Topic 8G: Antiviral agents

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❖ Structure of viruses

➢ Viruses can be viewed as protein packages transmitting foreign nucleic acid between host cells.

➢ Type of nucleic acid present depends on the virus types. All viruses contain one or more molecules of either RNA or DNA but not both.

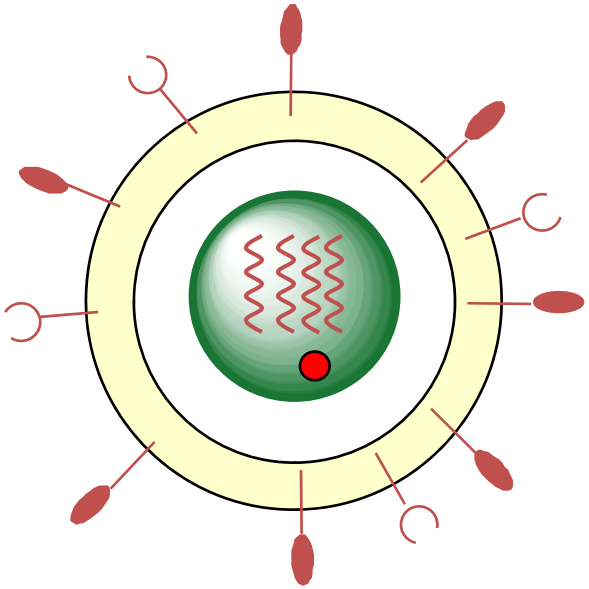
➢ So they can be defined as **RNA or DNA viruses**.

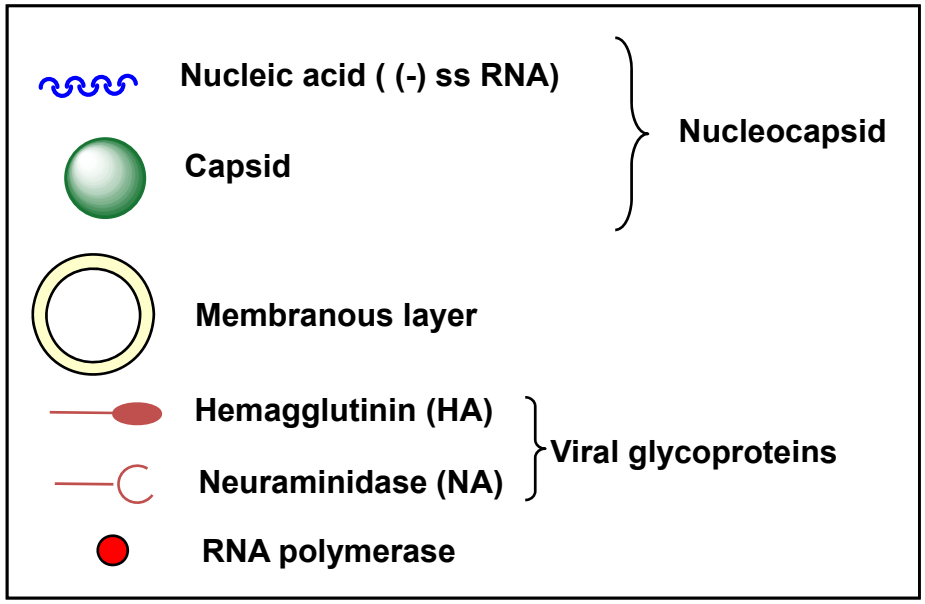
➢ Most RNA viruses contain single stranded RNA "ssRNA", but some viruses contain double-stranded RNA. If the base sequence of the RNA strand is identical to viral messenger RNA "mRNA" it is called the **positive** "+"; and thus can be immediately translated by the host cell.

➢ if it is complementary it is called the **negative** "-" strand. and thus must be converted to positive-sense RNA by an RNA polymerase before translation.

➢ the viral nucleic acid is contained and protected within a protein coat called the **capsid**. Capsids are made up of protein subunits called **protomers** which are generated in the host cell and can interact spontaneously to form the capsid in a process called **self-assembly**.

➢ capsids + nucleic acid known as **nucleocapsid**. The complete structure is known as a **virion**. In some viruses, the nucleocapsid may contain viral enzymes which are important to its replication in the host cells. E.g. flu virus contain an enzyme called **RNA-dependent RNA polymerase**.





❖ Life cycle of viruses

• **Adsorption**: a virion has to first bind to the outer surface of a host cell. By binding of specific receptor in the host cell that have an important cellular functions such as the binding of hormones.

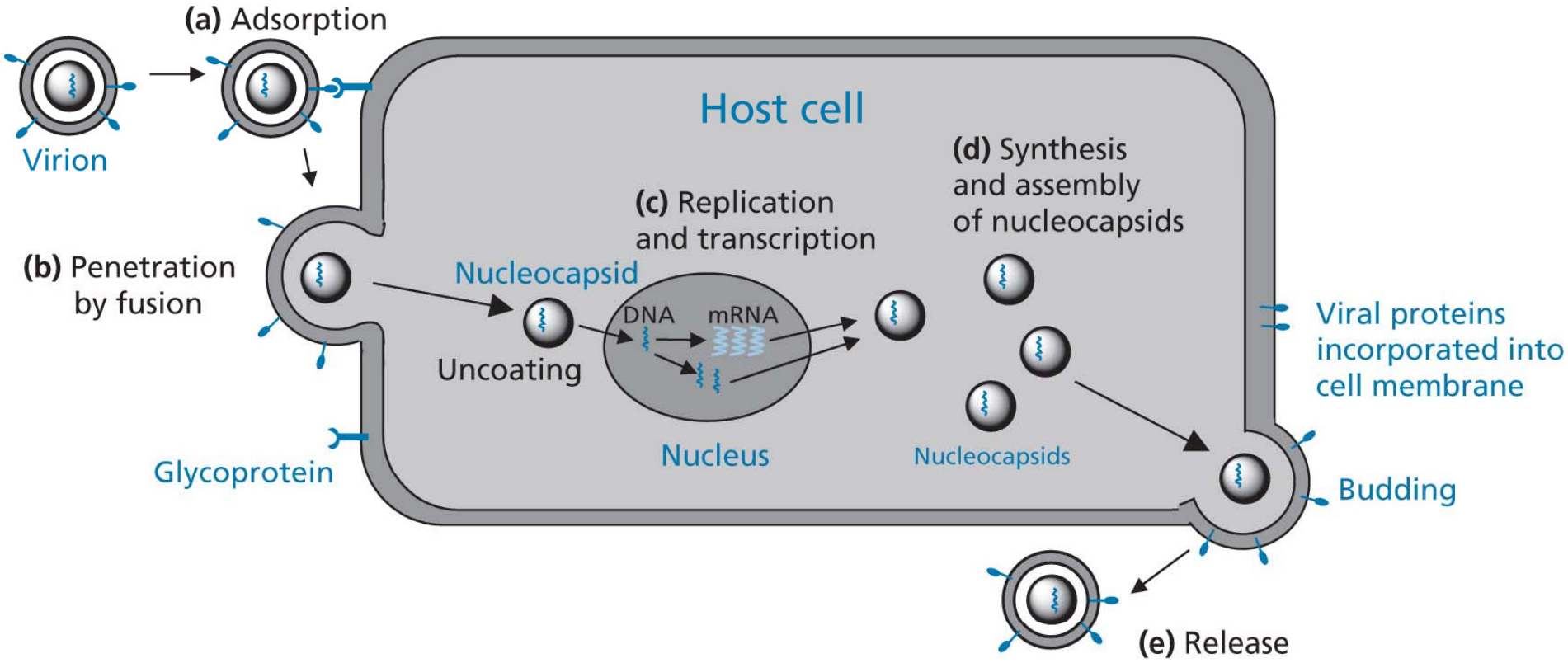
• **Penetration & uncoating**: different viruses introduce their nucleic acid into the host cell by different methods by injecting their nucleic acid through the cell membrane or enter the cell and then uncoated.

• **Replication & transcription**: viral genes can be defined as *early or late*. Early genes with viruses contain (+) strand, and late viruses contain negative strand.

• **Synthesis & assembly of nucleocapsids**: then the genes direct the synthesis of capsid proteins and these self-assemble to form capsid. Then viral nucleic acid is taken into the capsid to form nucleocapsid.

• **Virion release**: naked virions "those with no outer layers round the nucleocapsid" are released by cell lysis where the cell is destroyed.

Viruses with envelops are released by a process called **budding**.



❖ Antiviral drugs used against DNA viruses

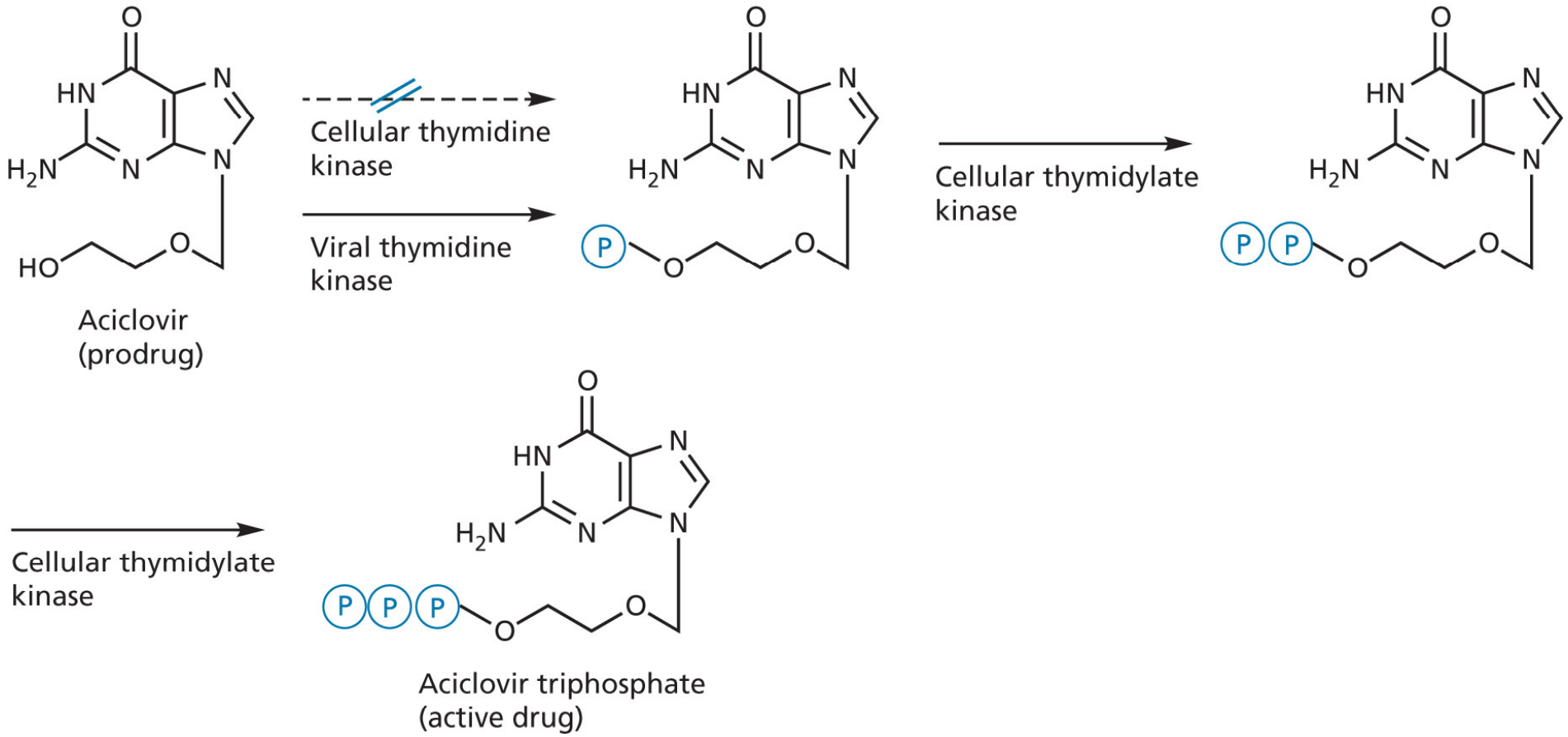
➢ Most of the drugs which are active against DNA viruses have been developed against herpesviruses to combat diseases such as cold sores, genital herpes, chicken pox, shingle, Burkitt´s lymphoma & kaposi sarcoma.

❑ Inhibitors of viral DNA polymerase

➢ **Aciclovir** was introduced in the market in 1981. it has a nucleoside like structure and contains the same nucleic acid base as deoxyguanosine but lacks the complete sugar ring.

➢ In virally infected cells, it is phosphorylated in three stages to form a triphosphate which is the active agent so aciclovir iteself is a prodrug.

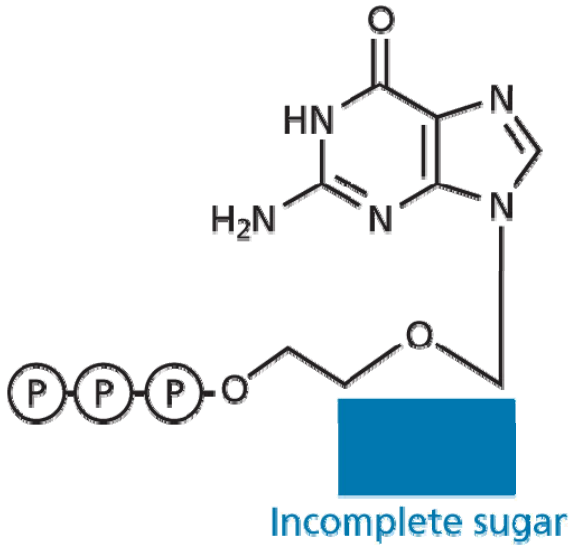
➢ Nucleotide triphosphates are the building blocks for DNA replication where a new DNA strand is constructed using a DNA template-catalysed by **DNA polymerase**.


Cellular thymidine kinase
Viral thymidine kinase
Cellular thymidylate kinase
Aciclovir (prodrug)
Cellular thymidylate kinase
Aciclovir triphosphate (active drug)

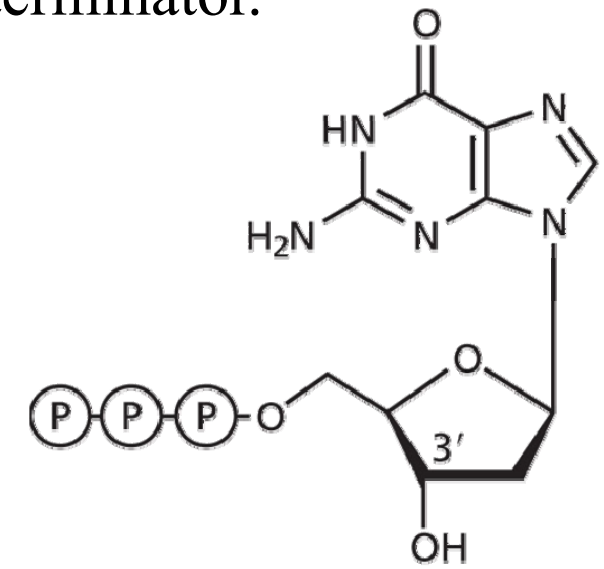
➢ Aciclovir triphosphate prevents DNA replication in two ways:

• it is sufficiently similar to the normal deoxyguanosine triphosphate building block that it can bind to DNA polymerase and inhibit it.

• DNA polymerase can catalyse the attachment of the aciclovir nucleotide to the growing DNA chain. Since the sugar unit is incomplete and lacks the required hydroxyl group normally present at position 3′ of the sugar ring. The nucleic acid chain cannot be extended any further. So Drug acts as a chain terminator.



Aciclovir triphosphate



Deoxyguanosine triphosphate

➢ What is to stop aciclovir triphosphate inhibiting DNA polymerase in normal uninfected cells?

➢ the explication lies in two points that it is only converted to the active triphosphate in infected cells:

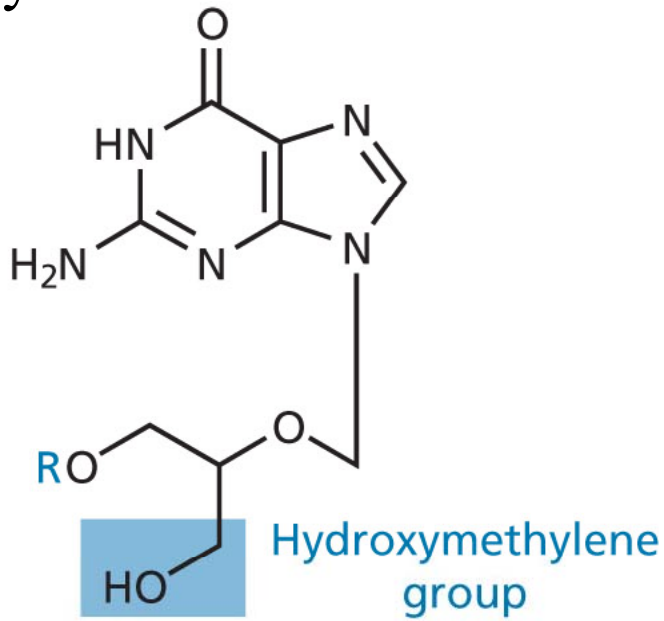
• Viral thymidine kinase is 100 times more effective at converting aciclovir to its monophosphate than host cell thymidine kinase. So in normal uninfected cells, aciclovir is a poor substrate for cellular thymidine kinase and remains as the prodrug.

• the selective action of aciclovir by a 50-fold against viral DNA polymerase relative to cellular polymerases.

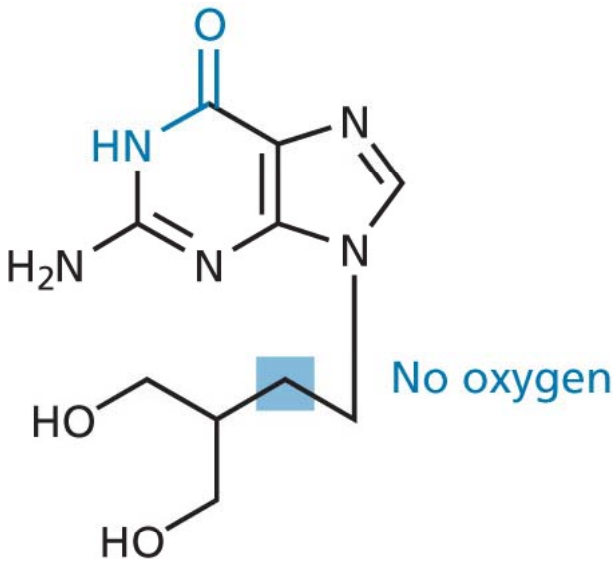
➢ The oral bioavailability of aciclovir is quite low (15-30%) and to overcome this, various prodrugs were developed to increase water solubility.

➢ **Ganciclovir** is an analogue of aciclovir which bears an extra hydroxymethylene group. It is effective against both α- & β-subfamilies of herpesvirus. Aciclovir is effective only against the α-subfamily of herpesvirus.

➢ **Penciclovir** is an analogue of ganciclovir which lack the oxygen atom. It has the same spectrum of aciclovir but has a better potency, a faster onset and a longer duration of action. But it is still has poor oral bioavailability.



