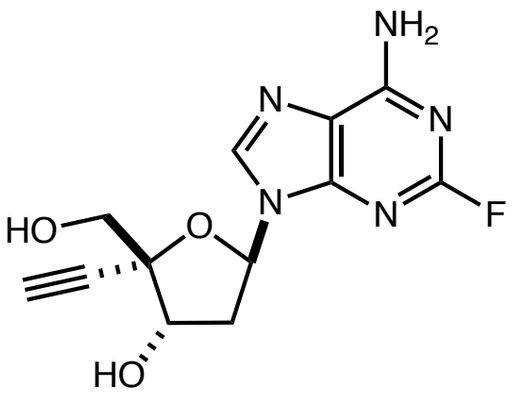


AZT
(Burroughs Wellcome)

*Nucleotides are **sp³ rich, chiral, heavily functionalized targets***

Introduction

Nuceloside Therapies



Islatravir
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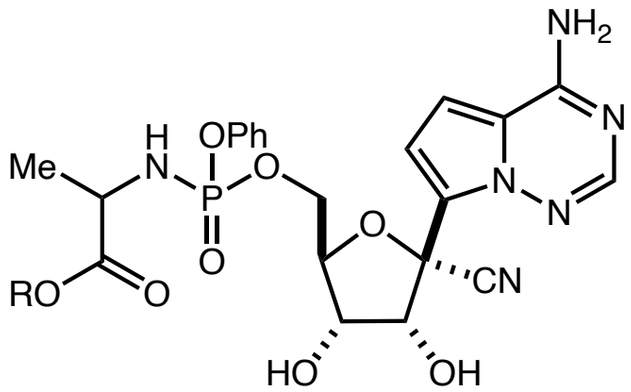
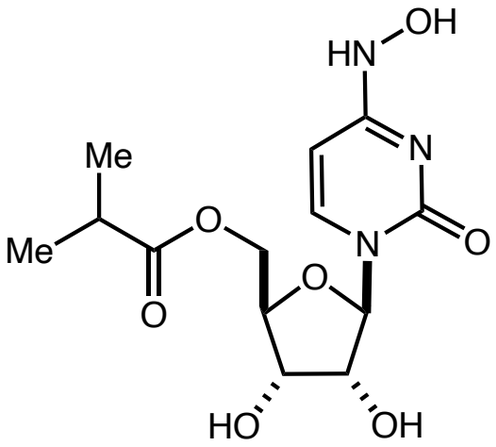


Remdesivir
(Gilead)



Molnupiravir
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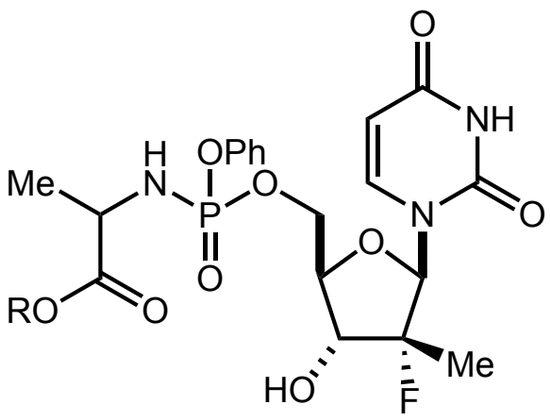
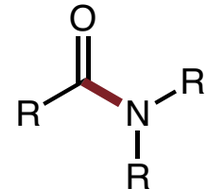


Sofosbuvir
(Gilead)

Synthetic Toolbox

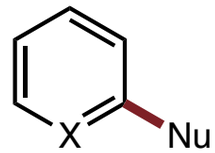
amides

peptide coupling



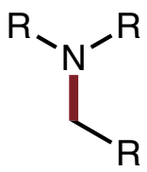
amino-pyridines

S_NAr



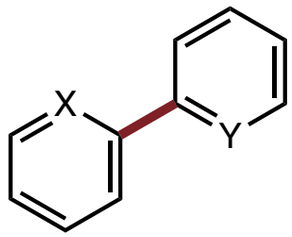
alkyl amines

reductive amination



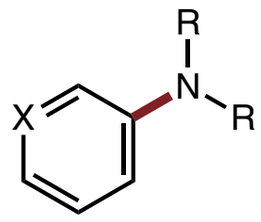
biaryl bonds

Suzuki-Miyura



anilines

Buchwald-Hartwig



*Many reactions we are very comfortable with are **less applicable** to making this class of therapies*

Introduction

Outline

I *Virology crash course*

II *Mechanism of Action*

III *Structure Activity Relationships (SAR)*

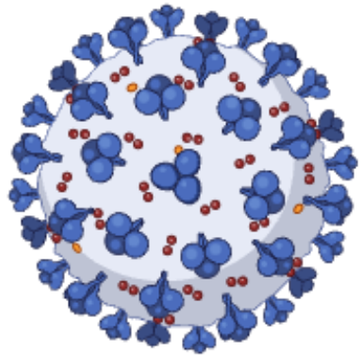
IV *Synthetic Strategies and Case Studies*

I Virology Crash Course

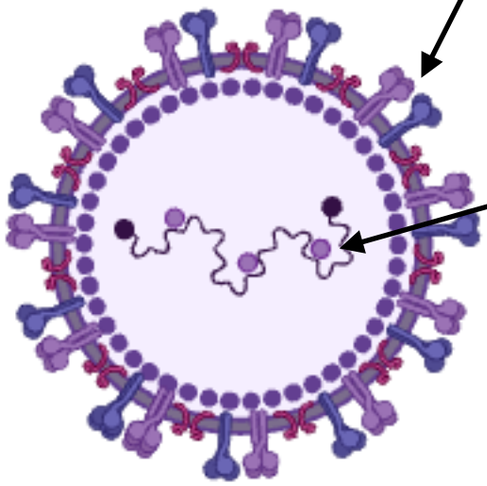
Virology Crash Course

Types of viruses

Zika virus



SARS-CoV-2

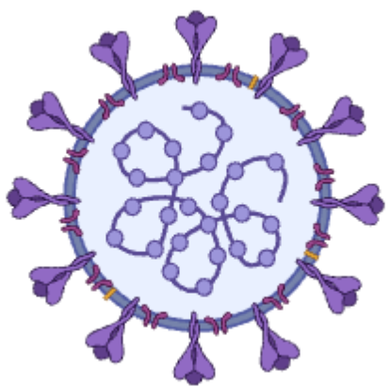


Protein envelope

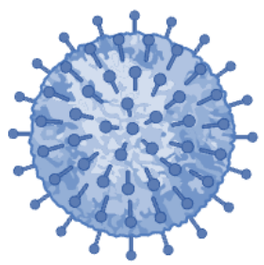
Genetic information

Respiratory syncytial virus

Influenza virus



MERS

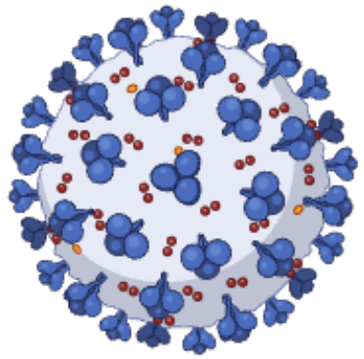


Epstein-Barr virus

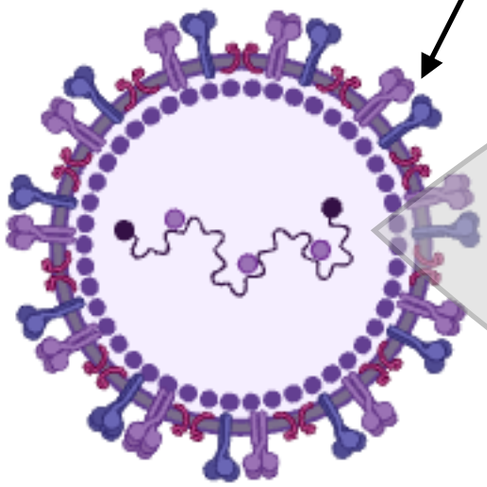
Virology Crash Course

Types of viruses

Zika virus

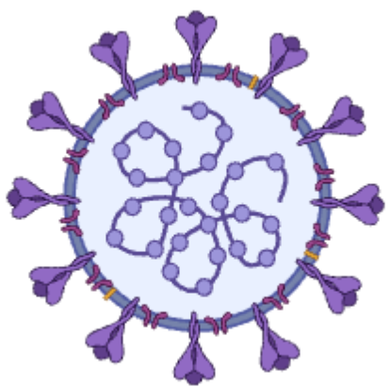


SARS-CoV-2

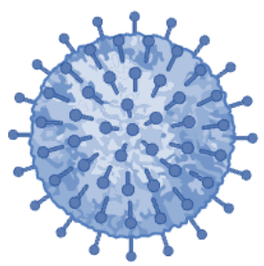


Respiratory syncytial virus

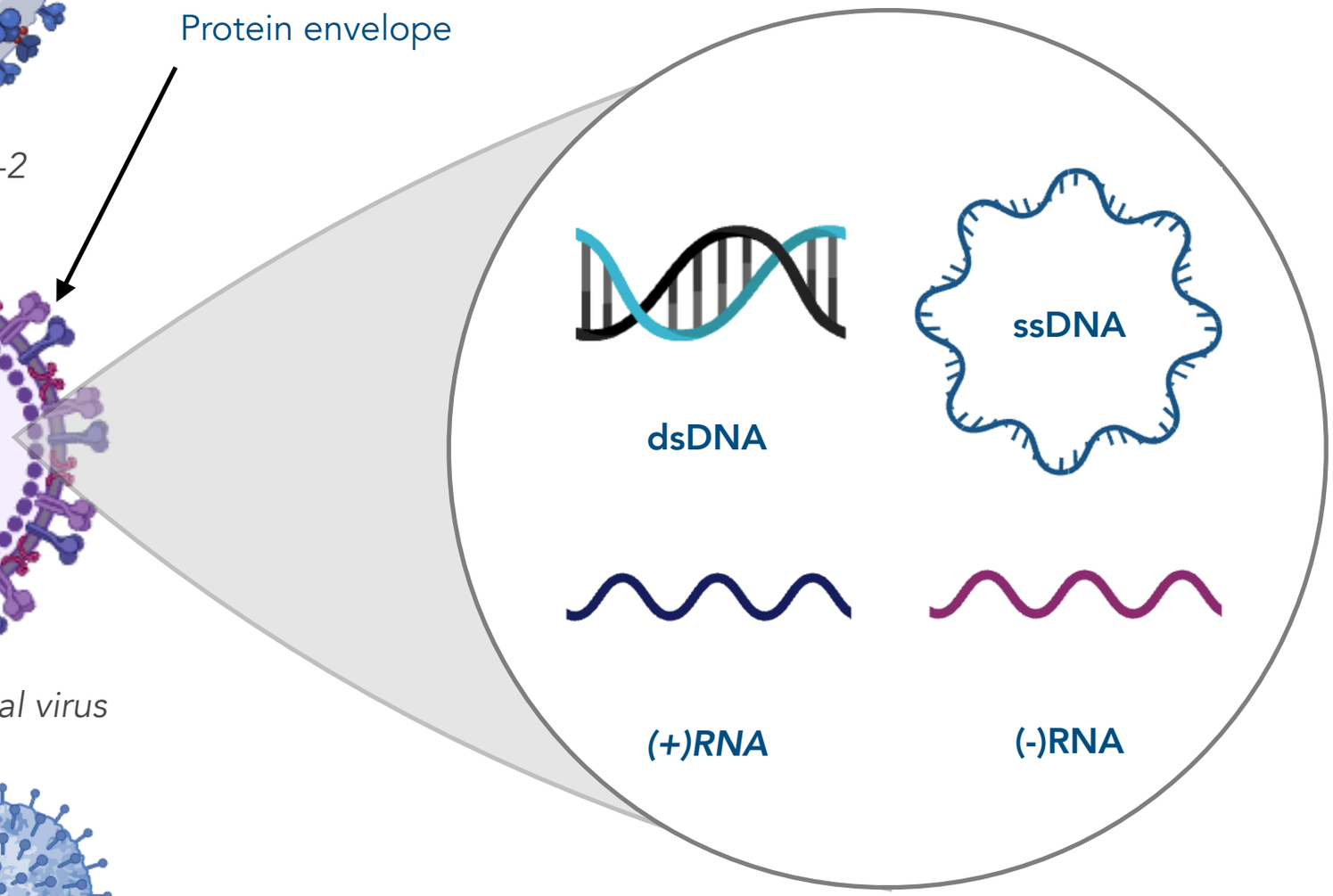
Influenza virus



MERS



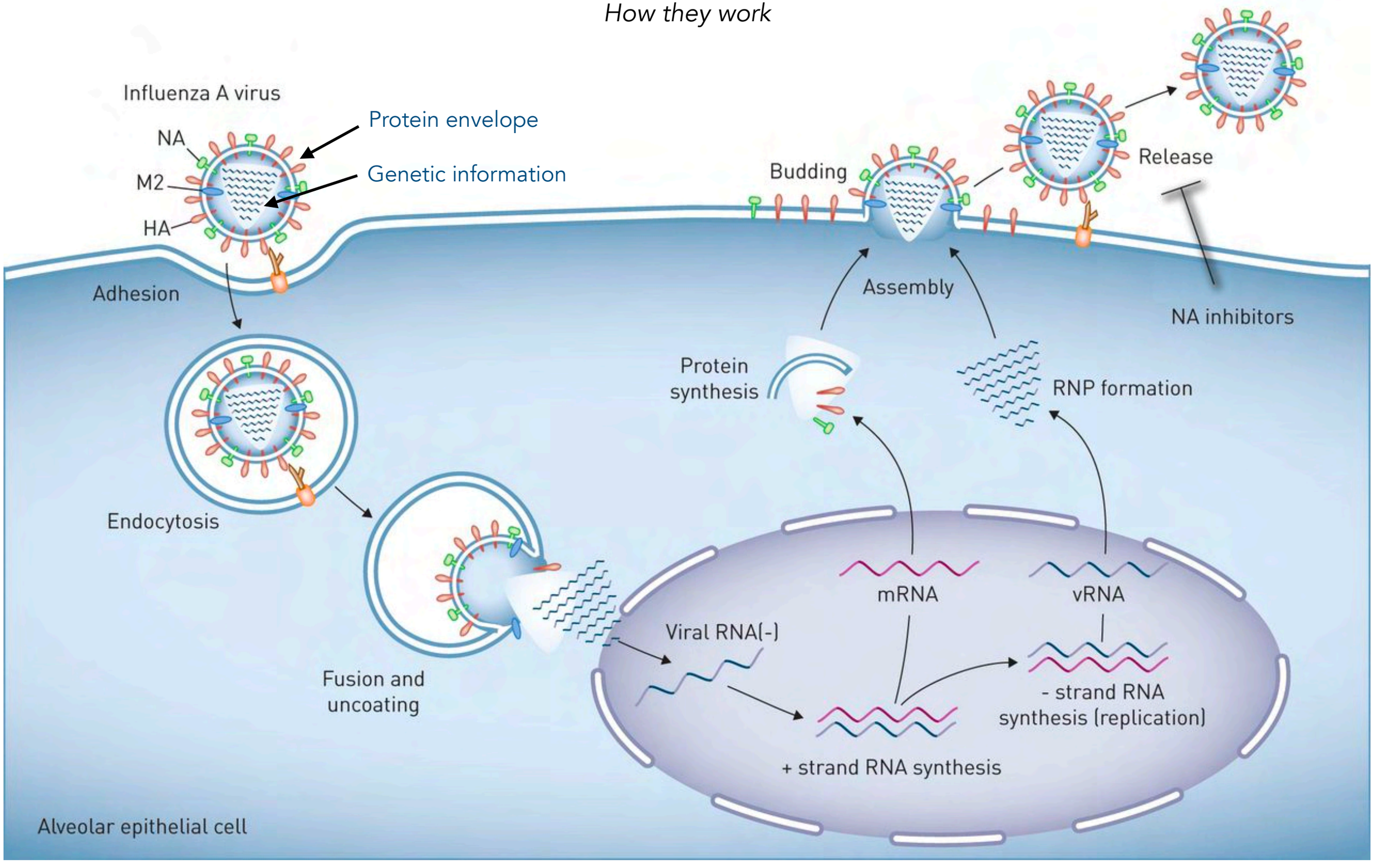
Epstein-Barr virus



*Viruses can carry their **genetic information in many different forms***

Virology Crash Course

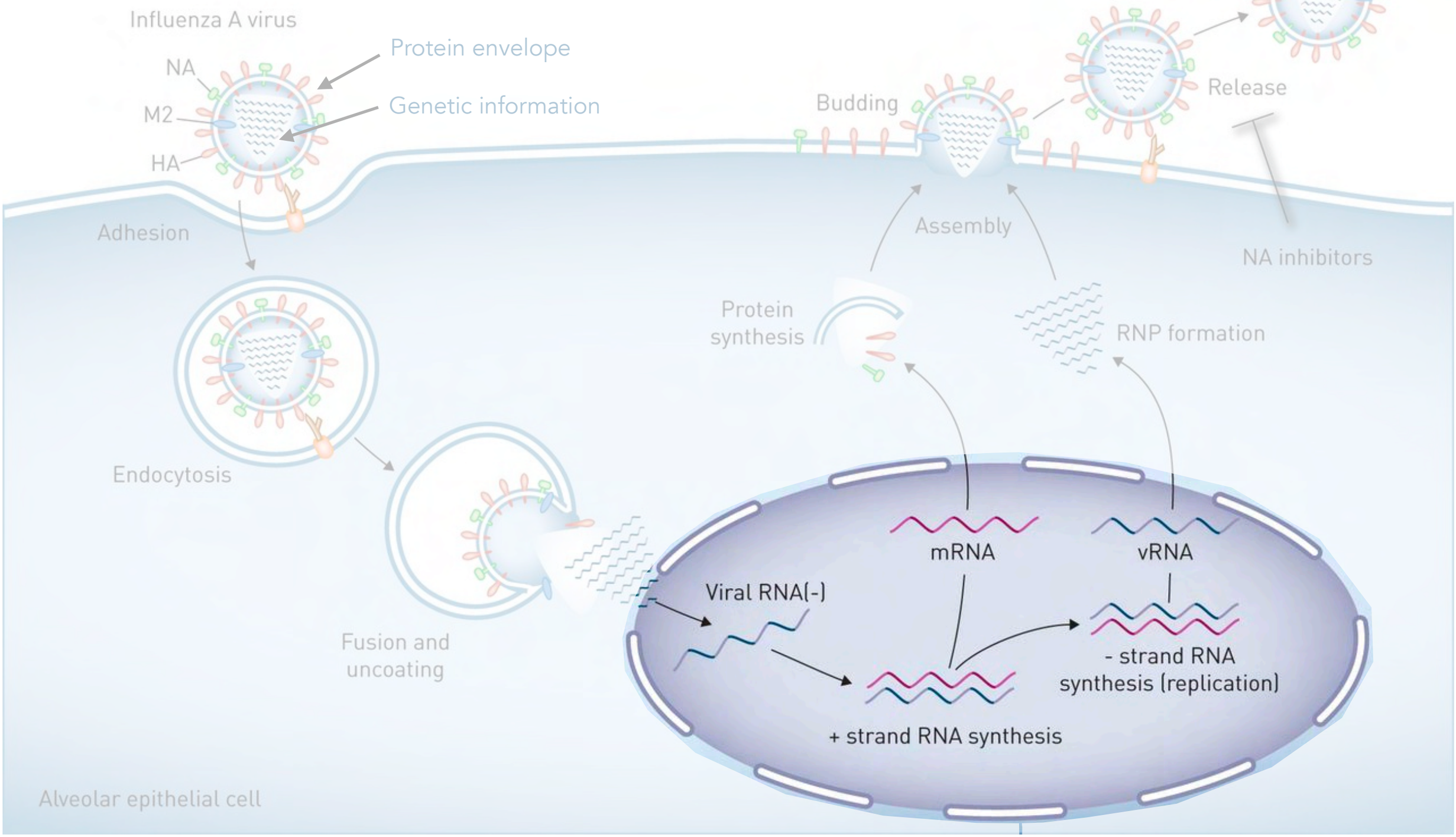
How they work



*Viruses are **obligate parasites** (they rely upon the cells natural machinery to replicate)*

Virology Crash Course

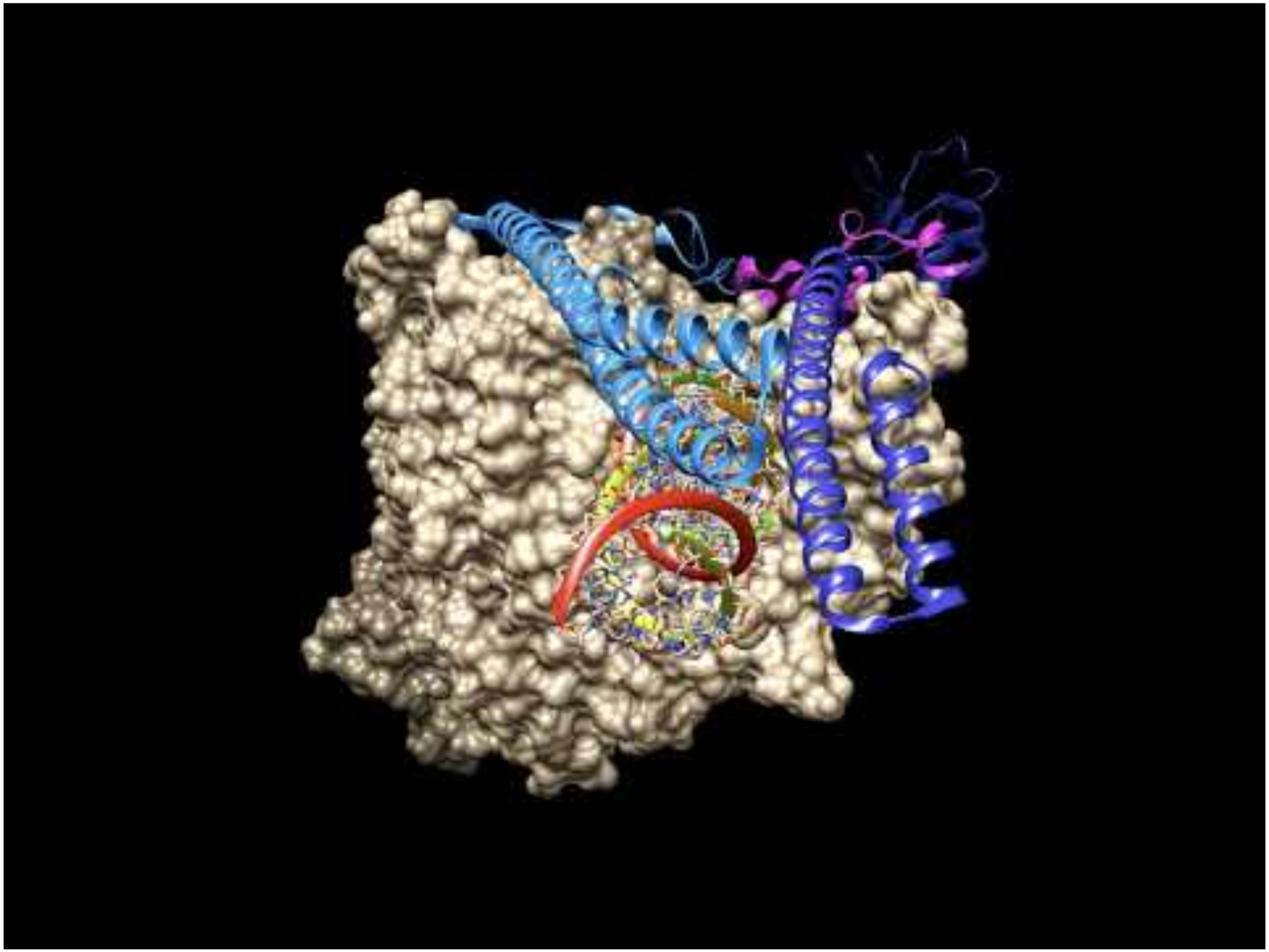
How they work



Nucleoside analogs ***interfere with viral replication*** *processes by* ***targeting the polymerase***

Virology Crash Course

Polymerase structure



The polymerase is responsible for ***replication*** *and often referred to as a* ***“right hand”***

Virology Crash Course

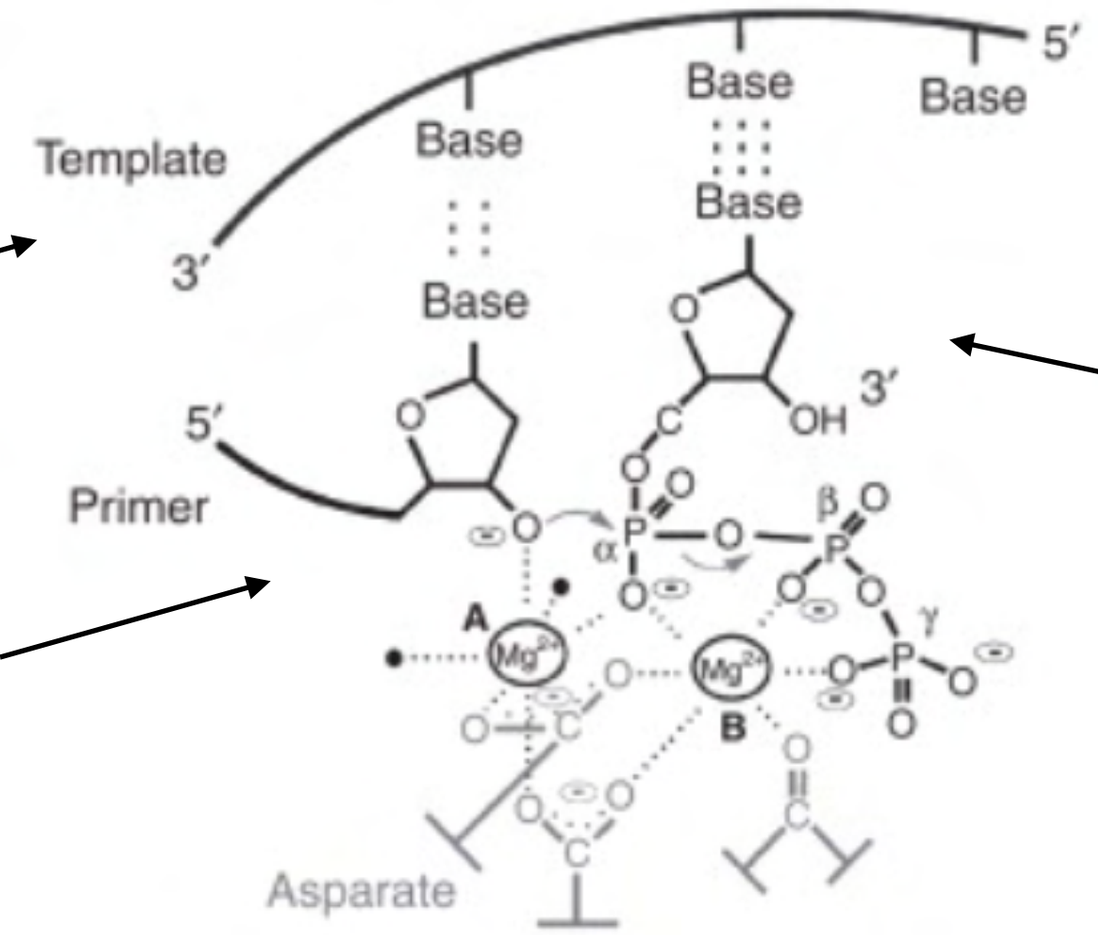
Types of polymerases

Class	Substrate	Product	"Classic" function
DNA dependent DNA polymerase (DdDp)	*DNA*	*DNA*	*Replication*
DNA dependent RNA polymerase (DdRp)	*DNA*	*RNA*	*Transcription*
RNA dependent DNA polymerase (RdDp or reverse transcriptase)	*RNA*	*DNA*	*Viral replication (DNA viruses)*
RNA dependent RNA polymerase (RdRp)	*RNA (+/-)*	*RNA (-/+)*	*Viral replication (RNA viruses)*

RdDp and RdRp** are frequently the targets of nucleoside antivirals given their role in **viral replication