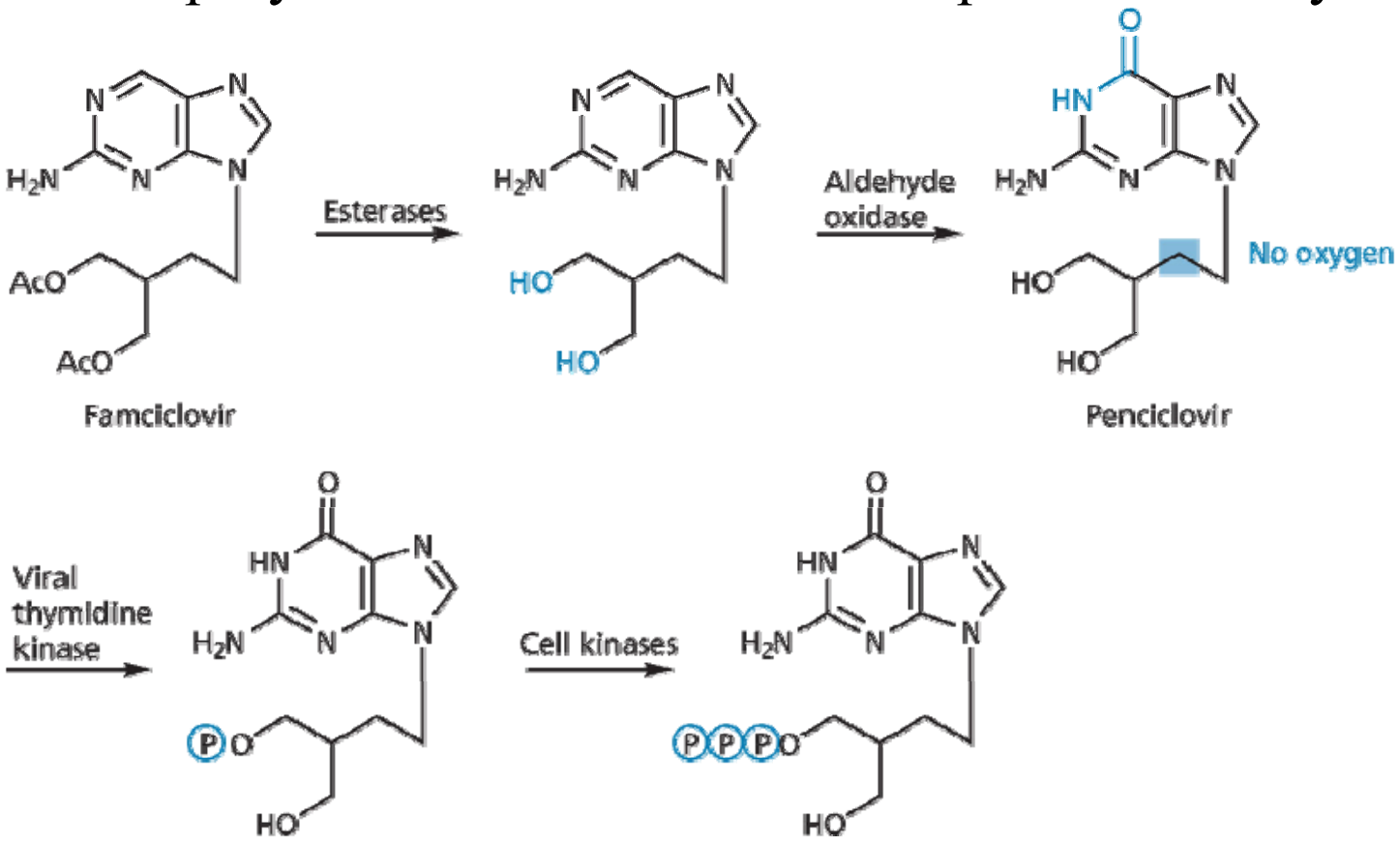
Ganciclovir R=H



Penciclovir

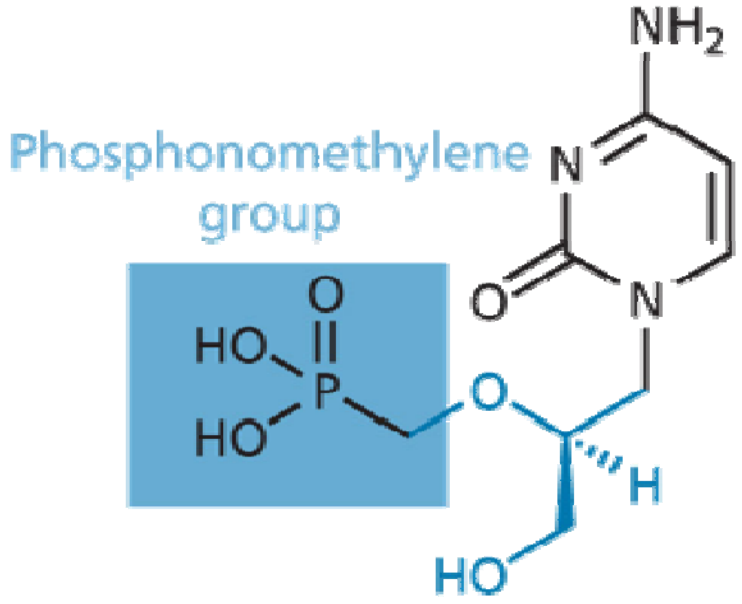
➢ **Famciclovir** is a prodrug of penciclovir where the two alcohol groups are masked as esters making the structure less polar, and leading to better absorption.

➢ Once absorbed, the acetyl groups are hydrolysed by esterases and the purine ring is oxidized by aldehyde oxidase in the liver to generate penciclovir. Phosphrylation reactions then take place in virally infected cells.

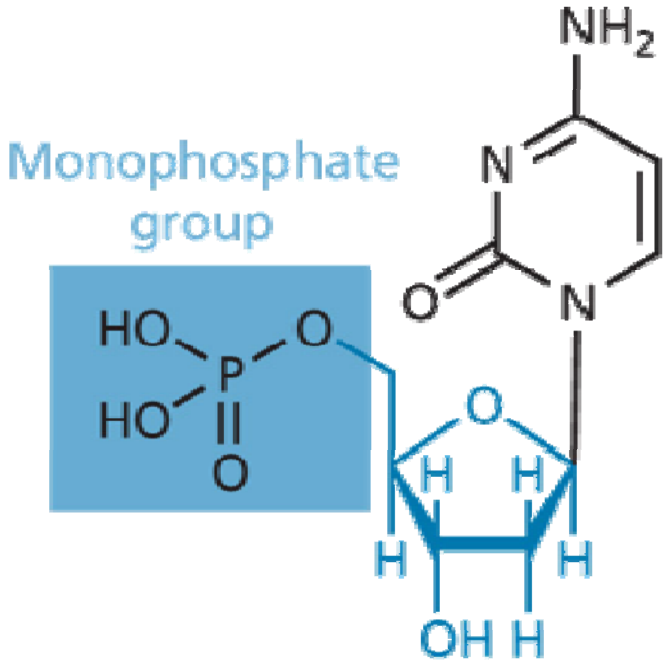


➢ Some viruses are immune from the action of the above antiviral agents because they lack the enzyme thymidine kinase. As a result, phosphorylation fails to take place.

➢ **Cidofovir** was designed to combat this problem. It is an analogue of deoxycytidine 5-monophosphate, where the sugar and phosphate groups have been replaced by an acyclic group & a phosphonomethylene group, respectively.



Cidofovir



Deoxycytidine monophosphate

- The phosphonomethylene gp acts as a bioisostere for the phosphate gp and is used coz the phosphate gp itself would be more susceptible to enzymatic hydrolysis.

- Since a phosphate equivalent is present. The drug does not require thymidine kinase to become activated.

- two more phosphorylation can now take place catalysed by cellular kinases to convert cidofovir to the active (triphosphate).

❑ Inhibitors of tubulin polymerization

➢ The plant product **podophyllotoxin** has been used clinically to treat genital warts.

❑ Antisense therapy

➢ **Fomivirsen** is the first and so far the only, DNA antisense molecule that has been approved as an antiviral agent.

➢ it consists of 21 nucleotides and a phosphonothioate backbone rather than a phosphate backbone to increase the metabolic stability of the molecule.

➢ the drug blocks the translation of viral RNA and is used against retinal inflammation in AIDS patients. Because of its high polarity it is administered as an ocular injection.

❖ Antiviral drugs acting against RNA viruses: HIV

❑ Structure & life cycle of HIV "Human immuno deficiency virus"

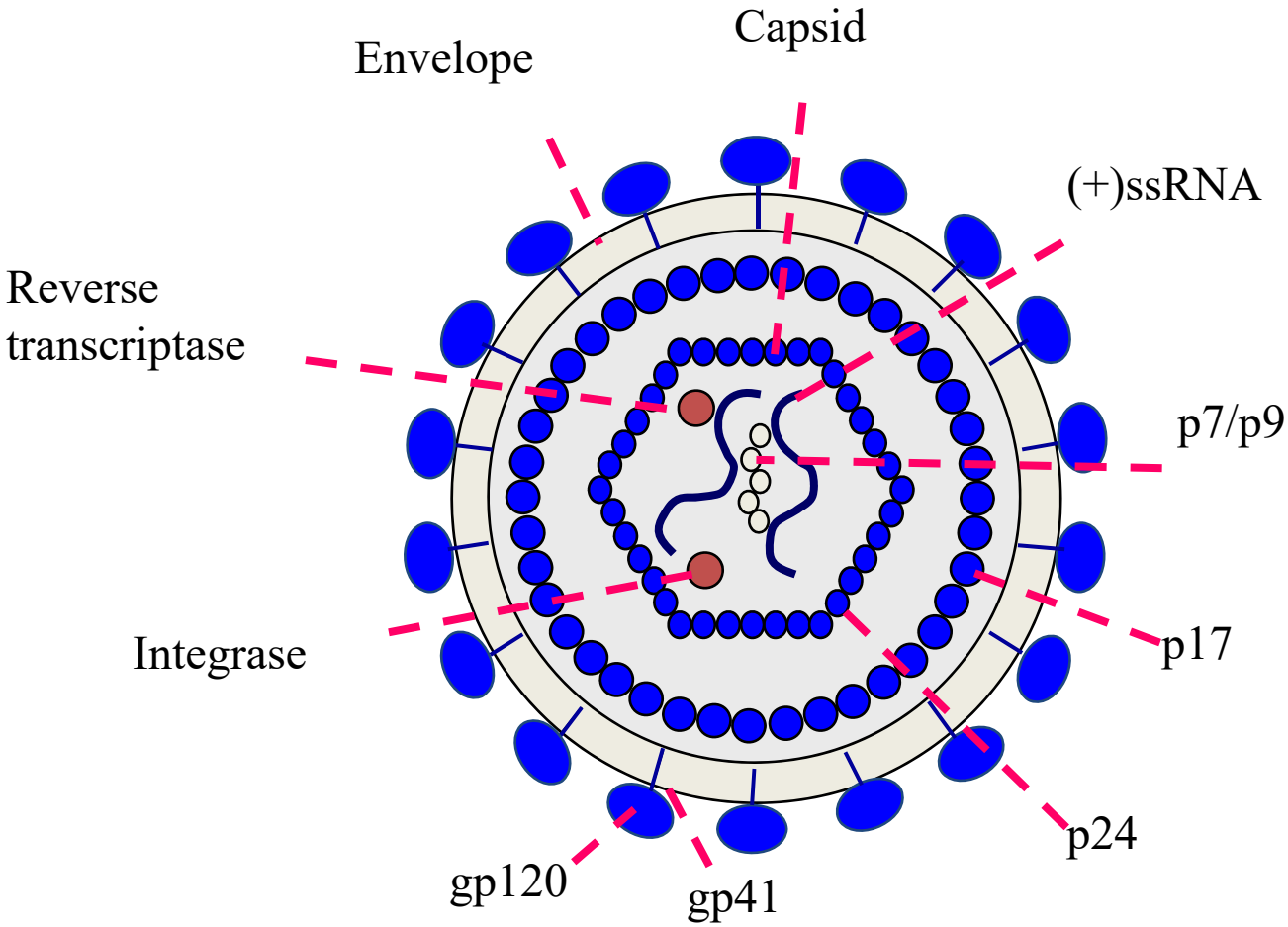
➢ HIV is an example of a group of viruses known as the retroviruses. There are two variants of HIV. HIV-1 is responsible for AIDS in america, europe and asia, whereas HIV-2 occurs mainly in western africa.

➢ At present, most clinically useful antiviral drugs act against two targets- the viral enzymes **reverse transcriptase & protease**.

➢ HIV is an RNA virus which contains two identical strands of (+) ssRNA within its capsid. Also present are the viral enzymes reverse transcriptase & integrase and other proteins called p7/p9.

➢ the capsid is made up of protein units known as p24 surrounded by a layer of matrix protein (p17). Then the membrane envelope.

➢ in the outer surface there are the viral glycoproteins **gp120 & gp41**. Both are essential for the process of adsorption & penetration. Gp120 interacts & binds with a transmembrane protein called **CD4** which is present on host T-cells & gp41 anchor the virus to the surface of the host cell.



- Once fusion has taken place, the HIV nucleocapsid enters the cell. Deintegration of the protein capsid then takes place, aided by the action of a viral enzyme called protease.

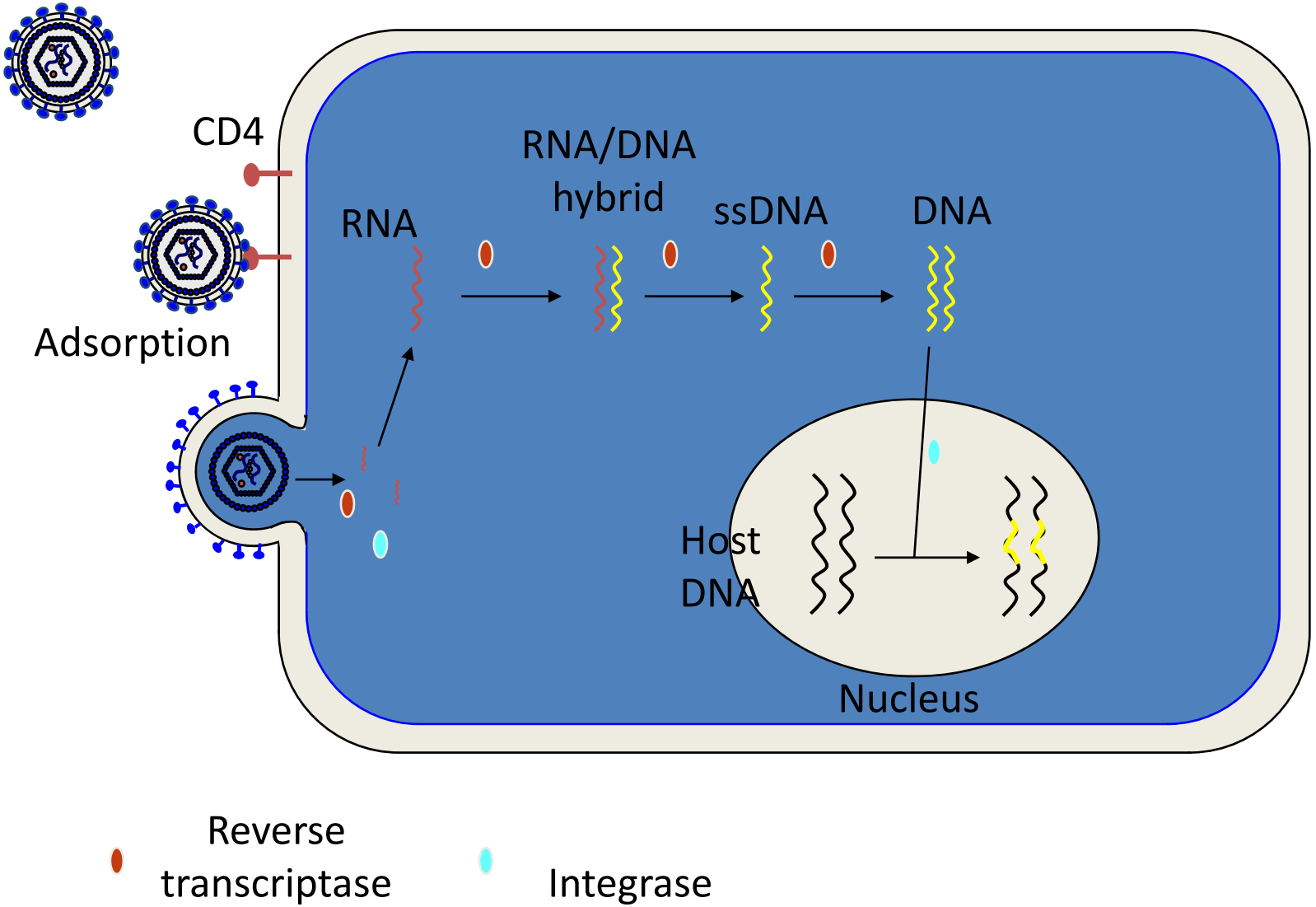
- Viral RNA & viral enzymes are then released into the cell cytoplasm. The released viral RNA is not capable of coding directly for viral proteins or of self-replication.

- Instead it is converted into DNA & incorporated into the host cell DNA. This conversion carried by HIV enzyme called reverse transcriptase (is a member of DNA polymerase family).

- Proviral DNA is now spliced into the host cell's DNA, a process catalysed by integrase-an enzyme also carried by the virion.

- once the proviral DNA has been incorporated into host DNA, it is called the provirus & can remain dormant in host cell DNA until activated by cellular processes.

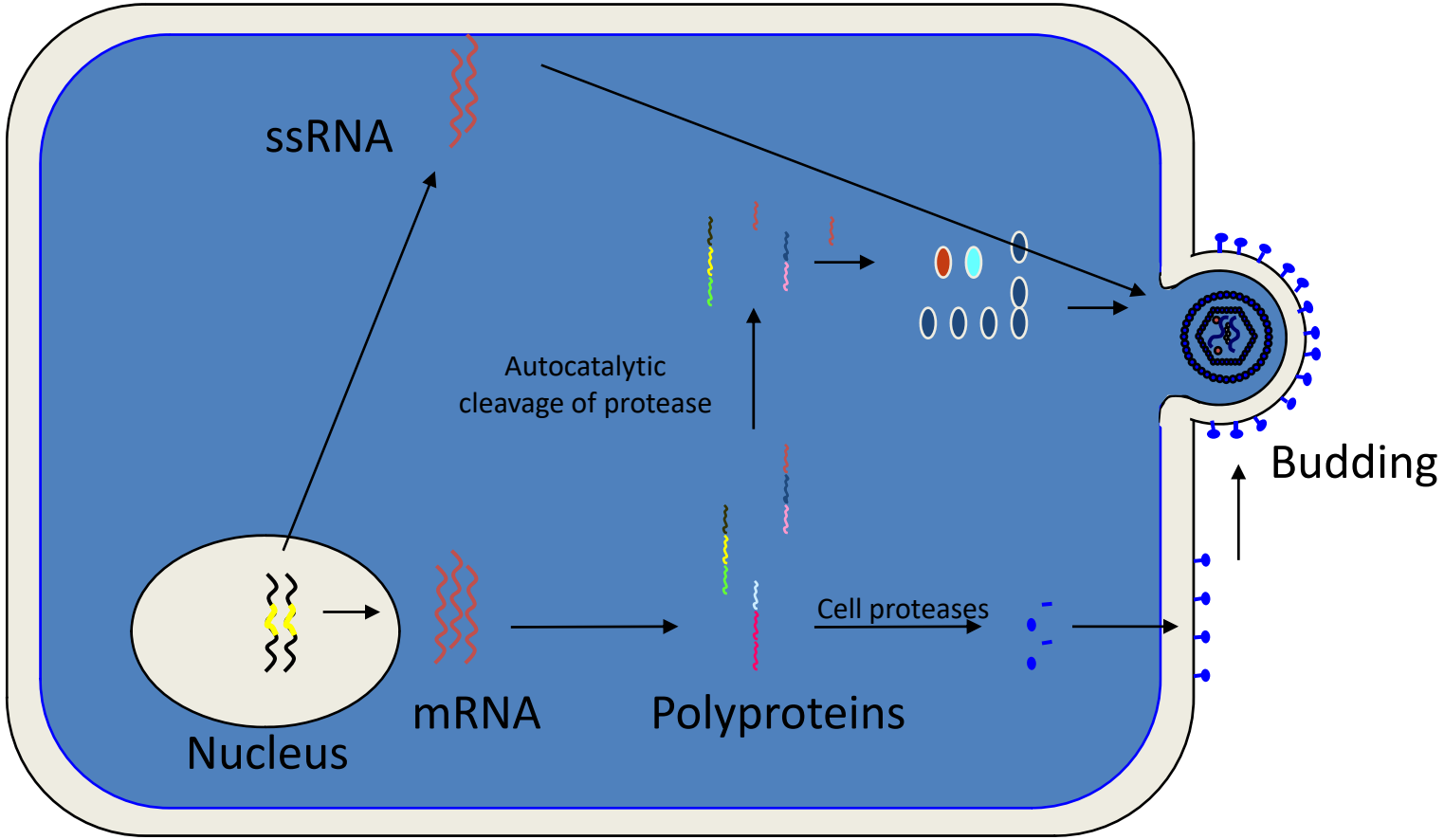
Infection


CD4
Adsorption
RNA
RNA/DNA
hybrid
ssDNA
DNA
Host
DNA
Nucleus
Reverse
transcriptase
Integrase

- When activated, transcription of viral genes takes place to produce viral RNA, some incorporate into new virions and the rest is used in translation to produce three large polyproteins

- Then the viral glycoprotein (gp120 & gp41) are incorporated into the cell membrane. Budding then takes place to produce an immature membrane-bound virus particle & a new nucleocapsids containing viral RNA, reverse transcriptase & integrase.

Replication and release



Reverse transcriptase

Integrase

Protease

Structural proteins

❖ Antiviral therapy against HIV

➢ Most drugs that have been developed act against the viral enzymes reverse transcriptase and protease.

➢ the main problem is the result of rapid resistance to antiviral drugs, but with experience, several studies showed that the combination of different drugs acting on both reverse transcriptase & protease have been successfully delayed the progress of AIDS "acquired immuno deficiency syndrome".

❖ Inhibitors of viral reverse transcriptase

❑ Nucleoside reverse transcriptase inhibitors "NRTIs"

➢ Since the enzyme reverse transcriptase is unique to HIV, it serves as an ideal drug target.

➢ Various nucleoside-like structures have proved useful as antiviral agents.

➢ The vast majority of these are not active themselves but are phosphorylated by three cellular enzymes to form active nucleotide triphosphate.

➢ the important difference between these agents and the previous ones is the requirement for all three phosphorylations to be carried out by cellular enzymes as HIV does not produce a viral kinase.

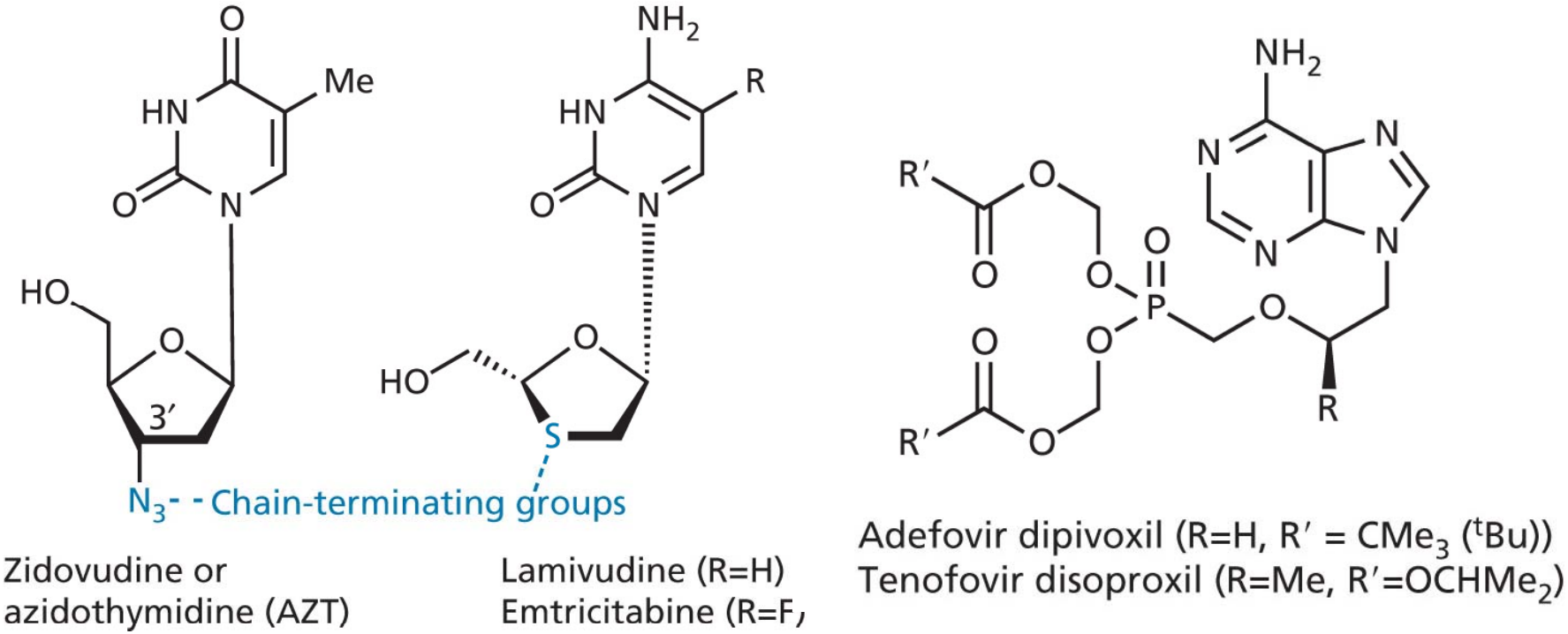
✓ **Zidovudine** was originally developed as an anticancer agent but was the 1st drug to be approved for use in the treatment of AIDS.

➢ it is analogue of deoxythymidine where the sugar 3′-hydroxyl group has been replaced by an azido group.

✓ Studies of the enzyme active site led to the development of **lamivudine & emtricitabine** "analogues of deoxycytidine where the 3′carbon has been replaced by sulfur".

✓ **Adefovir dipivoxil & Tenofovir disoproxil** prodrugs of modified nucleosides. Both structures contain a monophosphate group protected by two extended esters.

➢ Hydrolysis *in vivo* reveals the phosphate group which can then be phosphorylated to the triphosphate as we saw earlier.



Zidovudine or azidothymidine (AZT)

Lamivudine (R=H)
Emtricitabine (R=F,

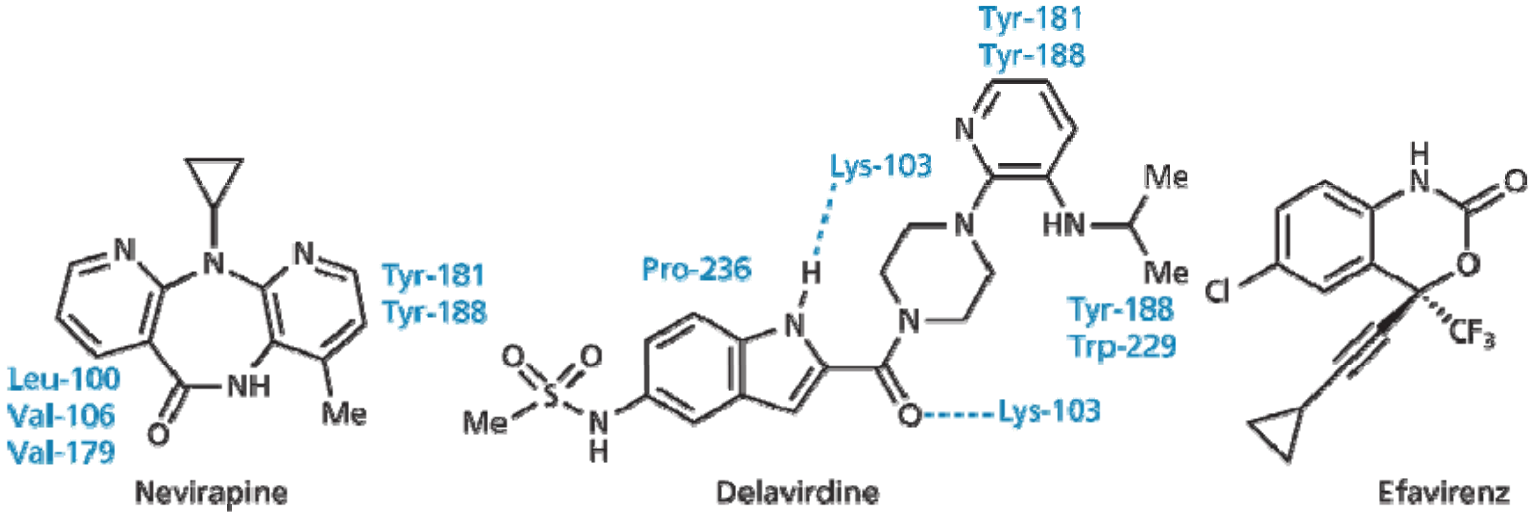
Adefovir dipivoxil (R=H, R′ = CMe_3 (^{t}Bu))
Tenofovir disoproxil (R=Me, R′=$OCHMe_2$)

❑ Non-nucleoside reverse transcriptase inhibitors "NNRTIs"

➢ they are generally hydrophobic molecules that bind to an allosteric binding site which is hydrophobic in nature results in an induced fit which locks the neighbouring substrate-binding site into an inactive conformation.

➢ The NNRTIs are non-competitive reversible inhibitors since the allosteric binding site is separate from the substrate binding site.

➢ they include 1st generation NNTRIs such as **nevirapine** & **delaviridine**, as well as 2nd generation drugs such as **efavirenz**.



❖ Protease inhibitors

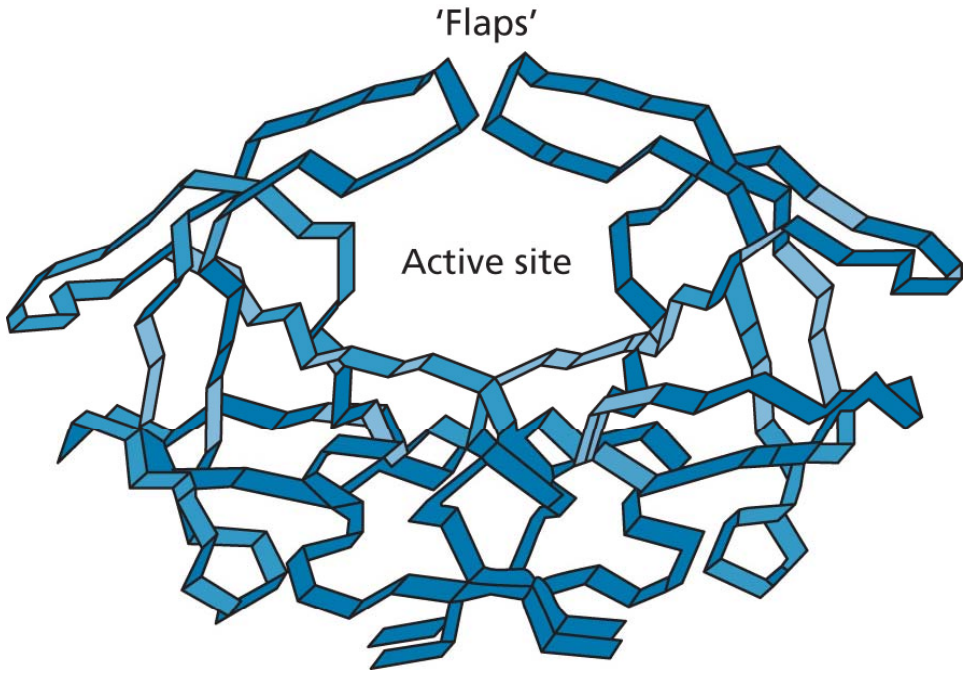
❑ The HIV protease enzyme

➢ the HIV protease enzyme is an example of an enzyme family called the aspartyl proteases- enzymes which catalyse the cleavage of peptide bonds and which contain an aspartic acid in the active site that is important to the catalytic mechanism.

➢ The HIV protease enzyme is a dimer made up of two identical protein units so it is **symmetrical**.

➢ the amino acids Asp-25, Thr-26 & Gly-27 from each monomer are located on the floor of the active site, & each monomer provides a flap to act as the ceiling.

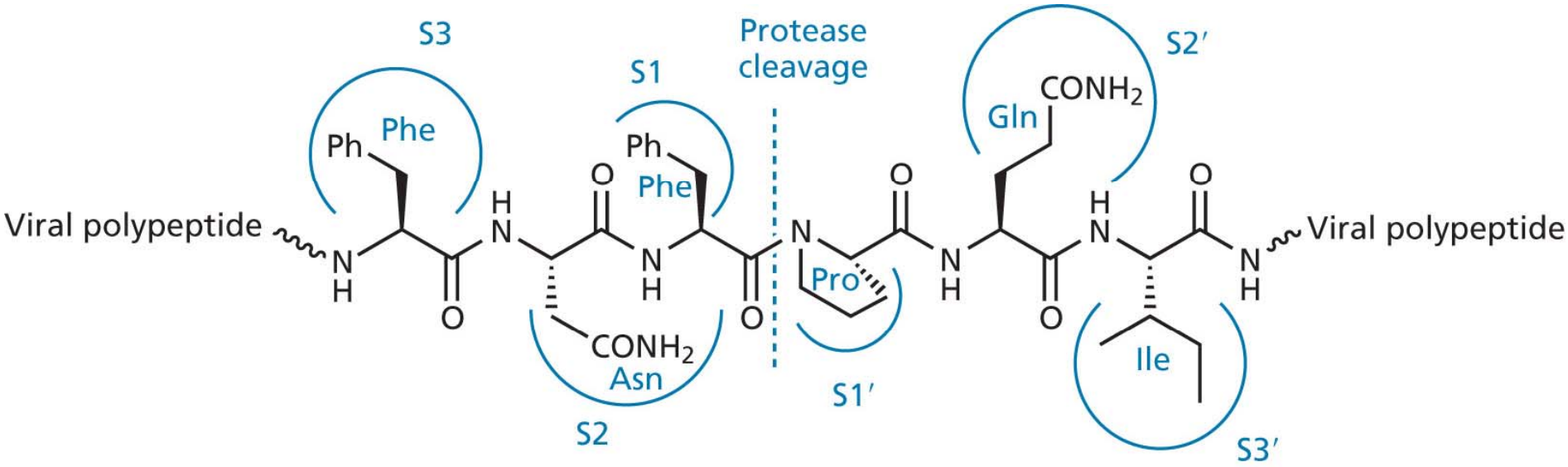
- the enzyme cleaves bonds between a proline residue & an aromatic residue “phenylalanine or tyrosine”.



- the cleavage of a peptide bond next to proline is unusual and does not occur with mammalian proteases so the chances are good of achieving **selectivity** against HIV protease over mammalian proteases.

- Moreover, the symmetrical nature of the viral enzyme & its active site is not present in mammalian proteases, again suggesting the possibility of drug **selectivity**.

Substrate binding and cleavage



Notes

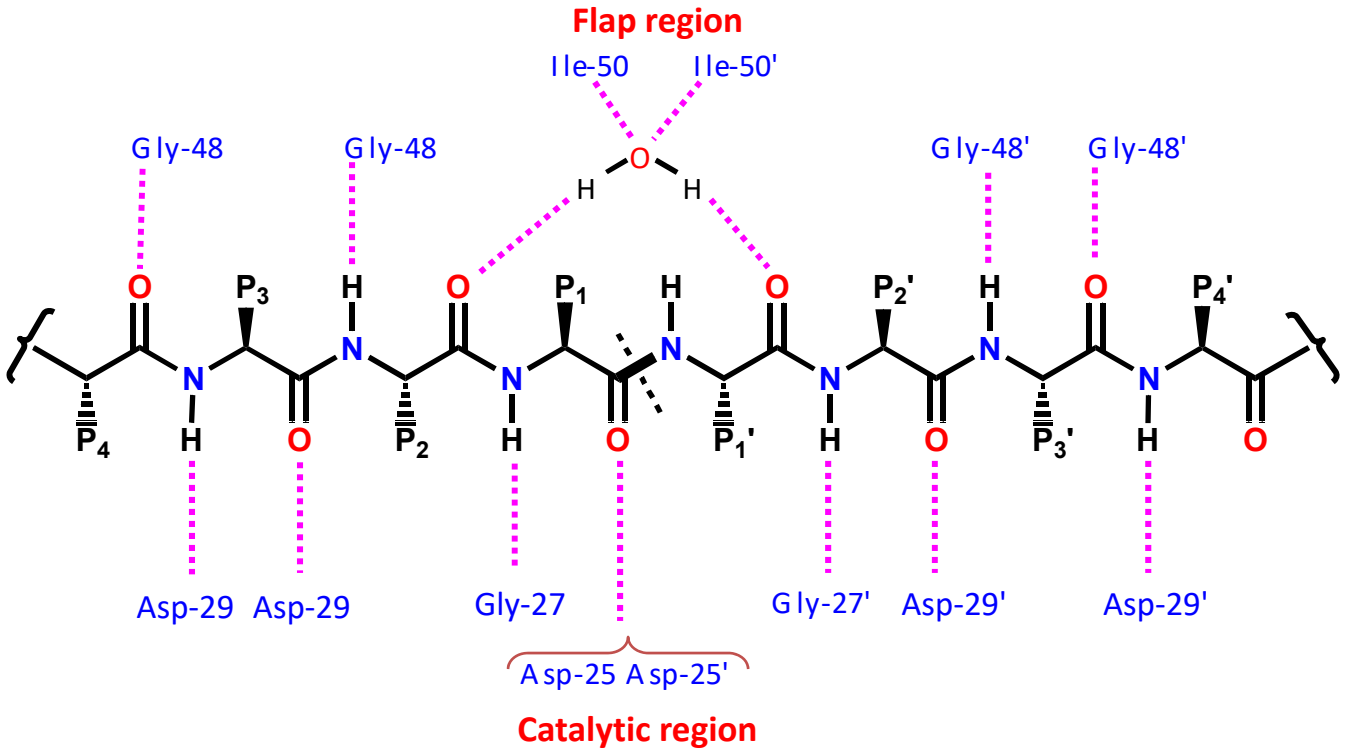
➢ There are eight binding subsites in the enzyme, four on each protein unit, located on either side of the catalytic region.

➢ these subsites accept amino acid residues of the substrate and are numbered S1-S4 on one side & S1´-S4´ on the other side

Binding interactions

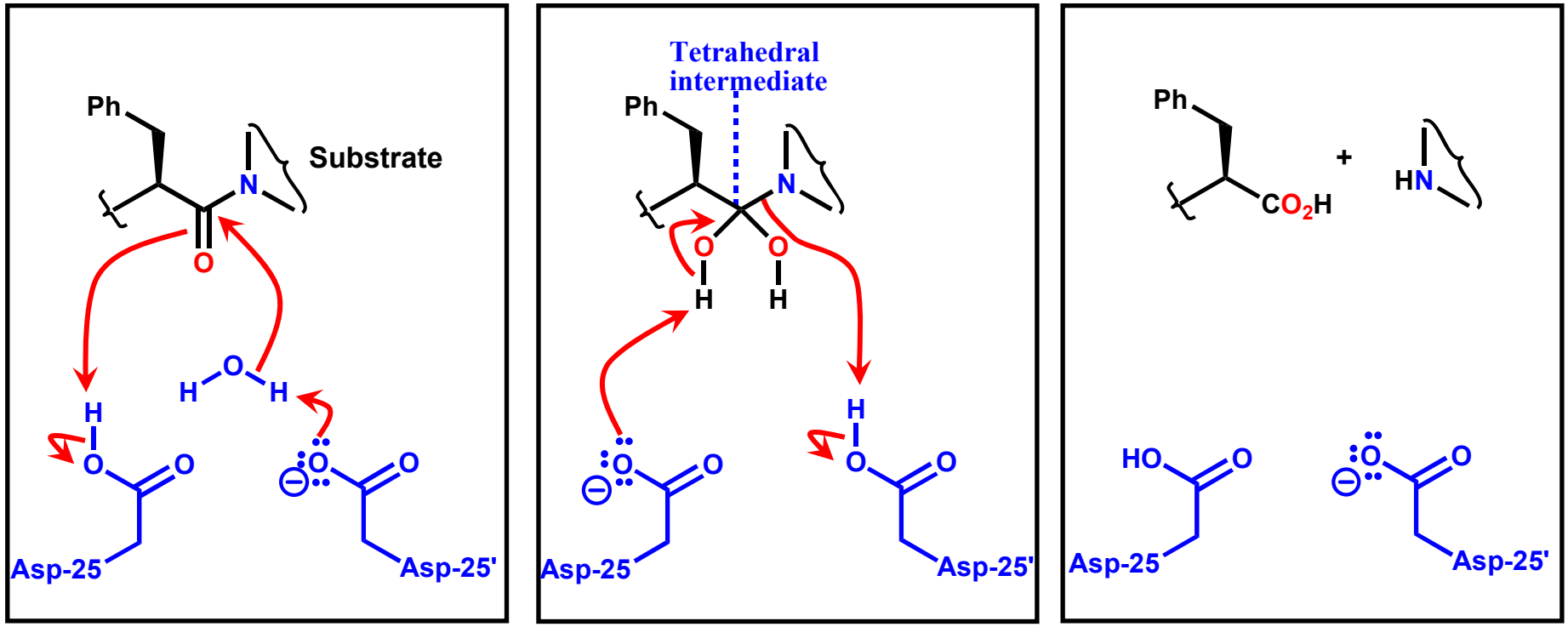
➢ The relevant residues on the substrate are numbered P1-P4 & P1′-P4′. The N & O of each peptide bond in the substrate′s peptide backbone is involved in H bonding interactions with the enzyme.

➢ A water molecule is present in the active site acts as a H bonding bridge to 2 isoleucine NH gps on the enzyme flaps. This H bonding network has the effect of closing the flaps over the active site once the substrate is bound.



➢ The aspartic acids Asp-25 & Asp-25′ are involved in the catalytic mechanism & are on the floor of the active site, each contributed from one of the protein subunits.

➢ the carboxylate residues of these asparates & a bridging water molecule are involved in the mechanism by which the substrate′s peptide bond is hydrolysed.



❑ Design of HIV protease inhibitors

➢ A similar hydrolytic mechanism to that takes place for a mammalian aspartyl protease called **renin**.

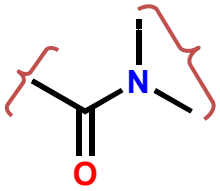
➢ the renin inhibitors were designed as antihypertensive agents. These agents act as **transition-state inhibitors**. Many of the strategies resulting from the development of renin inhibitors were adapted to the design of HIV protease inhibitors.

➢ In the HIV protease catalysed reaction, the transition state resembles the tetrahedral intermediate and it is unstable.