Virology Crash Course

Polymerase active site



Read 3′ to 5′

Other residues (not shown) assist in nucleotide recognition

Synthesized 5′ to 3′

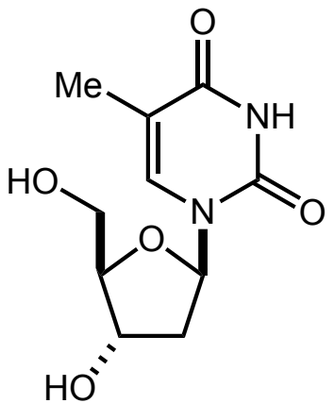
Key Asp residues bind Mg ions used to catalyze the chain elongation

Irrespective of the type of polymerase, the ***active site is structurally conserved***

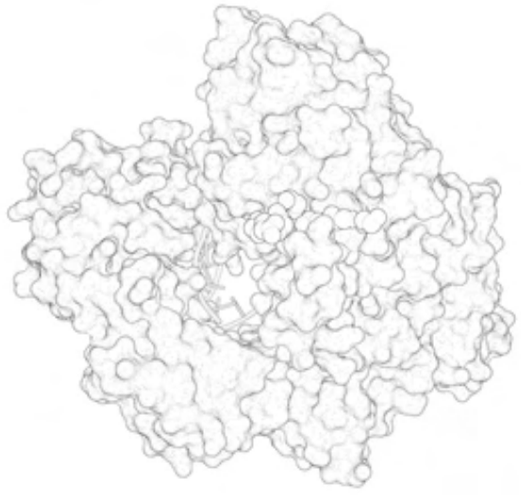
II Mechanism of Action

Mechanism of Action

Typical pathway



Nuceloside (T)



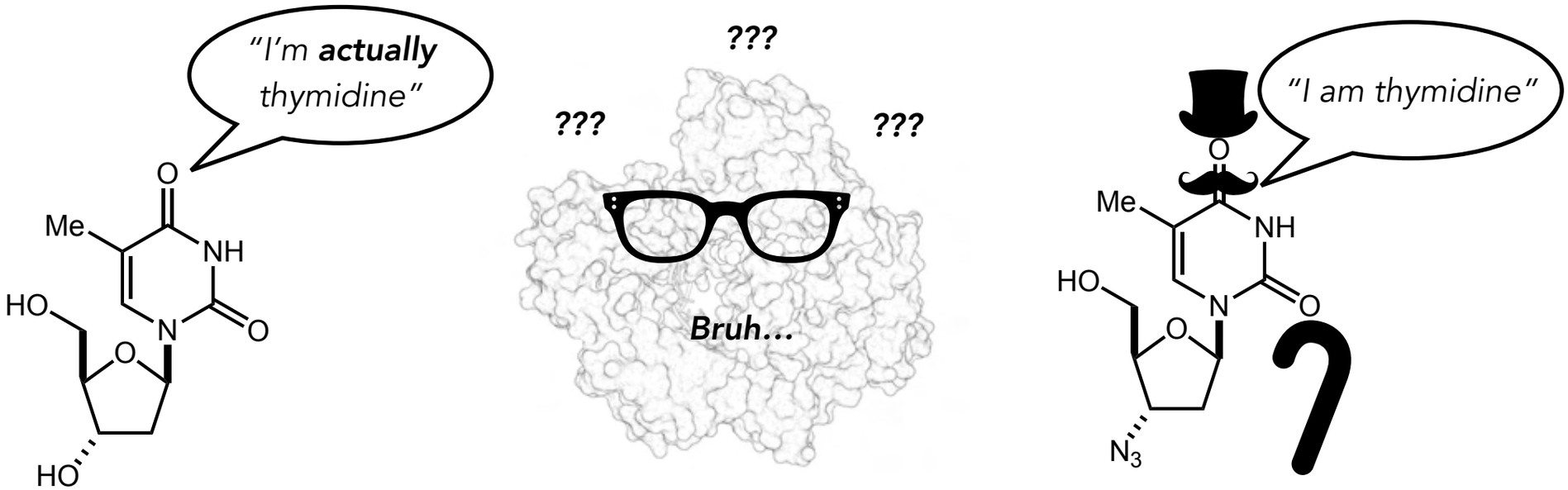
Polymerase

DNA

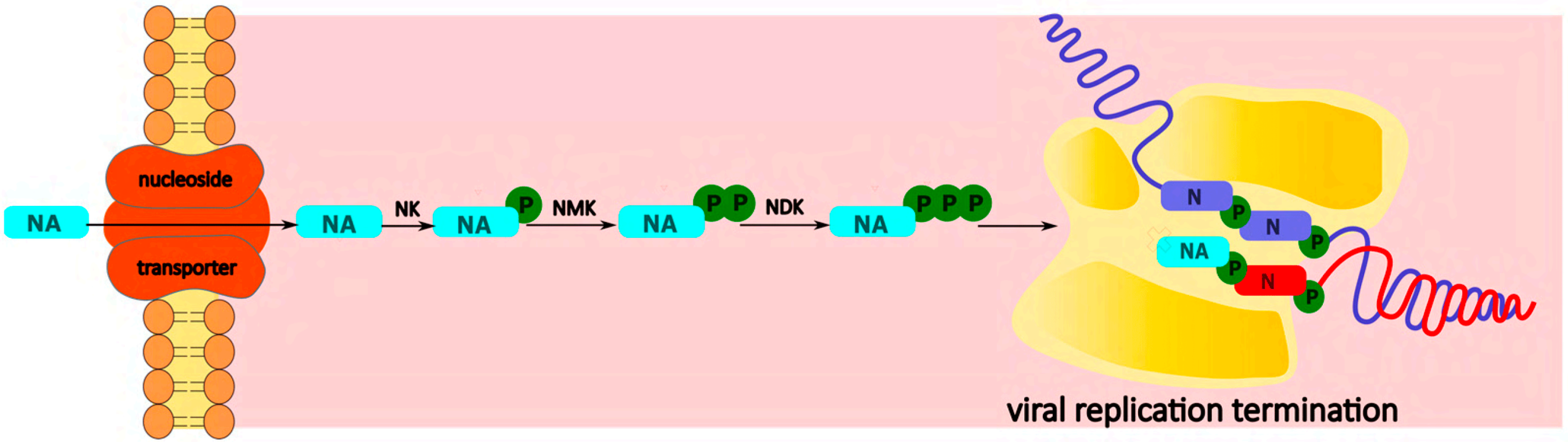
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Typical pathway



*Nucleoside antiviral slow or inhibit viral replication by **"tricking" the polymerase***



*Therapeutics must reach the **triphosphate form** to be recognized by the polymerase*

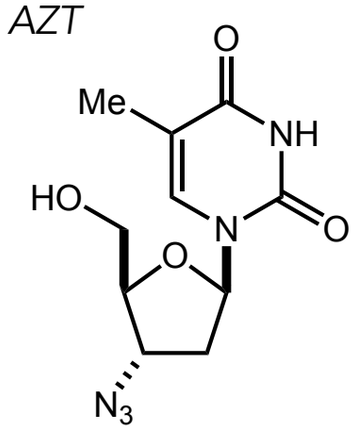
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Inhibition mechanisms

Chain Termination

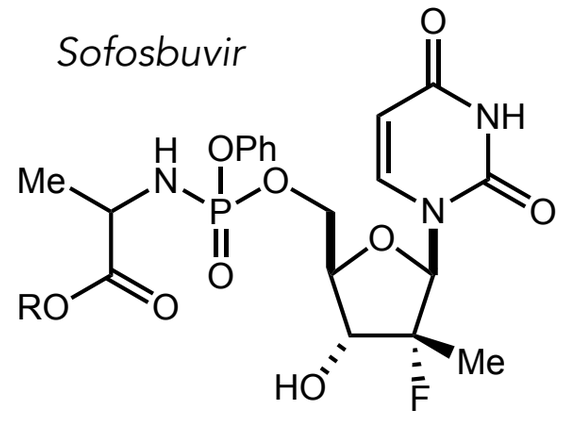
AZT



Obligate terminators, lack 3° OH

Disruption of Elongation

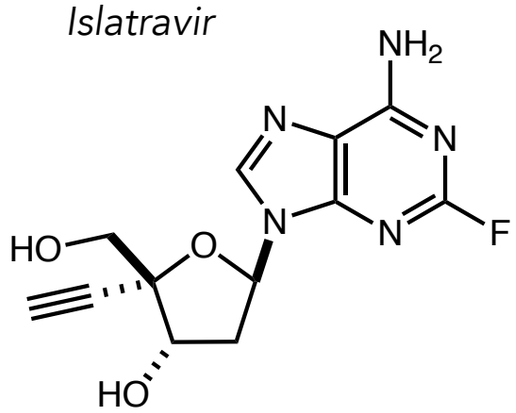
Sofosbuvir



Disrupts key H-bonding networks

Translocation Inhibitors

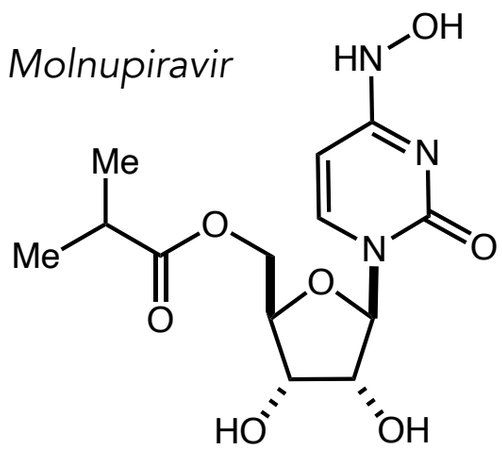
Islatravir



Bind strongly to the RdRP catalytic site

Lethal Mutagenesis

Molnupiravir



Incorporation leads to downstream toxicity

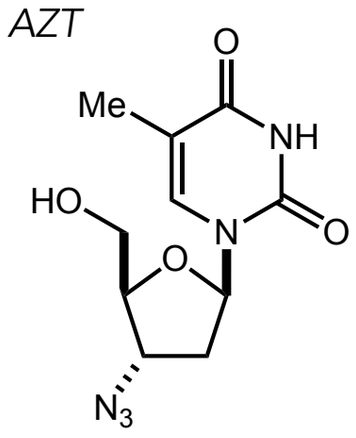
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Inhibition mechanisms

Chain Termination

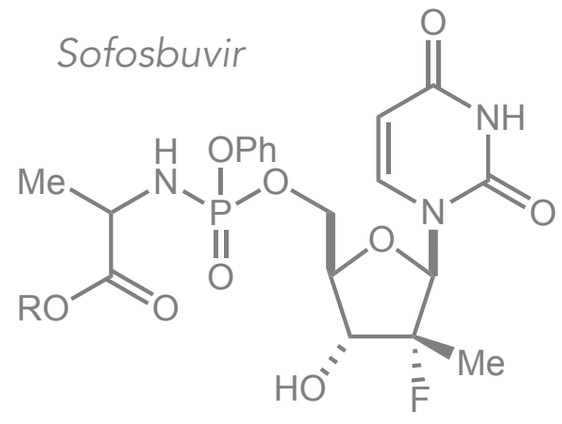
AZT



Obligate terminators, lack 3° OH

Disruption of Elongation

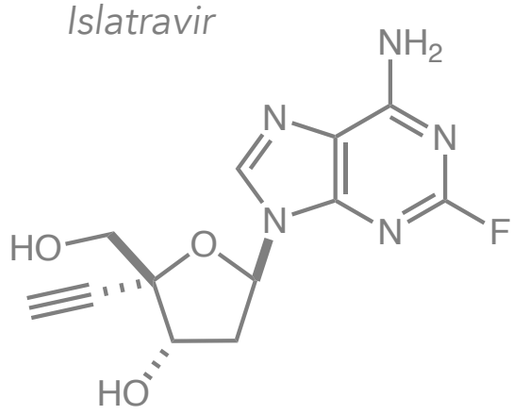
Sofosbuvir



Disrupts key H-bonding networks

Translocation Inhibitors

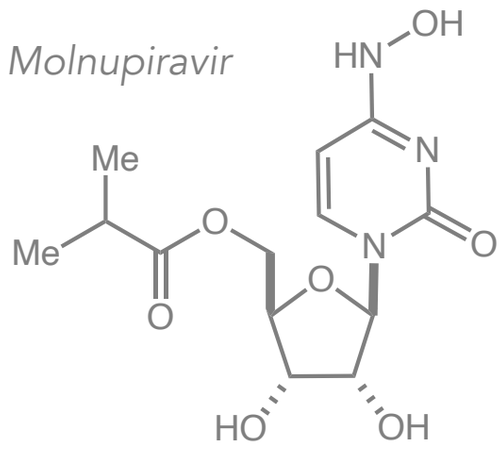
Islatravir



Bind strongly to the RdRP catalytic site

Lethal Mutagenesis

Molnupiravir



Incorporation leads to downstream toxicity

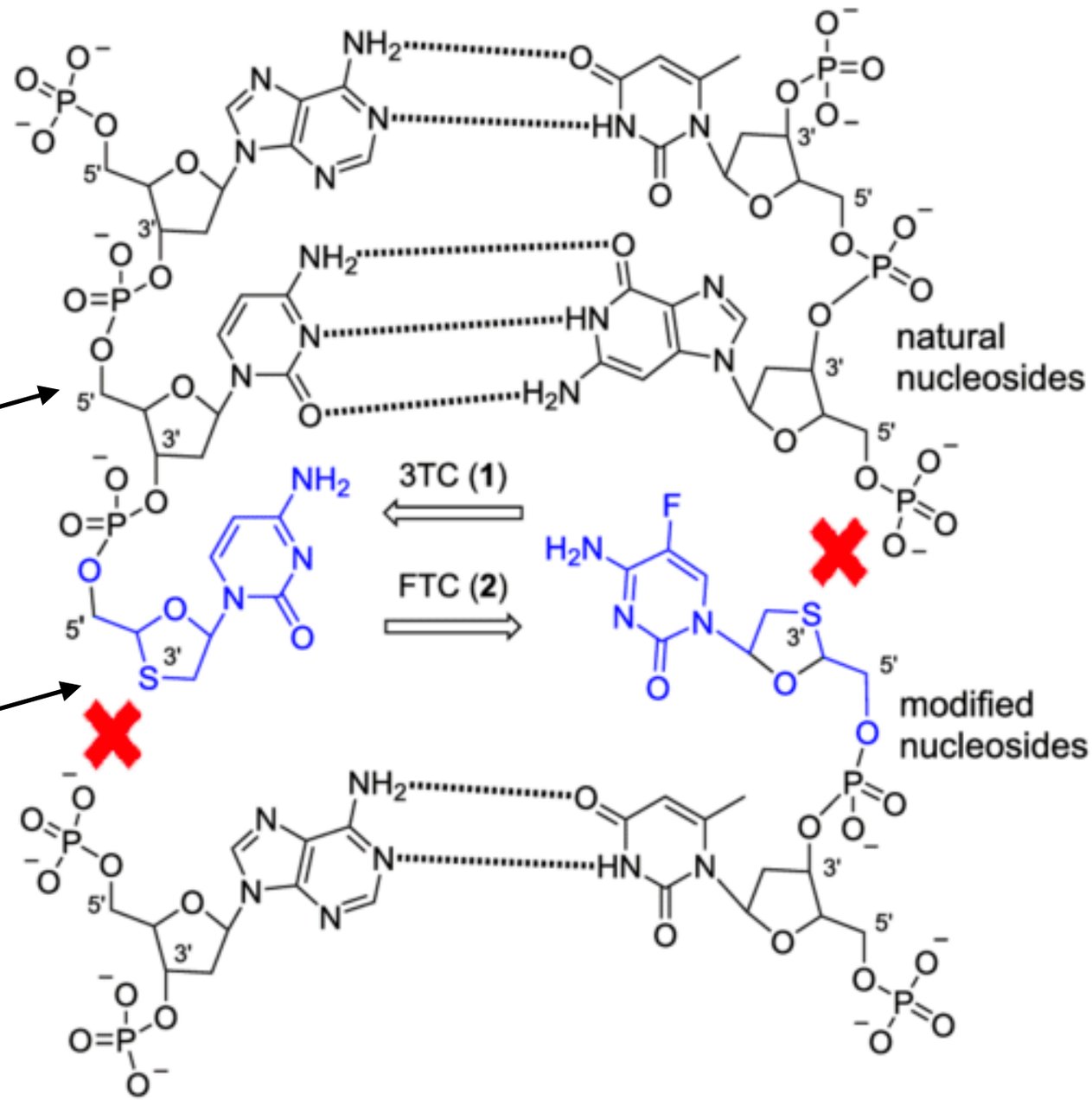
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Chain Termination

Contains 5′ OH for incorporation

Lacks 3′ OH for elongation

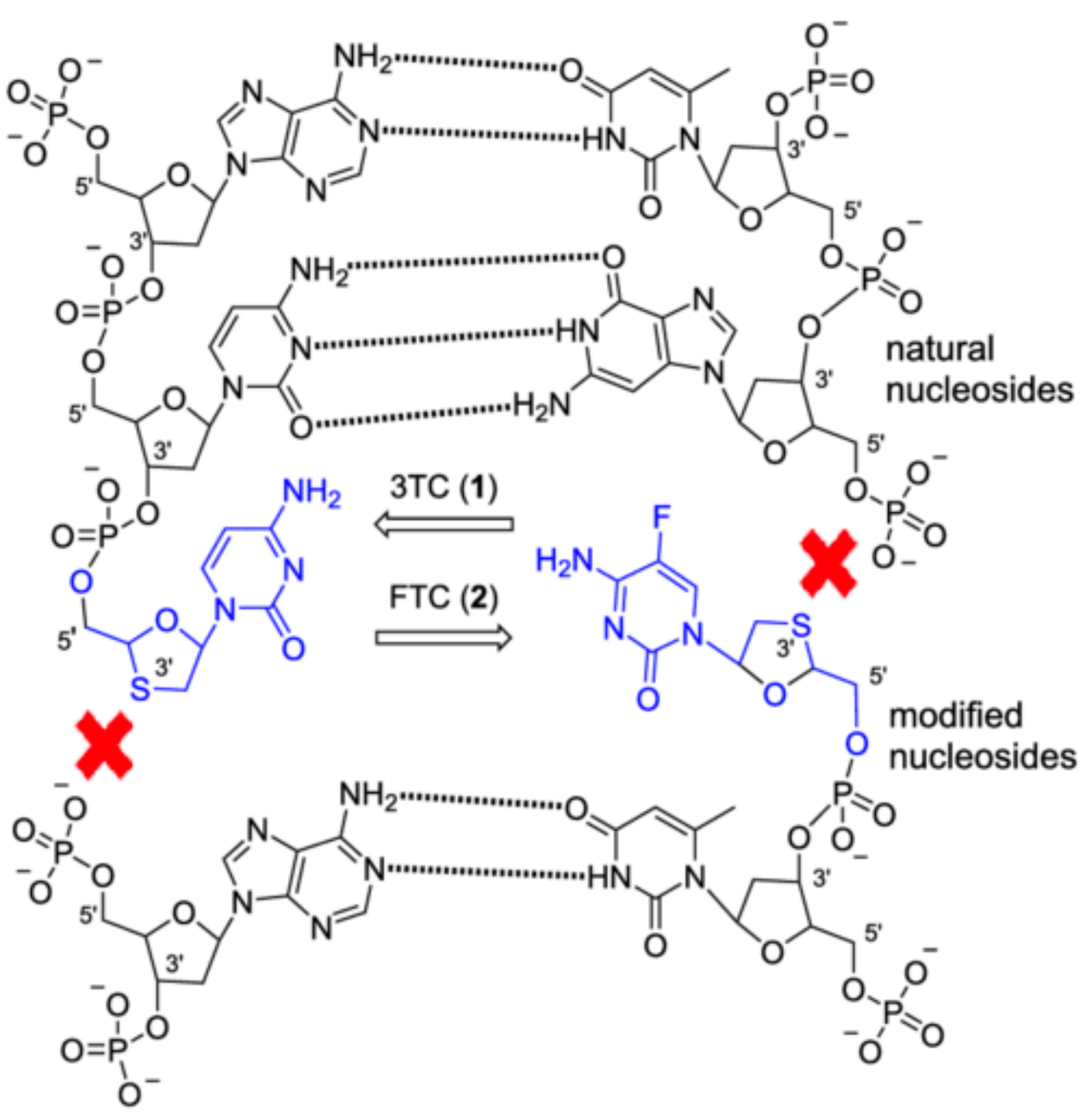


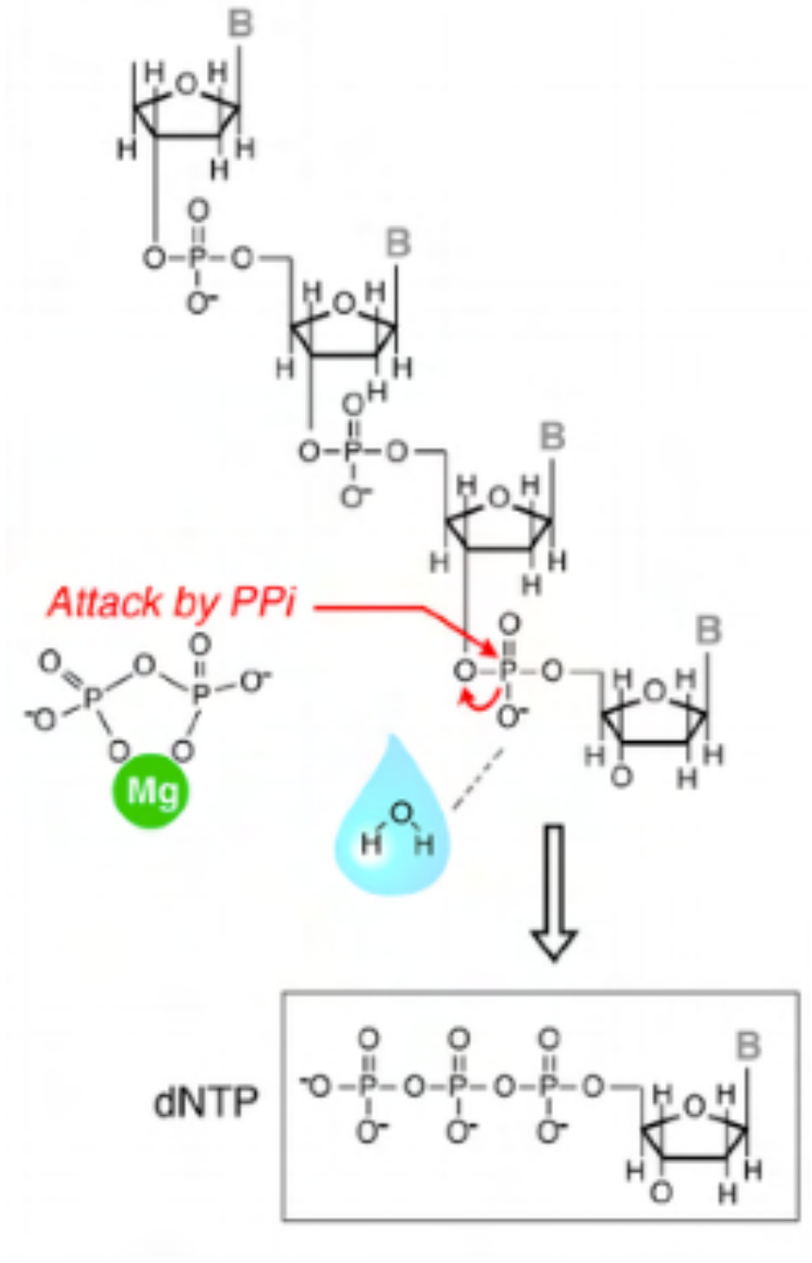
Chain terminators *function by preventing the installation of the next base through removal of the 3° hydroxyl*

Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Chain Termination





However these therapies are vulnerable to ***pyrophosphorolysis which can remove the therapy from the chain***

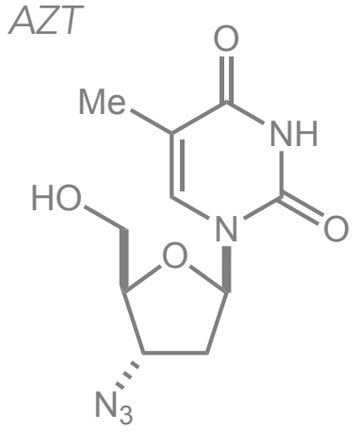
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Inhibition mechanisms

Chain Termination

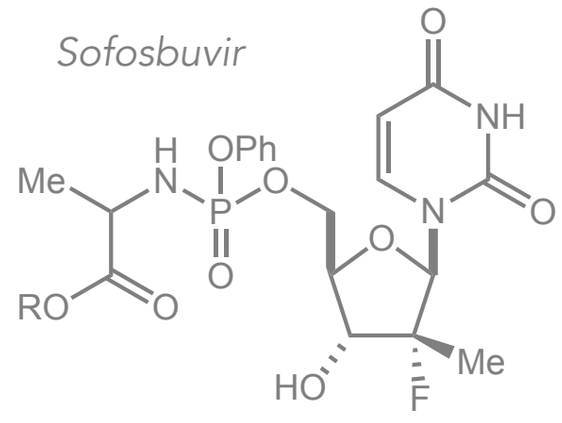
AZT



Obligate terminators, lack 3° OH

Disruption of Elongation

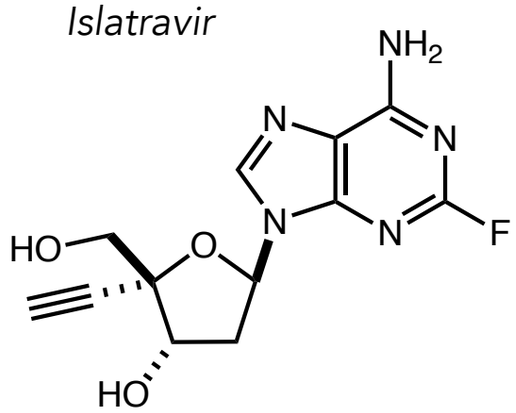
Sofosbuvir



Disrupts key H-bonding networks

Translocation Inhibitors

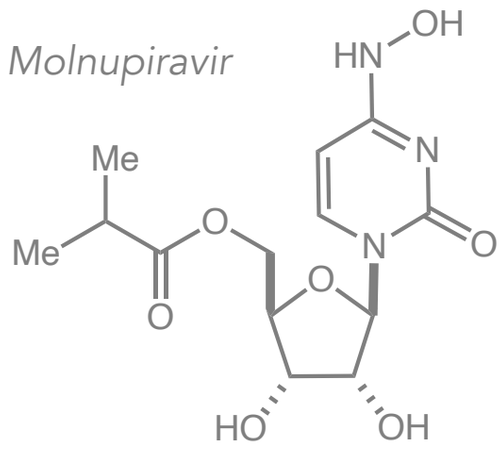
Islatravir



Bind strongly to the RdRP catalytic site

Lethal Mutagenesis

Molnupiravir



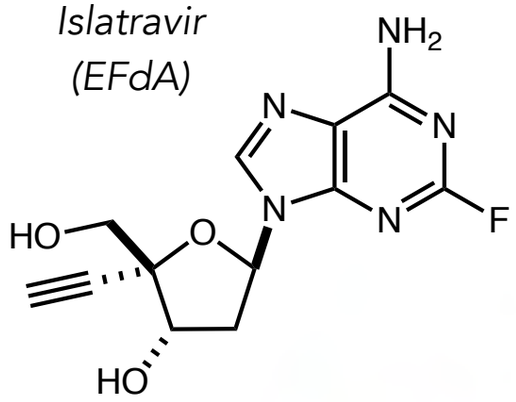
Incorporation leads to downstream toxicity

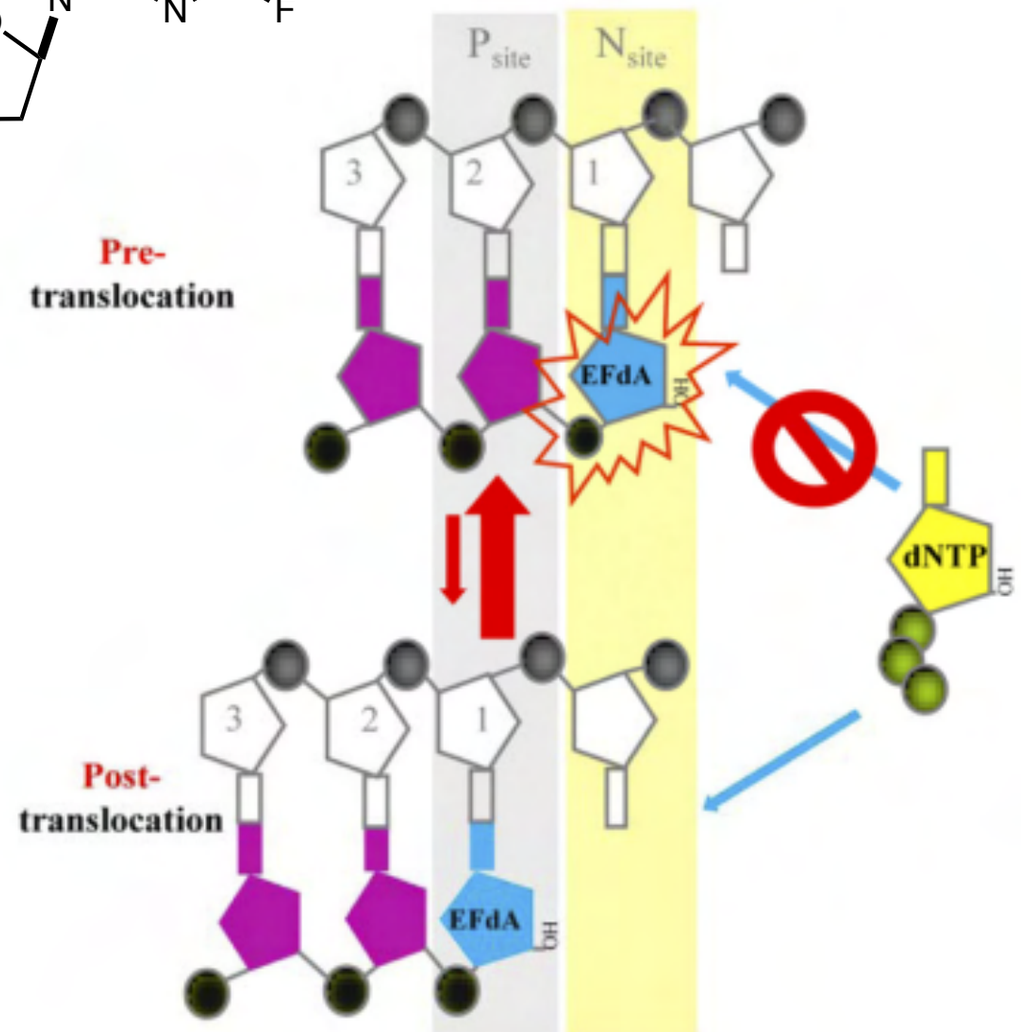
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

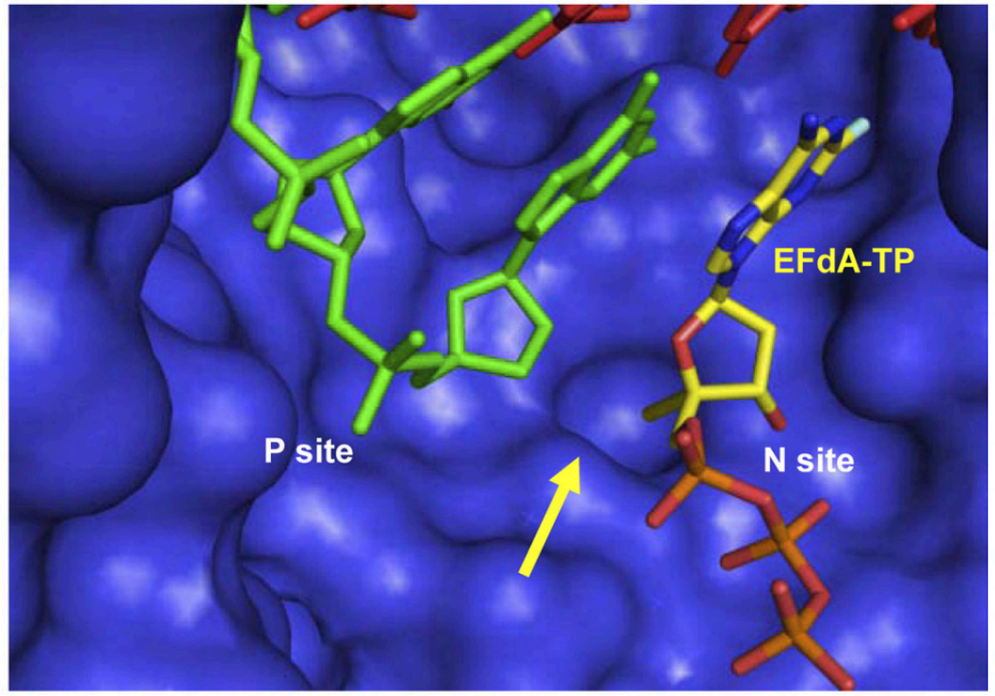
Translocation inhibitors

Islatravir
(EFdA)

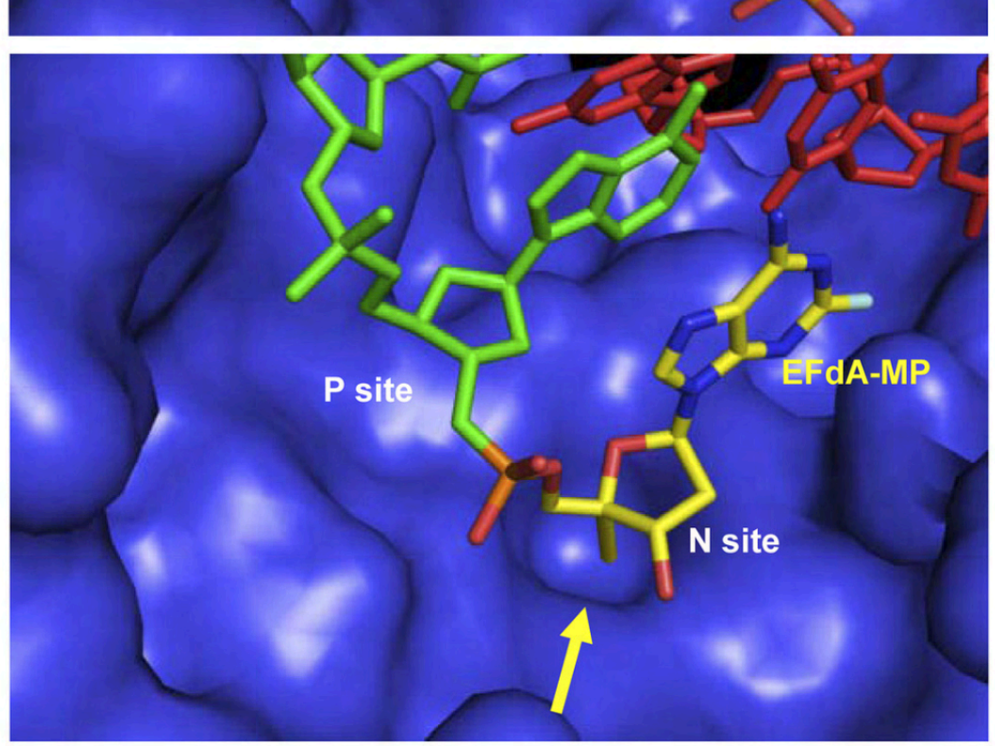




Pre-Incorperation



Pre-Translocation



Translocation inhibitors *function by binding tightly to the polymerase active site through optimized interactions*

Michailidis, E.; *et. al.*; *J. Biol. Chem.* **2009**, *284*, 35681-35691.

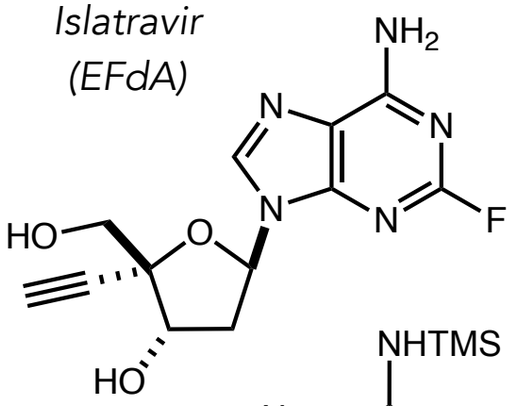
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Translocation inhibitors

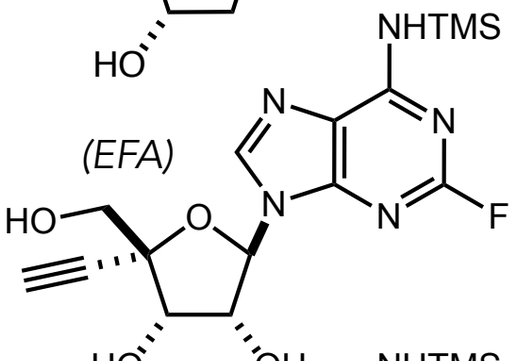
HIV-1 replication in PBMCs

Islatravir (EFdA)



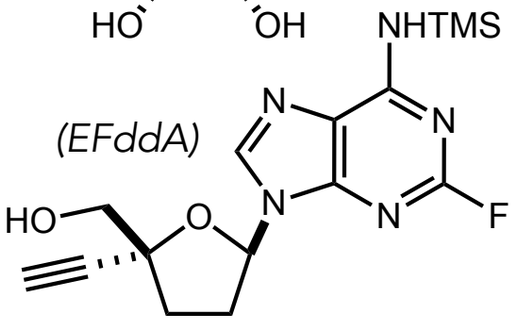
EC_{50} = 0.05 nM

(EFA)

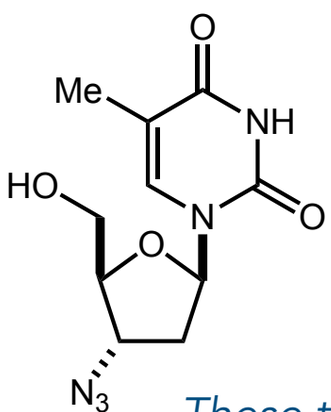


EC_{50} = 11 nM

(EFddA)

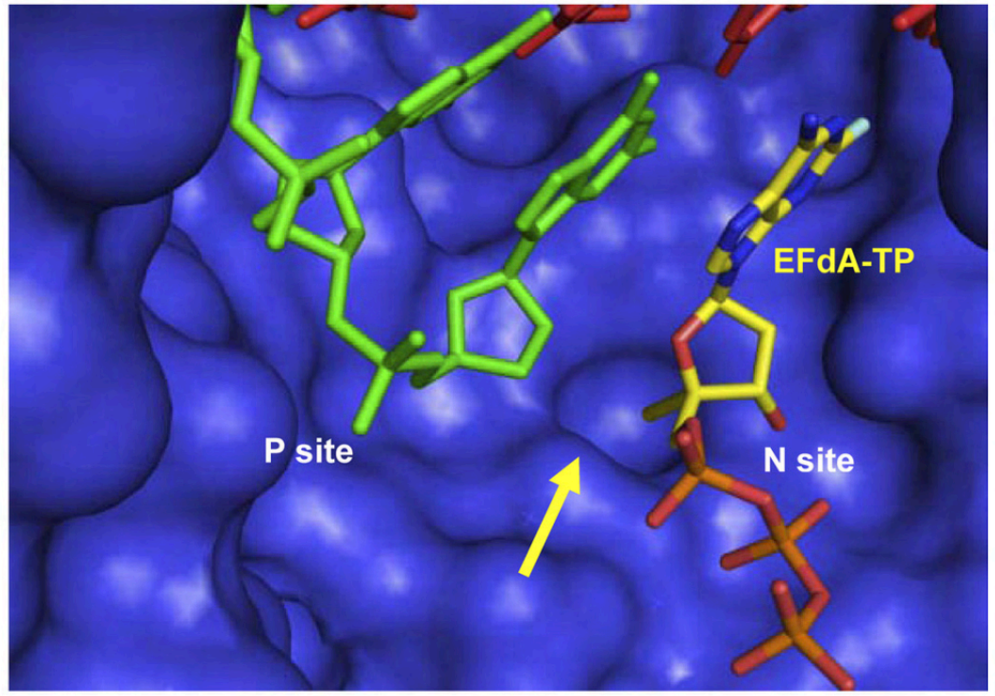


EC_{50} = 570 nM

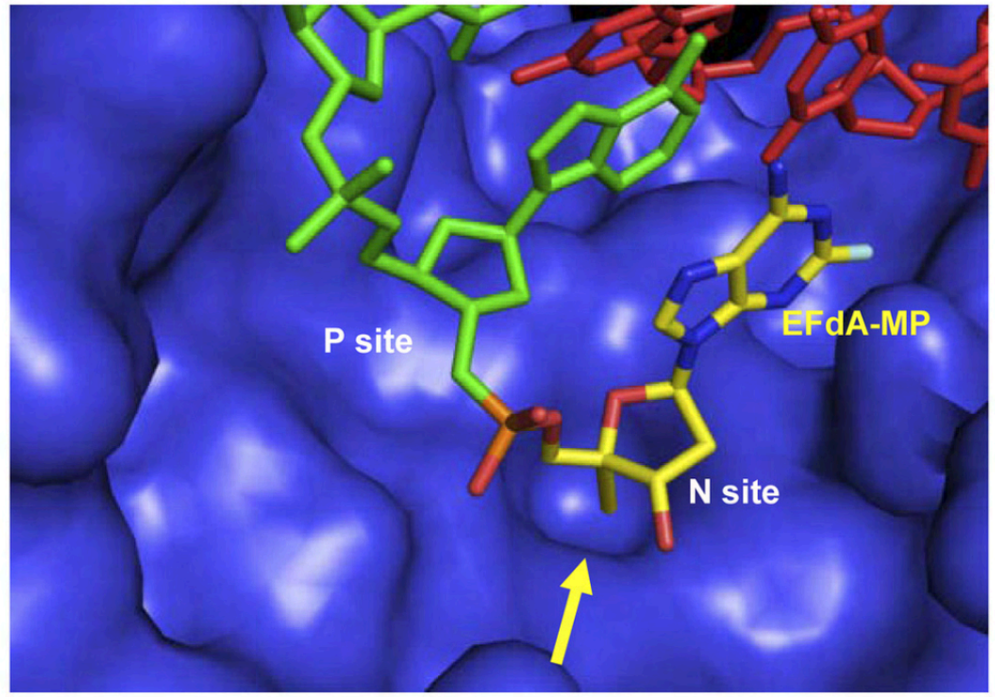


(AZT) *EC_{50} = 22 nM*

Pre-Incorperation



Pre-Translocation



*These types inhibitors are much **newer to the field** are promising HIV treatments*

Michailidis, E.; *et. al.*; *J. Biol. Chem.* **2009**, *284*, 35681-35691.

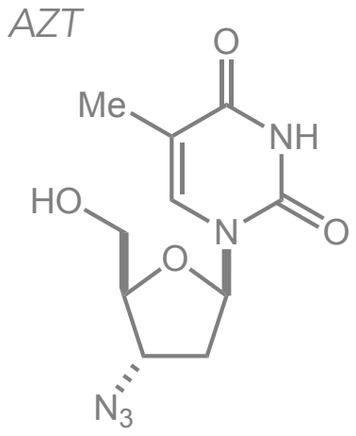
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Inhibition mechanisms

Chain Termination

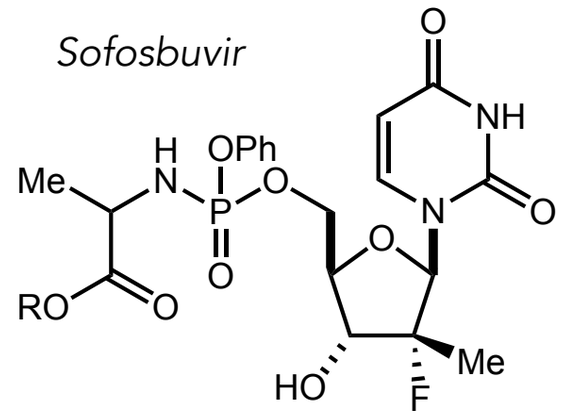
AZT



Obligate terminators, lack 3° OH

Disruption of Elongation

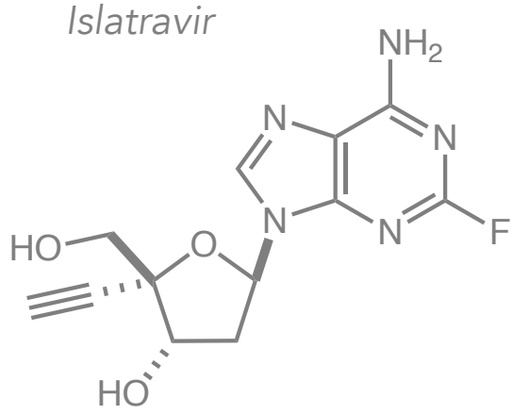
Sofosbuvir



Disrupts key H-bonding networks

Translocation Inhibitors

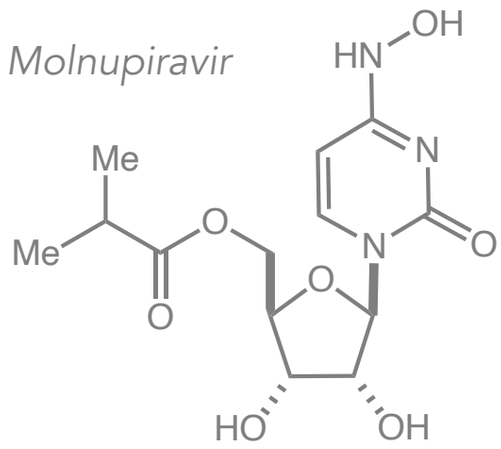
Islatravir



Bind strongly to the RdRP catalytic site

Lethal Mutagenesis

Molnupiravir

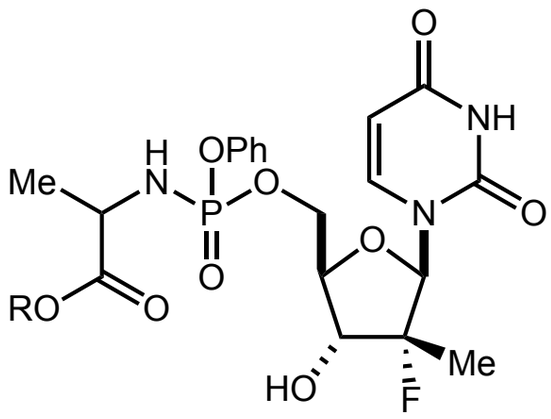


Incorporation leads to downstream toxicity

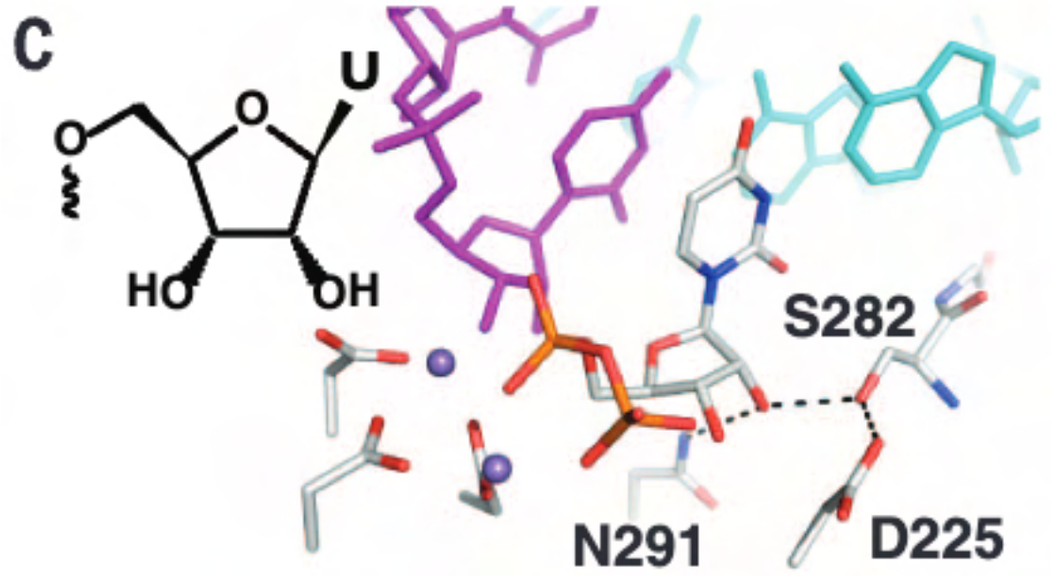
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

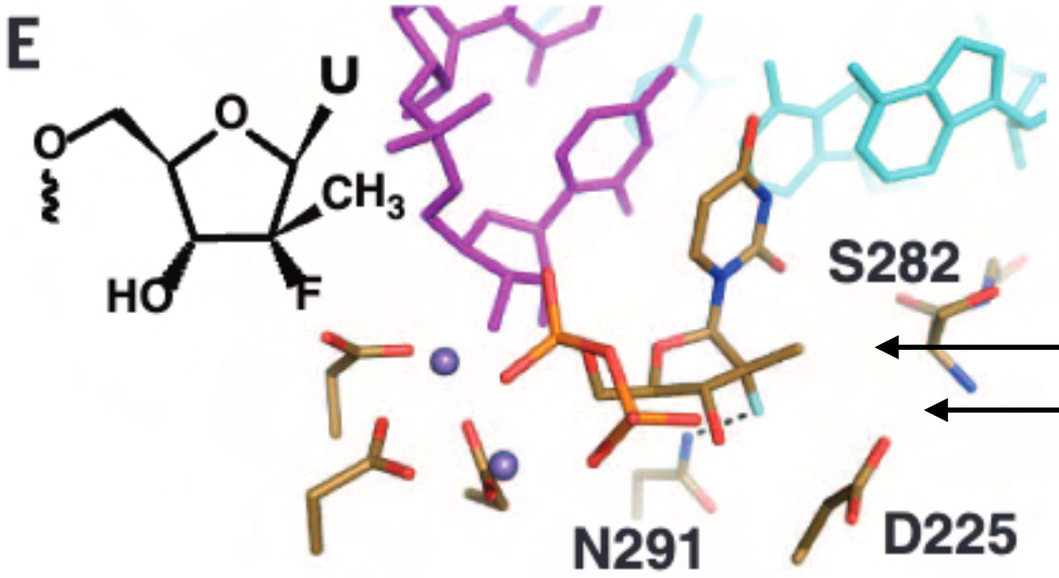
Mechanism of Action

Disruption of Elongation

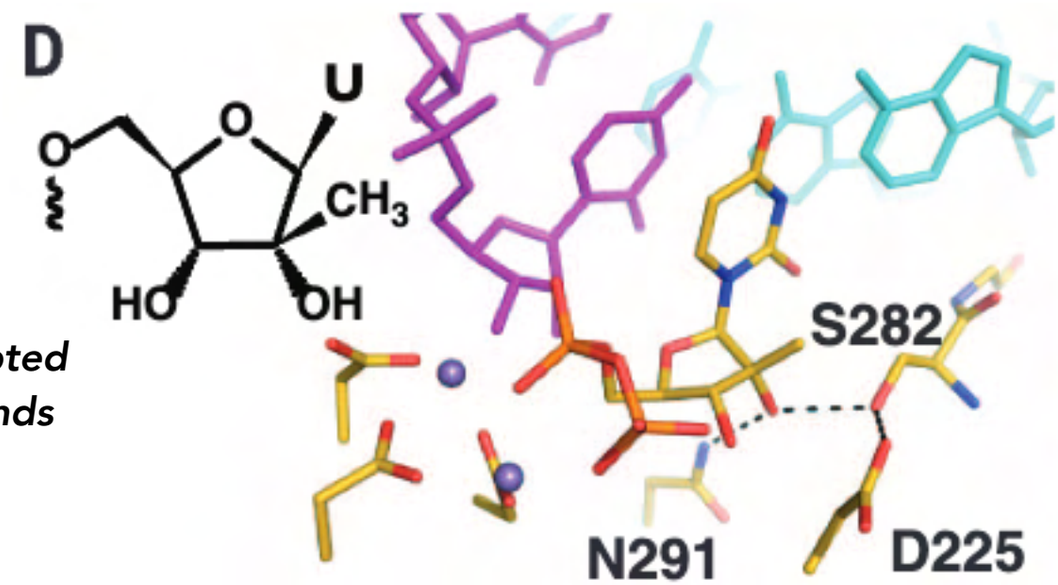


Sofosbuvir
(HCV inhibitor)





Disrupted H-bonds



*By **disrupting key hydrogen bonding interactions** after incorporation, these drugs **prevent further elongation***

Appleby, T. C.; *et. al.*; *Science.* **2015**, *347*, *771-775.*

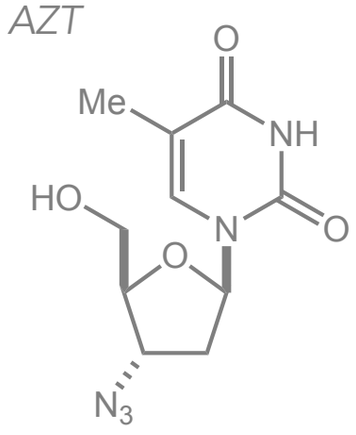
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

Mechanism of Action

Inhibition mechanisms

Chain Termination

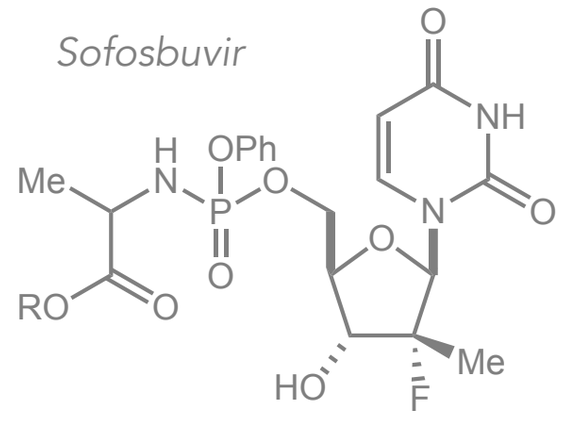
AZT



Obligate terminators, lack 3° OH

Disruption of Elongation

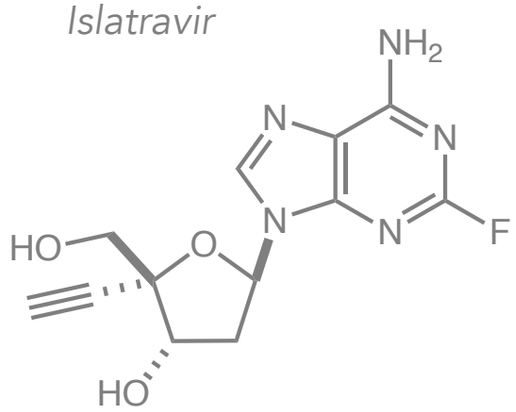
Sofosbuvir



Disrupts key H-bonding networks

Translocation Inhibitors

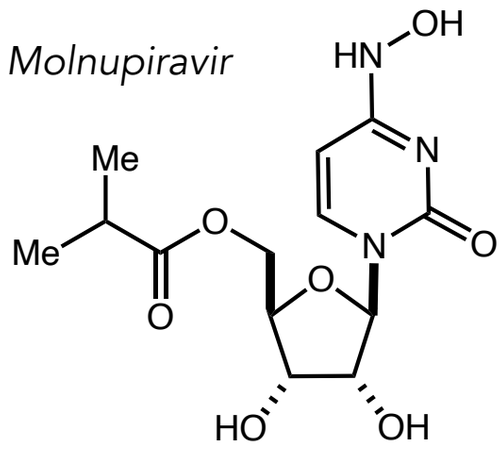
Islatravir



Bind strongly to the RdRP catalytic site

Lethal Mutagenesis

Molnupiravir



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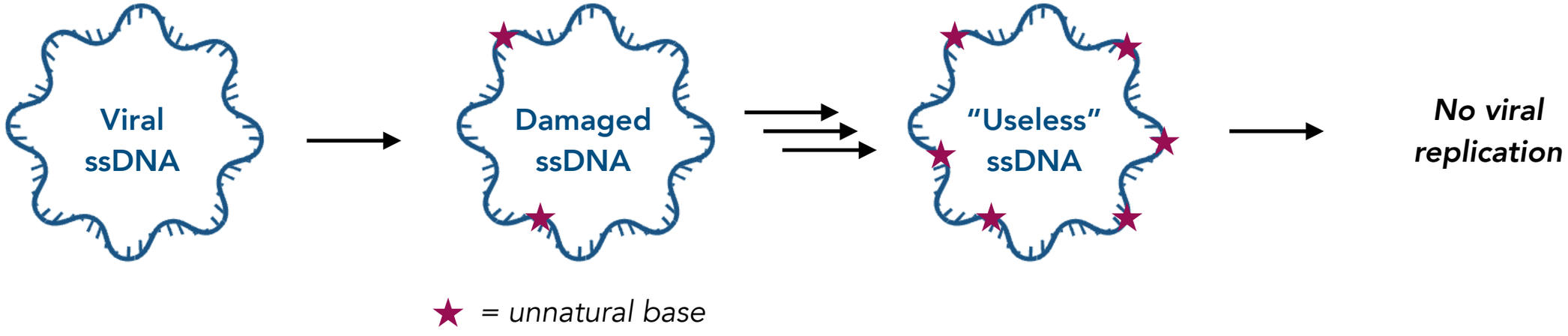
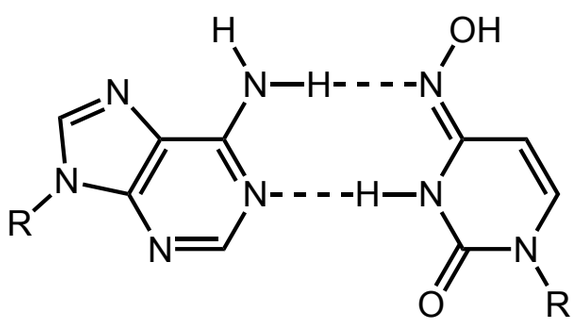
Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

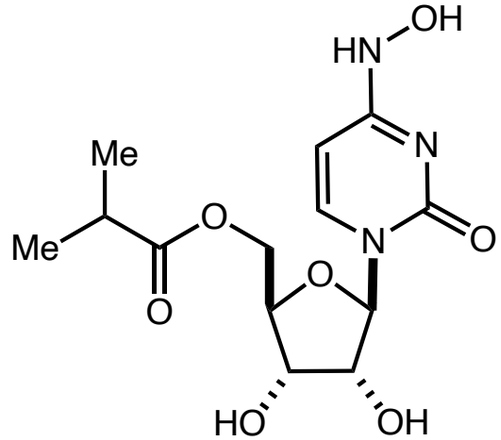
Mechanism of Action

Lethal Mutagenesis





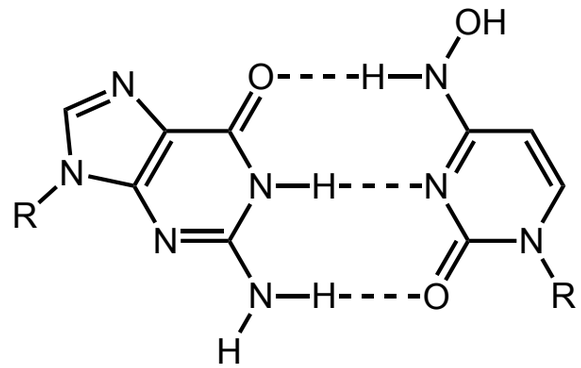
Mimicking U



Molnupiravir

-Not recognized by proof reading enzymes

-Interpreted as C or U (tautomers)



Mimicking C

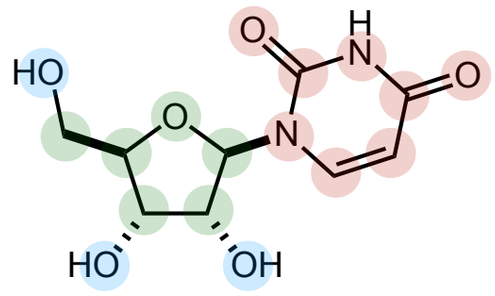
Lethal mutagenesis relies upon ***incorporation into the genome*** *rather than preventing its replication*

Kamzeeva, P. N.; Aralov, A. V.; Alferova, V. A.; Korshun, V. A.; *Curr. Issues Mol. Biol.* **2023**, *45*, 6851-6879.