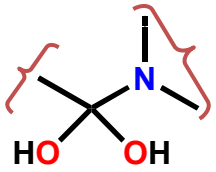
➢ so it is necessary to design an inhibitor which contains a **transition-state isostere** that mimic tetrahedral centre but stable to hydrolysis.

➢ Hydroxyethylamine isostere proven to be effective. Since has a hydroxyl gp which mimics one of the hydroxyl gps of the tetrahedral intermediate and binds to the aspartate residues in the active site

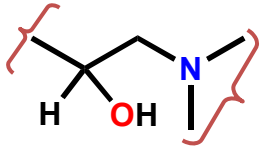
➢ R-configuration is preferred in this group.



Amide



Tetrahedral intermediate



Hydroxyethylamine transition-state isostere

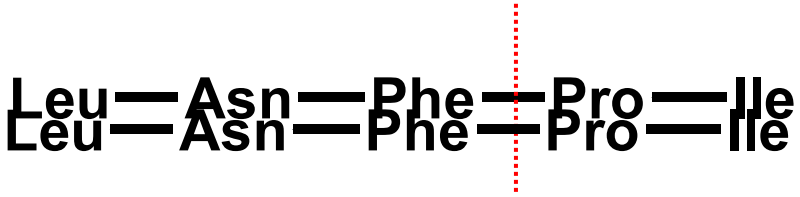
1. Saquinavir

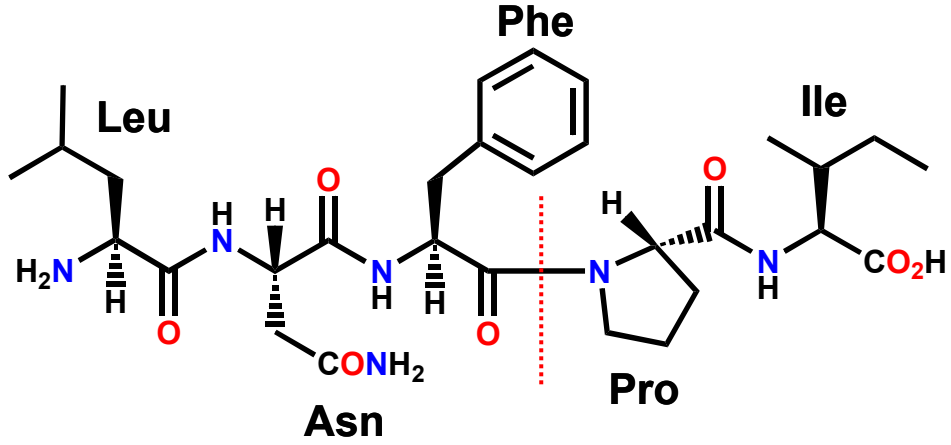
➢ It was developed by Roche and as the 1st PI to reach the market it serves as the benchmark for all other PIs.

• *Pol* is a viral polypeptide necessary for the budding and it is a good substrate for HIV protease.

• Includes a pentapeptide sequence containing the susceptible Phenylalanine-Proline linkage.

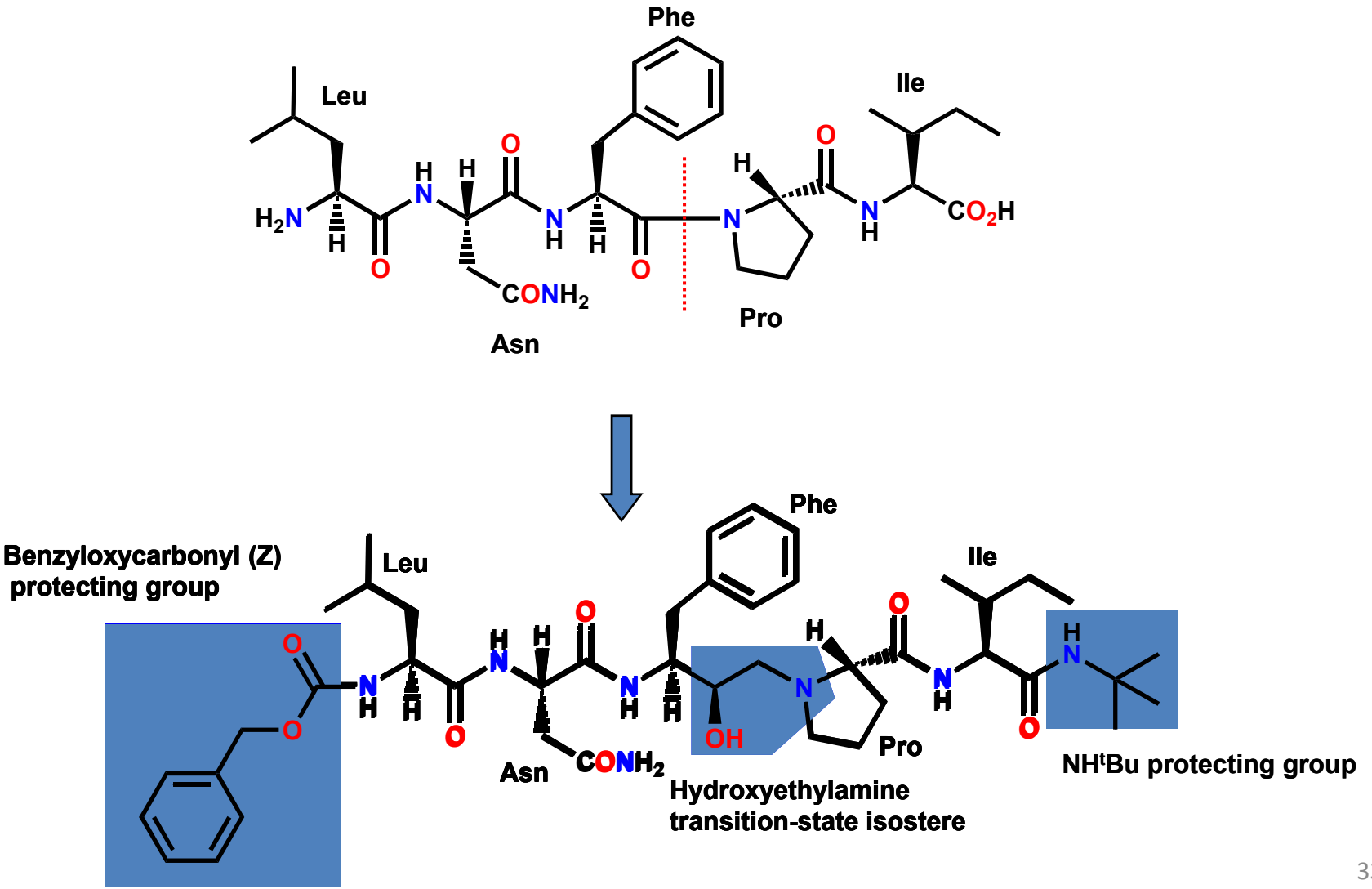
• the peptide link normally hydrolysed in this sequence is between Phe & Pro and so this link was replaced by a hydroxyethylamine transition-state isostere to give a successfully inhibitor.





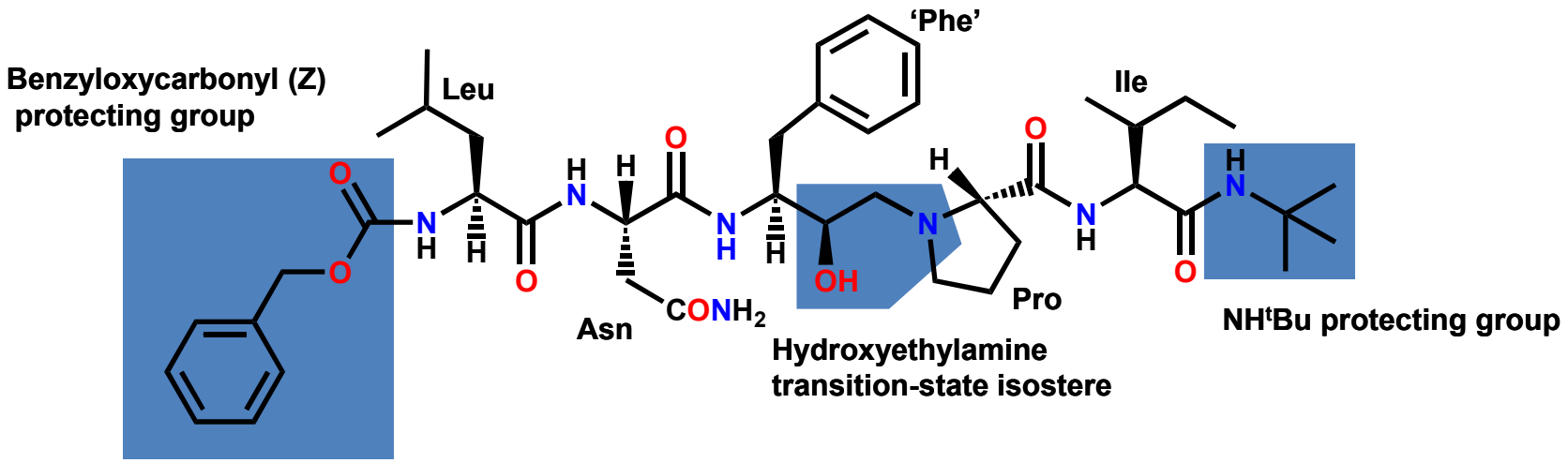
1. Saquinavir

Lead compound



1. Saquinavir

Lead compound



Notes

- Weak inhibitor "IC_{50} = 750 nM"
- Stable to enzyme-catalysed reaction
- 5 side chains fit subsites S3-S2'
- 'Phe' = equivalent side chain to Phe

Disadvantages

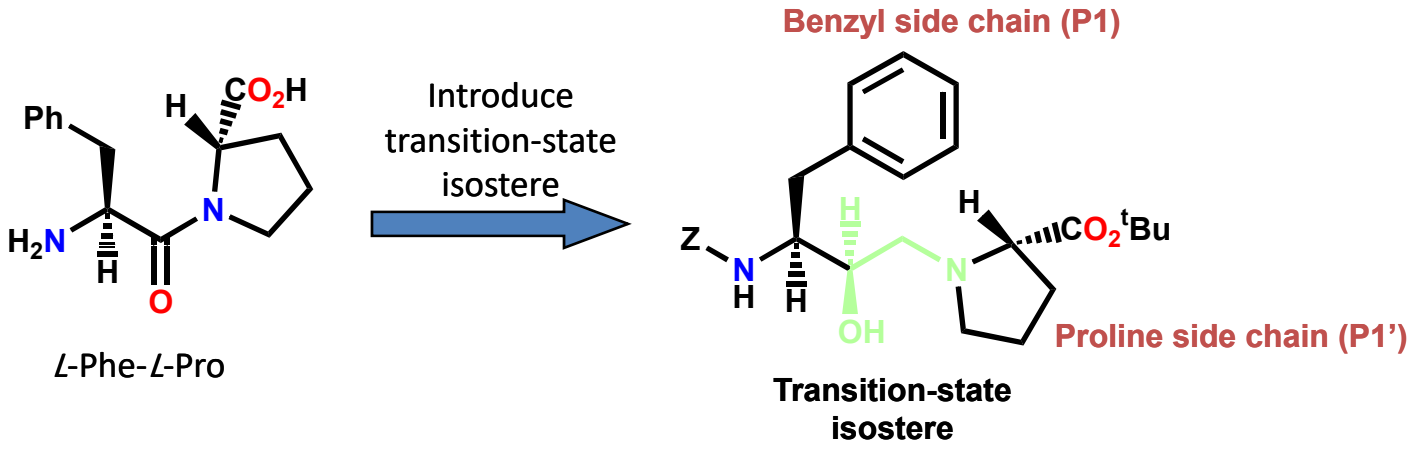
- High molecular weight
- High peptide character
- Poor oral bioavailability

➢ Roche team set out to identify a smaller inhibitor, starting from the simplest possible substrate for the enzyme-phe-pro.

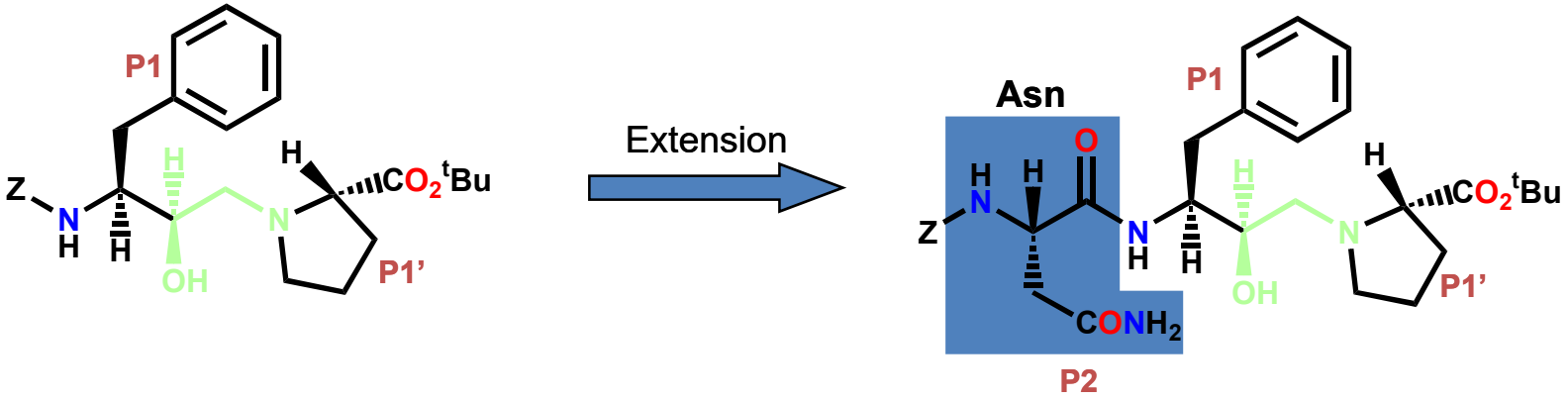
➢ the peptide link was replaced by the hydoxylamine transition-state isostere & the resulting N- & C- protected strucutre.

➢ it has showed weak inhibitory activity "IC_{50} 6500 nM".

➢ Side chains (P1 and P1') occupy enzyme subsites (S1 and S1')



- **Extension strategy - add an extra amino acid**
- **Aim: to increase binding interactions**



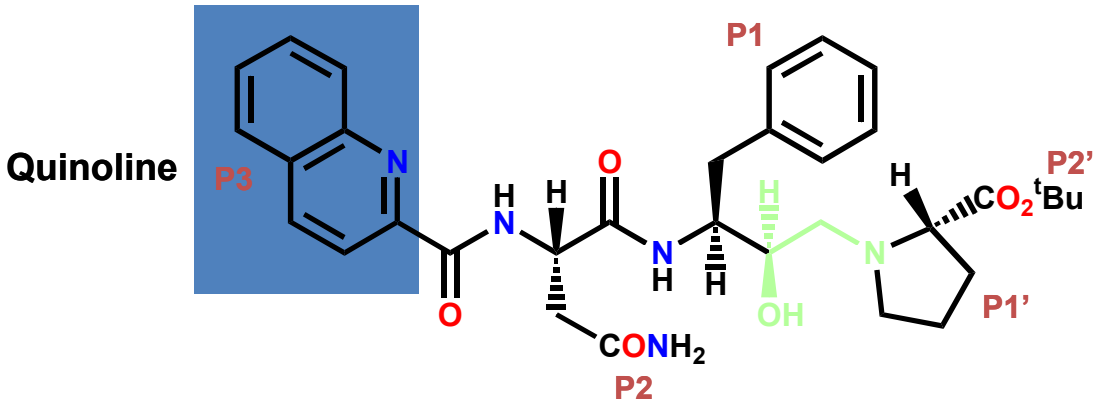
- Side chain of Asn (P2) occupies subsite (S2)
- Results in increased binding
- 40 fold increase in inhibition (IC_{50} 140 nM)

- **This structure was adopted as the new lead compound and the residues P1 & P2 were varied to find the optimum gps for the S1 & S2.**

- **The benzyl gp & the aspragine side chain were the optimum groups.**

➢ An X-ray crystallographic study of the enzyme-inhibitor complex was carried out & revealed that the protecting gp "Z" the S3 subsite which proved to be a large hydrophobic pocket.

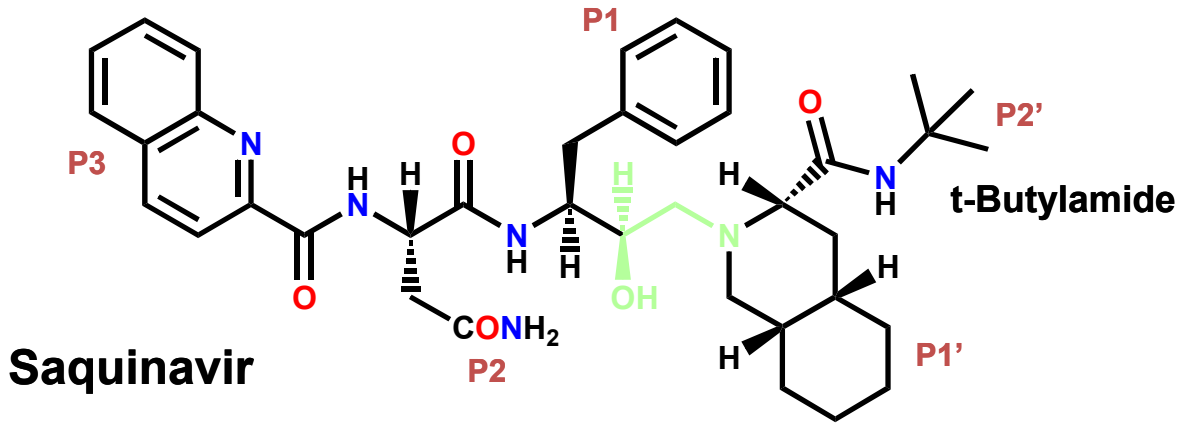
➢ So, the protecting gp was repalced with larger quinoline ring system which could occupy the subsite more fully & this led to a sixfold increase in activity "IC_{50} = 23 nM".



➢ Variation also carried out on the carboxyl half of the molecule.

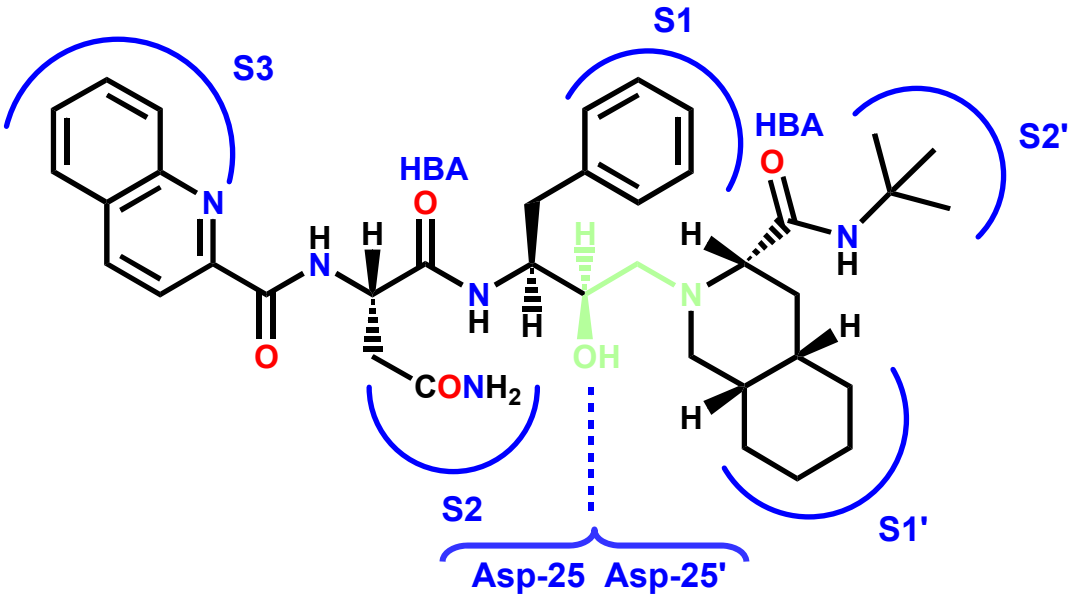
➢ Proline fits into the S1′ pocket but it was found that it could be replaced by a bulkier decahydroisoquinolone ring system.

➢ the t-butyl ester protecting gp was found to occupy the S2′ subsite & could be replaced by a t-butylamide gp which proved more stable in animal studies.