III Structure-Activity-Relationships (SAR)

Structure Activity Relationships (SAR)

Sites of potential modification

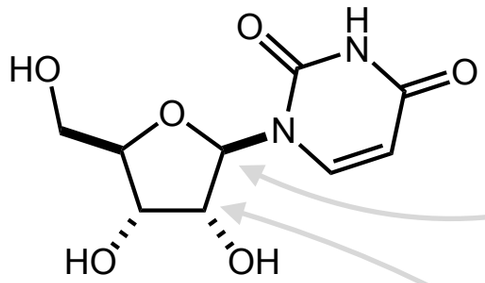


Uracil nucleobase

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification



1′ carbon

- Nitrile

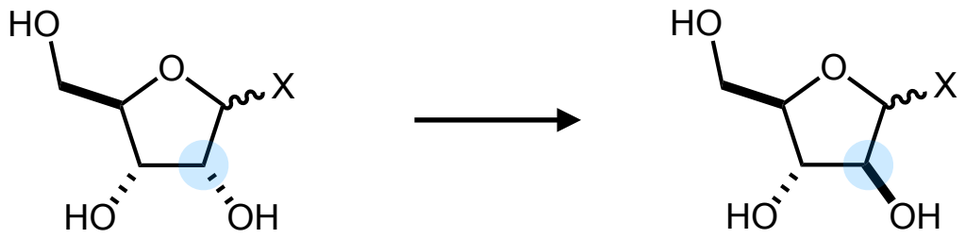
2′ carbon

- Epimerization
- Nitrile/Fluorine/Methyl

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

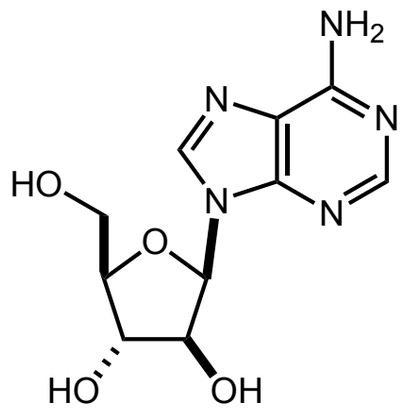
Structure Activity Relationships (SAR)

1′/2′ carbon modifications

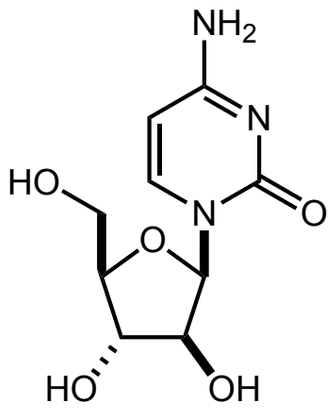


Ribose Arabinose

Epimerization *of 2′ alcohol was one of the first modifications pursued (~1950s)*

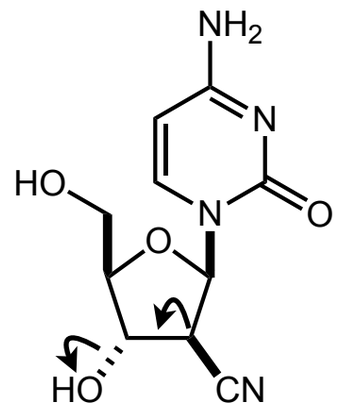


Ara-A
(1976 approval, antiviral)



Ara-C
(1969 approval, chemotherapy)

6 cell lines, IC_{50} = 0.09-4.5 µg/mL



CNDAC
(Experimental chemotherapy)

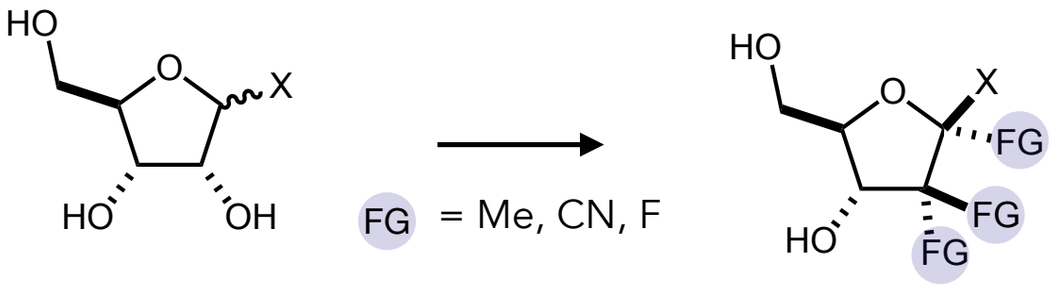
14 cell lines, IC_{50} = 0.04-6.8 µg/mL

CNDAC is under investigation as a ***more powerful chemotherapy*** *enabling DNA damage through* ***β-elimination***

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

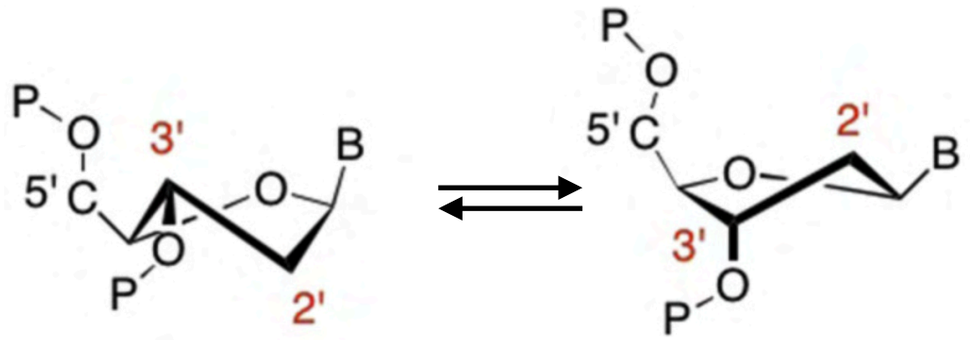
Structure Activity Relationships (SAR)

1′/2′ carbon modifications



Ribose

Ribose derivative

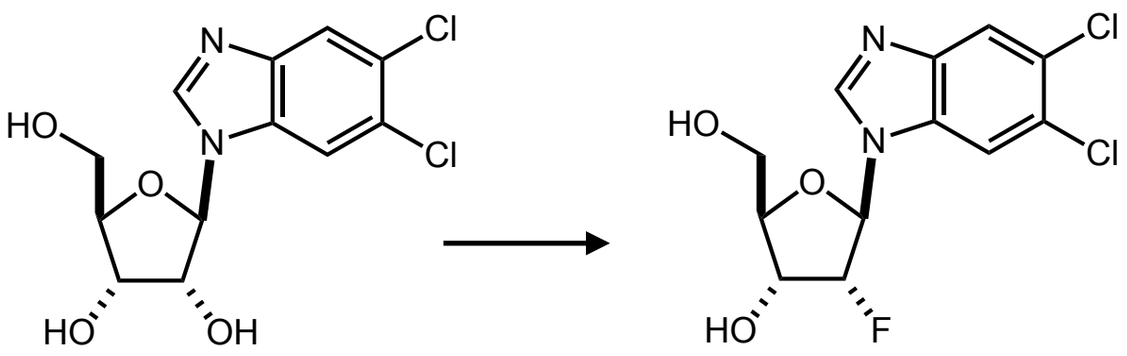


“North” Conformation
(2′-exo/3′-endo)

Favored by DdDp and RdDp

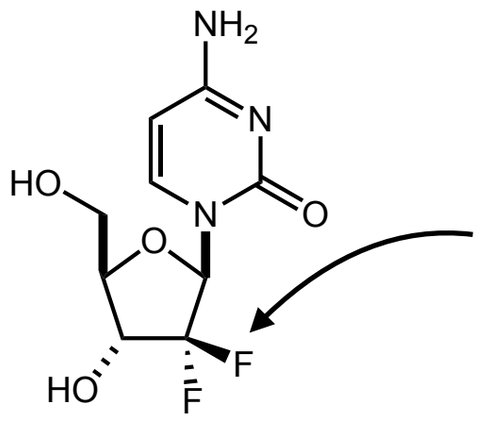
“South” Conformation
(2′-endo/3′-exo)

Favored by kinases



$T_{1/2}$ < 1 hr

Improved $T_{1/2}$



Unique ribose conformation enabled by difluoro motif

Gemcitabine

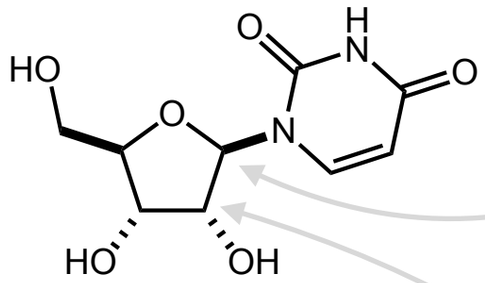
A range of functional groups can stabilize the C-N bond, ***reducing rates of glycolysis***

Fluorine can ***lock the sugar into one conformation,*** *affecting its interaction with proteins*

Gudmundsson, K. S.; Freeman, G. A.; Drach, J. C.; Townsend, L. B.; *J. Med. Chem.* **2000**, *43*, 2473-2478.
Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.

Structure Activity Relationships (SAR)

Sites of potential modification



1′ carbon

- Nitrile

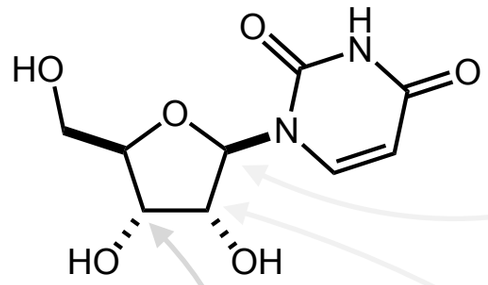
2′ carbon

- Epimerization
- Nitrile/Fluorine/Methyl

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification



1′ carbon

- Nitrile

2′ carbon

- Epimerization
- Nitrile/Fluorine/Methyl

3′ carbon

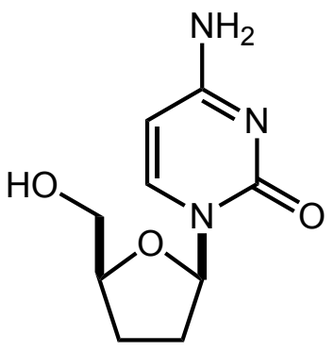
- Removal of OH
- Nitrile/Fluorine/Methyl

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

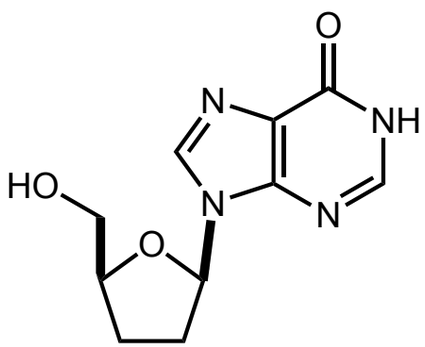
3’ carbon modifications

First generation HIV treatments



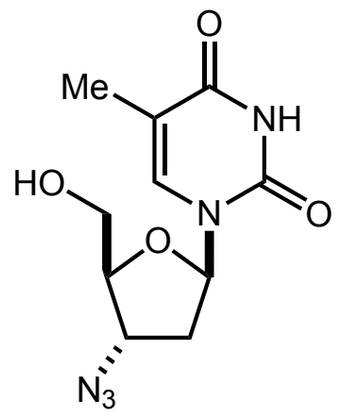
Zalcitabine

IC_{50} = 0.60 μM



Diadanosine

IC_{50} = 0.049 μM

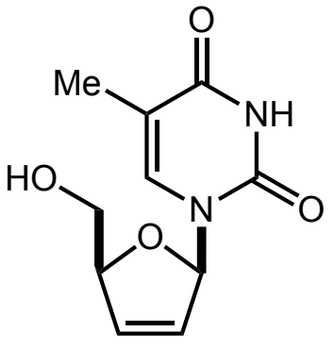


AZT

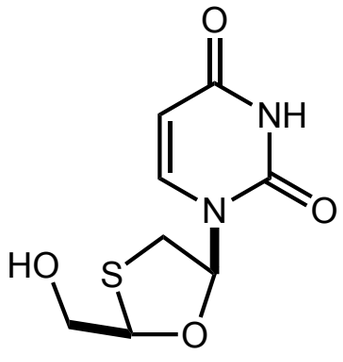
IC_{50} = 0.03 μM

By 1992, resistance was well documented for all 3 drugs

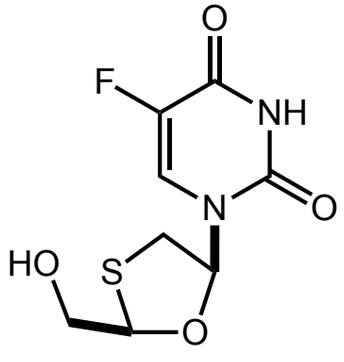
Modern HIV treatments



Stavudine



Lamivudine



Emtricitabine

Less side effects and reduced toxicity

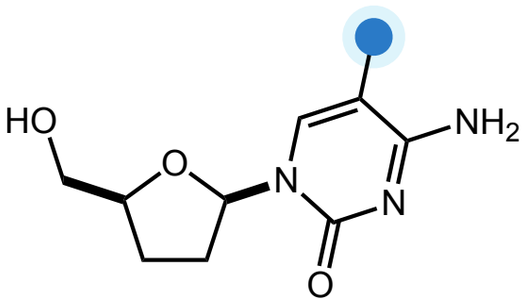
Removal of 3’ hydroxyl *stops the polymerase via* ***chain termination*** *(no site for next nucleobase)*

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.

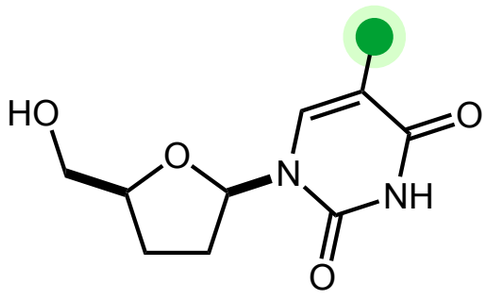
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

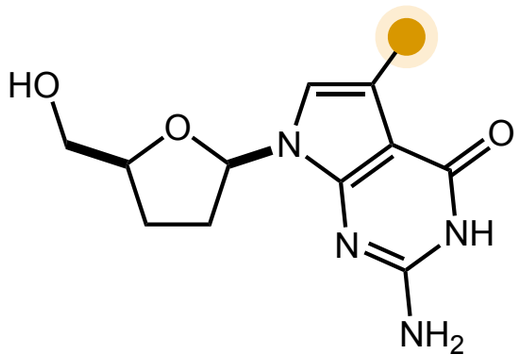
3’ carbon modifications



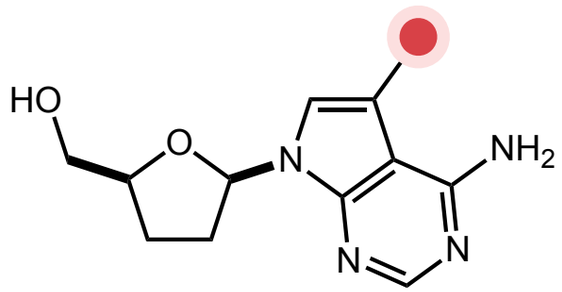
ddCytosine



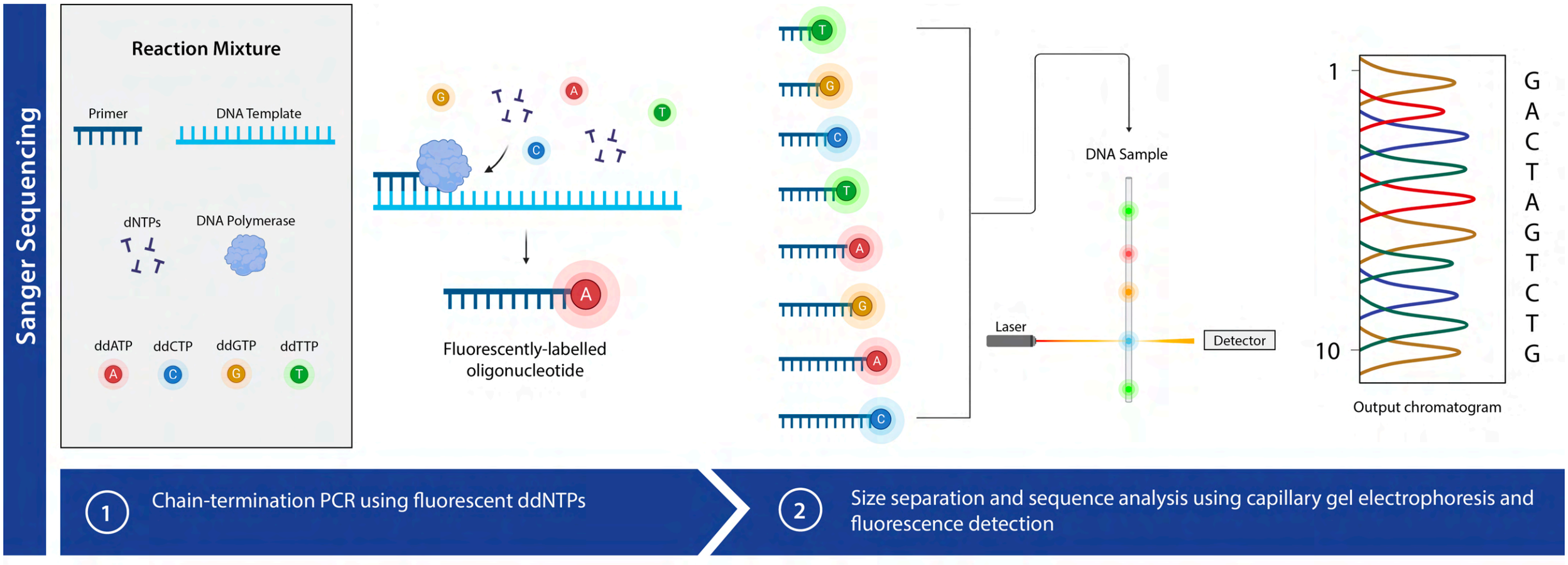
ddThymidine



ddGuanosine



ddAdenosine

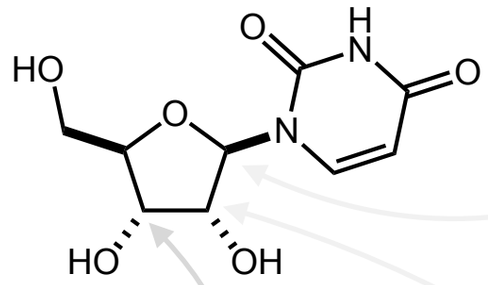


*Furthermore **Sanger sequencing** was enabled through the use of **2/3° deoxy nucleosides***

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification



1′ carbon

- Nitrile

2′ carbon

- Epimerization
- Nitrile/Fluorine/Methyl

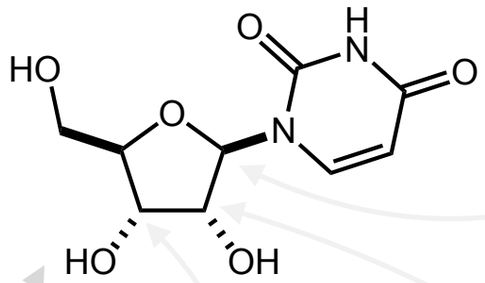
3′ carbon

- Removal of OH
- Nitrile/Fluorine/Methyl

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification



1′ carbon
- Nitrile

Other strategies
- Backbone removal
- Ring size

3′ carbon
- Removal of OH
- Nitrile/Fluorine/Methyl

2′ carbon
- Epimerization
- Nitrile/Fluorine/Methyl

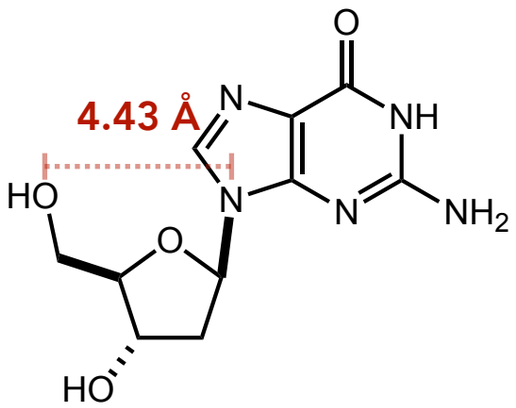
Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

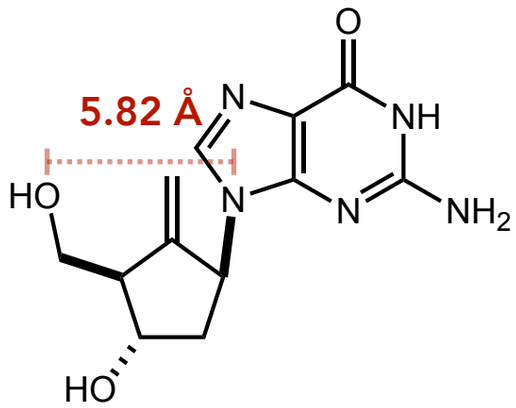
Other modifications

Zahler Hypothesis

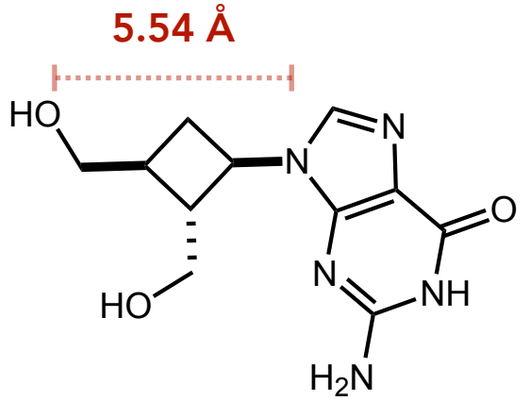
*"The **identity** of the 5' and nucleobase linker doesn't matter, only its **length**"*



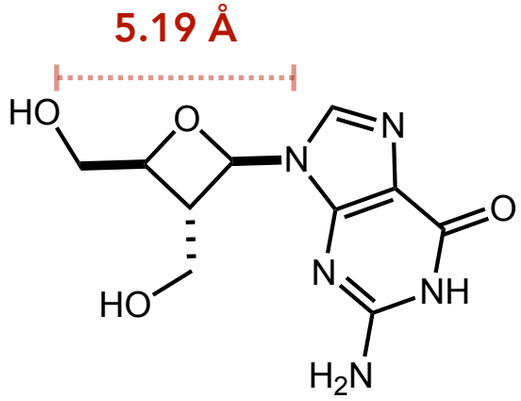
deoxy guanosine
(Nucleobase)



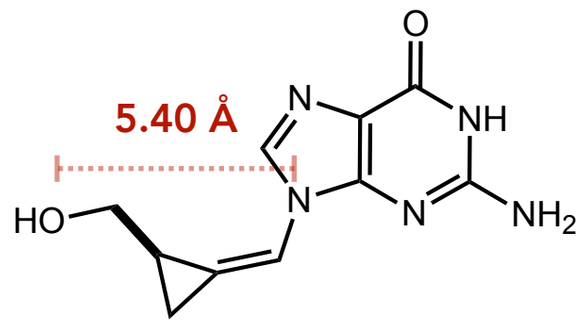
Entecavir
(Approved HBV treatment)



Lobucavir
(Broad spectrum experimental antiviral)



Oxetanocine G
(Experimental HIV treatment)



Synguanol
(Experimental EBV treatment)

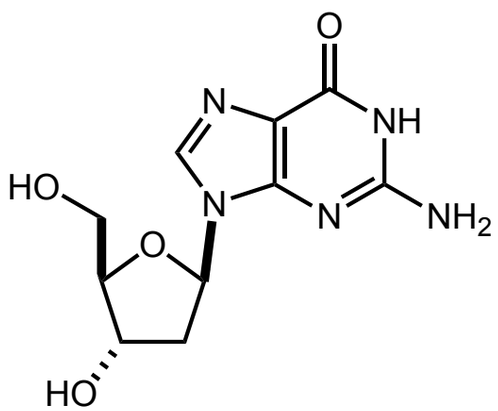
Altering ring size *can lead to new inhibitors but none have made it out of the clinic*

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162,* 5-21.

DFT using B3YLP 6-31G'+ (2d, 2p), GD3BJ dispersion, SMD solvation model in water

Structure Activity Relationships (SAR)

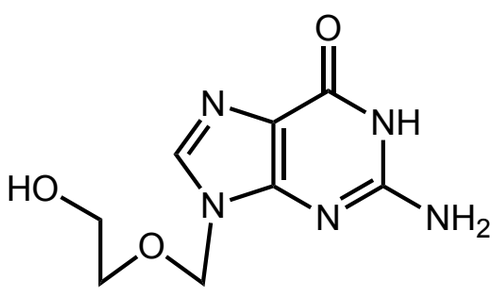
Other modifications



deoxy guanosine

(Nucleobase)

Backbone Removal

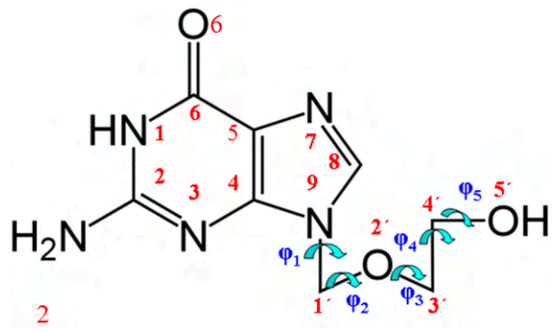


Acyclovir

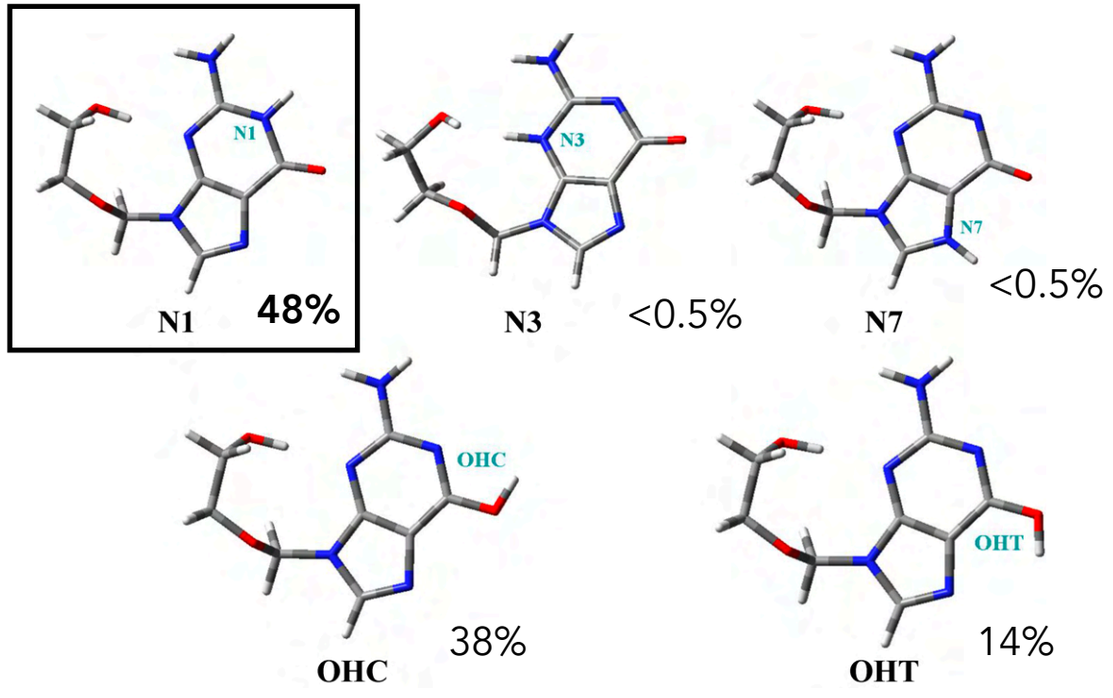
(HSV-1/2 replication inhibitor)

>3000x rate of phosphorylation by viral thymidine kinases

100x selectivity for viral DNA polymerase vs cellular



"Acyclovir's inherent flexibility allows for optimized interactions in the target enzyme binding sites"

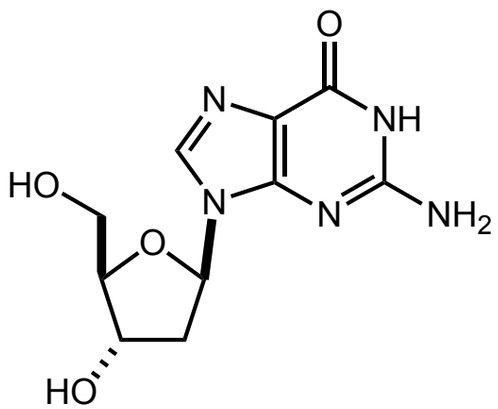


Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.

Alvarez-Ros, M. C.; Palafox, M. A.; *Pharmaceuticals* **2014**, *7*, 695-722.

Structure Activity Relationships (SAR)

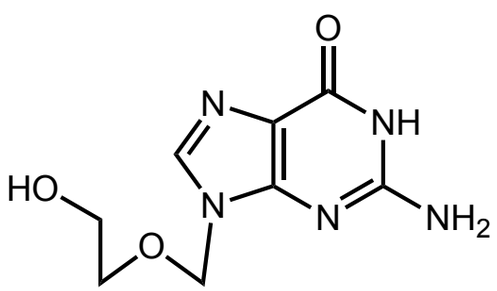
Other modifications



deoxy guanosine

(Nucleobase)

Backbone Removal



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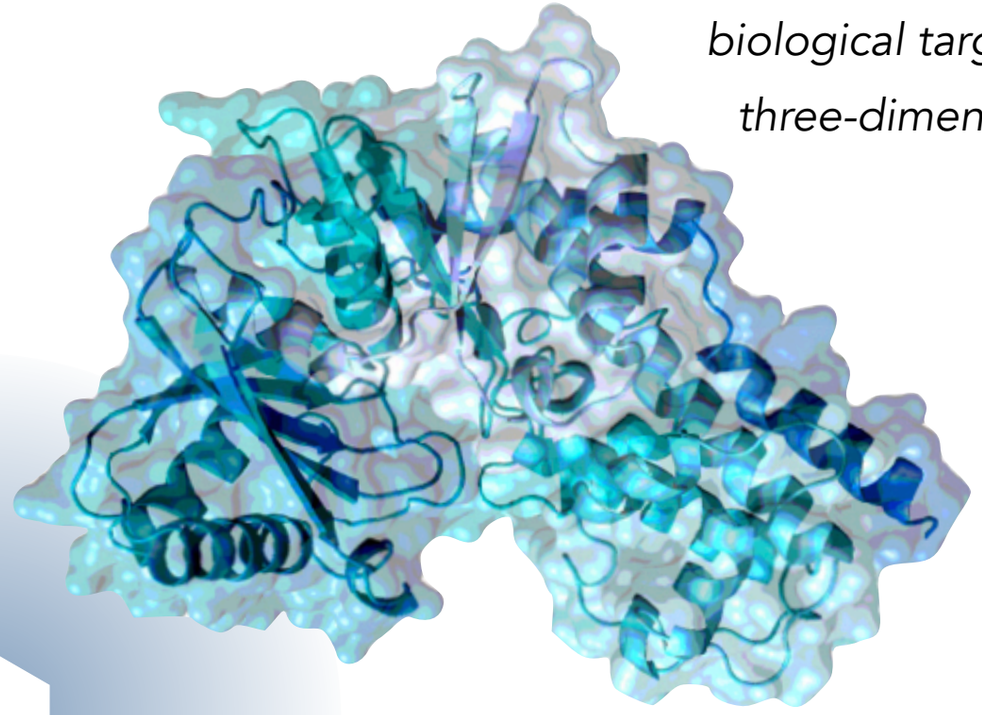
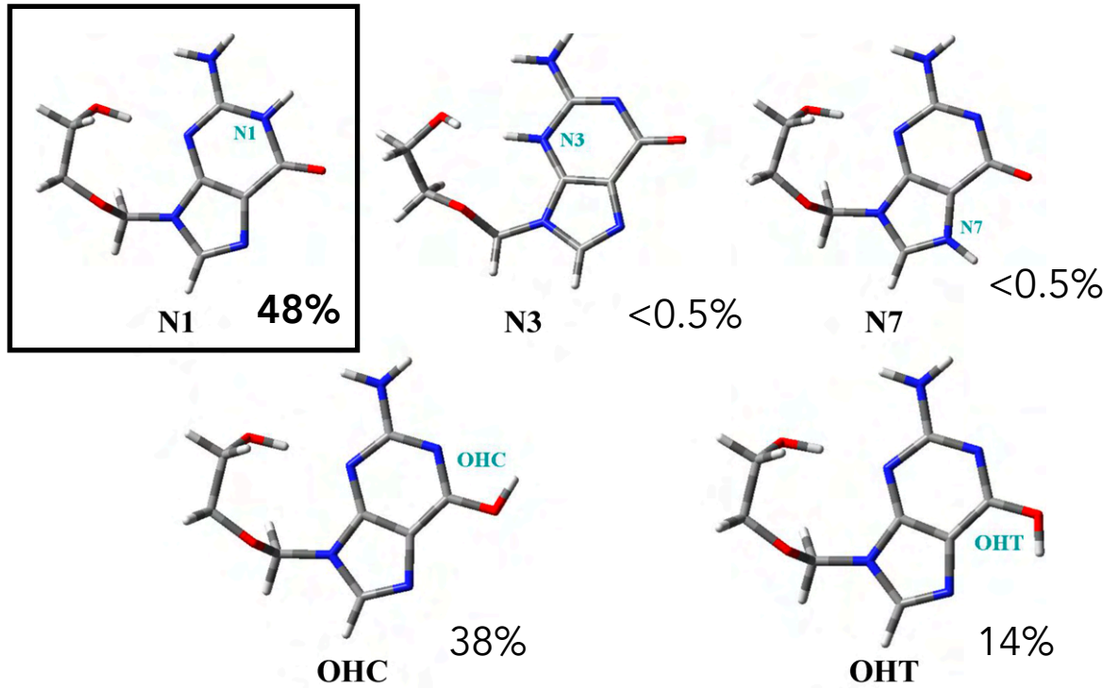


biological targets are three-dimensional



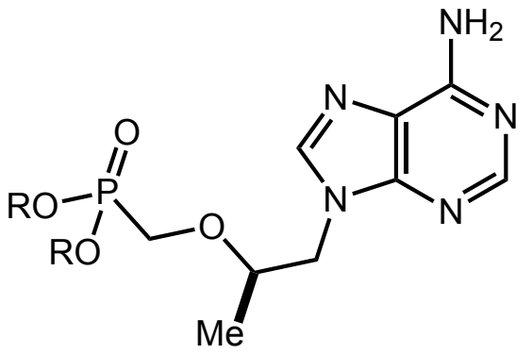
*Removal of 2′/3′ carbon can enable **conformational flexibility** leading to favorable interactions*

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.

Alvarez-Ros, M. C.; Palafox, M. A.; *Pharmaceuticals* **2014**, *7*, 695-722.

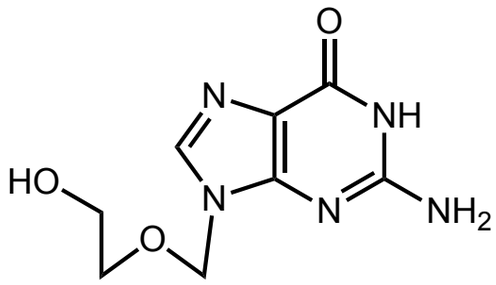
Structure Activity Relationships (SAR)

Other modifications



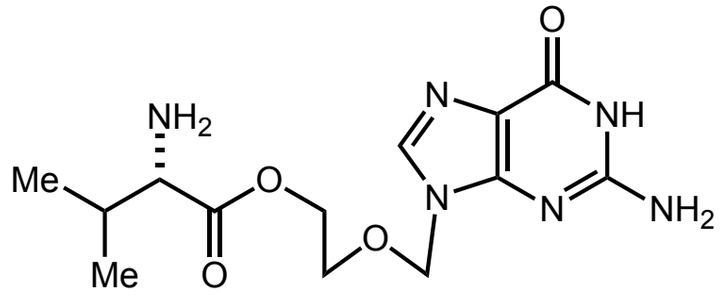
Tenofovir

(HIV replication inhibitor)



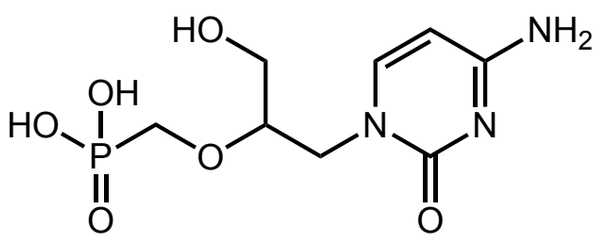
Acyclovir

(HSV-1/2 replication inhibitor)



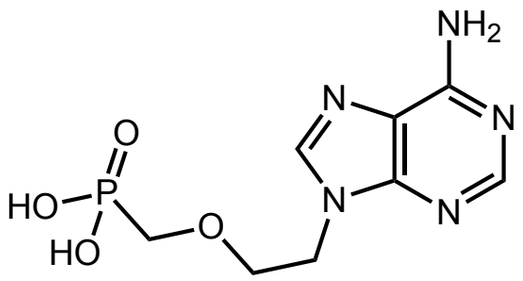
Valacyclovir

(HSV replication inhibitor)



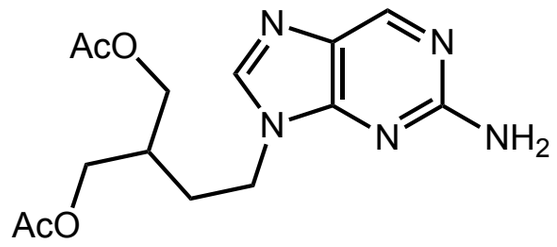
Cidofovir

(CMV replication inhibitor)



Adefovir

(Hep B replication inhibitor)



Famciclovir

(HSV replication inhibitor)

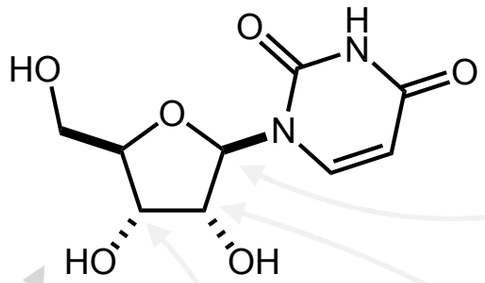
This strategy has been successfully applied to enable many new antiviral therapies, ***some still in use today***

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.

Alvarez-Ros, M. C.; Palafox, M. A.; *Pharmaceuticals* **2014**, *7*, 695-722.

Structure Activity Relationships (SAR)

Sites of potential modification



1′ carbon
- Nitrile

Other strategies
- Backbone removal
- Ring size

3′ carbon
- Removal of OH
- Nitrile/Fluorine/Methyl

2′ carbon
- Epimerization
- Nitrile/Fluorine/Methyl

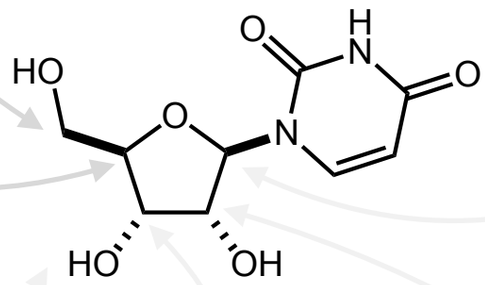
Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification

5′ carbon
- Phosphonate prodrugs
- McGuigan Protide
- Removal of OH

4′ carbon
- Alkyne



1′ carbon
- Nitrile

Other strategies
- Backbone removal
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3′ carbon
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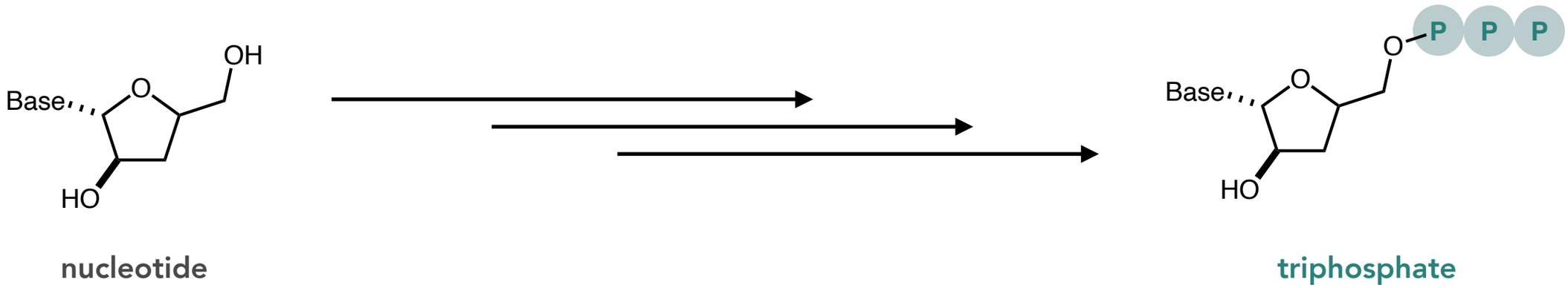
2′ carbon
- Epimerization
- Nitrile/Fluorine/Methyl

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

4′/5′ carbon modifications



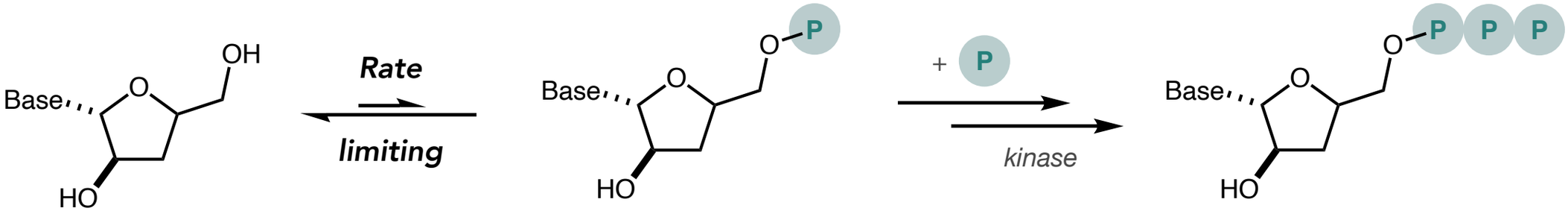
nucleotide **triphosphate**

*Nucleotide analogs must reach the **triphosphate form** to interact with the polymerase*

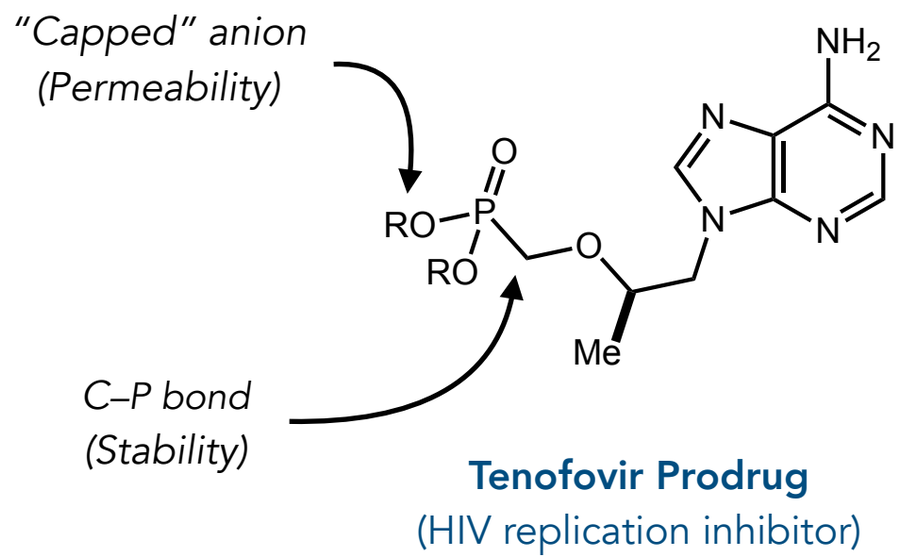
Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162,* 5-21.

Structure Activity Relationships (SAR)

4′/5′ carbon modifications

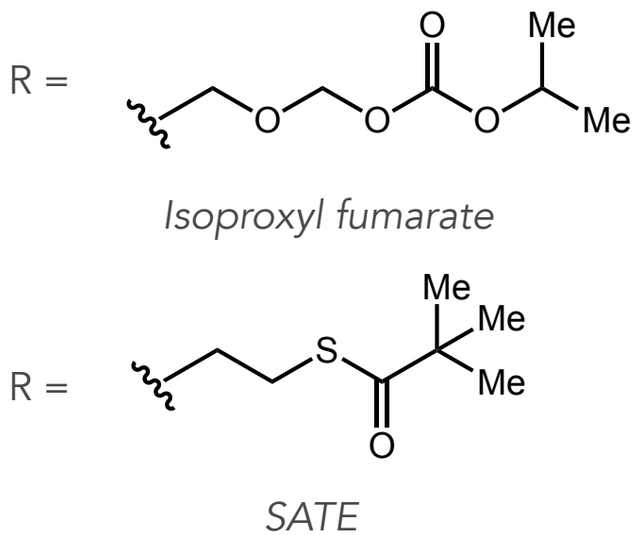


nucleotide **monophosphate** **triphosphate**



Tenofovir Prodrug
(HIV replication inhibitor)

Common prodrugs



*5′ modifications are often utilized to **improve the pharmacokentic profile** through prodrug approaches*

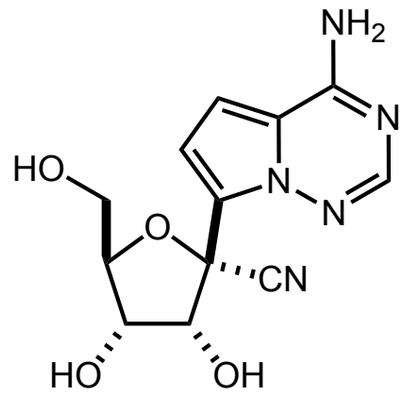
Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162,* 5-21.

Structure Activity Relationships (SAR)

4′/5′ carbon modifications

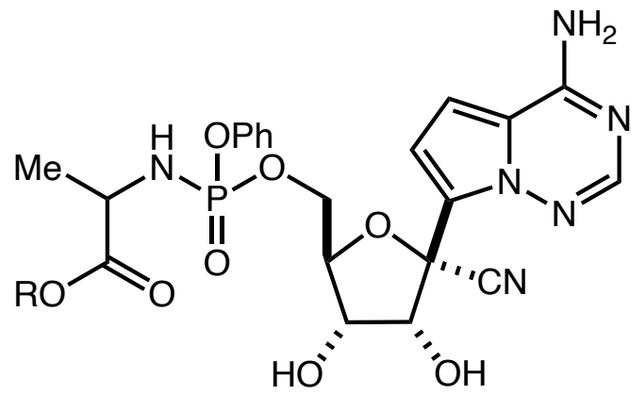
Prof. Chris McGuigan
(Cardiff University)

McGuigan Protide
(P(V) prodrug)

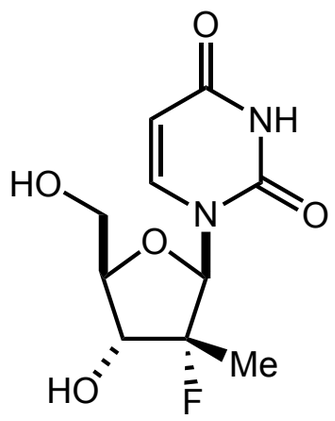


Remdesivir Parent
(EC_{50} = 780 nm vs EBOV)

+ P(V) prodrug

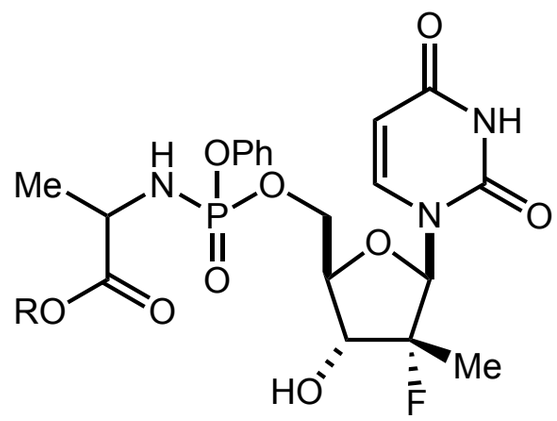


Remdesivir Protide
(EC_{50} = 60 nM vs EBOV)



Sofosbuvir Parent
(EC_{50} = 5.4 μm vs HCV)

+ P(V) prodrug



Sofosbuvir Parent
(EC_{50} = 0.42 μm vs HCV)

Protides *are an emerging* ***orthogonal P(V) prodrug*** *that also can improve pharmacokentic profiles*

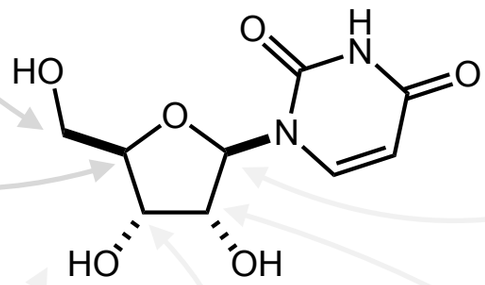
Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification

5′ carbon
- Phosphonate prodrugs
- McGuigan Protide
- Removal of OH

4′ carbon
- Alkyne



1′ carbon
- Nitrile

Other strategies
- Backbone removal
- Ring size

3′ carbon
- Removal of OH
- Nitrile/Fluorine/Methyl

2′ carbon
- Epimerization
- Nitrile/Fluorine/Methyl

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

Structure Activity Relationships (SAR)

Sites of potential modification

5′ carbon
- Phosphonate prodrugs
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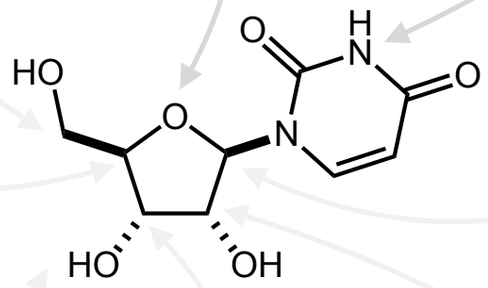
- Removal of OH

Heteroatom
- Conversion to S or N
- Removal/alkene

Base
- OCN to OCC
- N transmutation
- Prodrug installation



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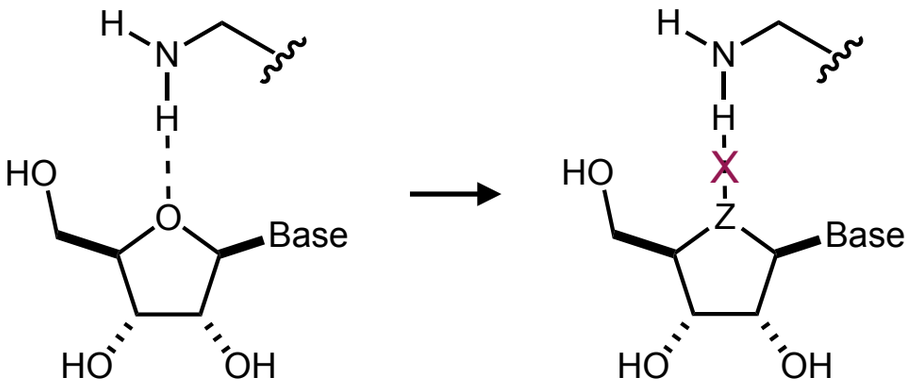
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

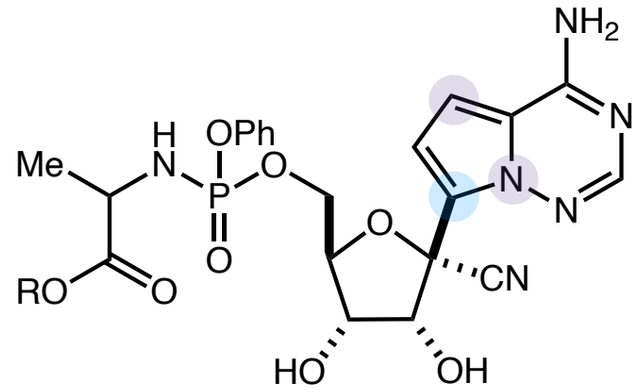
Structure Activity Relationships (SAR)

Heteroatom and nucleobase modifications



Key interaction *Z = S, Se*

*Altering the **heteroatom disrupts H-bonding***

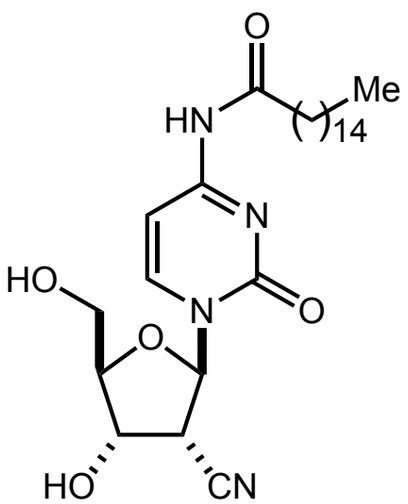


Remdesivir
(Gilead)

*C–C nucleotide analogs prevent **glycolysis***

***Translocating N atoms** can alter sites of H-bonding*

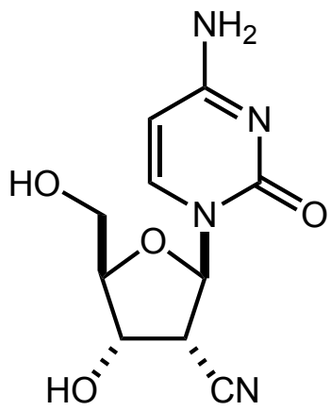
Sapacitabine
(Phase III clinical trials)



Lypophillic chain

Enzymatic cleavage

In vivo



+ cLogP

+ Bioavilibity

+ Lower dose

***Prodrugs** can also be utilized on the base backbone*

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

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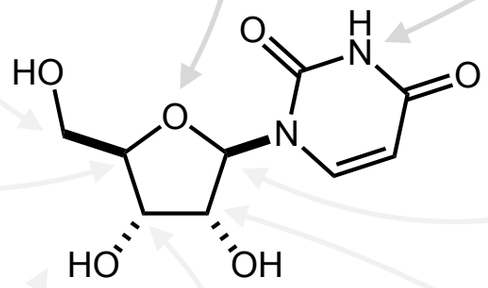

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Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

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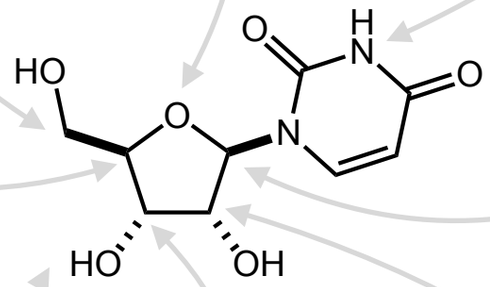

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Other strategies
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3′ carbon
- Removal of OH
- Nitrile/Fluorine/Methyl

2′ carbon
- Epimerization
- Nitrile/Fluorine/Methyl

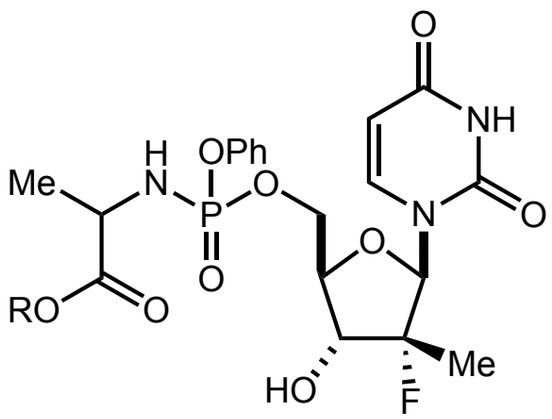
*There are **many possible sites for modification** modern nucleoside therapies often **utilize a combination of these***

Seley-Radtke, K. L.; Yates, M. K.; *Antiviral Research* **2018,** *154*, 68-86.
Yates, M. K.; Seley-Radtke, K. L.; *Antiviral Research* **2019**,*162*, 5-21.