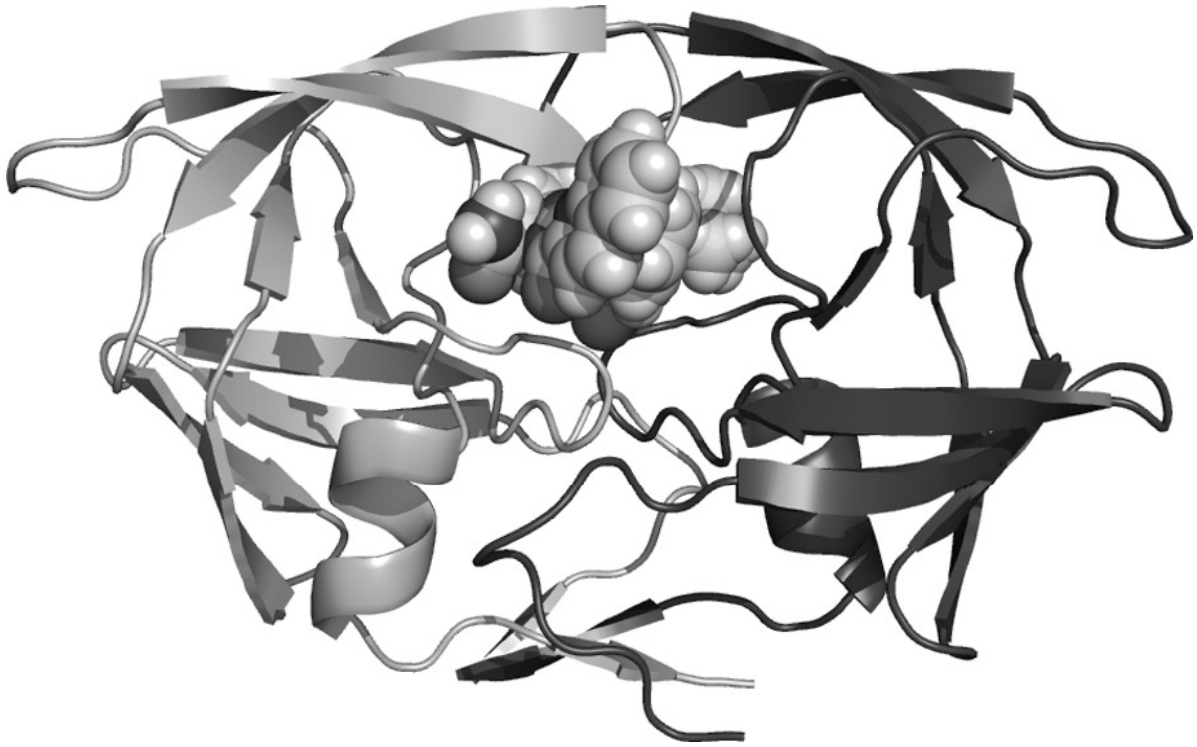
•60 fold increase in activity (IC_{50} 0.4 nM)
•*R*-Stereochemistry is essential for the transition-state isostere
•Saquinavir was the first protease inhibitor to reach the clinic

Binding interactions



Notes

- Studied by X-ray crystallography
- Five subsites are occupied (S3-S2')
- The S3' subsite is inaccessible
- The transition state isostere interacts with the catalytic aspartates by hydrogen bonding interaction
- Carbonyls act as HBAs to the bridging water molecule in the flap region



Disadvantages

- **Poor oral bioavailability**
- **Susceptible to drug resistance**
- **Subsequent research on more modern protease inhibitors aims to reduce molecular weight and peptide character**

2. Ritonavir and Lopinavir

Notes

- Developed by Abbott Pharmaceuticals

- Designed to take advantage of the symmetrical nature of the active site

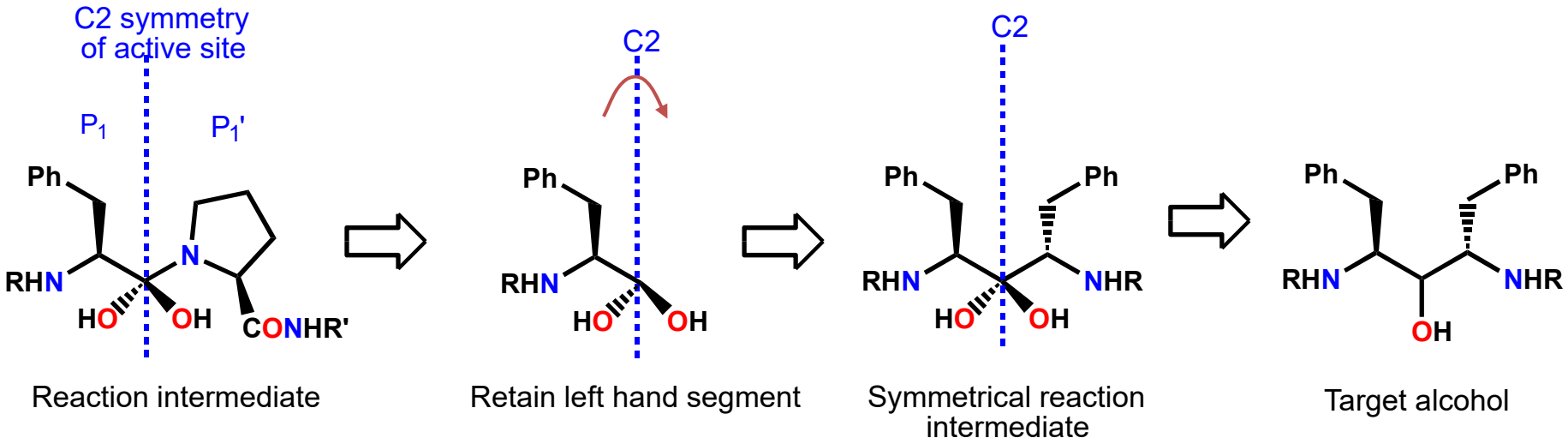
- Symmetrical inhibitors should be capable of binding left to right or right to left

- Symmetrical inhibitors likely to show greater selectivity over mammalian proteases

- Symmetrical inhibitors likely to be more resistant to hydrolytic breakdown by peptidases

- Lead compound designed to have C2 symmetry

De novo design of a symmetrical lead compound



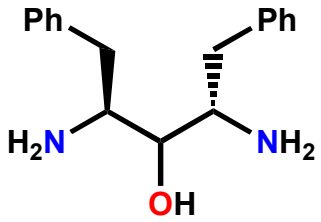
Reaction intermediate | Retain left hand segment | Symmetrical reaction intermediate | Target alcohol

Notes

- ***De novo*** **design is based on the enzyme-catalysed reaction intermediate**
- **Benzyl group is retained - strong binding group to S1**
- **Symmetrical reaction intermediate contains two benzyl groups**
- **Remove one alcohol group to stabilise the molecule**
- **Molecular modelling confirms that molecule should bind to the active site**
- **The target alcohol is synthesised and tested**

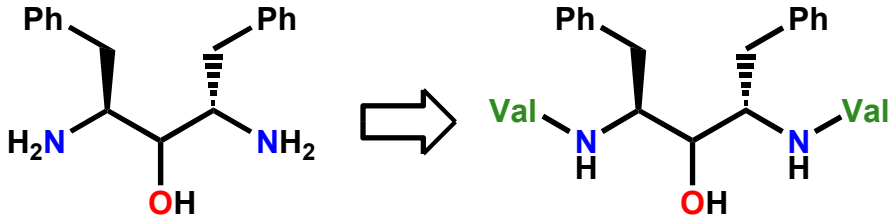
2. Ritonavir and Lopinavir

Drug Design



Target alcohol (I)
IC_{50} > 10,000 nM

- Target alcohol (I) acts as a weak enzyme inhibitor
- Inactive *in vitro*
- But still represents the successful *de novo* design of a lead compound



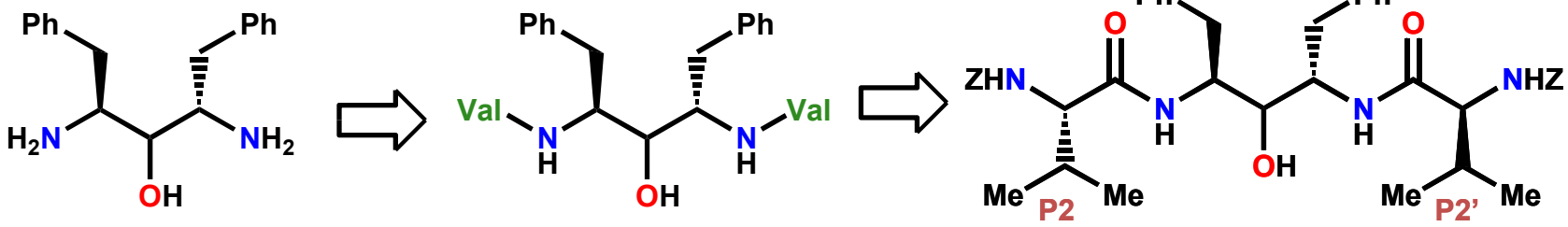
Target alcohol (I)
IC_{50} > 10,000 nM

(II)
IC_{50} 590 nM

Activity increases with the addition of valines

2. Ritonavir and Lopinavir

Drug Design



Target alcohol (I)
IC_{50} > 10,000 nM

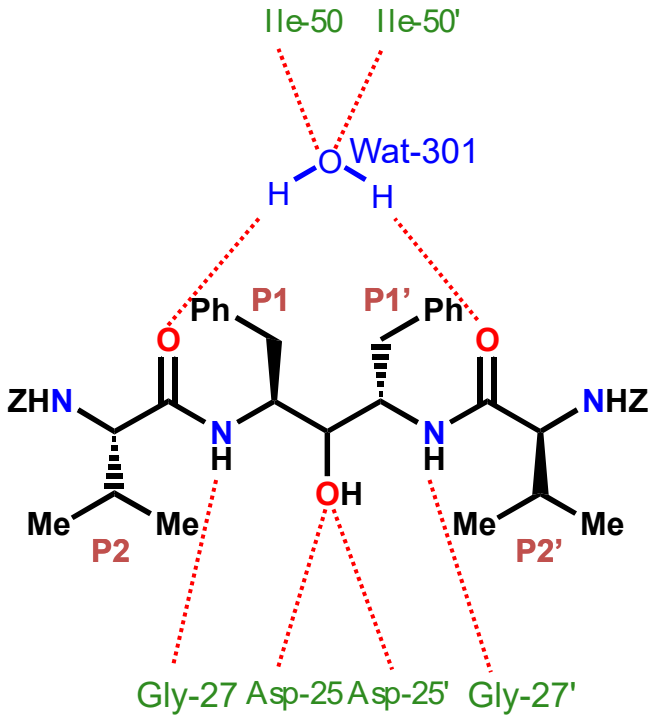
(II)
IC_{50} 590 nM

A74704
IC_{50} 3 nM

- **Activity increases with the addition of protecting groups**
- **Resistant to proteolytic breakdown**

2. Ritonavir and Lopinavir

Binding interactions for A74704
X-ray crystallography

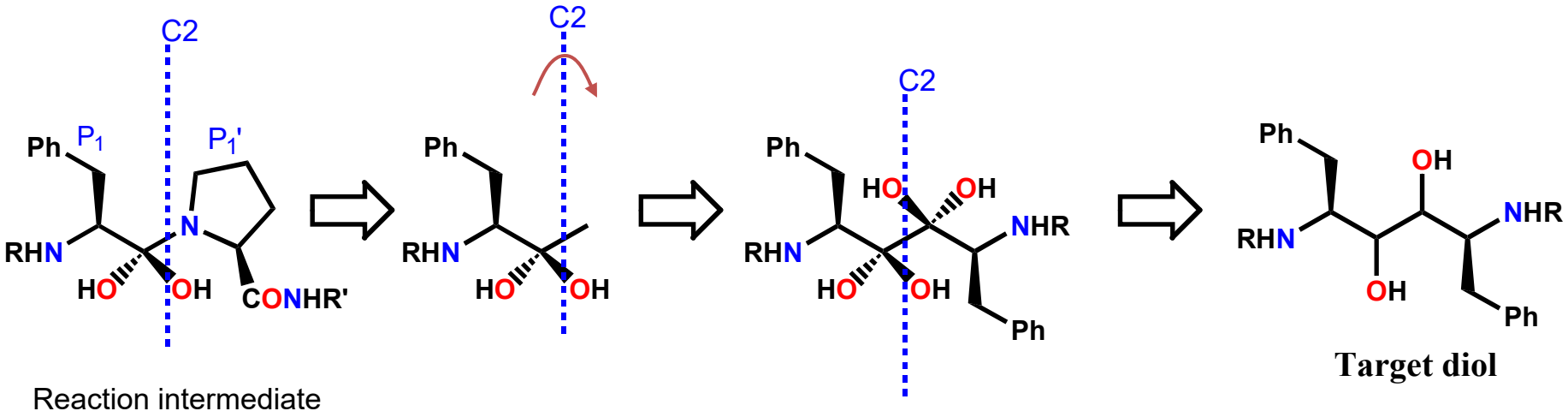


Notes

- **Symmetrical binding pattern**
- **Binding interactions to Gly-27 and Gly-27' are not optimum**
- **Increasing the distance between relevant NH groups may be beneficial**

2. Ritonavir and Lopinavir

De novo design of symmetrical lead compound II



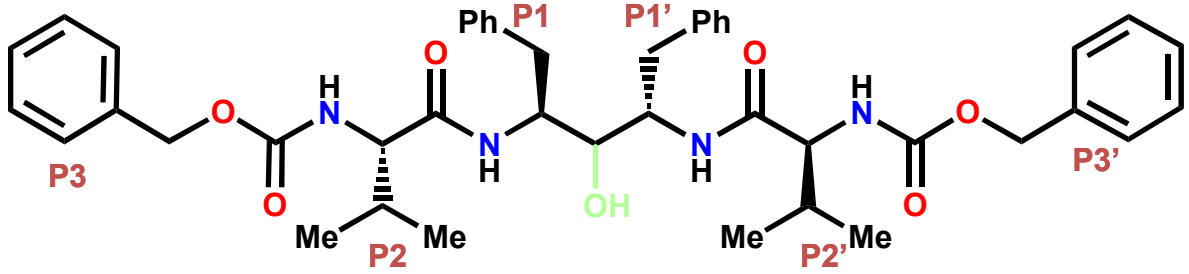
Reaction intermediate

Target diol

Notes

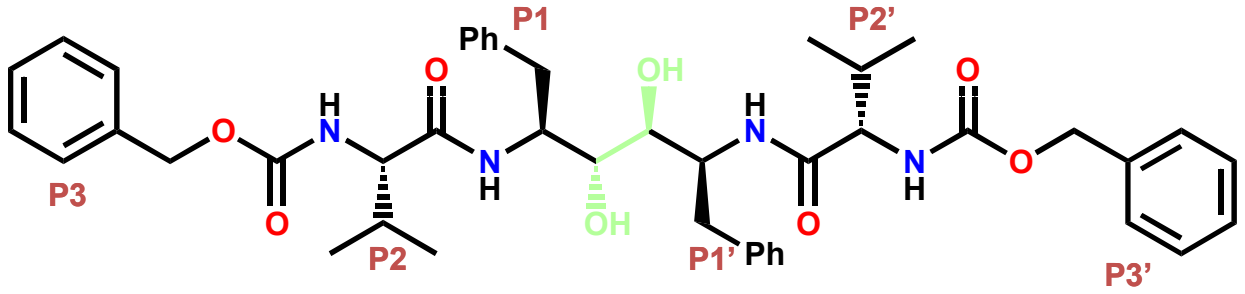
- ***De novo*** **design is based on the enzyme-catalysed reaction intermediate**
- **Axis of symmetry is designed to go through the centre of a bond rather than the reaction centre**
- **Allows the introduction of an extra atom**
- **Increases the separation of the NH groups**

Comparison of A74704 and its diol equivalent



A74704

IC_{50} 3 nM



Diol equivalent

IC_{50} 0.22 nM

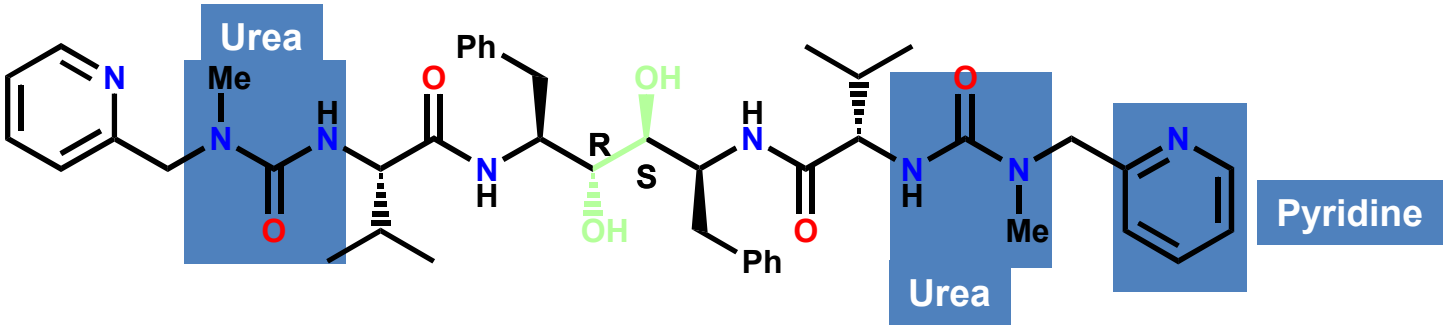
Notes

- **Diol equivalent of A74704 shows 10-fold greater activity**
- **Poor water solubility**
- **Terminal portions are exposed to solvent (crystal structure)**
- **Possible to add more polar groups to increase solubility**

2. Ritonavir and Lopinavir

Drug design

A77003



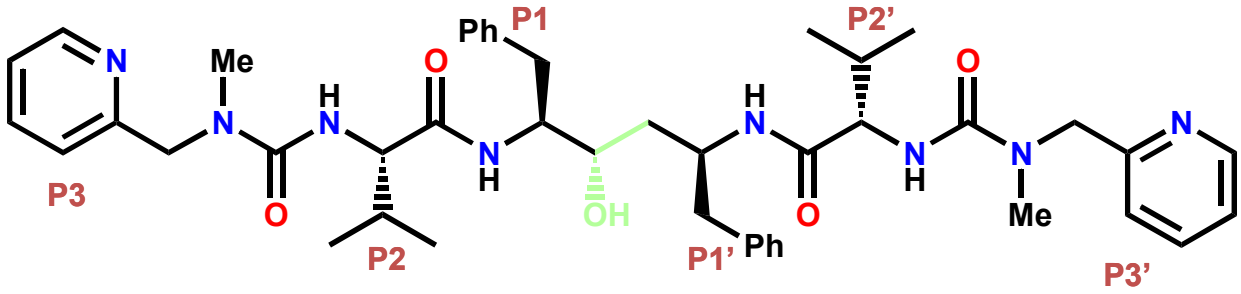
Notes

- **Pyridines and ureas increase polarity and water solubility**
- **Poor oral bioavailability**
- **Entered clinical trials as an intravenous agent**
- **Does not bind as predicted by molecular modelling**
- **Asymmetric binding is observed in the crystal structure**
- ***R*-OH forms two hydrogen bonds to both catalytic aspartates**
- ***S*-OH forms only one hydrogen bond**
- **Remove *S*-OH to avoid energy penalty involved in desolvation**

2. Ritonavir and Lopinavir

Drug design

A78791

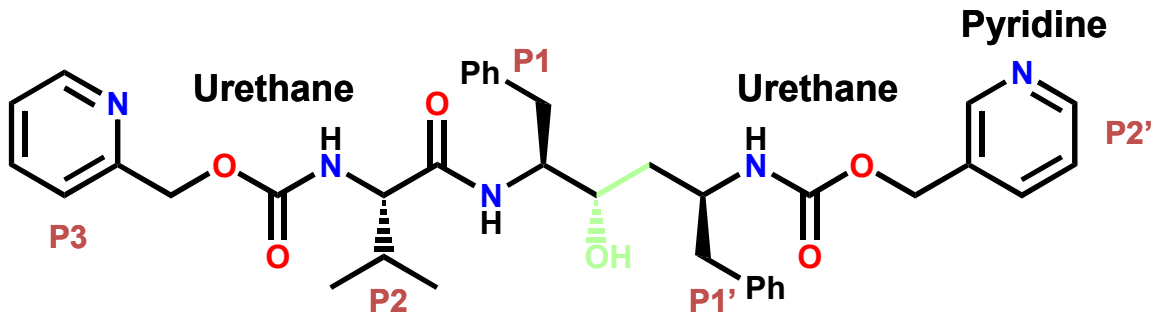


- Improved activity (K_i 17 pM)
- Similar binding mode to A 77003

2. Ritonavir and Lopinavir

Drug design

A80987



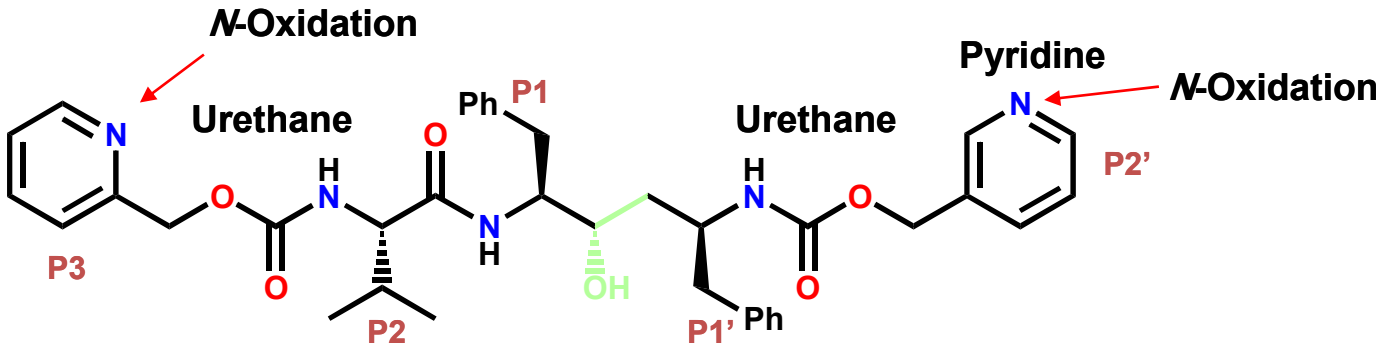
Notes

- **Modifications aimed at varying molecular weight, aqueous solubility and hydrogen bonding**
- ***N*-Methylureas found to be good for water solubility and oral bioavailability**
- **Urethanes good for plasma half life and potency**
- **Allows for fine tuning**
- **Urea replaced by urethane, valine removed from right hand segment**
- **Molecule no longer symmetrical, but smaller**
- **Retained activity and improved oral bioavailability**
- **Relatively short plasma lifetime**
- **Binds strongly to plasma proteins**