IV Synthetic Strategies

Synthetic Strategies

General retrosynthesis

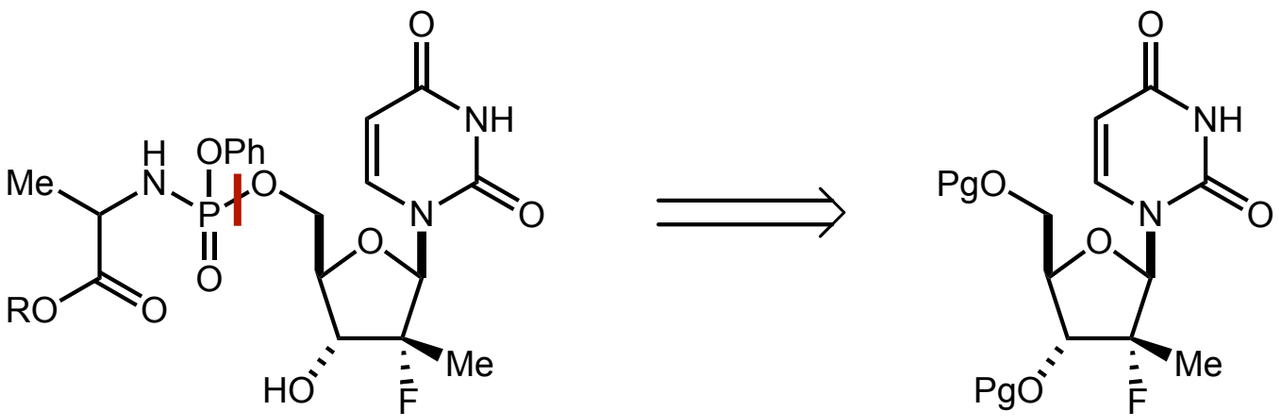


Sofosbuvir
(Gilead)

Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

General retrosynthesis



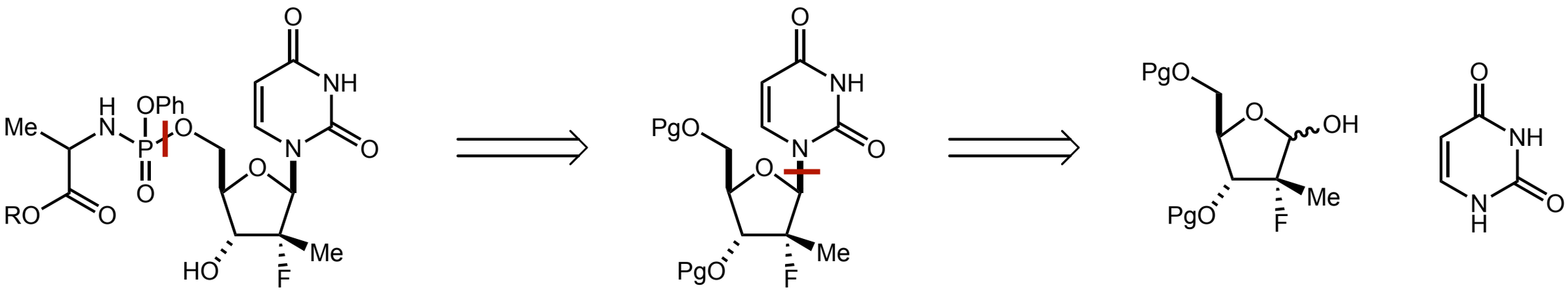
Sofosbuvir
(Gilead)

Remove *the phosphorus prodrug motif and* ***protect*** *free alcohols*

Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

General retrosynthesis



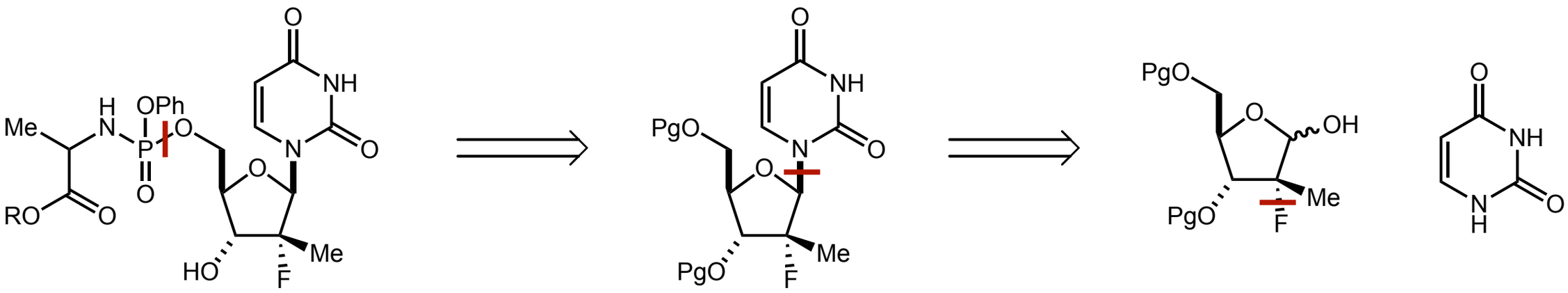
Sofosbuvir
(Gilead)

*Disconnect the **glycoside bond***

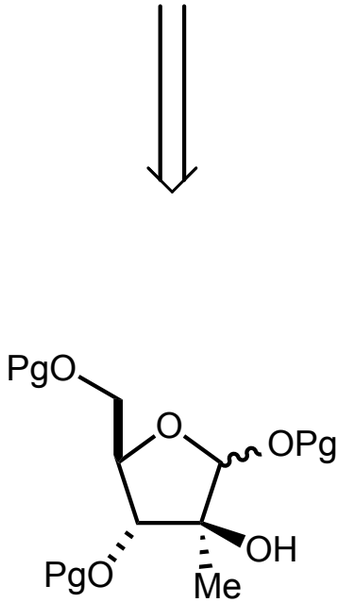
Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

General retrosynthesis



Sofosbuvir
(Gilead)

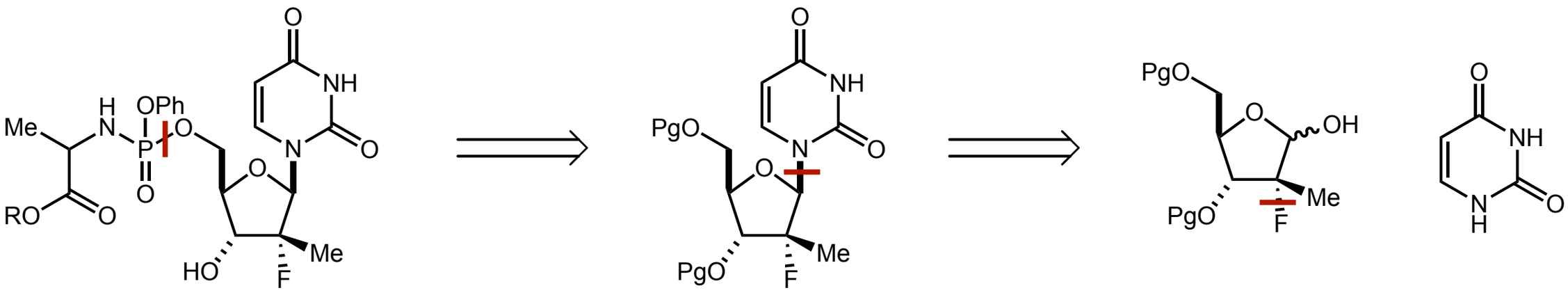


Install any functionality (halides/nitriles) using ***substitution chemistry***

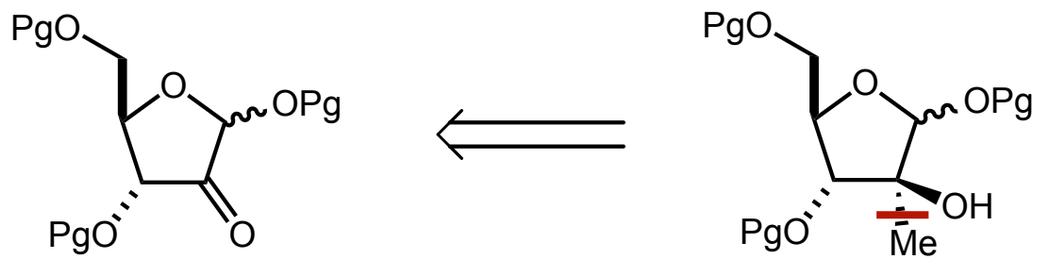
Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

General retrosynthesis



Sofosbuvir
(Gilead)

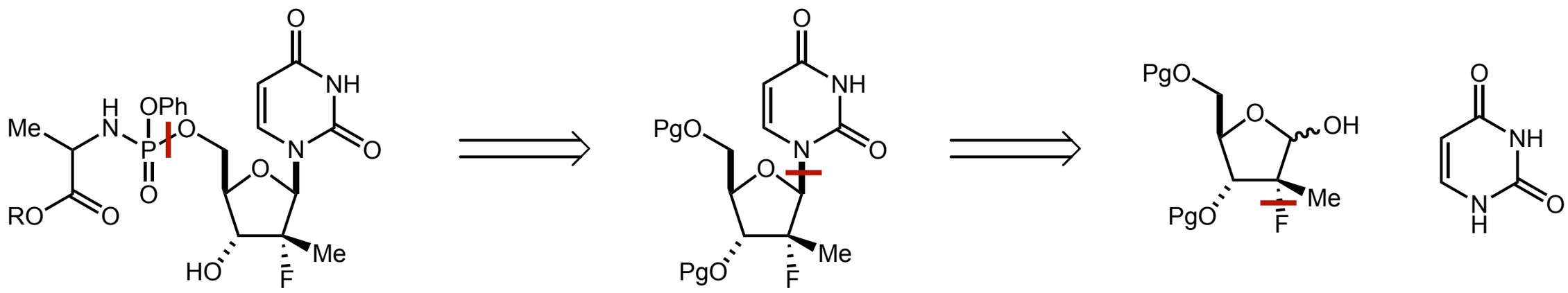


*Utilize **Grignard additions** to incorporate any carbon functionalities*

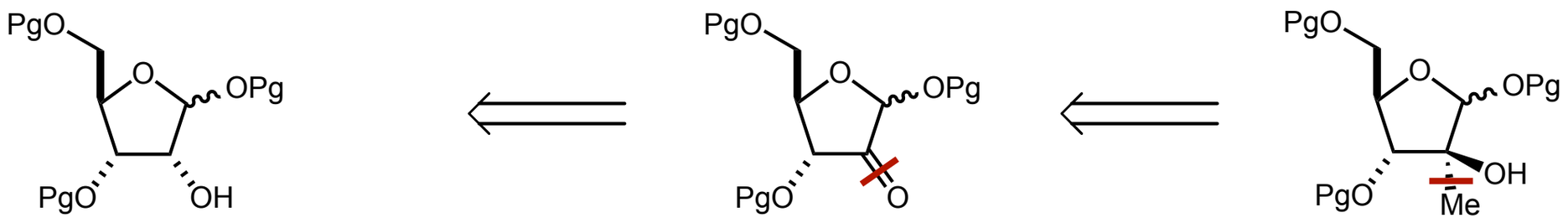
Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

General retrosynthesis



Sofosbuvir
(Gilead)

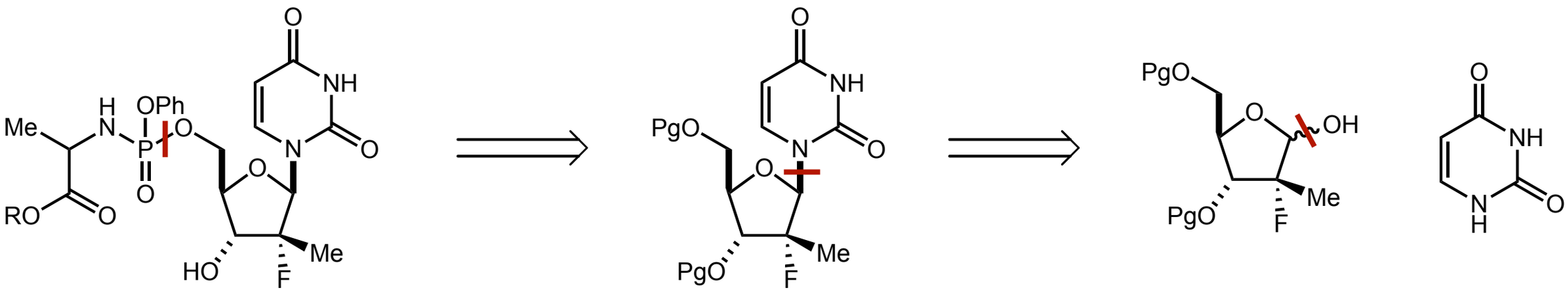


*Return to an **unfuctionalized, protected furanose***

Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

General retrosynthesis



Sofosbuvir
(Gilead)

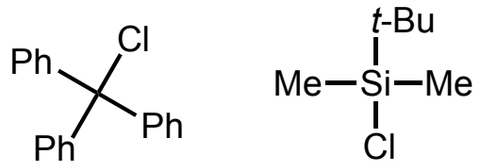
Feedstock chemicals

*Alternatively one can consider glycosylation following a **de novo sugar synthesis** but this is often less efficient*

Barth, R.; Rose, C. A.; Schone, O.; *Topics in Heterocyclic Chemistry*, **2015**, 55-88.

Synthetic Strategies

A quick note on protecting groups

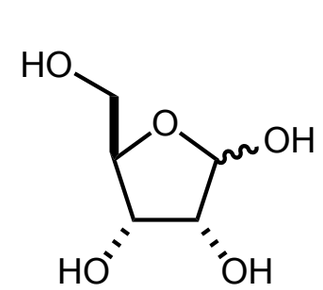


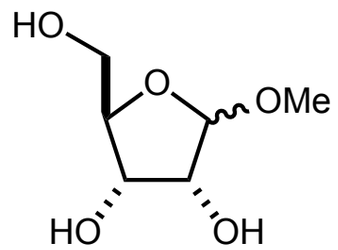
Trityl-Cl

TBS-Cl

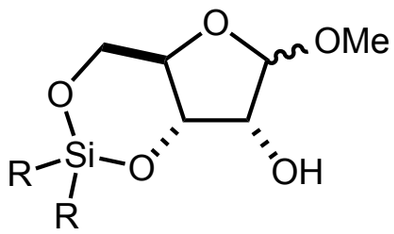
Easy to differentiate
(1° vs 2° OHs)

Some selective strategies
(Anomeric OH)





Acetal formation



DTBS group

Ribose

Harder to differentiate
(Both 2° OHs)

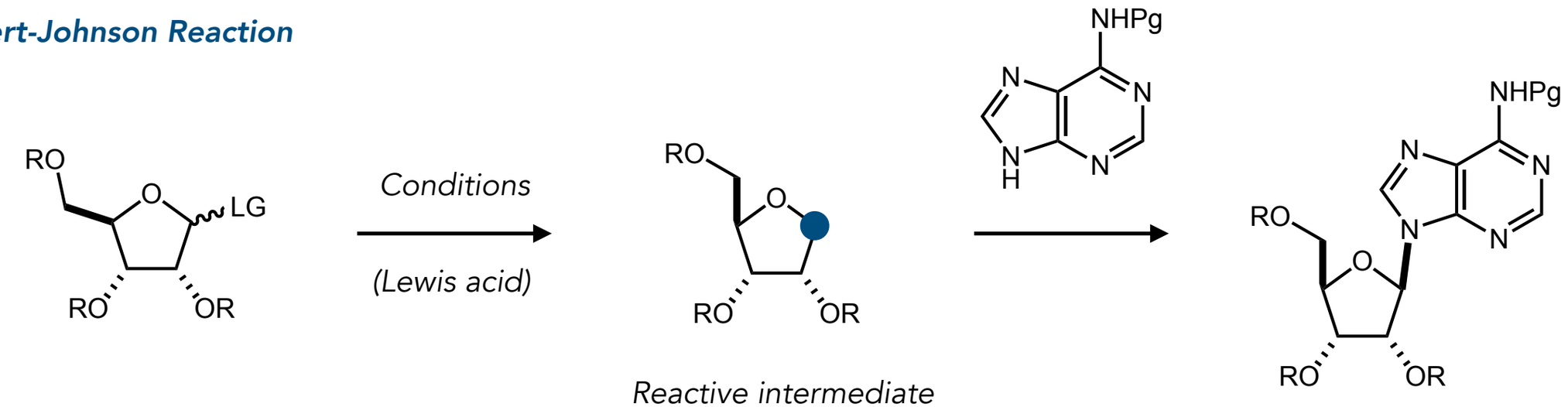
*There are **MANY** options for sugar protecting groups. Each have their own pros and cons.*

Rajapaksha, D. G.; *et. al.; Med. Chem. Res.* **2023,** *32*, 1315-1333.

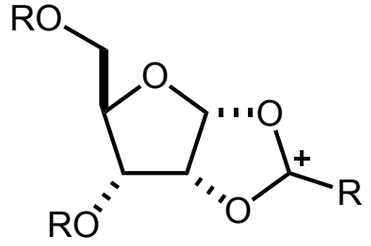
Synthetic Strategies

Glycosylation Methods

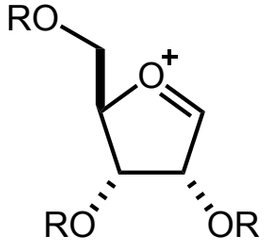
Silyl-Hilbert-Johnson Reaction



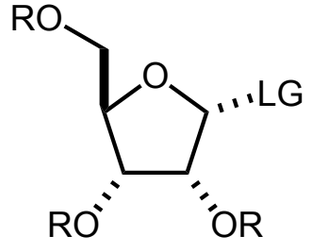
Reactive intermediate



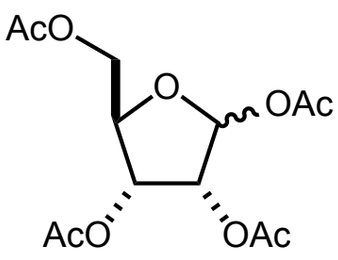
Type I intermediate
(S_N2, anchimeric)



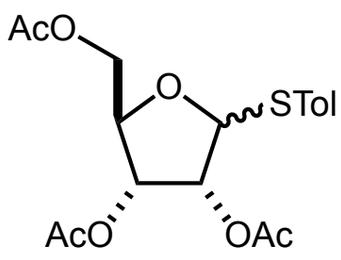
Type II intermediate
(S_N1, oxocarbenium)



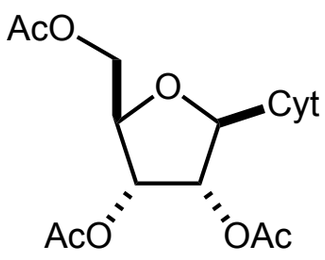
Type III intermediate
(S_N2, non anchimeric)



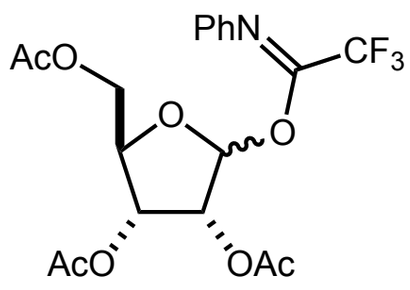
Acetates



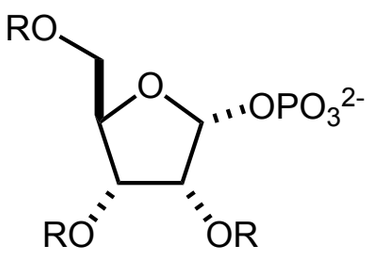
Thioglycosides



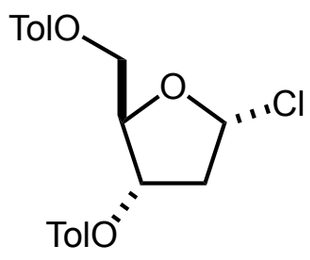
Cytosine



CF_3–acetimidates



Phosphates

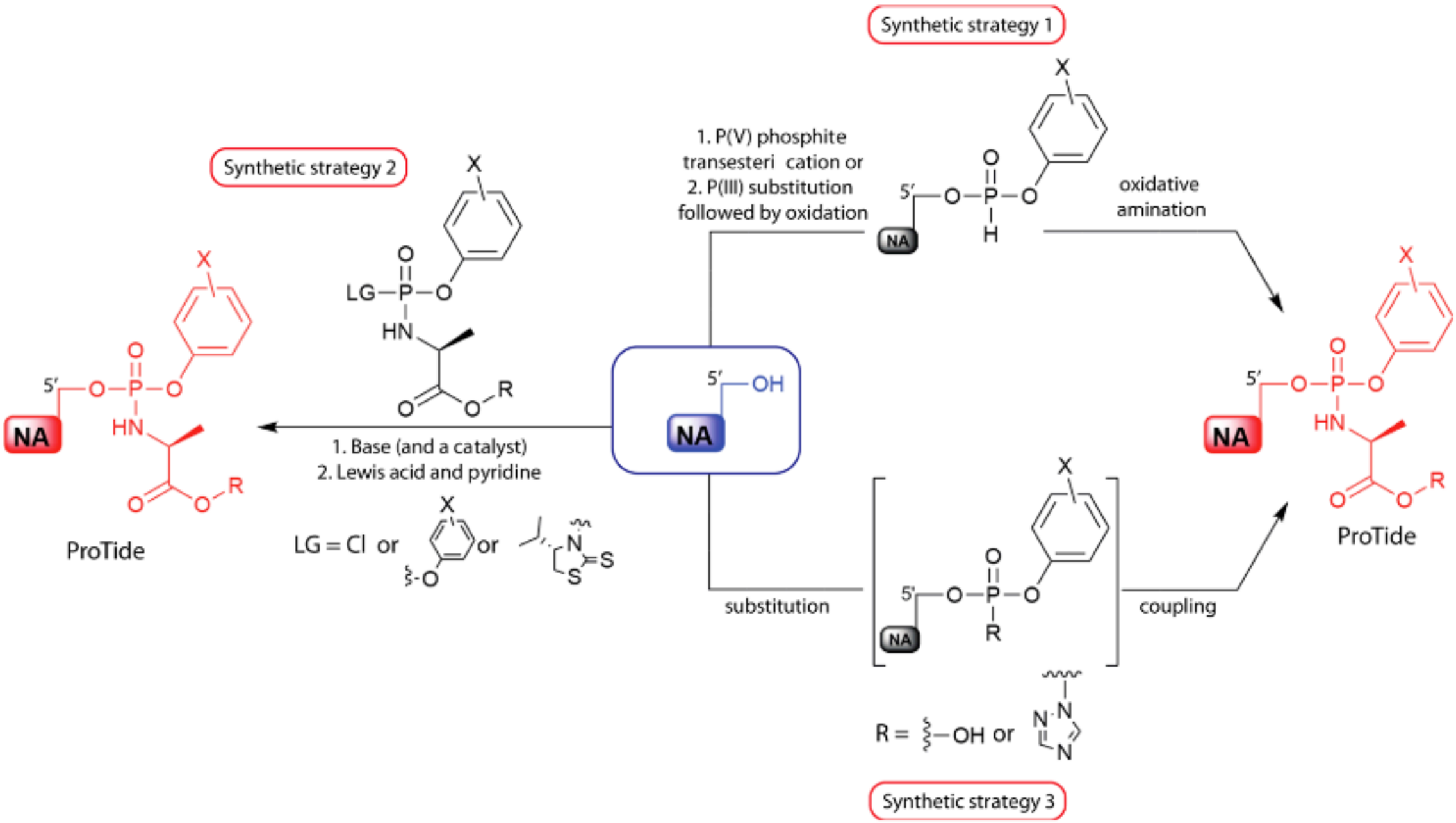


Halogens

Kaspar, F.; *et. al.*; *Green Chem.* **2021,** *23*, 37-50.
Rajapaksha, D. G.; *et. al.; Med. Chem. Res.* **2023,** *32*, 1315-1333.

Synthetic Strategies

Protide Synthesis



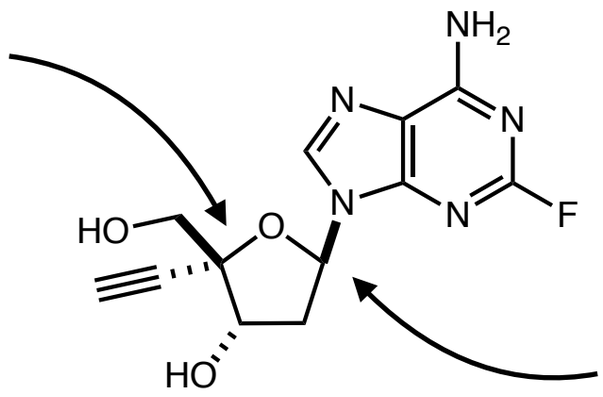
There are 3 main strategies to install protides. ***Strategy 2*** *is used most frequently due to high* ***diasteroselectivity***

Ross, B. S.; *et. al.*; *J. Org. Chem.* **2011**, *76*, 8311-8319.
Rajapaksha, D. G.; *et. al.; Med. Chem. Res.* **2023,** *32*, 1315-1333.