2. Ritonavir and Lopinavir

Drug design

A80987



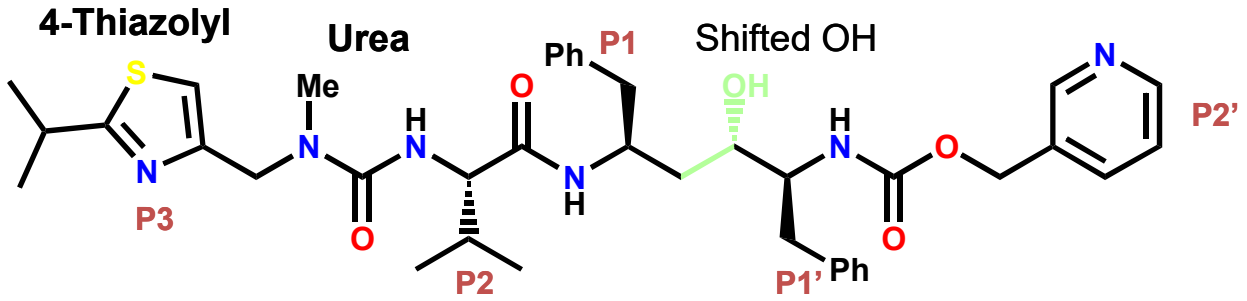
Notes

- **Pyridine rings susceptible to metabolism (*N*-oxidation)**
- **Steric shields and electron-withdrawing substituents fail to block metabolism**
- **Replace pyridine rings with alternative heterocycles (bio-isosteres)**

2. Ritonavir and Lopinavir

Drug design

A83962



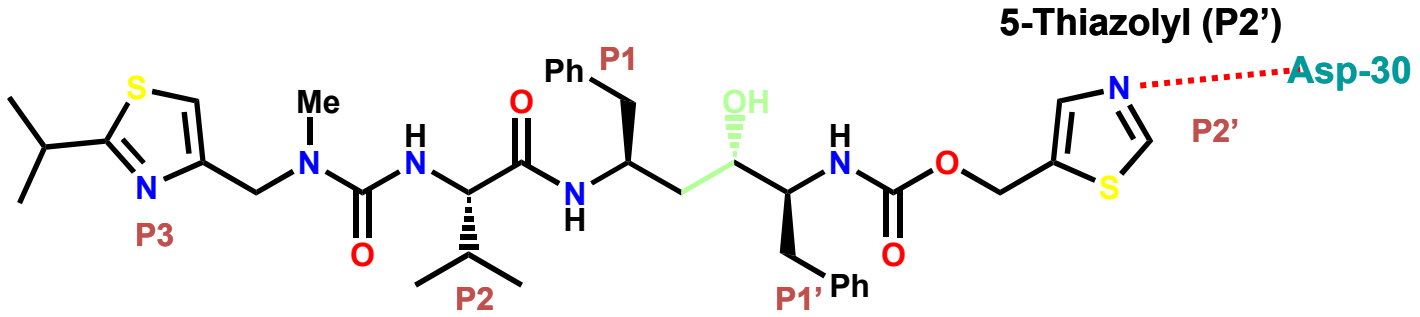
Notes

- **Thiazolyl ring used as a bio-isostere for the pyridine ring (P3)**
- **Thiazolyl ring is bad for water solubility**
- **Replacing a urethane group with *N*-methyl urea restores water solubility**
- **Activity improved by having an alkyl substituent on the thiazolyl ring and by shifting the OH group**
- **A83962 shows 8-fold increase in activity relative to A80987**

2. Ritonavir and Lopinavir

Drug design

Ritonavir



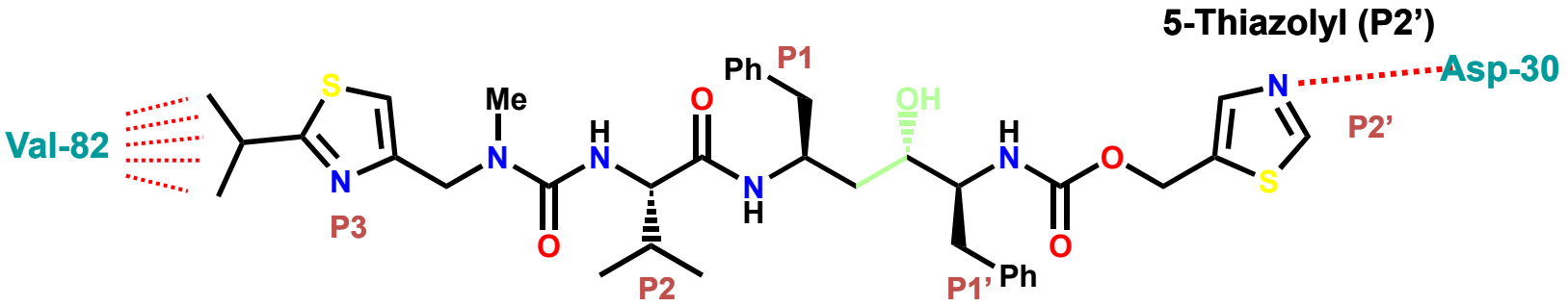
Notes

- **Thiazolyl ring is used as a bio-isostere for the pyridine ring (P2')**
- **Improved activity and better oral bioavailability**
- **Thiazolyl nitrogen forms a hydrogen bond to Asp-30**
- **20 x more stable to metabolism than A80987**
- **Therapeutic levels last 24 hours following oral administration**

2. Ritonavir and Lopinavir

Drug design

Ritonavir



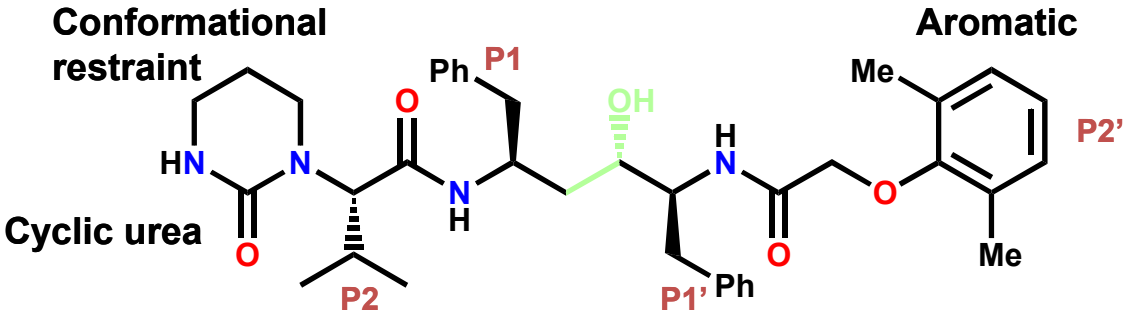
Notes

- Drug resistance arises when ritonavir is used alone
- Due to mutation of Val-82 to Ala, Thr or Phe
- Disrupts an important hydrophobic interaction between Val-82 and the isopropyl group

2. Ritonavir and Lopinavir

Drug design

Lopinavir



Notes

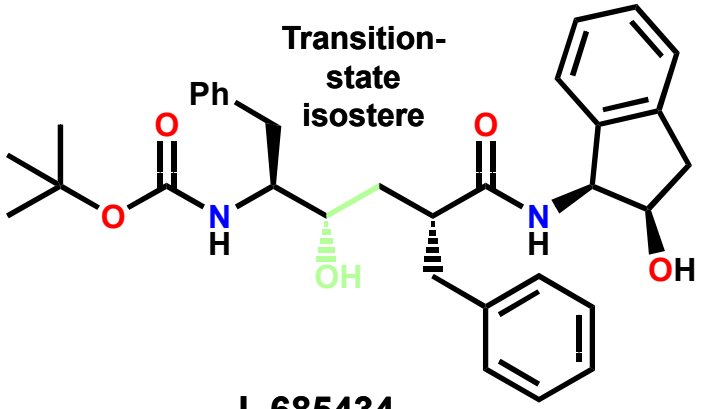
- P3 thiazolyl group removed and replaced with a cyclic urea
- Permits enhanced hydrogen bonding interactions with the S2 subsite
- Compensates for the loss of bonding interactions due to the thiazolyl group
- No interaction with Val-82
- Active against ritonavir-resistant strains

3. Indinavir

Drug design

Designed by Merck using a hybridisation strategy

- **Link one half of one inhibitor with one half of another**
- **Takes advantage of the symmetrical nature of the active site**
- **Hybrid formed from P' halves of saquinavir and L 685434**

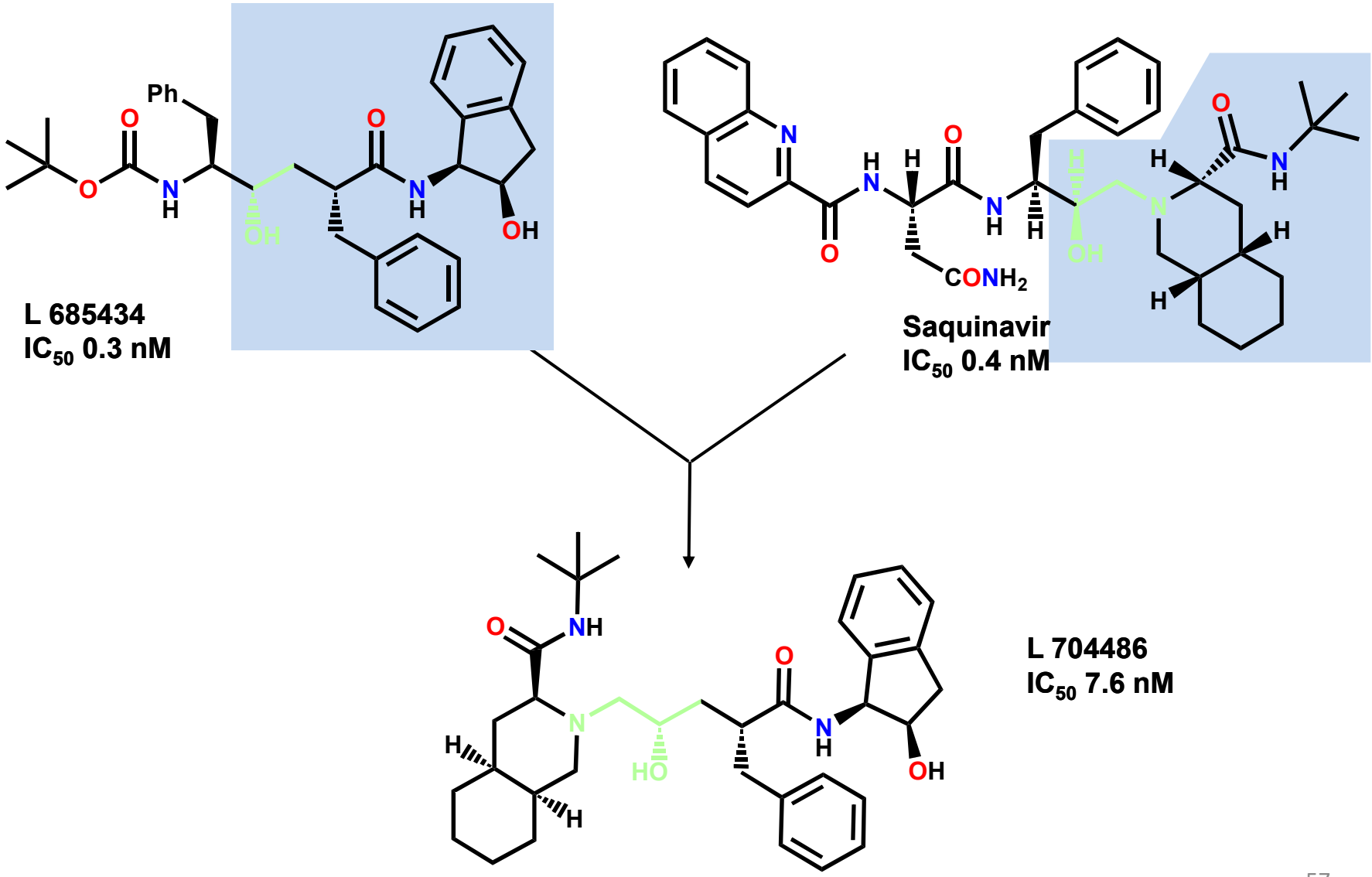


L 685434

- **Contains hydroxyethylene transition-state isostere**
- **Potent inhibitor (IC_{50} 0.3 nM)**
- **Poor bioavailability**
- **Liver toxicity**

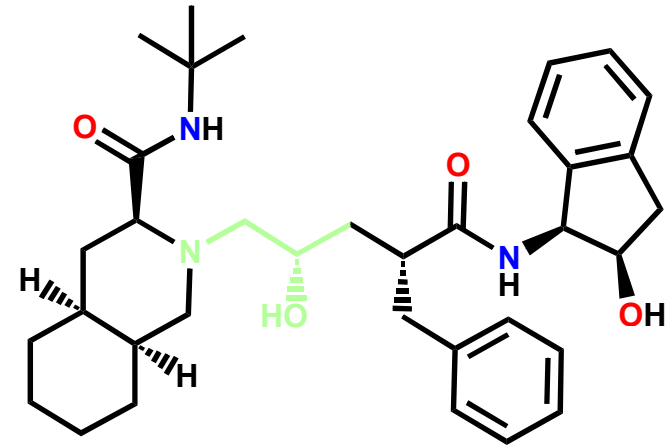
3. Indinavir

Drug design



3. Indinavir

Drug design



L 704486
IC_{50} 7.6 nM

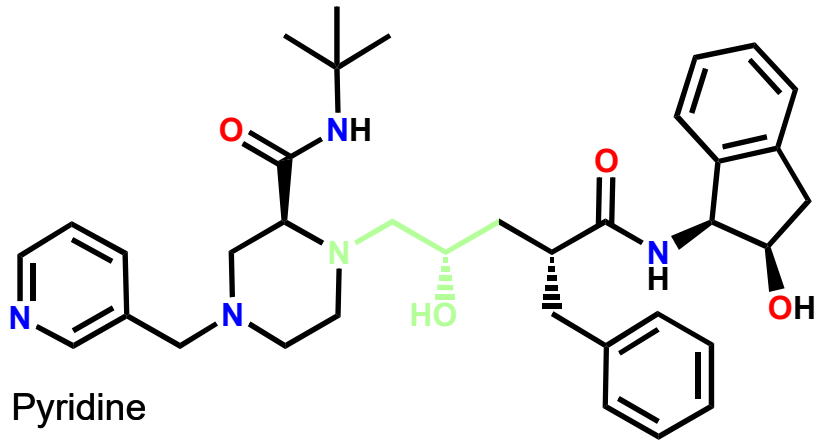
P' from saquinavir

P' from L685434

- **P' half of saquinavir is good for water solubility**
- **P' half of L685434 lacks peptide character**
- **L 704486 is less active but still potent**
- **Oral bioavailability 15%**

3. Indinavir

Drug design



Pyridine

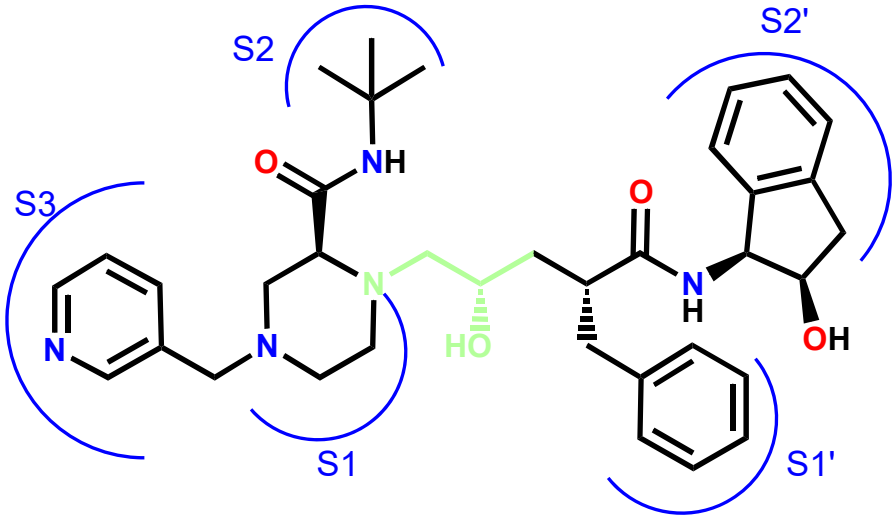
Indinavir
IC_{50} 0.56 nM
K_i 0.34 nM
EC_{95} 0.10 μM

Notes

- **P half of L704486 is modified**
- **Decahydroisoquinoline ring is replaced with a piperazine ring**
- **Better water solubility and oral bioavailability**
- **Allows further substitution. Pyridine ring was introduced**
- **Pyridine ring is lipophilic and interacts with the lipophilic subsite S3**
- **Pyridine ring contains nitrogen**
- **Improves water solubility and oral bioavailability**
- **Potent inhibitor with negligible activity against mammalian proteases**
- **Less highly bound to plasma proteins compared to saquinavir**
- **Reached market in 1996**

3. Indinavir

Binding interactions



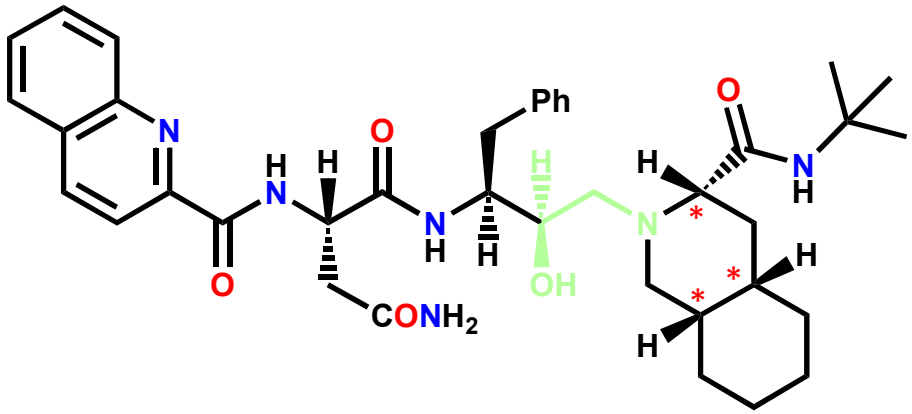
4. Amprenavir and darunavir

Notes

•Designed by Vertex Pharmaceuticals

•Designed as a non-peptide-like protease inhibitor

•Lead compound used was saquinavir



Saquinavir

• Squinavir suffers from high MWt. and high peptide character

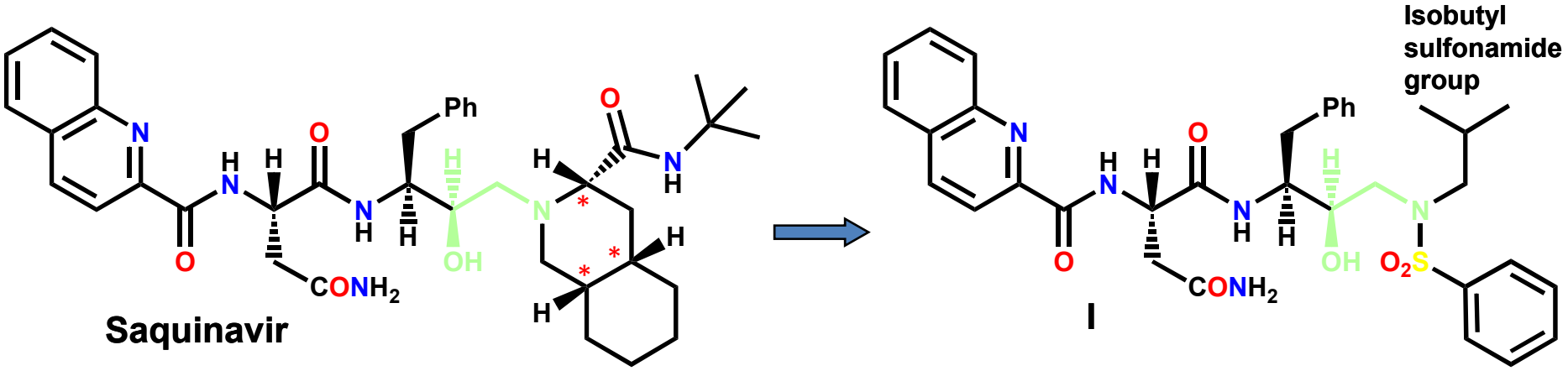
•Bad for oral bioavailability

•Six asymmetric centres present

•Three asymmetric centres in the decahydroisoquinoline ring)

4. Amprenavir and darunavir

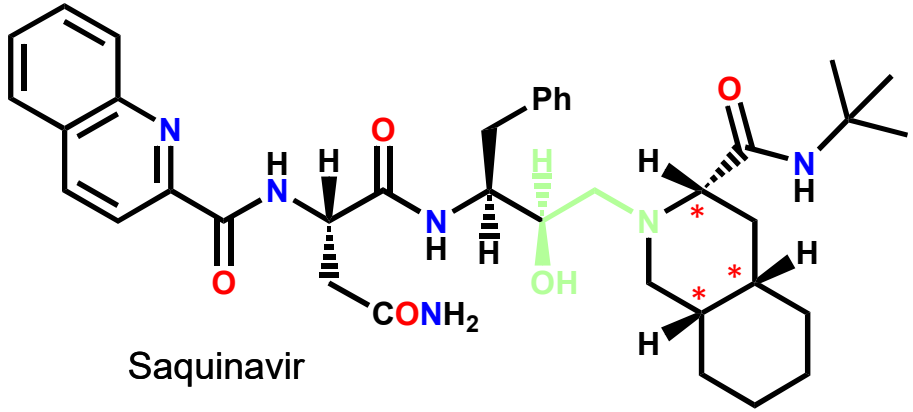
Drug design

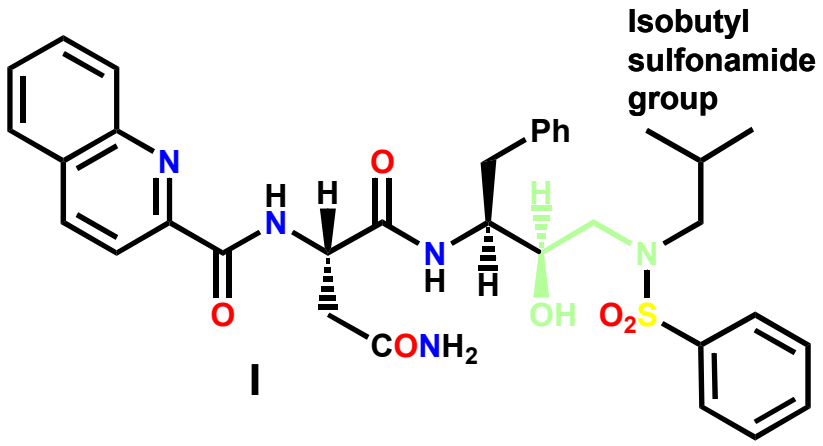


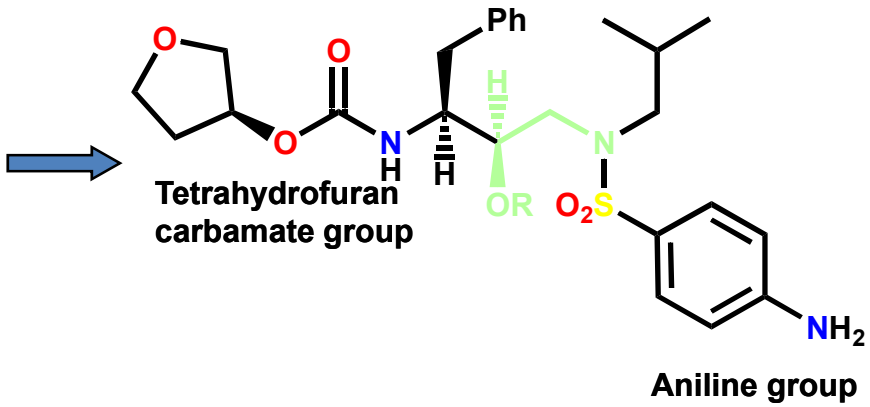
- P' half of saquinavir is replaced with an isobutyl sulfonamide group
- Three asymmetric centres are removed
- Permits easier synthesis of analogues

4. Amprenavir and darunavir

Drug design



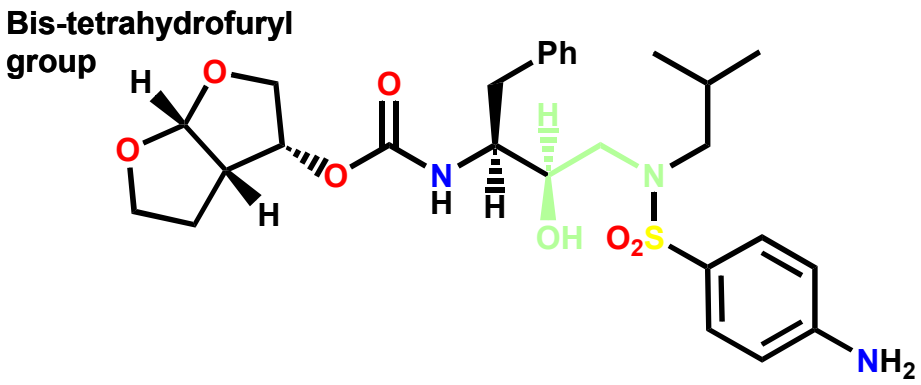




Amprenavir (R=H) (IC_{50} 12-80 nM)
Fosamprenavir (R=Phosphate)

- P half is replaced with a tetrahydrofuran carbamate group
- Good binding group for the S2 subsite
- Aniline group added for water solubility
- Good oral bioavailability (40-70%)
- 90% plasma protein bound
- Fosamprenavir is the phosphate prodrug

4. Amprenavir and darunavir



Darunavir

Notes

- **Bis-tetrahydrofuryl ring is a better binding group for the hydrophobic S2 subsite**
- **Fills the S2 subsite more fully**
- **Ring oxygens form hydrogen bonds to the protein backbone rather than amino acid side chains**
- **Mutations are less likely to lead to resistance**

ANTIVIRAL DRUGS
Agents for the treatment of influenza

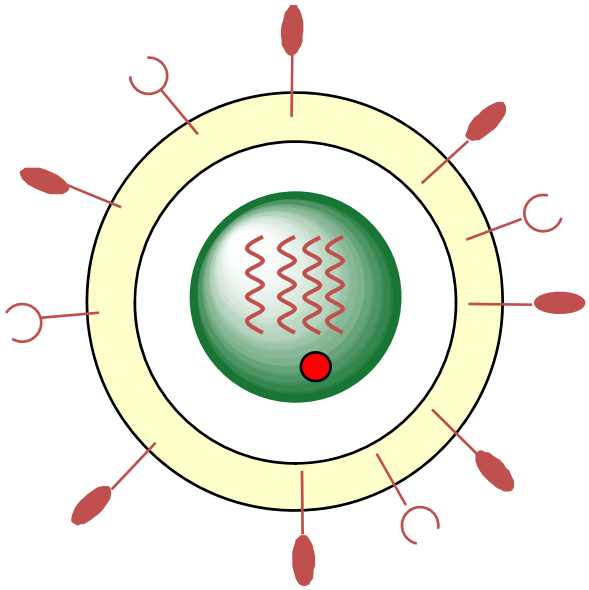
1. Influenza

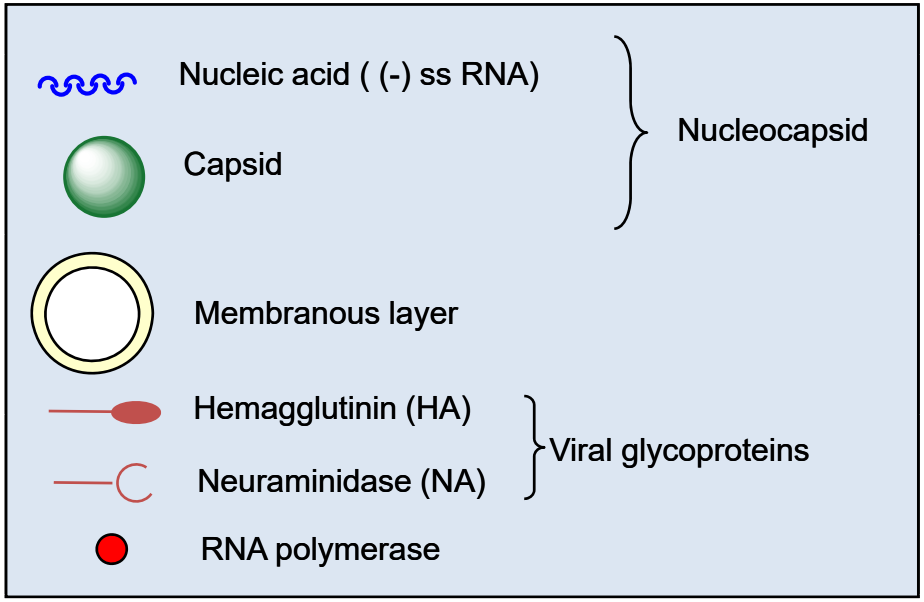
Notes

- Respiratory disease caused by the flu virus
- Airborne transmission
- Infects epithelial cells of airways
- 20 million deaths in 1918-19 Spanish flu pandemic
- Asian flu (1957); Hong Kong flu (1968); Russian flu (1977)
- 1997 outbreak in Hong Kong (6 deaths from 18 infected)
- Humans, poultry and pigs living in close proximity encourage species crossover of infections

2. The Flu Virus

Structure





Notes

- RNA virus, (-) single stranded RNA as genetic material and a viral enzyme RNA polymerase
- Surrounding the nucleocapsid there is a membrane envelope derived from host cells contains 2 viral glycoproteins called nueraminidase “NA” & haemagglutinin “HA”

2. Haemagglutinin & Neuraminidase

Notes

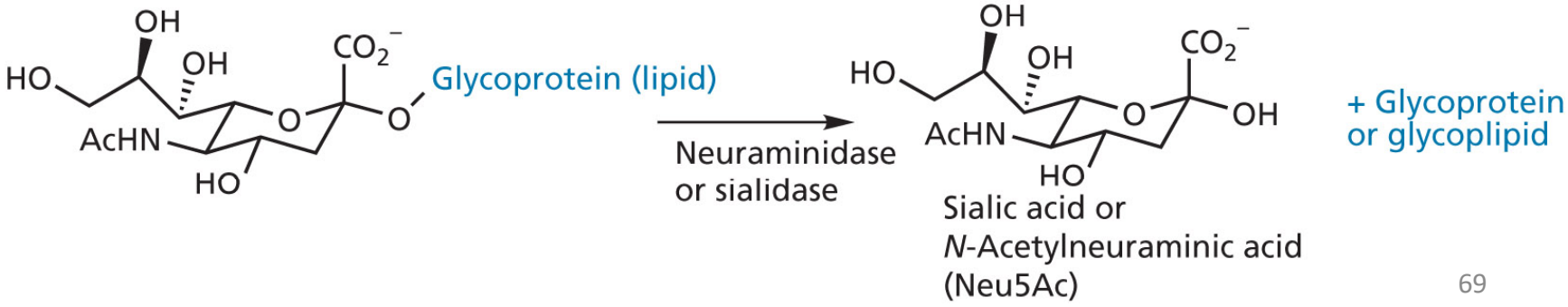
- **Viral glycoprotein**
- **Spike-like objects projecting 10 nm from surface**
- **Crucial to adsorption**
- **Binds to cellular glycoconjugates containing sialic acid**
- **Allows flu virus to recognise and enter host cells**

➢ In order to reach the epithelial host cells of the upper respiratory tract, the virus has to negotiate a layer of protective mucus.

➢ The mucosal secretions are rich in glycoproteins & glycolipids which bear a terminal sugar substituent called **sialic acid** "also called N-acetylneuraminic acid".

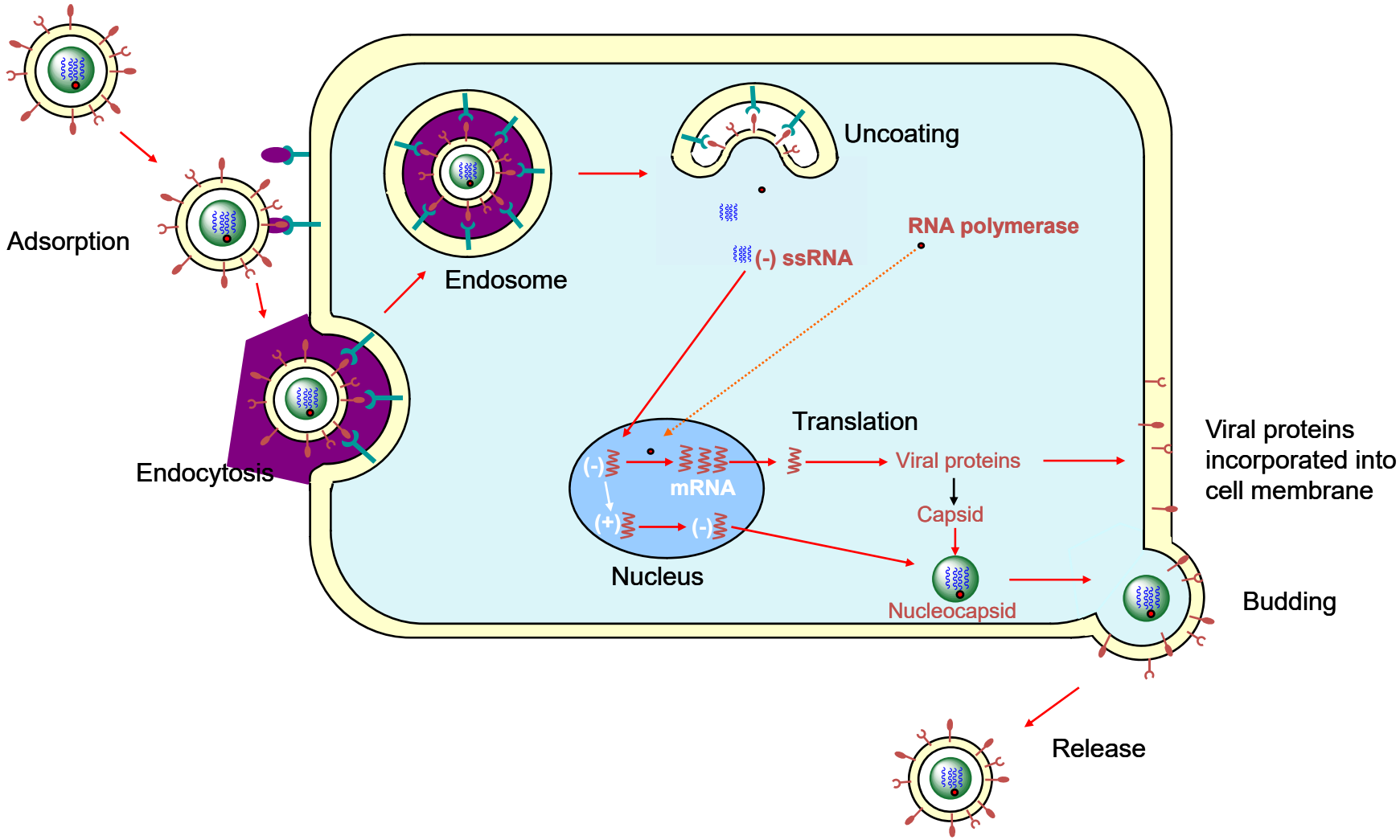
➢ Neuraminidase "also called sialidase" is an enzyme which is capable of cleaving the sialic acid sugar moiety from these glycoproteins and glycolipids thus degrading the mucus layer & allowing the virus to reach the surface of epithelial cells.

➢ Enzyme inhibitors block release and prevent infection of new host cells



3. The Flu Virus

Life cycle



4. Antigens and vaccination

- Since HA & NA act as antigens it is possible to prepare vaccines which allow the body to gain immunity from the flu virus.

- Vaccines lose protection with time due to the flu virus is adapt at varying the amino acids present in HA & NA thus making these antigens unrecognizable to the antibodies-process called **antigenic variation**.

- there are nine antigenic variants of NA.

- There are three groups of flu virus, classified as A, B & C. Antigenic variation does not appear to take place with influenza C, & occurs slowly with influenza B.

- With influenza A variation occurs almost yearly.

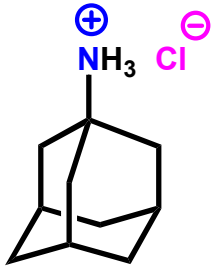
- if the variation is small, it is called **antigenic drift**. If it is large, it is called **antigenic shift** and it is this that can lead to the more serious epidemic and pandemics.

- there are two influenza A virus subtypes which are epidemic in humans-those with H1N1, H3N2, H5N1 antigens “H & N stand for HA & NA respectively”.

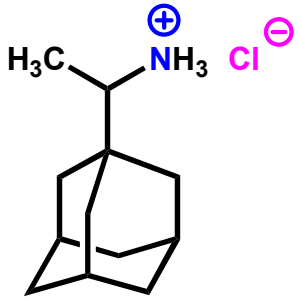
- Vaccination is the preferred method for preventing flu.

- Antiviral drugs are important if vaccination fails.

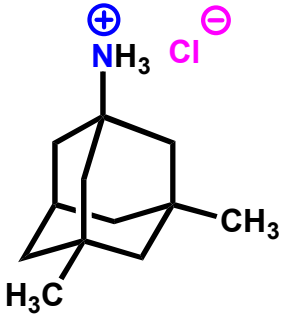
5. Ion channel disrupters



Amantadine



Rimantadine



Memantine

Adamantanes

- Earliest effective drugs versus flu
- Block a viral ion channel protein at low concentration
- Buffer the pH of the endosome at high concentration
- Prevent the acidic conditions required for the viral membrane to fuse with the endosome membrane
- Resistance problems and side effects

6. Neuraminidase inhibitors

- Neuraminidase is a mushroom shaped tetrameric glycoprotein anchored to the viral membrane by a single hydrophobic sequence.

- X-ray crystallographic studies have shown that the active site is a deep pocket located centrally on each protein subunits.

- There are two main types of the enzyme "corresponding to the influenza A & B" & various subtypes.

- Due to the ease with which mutations occur, there is a wide diversity of amino acids making up the various types & subtypes of the enzyme.

- However, the 18 amino acids making up the active site itself are constant, this will make the possibility to design inhibitor that inhibit all strains of the flu virus.