Synthetic Strategies

Process Case Study: Islatravir

challenging sterodefined 4' quaternary center



nontrivial diastereoselective glycosylation w/out 2' substituent

Islatravir
(Merck)

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

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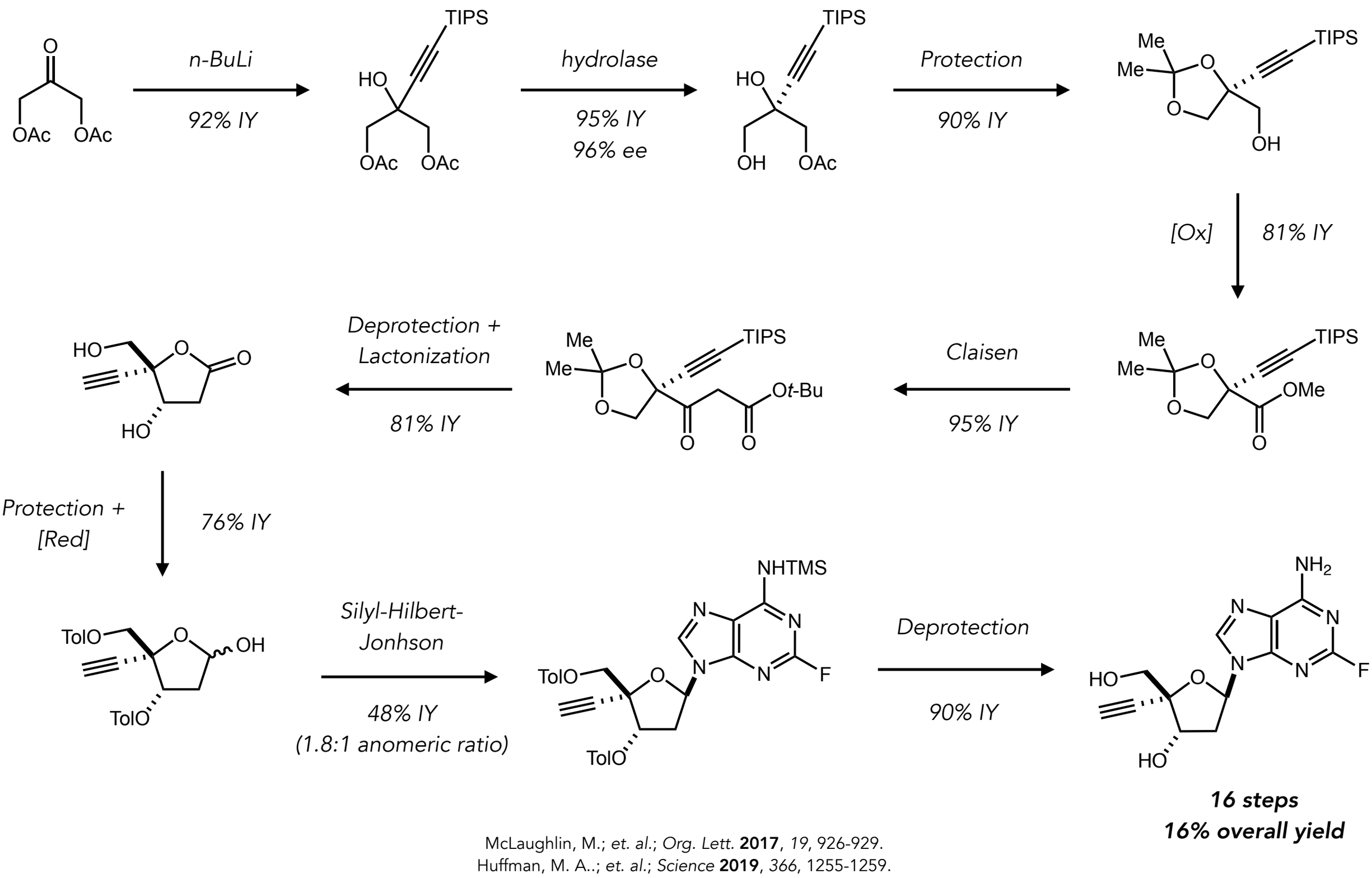

16 steps
16% overall yield

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

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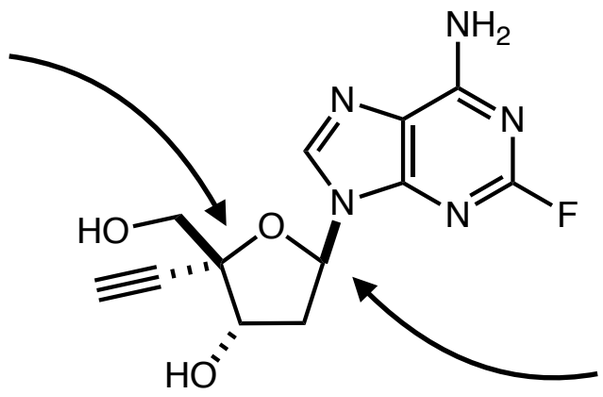

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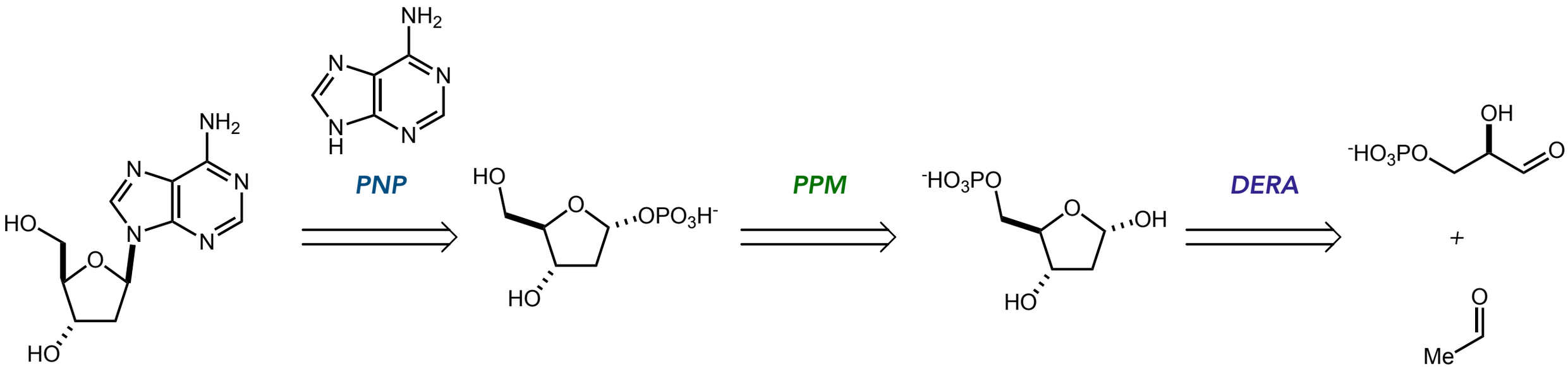
Synthetic Strategies

Process Case Study: Islatravir



Nucleoside salvage pathway

PNP *= Purine nucleoside phosphorylase*

PPM *= Phosphopenomutase*

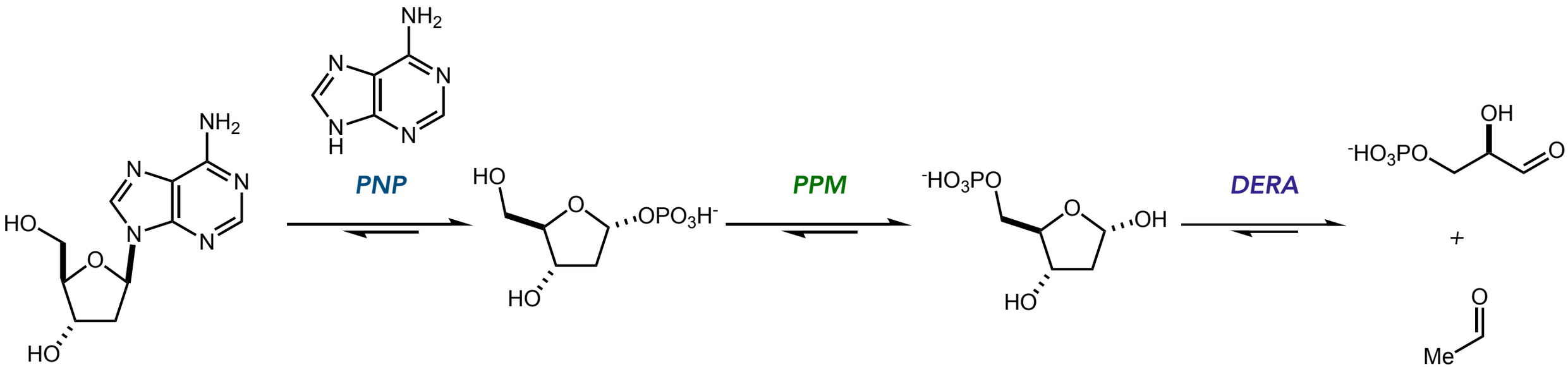
DERA *= Deoxyribose 5-phosphate Aldolase*

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.

Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

Process Case Study: Islatravir



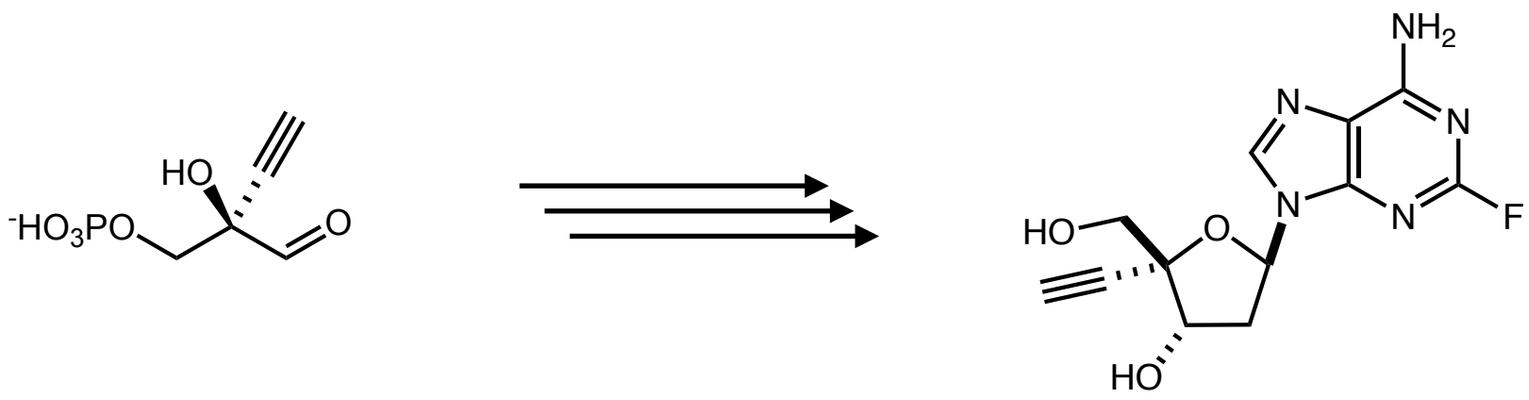
Nucleoside salvage pathway

PNP *= Purine nucleoside phosphorylase*

PPM *= Phosphopenomutase*

DERA *= Deoxyribose 5-phosphate Aldolase*

*Each step is **reversible and in equilibrium** (but heavily favors the **deconstructed nucleoside**)*



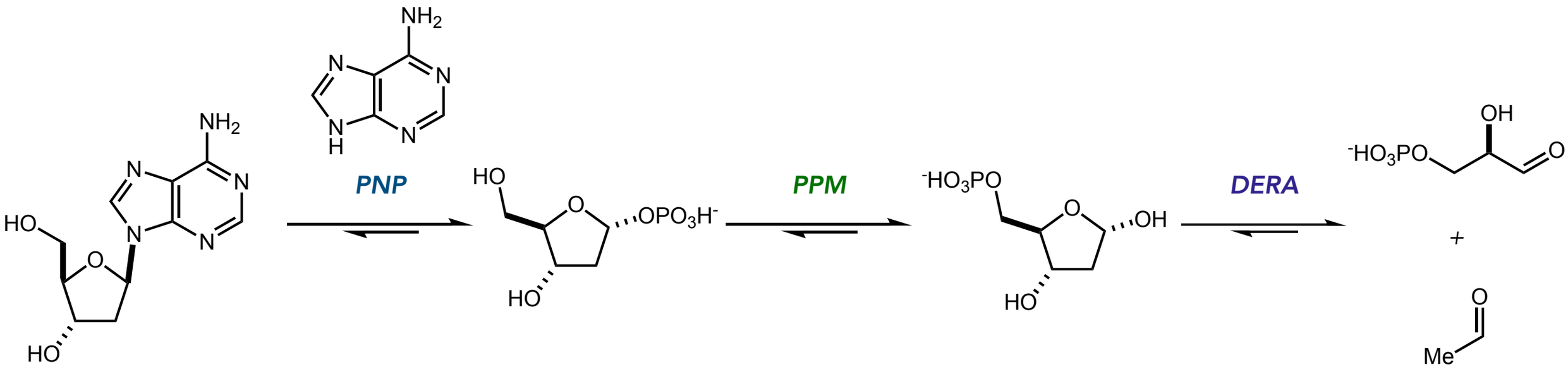
*Can these enzymes be **reversed** and utilized to make Islatravir using directed evolution?*

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.

Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

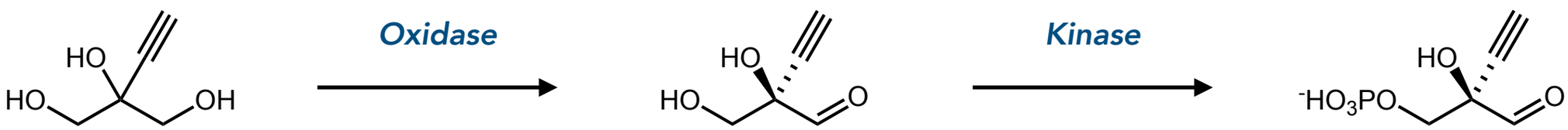
Process Case Study: Islatravir



Nucleoside salvage pathway

PNP = *Purine nucleoside phosphorylase*
PPM = *Phosphopenomutase*
DERA = *Deoxyribose 5-phosphate Aldolase*

*Each step is **reversible and in equilibrium** (but heavily favors the **deconstructed nucleoside**)*

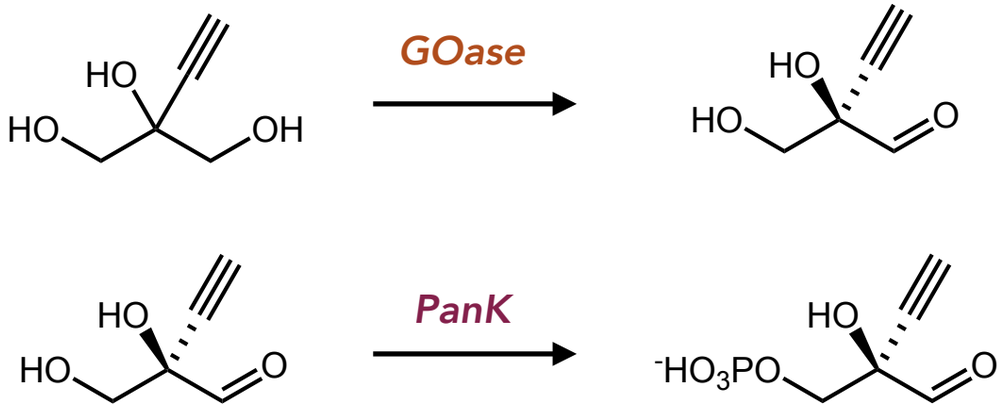


*Furthermore can the **starting material** be prepared through 2 enzymatic steps (dsymm [ox] + selective phos)*

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

Process Case Study: Islatravir



Starting enzyme

33% conversion
8:92 (R:S)
100% loading

<1% conversion
5:1 (R:S)
10% loading

97% conversion
98:1:1 dr
5% loading

0.5% conversion
N/A
0.5% loading

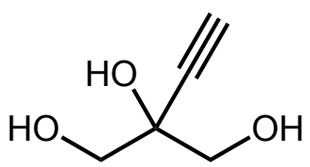
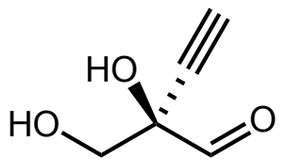
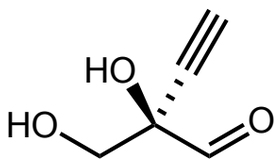
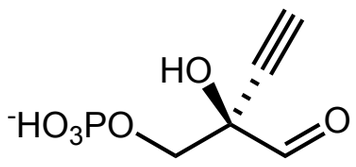
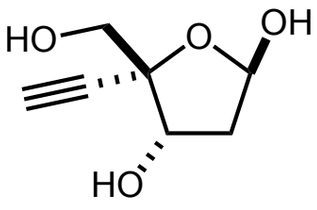
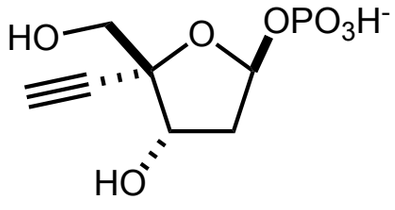
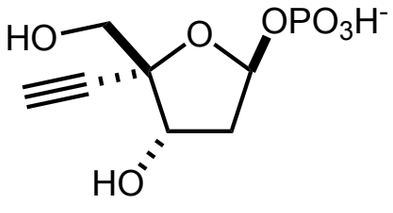
0.18% conversion
>99.5:0.5 dr
0.5% loading

Islatravir

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

Process Case Study: Islatravir

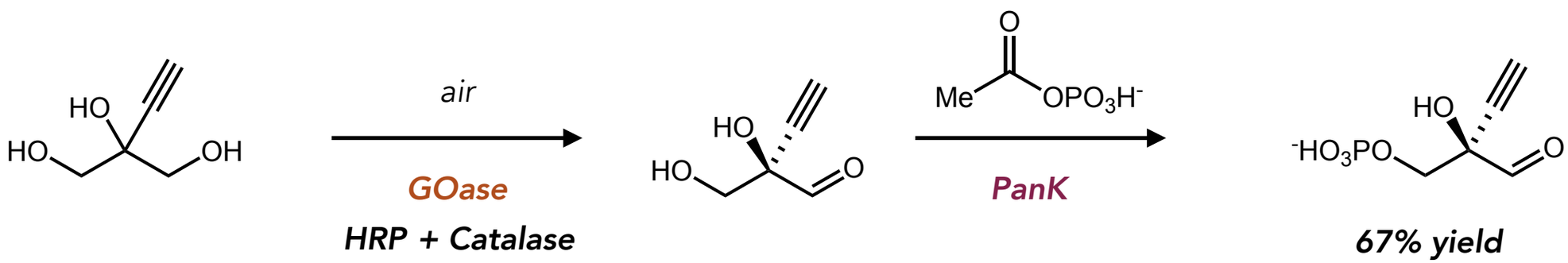
	***GOase***		***12***	***33% conversion*** *8:92 (R:S)* *100% loading*	***80% conversion*** *90:10 (R:S)* *20% loading*
	***PanK***		***3***	***<1% conversion*** *5:1 (R:S)* *10% loading*	***>95% conversion*** *5:1 (R:S)* *10% loading*
	PNP		***2***	***97% conversion*** *98:1:1 dr* *5% loading*	***97% conversion*** *98:1:1 dr* *0.2% loading*
	***PPM***		***2***	***0.5% conversion*** *N/A* *0.5% loading*	***34% conversion*** *N/A* *0.5% loading*
	***DERA***	***Islatravir***	***4***	***0.18% conversion*** *>99.5:0.5 dr* *0.5% loading*	***62% conversion*** *>99.5:0.5 dr* *0.5% loading*

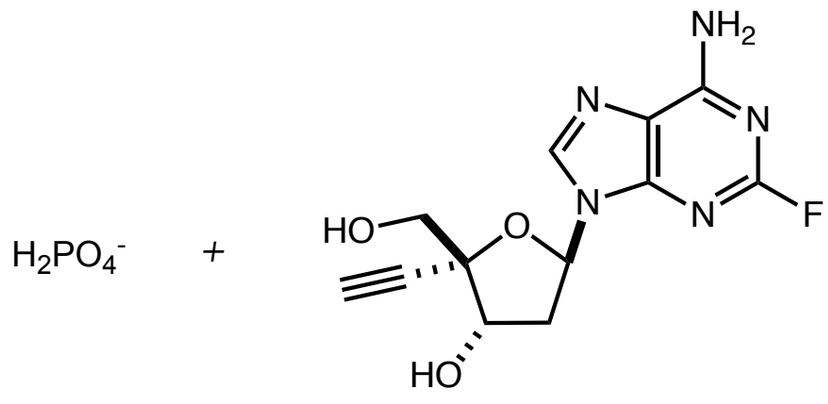
Directed evolution *allowed for the improvement of these initial hits through various mutations*

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

Process Case Study: Islatravir



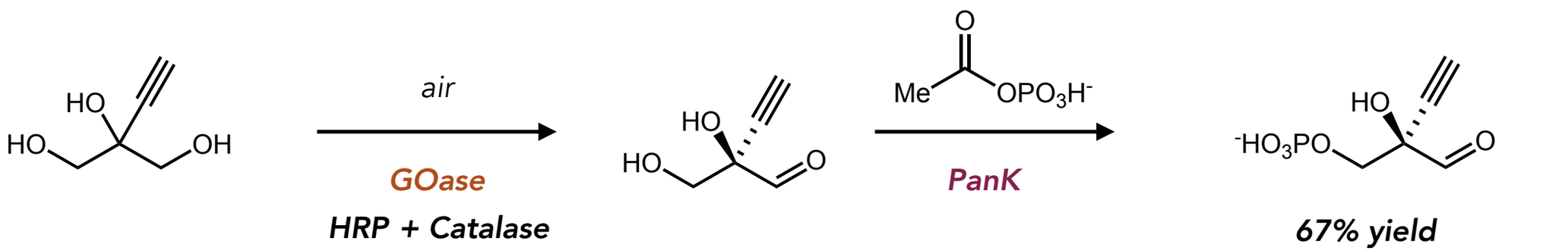


Hooking up the full process, the first two steps worked well but the ***final 3 stall in equilibrium***

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

Process Case Study: Islatravir



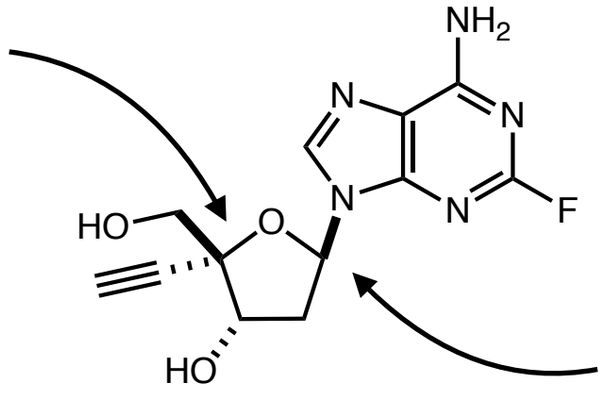
9 enzymes *in one cascade, reliant on Le Chatelier's Principle to drive equilibrium to Islatravir*

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

Synthetic Strategies

Process Case Study: Islatravir

challenging sterodefined 4′ quaternary center



nontrivial diastereoselective glycosylation w/out 2′ substituent

Islatravir
(Merck)

Best Route

16 steps
16% overall yield

⟹

Final Merck Process Route

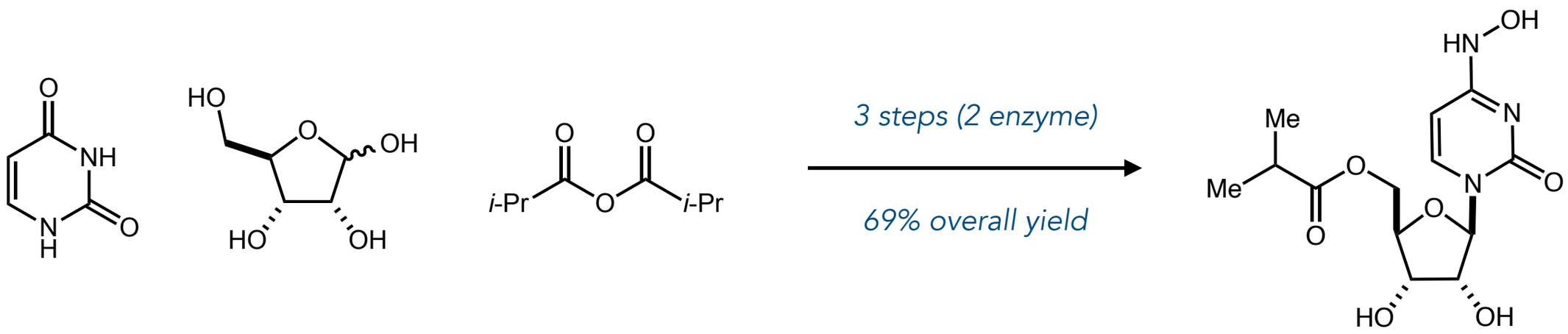
4 steps
51% overall yield

*Perhaps the most impressive application of **enzymatic cascades thus far** to process chemistry*

McLaughlin, M.; *et. al.*; *Org. Lett.* **2017**, *19*, 926-929.
Huffman, M. A..; *et. al.*; *Science* **2019**, *366*, 1255-1259.

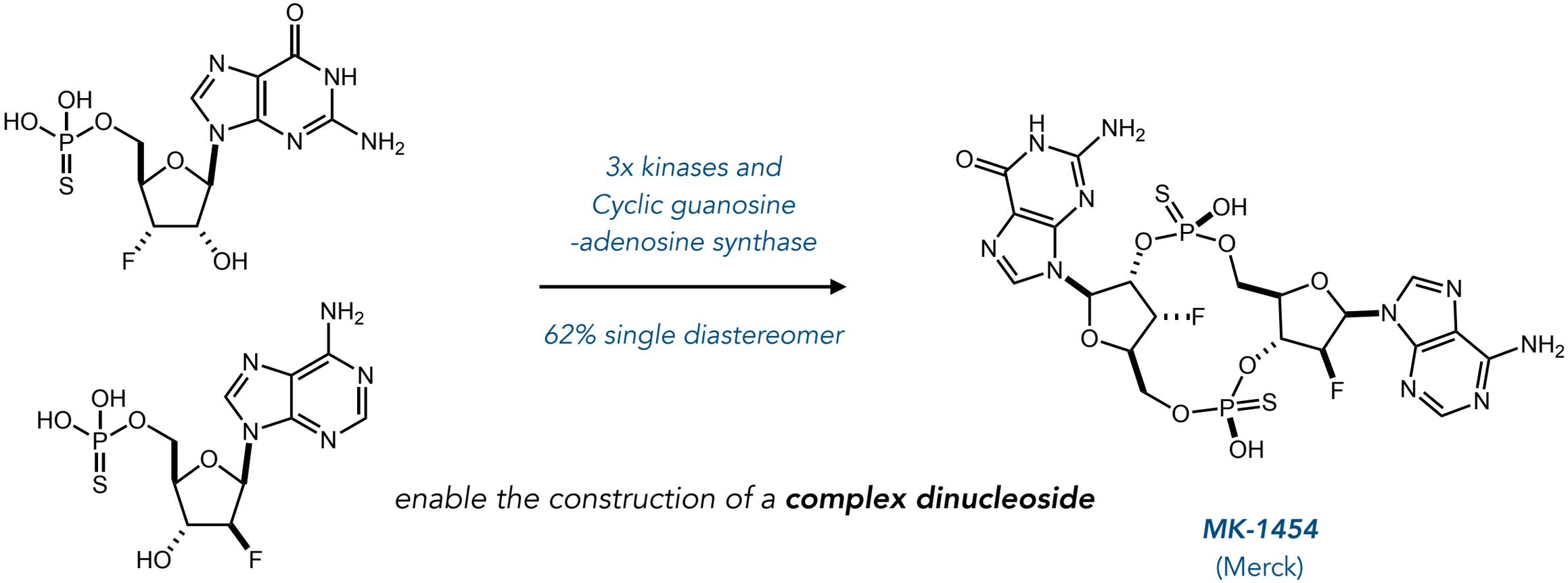
Synthetic Strategies

Other process scale enzymatic cascades



entire process development done in ***~6 months***

Molnupiravir
(Merck)



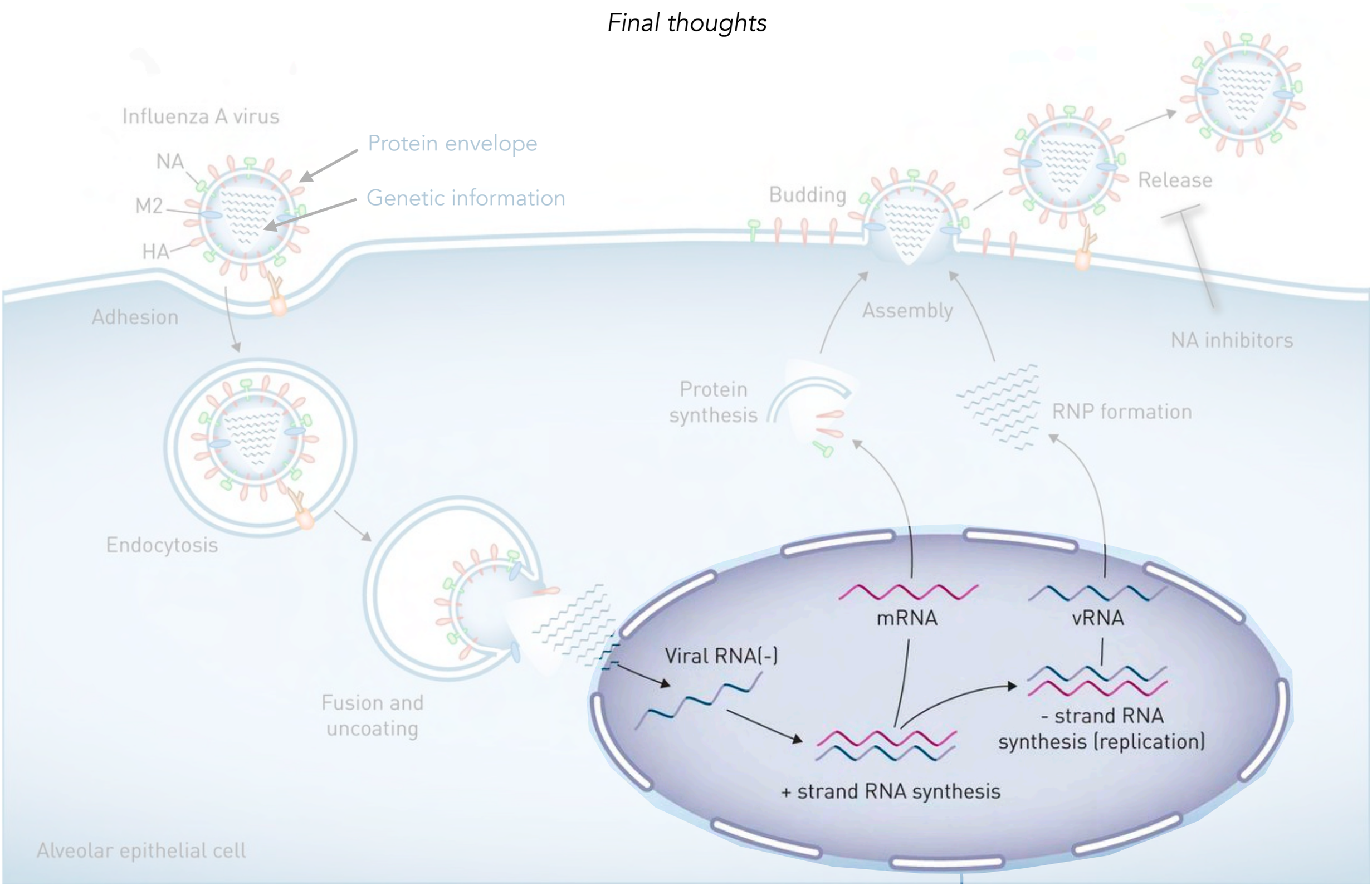
enable the construction of a ***complex dinucleoside***

MK-1454
(Merck)

McIntosh, J. A.; *et. al.*; *ACS Cent. Sci.* **2021**, *7*, 1980-1985.
McIntosh, J. A..; *et. al.*; *Nature* **2022**, *603*, 439-444.

Conclusion and Outlook

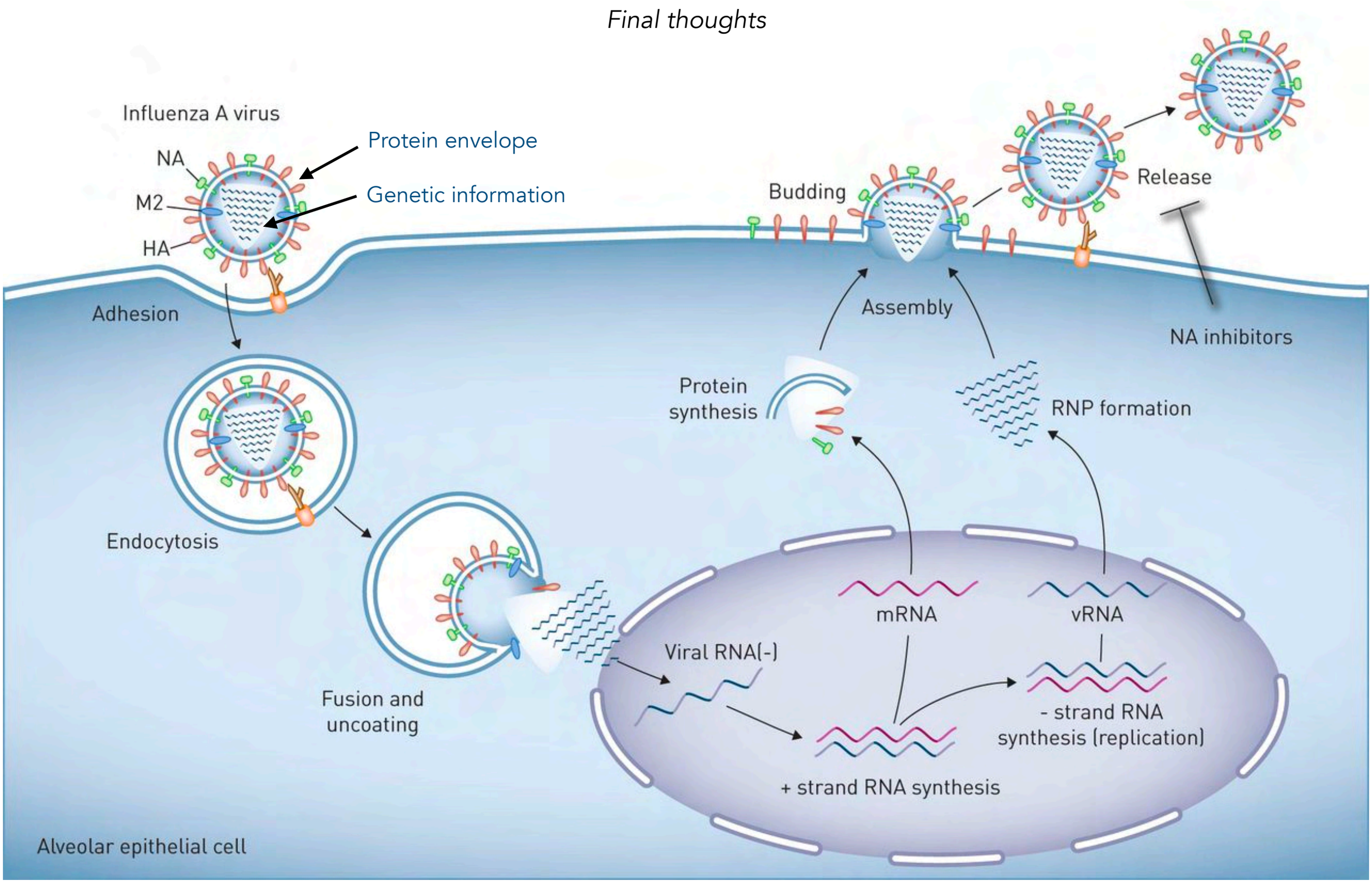
Final thoughts



Nucleoside analogs can ***interfere with viral replication processes…***

Conclusion and Outlook

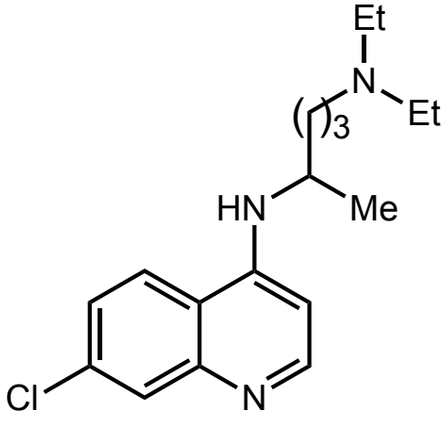
Final thoughts



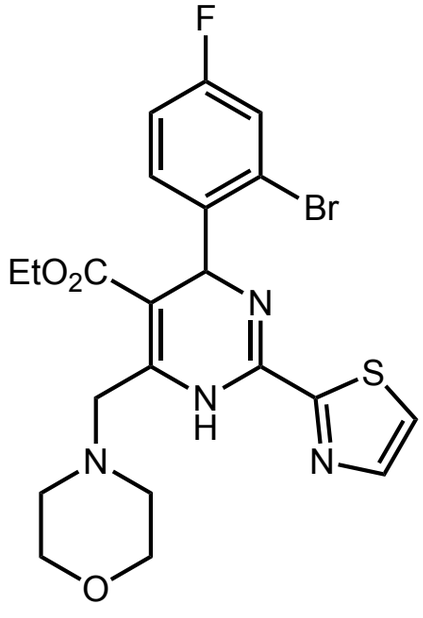
… *but they are just* ***one tool we have in our arsenal*** *for fighting viruses*

Conclusion and Outlook

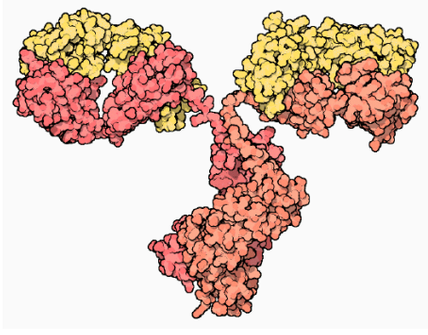
Final thoughts



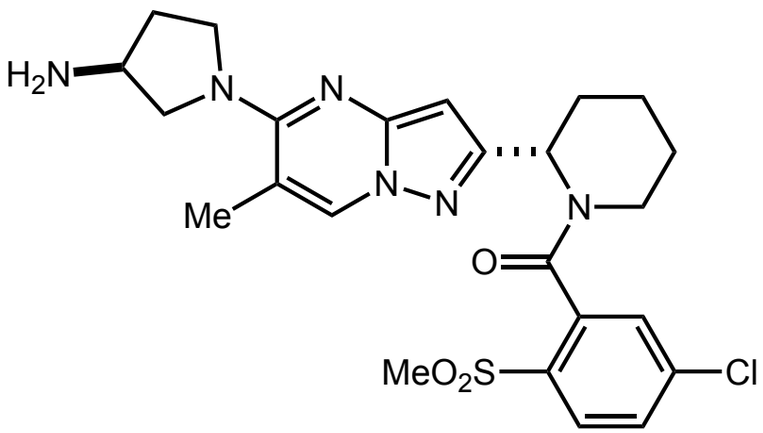
Chloroquine
(Cell entry inhibitor)



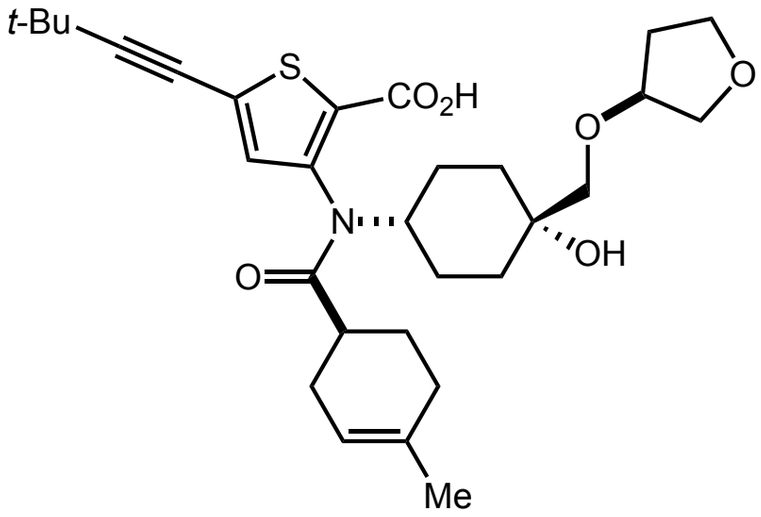
Morphothiadin
(Capsid assembly inhibitor)



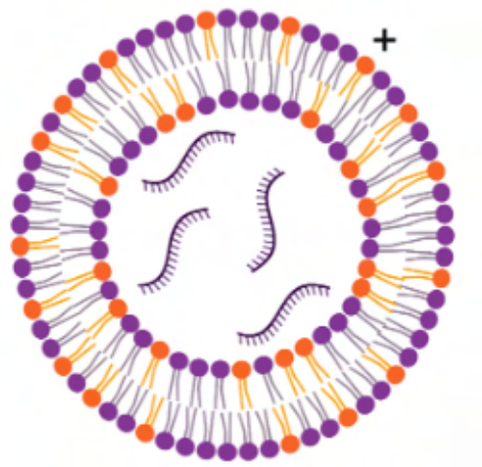
Tixagevimab
(Monoclonal antibody)



Presatovir
(Cell entry inhibitor)



Radalbuvir
(Non nucleoside polymerase inhibitor)

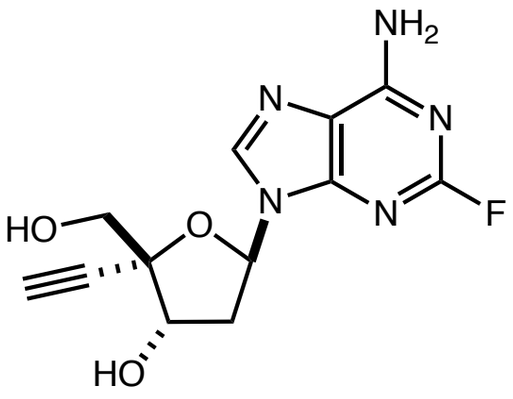


COVID-19 mRNA vaccine
(Vaccine)

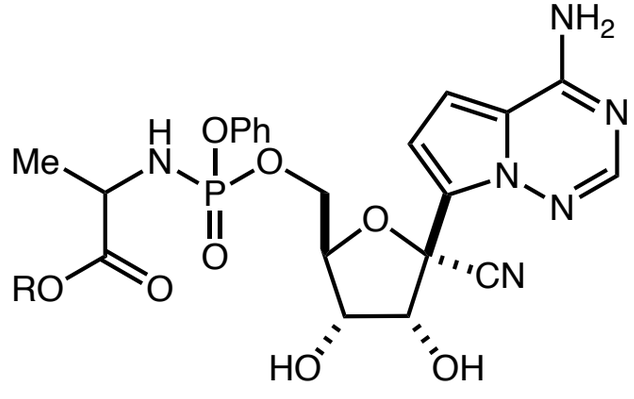
... but they are just ***one tool we have in our arsenal*** *for fighting viruses*

Conclusion and Outlook

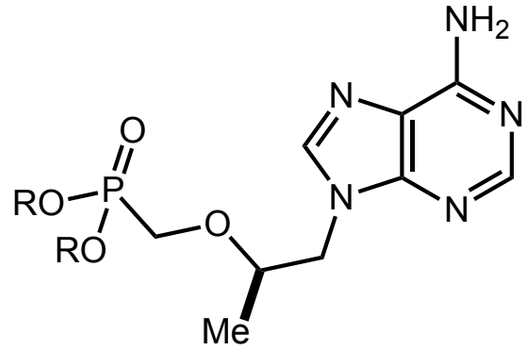
Final thoughts



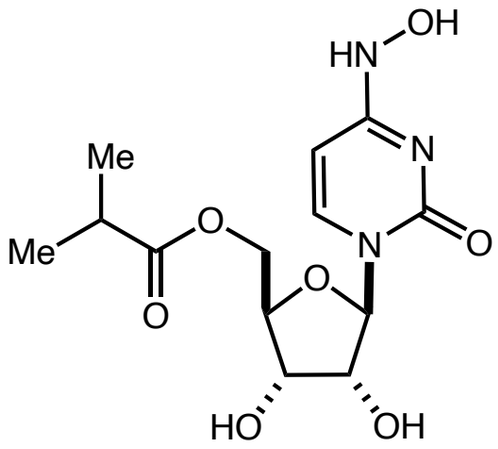
Islatravir
(Merck)



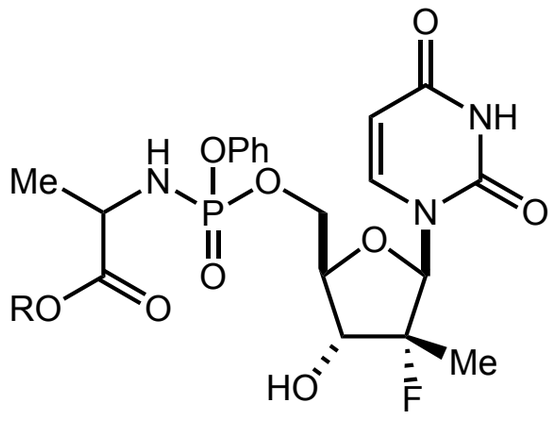
Remdesivir
(Gilead)



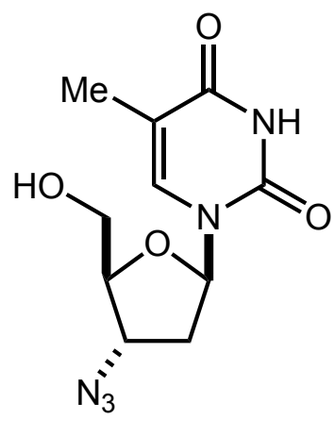
Tenofovir
(Gilead)



Molnupiravir
(Merck)



Sofosbuvir
(Gilead)

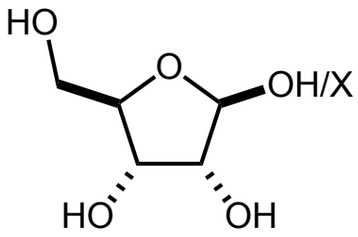


AZT
(Burroughs Wellcome)

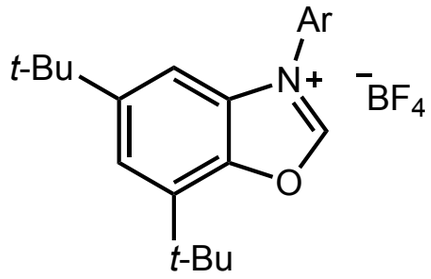
The synthesis of these complex targets ***is far from being a solved problem***

Conclusion and Outlook

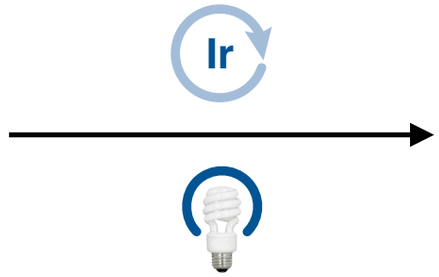
Final thoughts

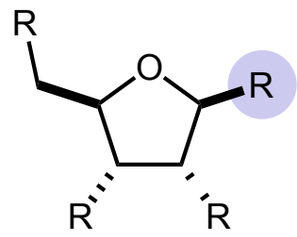


Sugar/Nucleoside

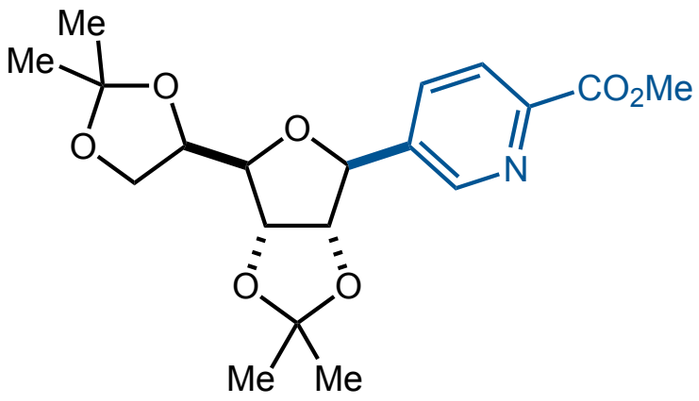


NHC

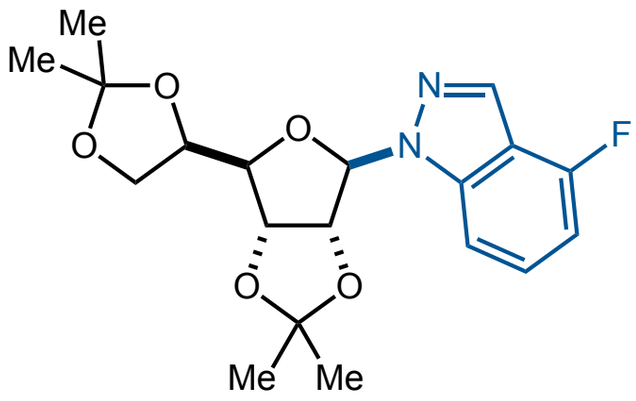




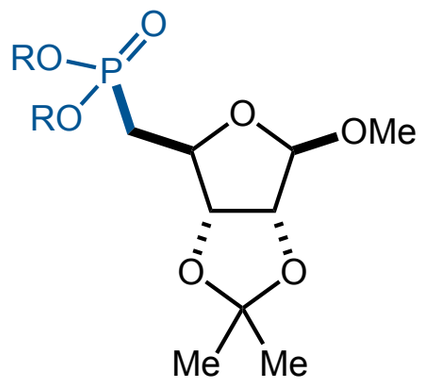
Functionalized Sugar/Nucleoside



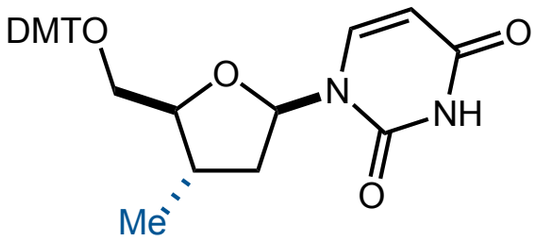
Arylation *92% yield*



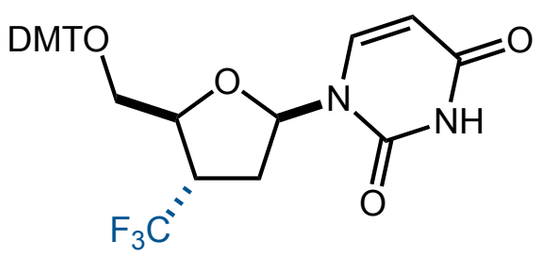
Amination *64% yield*



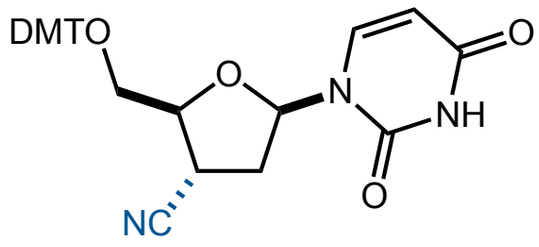
Phosphonylation *89% yield*



Methylation *44% yield*



Trifluoromethylation *38% yield*



Cyanation? *xx% yield*

Our own group's chemistry has the potential to ***drastically impact nucleoside analog discovery***

Conclusion and Outlook

Key Resources

Part II Mechanism of action

"Recent Advances in Molecular Mechanisms of Nucleoside Antivirals"
DOI: 10.3390/cimb45080433

Part III SAR

"The evolution of antiviral nucleoside analogues: A review for chemists and non chemists. Part I and II"
DOI: 10.1016/j.antiviral.2018.04.004 and 10.1016/j.antiviral.2018.11.016

Part IV Synthesis and Case Studies

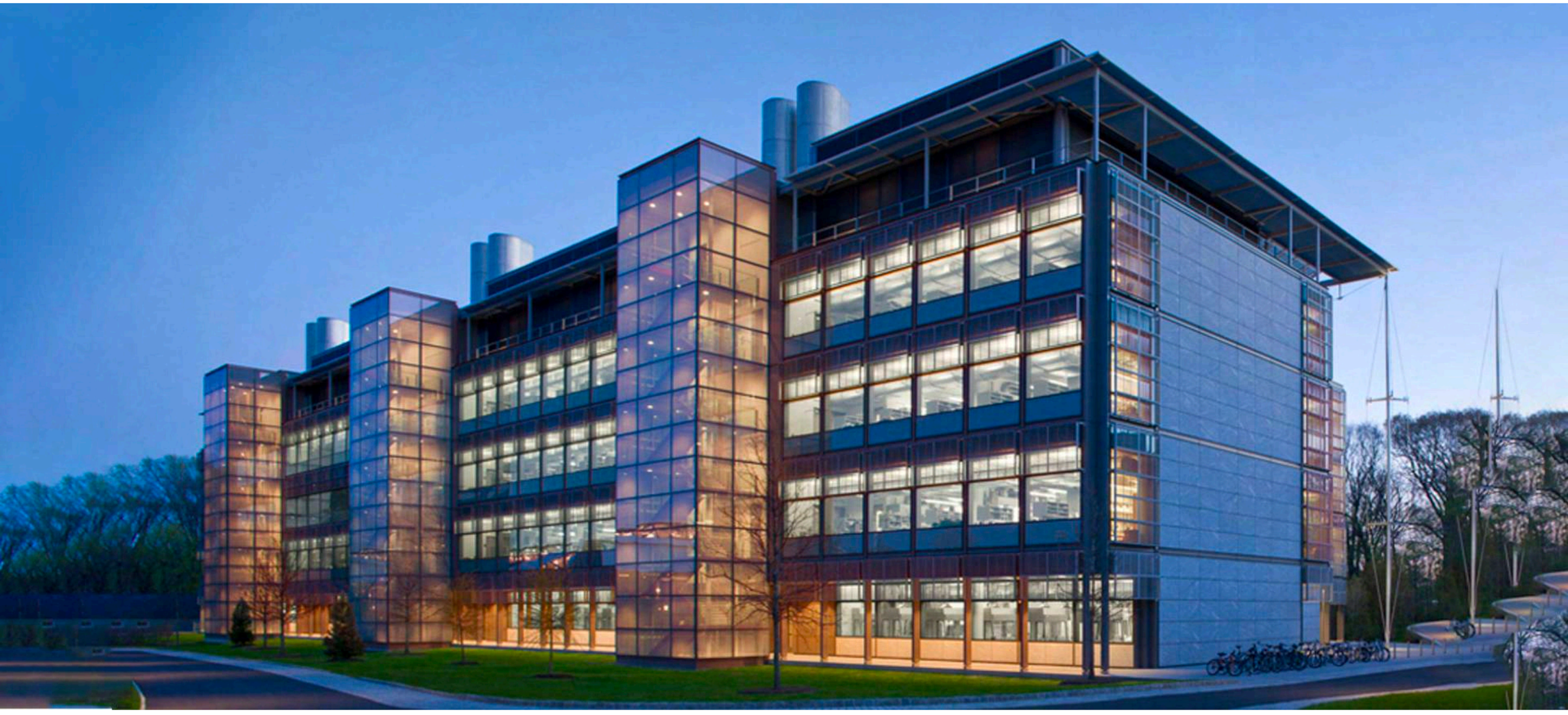
"A guide for the synthesis of key nucleoside scaffolds in drug discovery"
DOI: 10.1007/s00044-023-03096-w

"Design of an in vitro biocatalytic cascade for the manufacture of islatravir"
DOI: 10.1126/science.aay8484

Acknowledgments

David MacMillan *The MacMillan Group*

Thank you



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