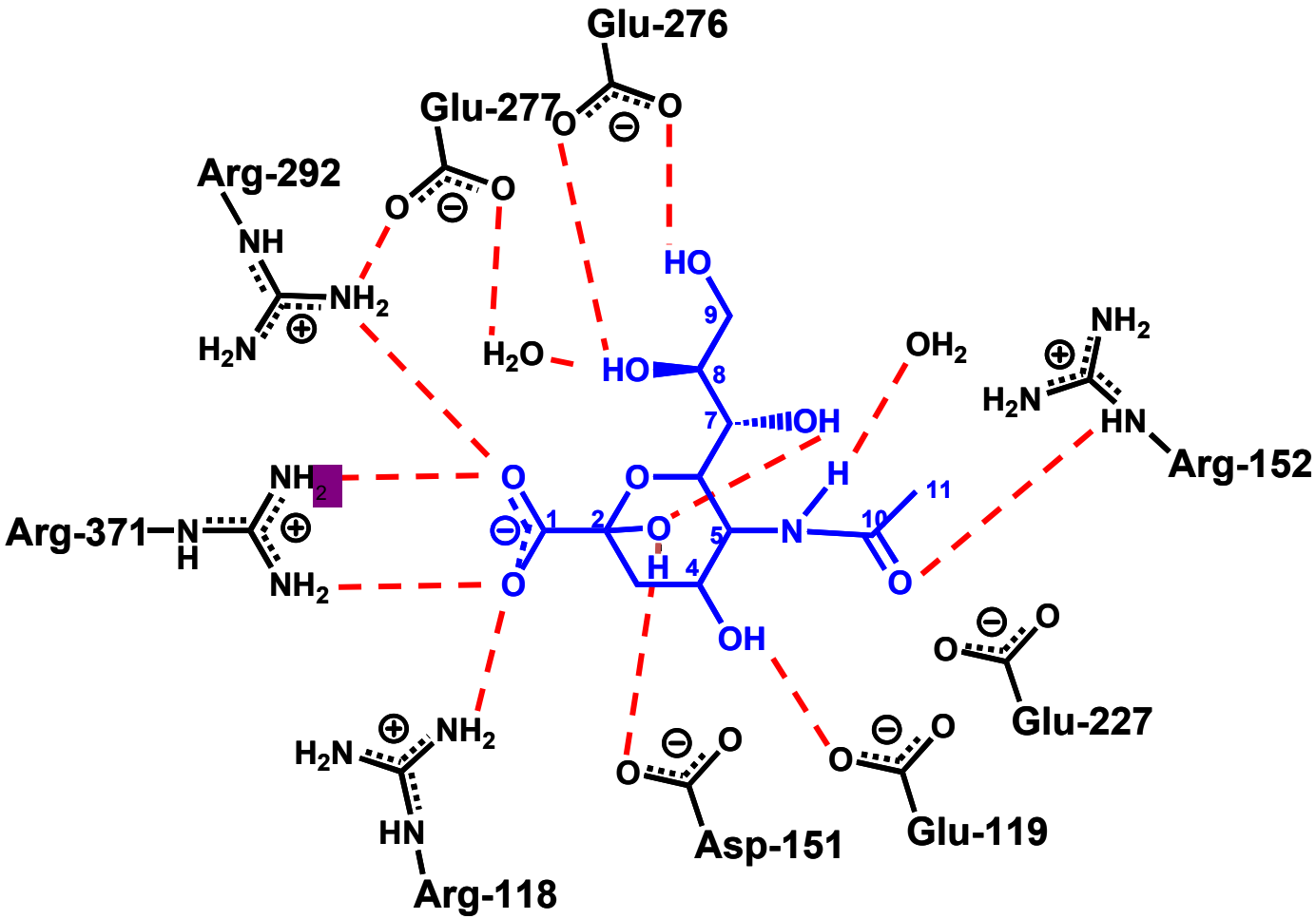
- it has been observed that the active site is quite different in structure from the active sites of comparable bacterial or mammalian enzymes, so there is a strong possibility to design selective antiviral drugs.

- the enzyme has been crystallized with sialic acid bound to the active site and the structure determined by X-ray crystallography.

- Binding interactions identified (ionic and H-bonding).

6. Neuraminidase

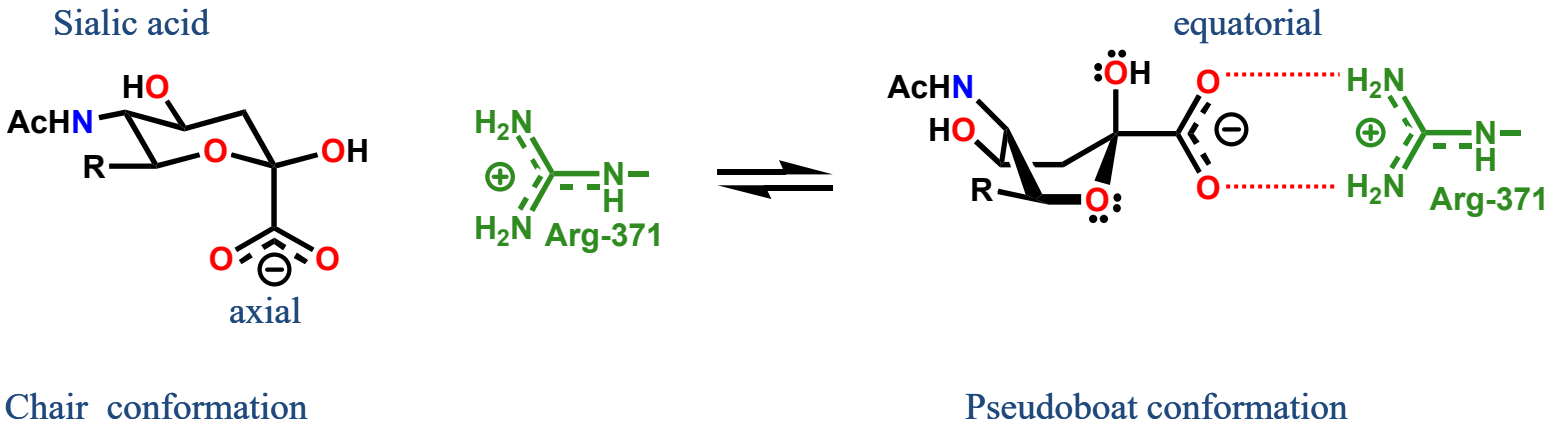
Binding interactions in the active site



6. Neuraminidase

Binding interactions in the active site

- Ionic interactions are very important
- Carboxylate group of the sialic acid interacts with 3 Arg residues
- Sialic acid is distorted to allow interactions
- Carboxylate ion is in equatorial position (pseudoboat conformation)



Chair conformation

Pseudoboat conformation

6. Neuraminidase

Binding interactions in the active site

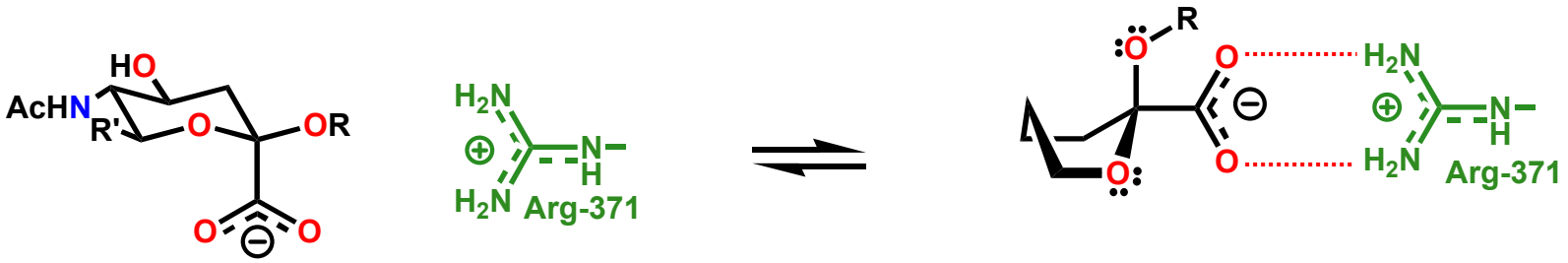
- Three other important binding groups
  - glycerol side chain
  - hydroxyl group at C-4
  - acetamido substituent

- Pyranose ring binds to floor of active site (vdw interactions)

- Hydroxyl group at C-2 is axial in the pseudoboat and forms hydrogen bonding interactions to Asp-151 as well as an intramolecular hydrogen bond to the hydroxyl gp at C-7.

6. Neuraminidase

Enzymatic mechanism of hydrolysis “4 steps”



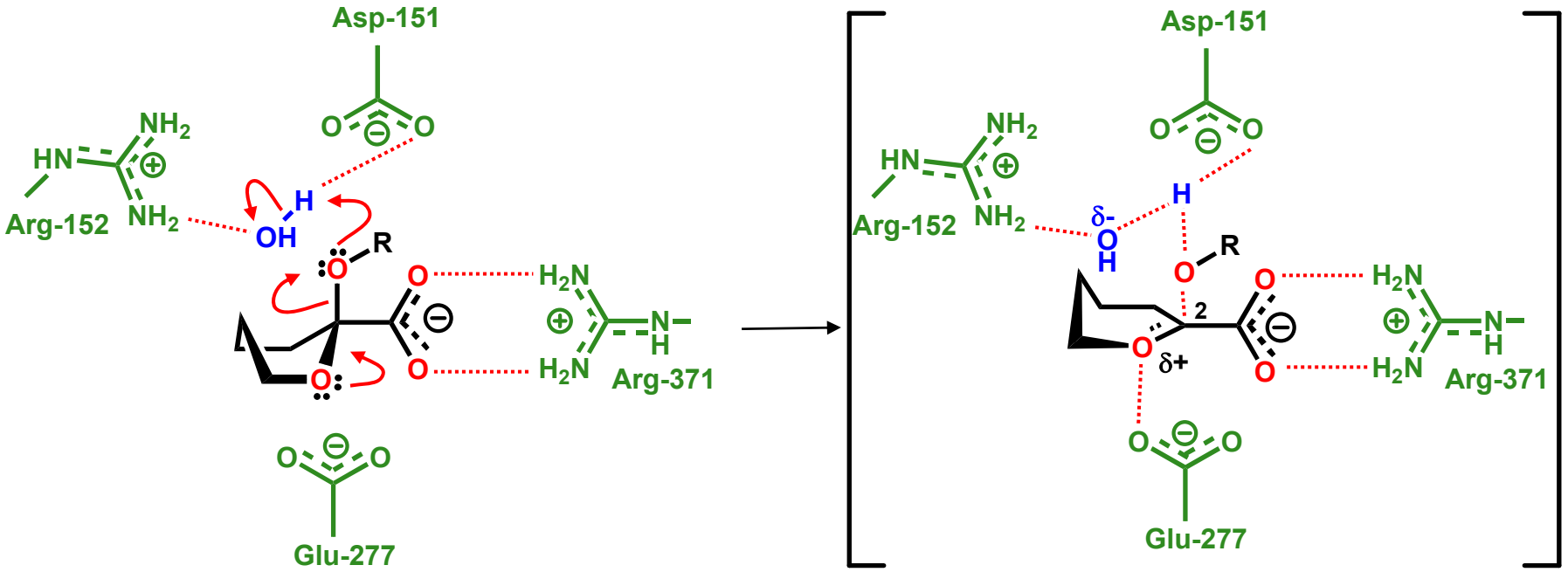
Sialoside

Stage 1 - Binding

- Binding of a sialoside substrate (glycolipid or glycoprotein)
- Sialic acid moiety adopts a pseudoboat conformation
- Less stable conformation allows greater binding interactions

6. Neuraminidase

Enzymatic mechanism of hydrolysis

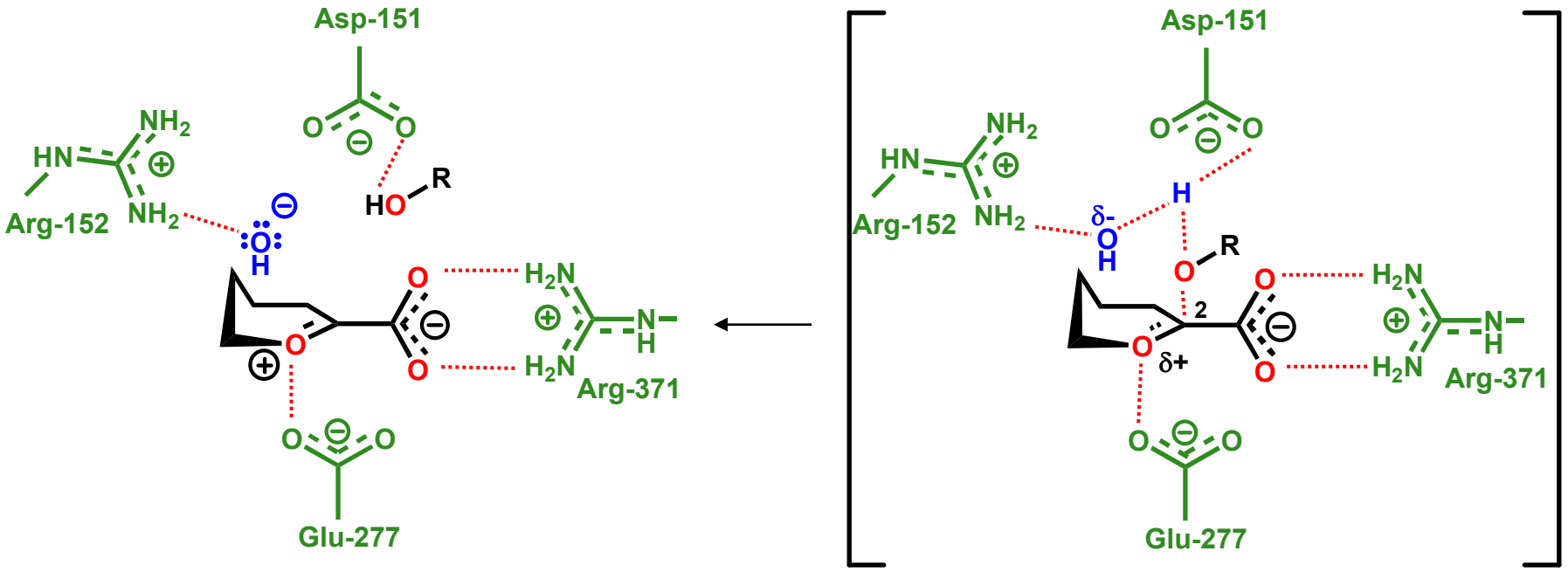


Stage 2 - Proton donation from activated water

- Formation of endocyclic sialosyl cation transition-state intermediate
- Asp-151 and Arg-152 activate water and aid the protonation mechanism
- Glu-277 stablises the partial charge on the glycosidic oxygen

6. Neuraminidase

Enzymatic mechanism of hydrolysis

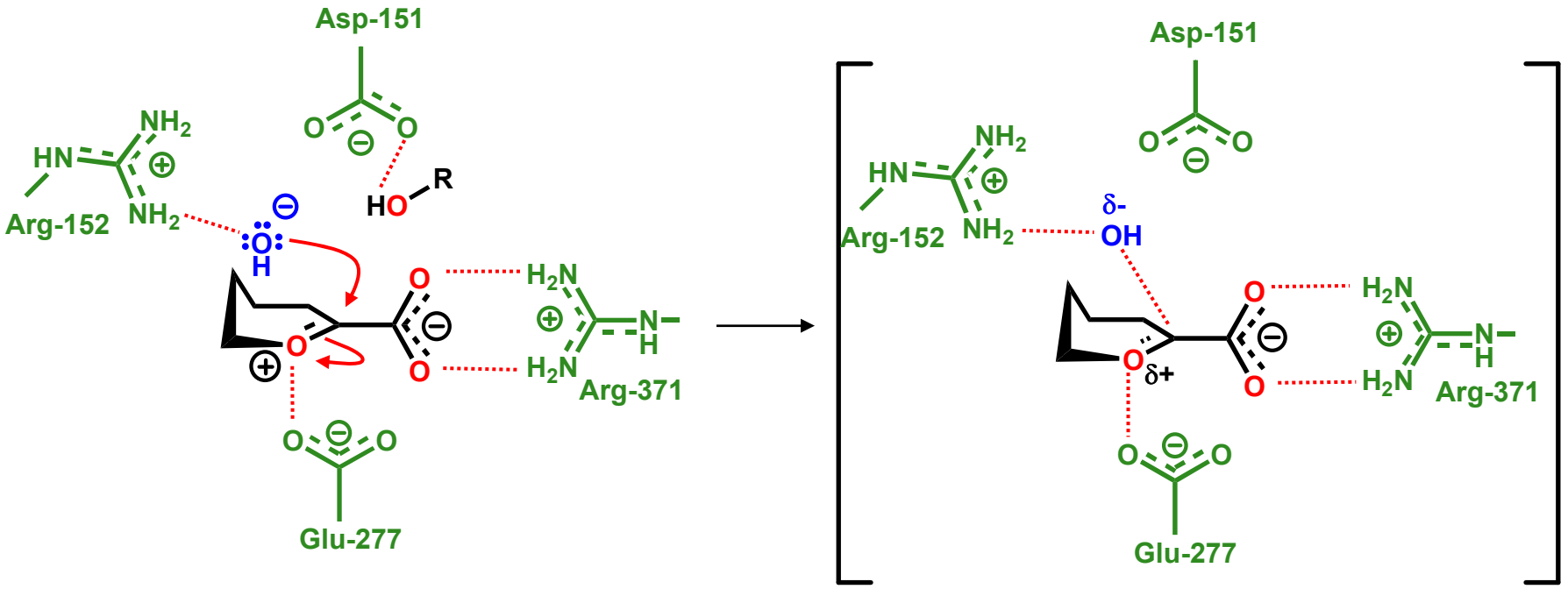


Stage 2 - Proton donation from activated water

- **HOR is a better leaving group than $^{-}$OR**
- **Glu-277 stablises charge on glycosidic oxygen**

6. Neuraminidase

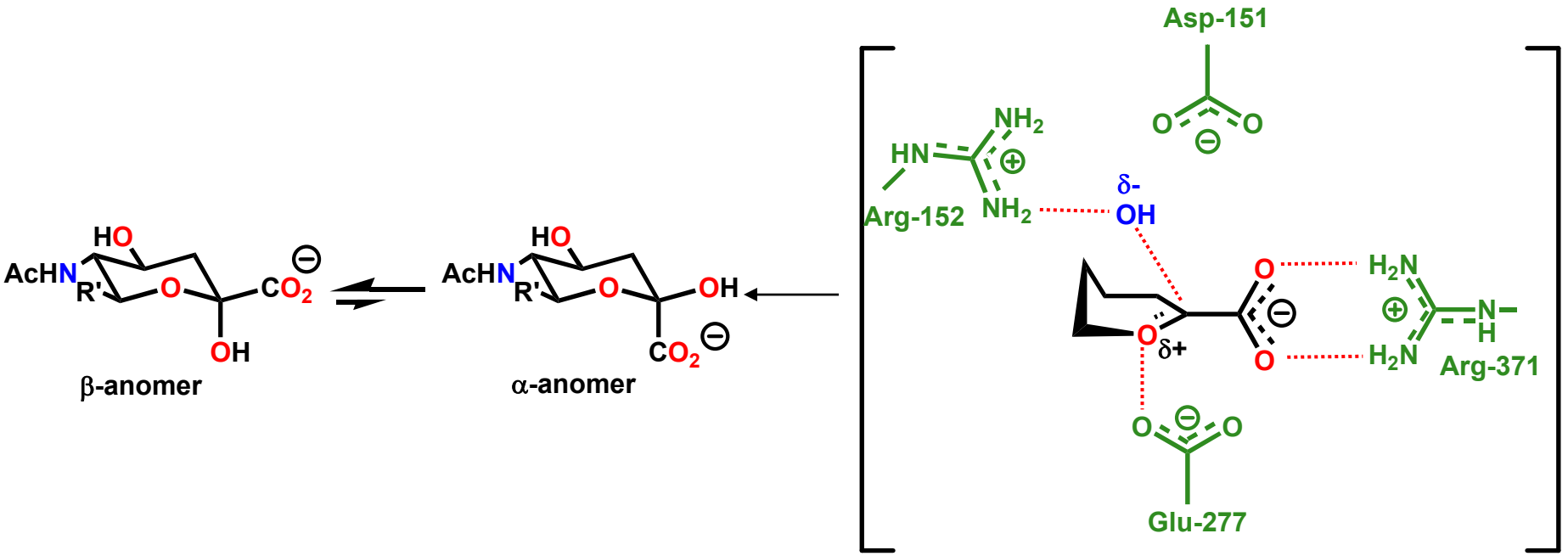
Enzymatic mechanism of hydrolysis



Stage 3 - Formation of sialic acid

6. Neuraminidase

Enzymatic mechanism of hydrolysis



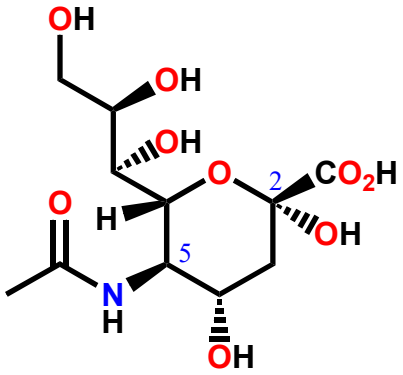
Stage 4 - Formation and release of sialic acid

α-Anomer of sialic acid is released

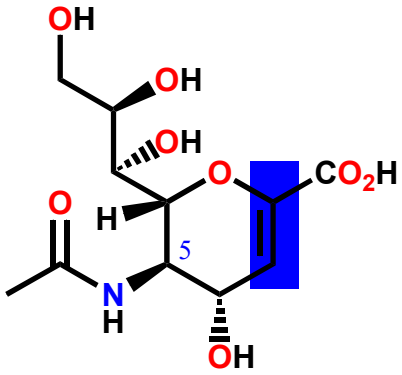
Equilibrates to the more stable β-anomer

7. Neuraminidase inhibitors - zanamivir

Lead compound - Neu5Ac2en



Sialic acid



Neu5Ac2en
K_i(M) 4x10^{-6}; IC_{50} 5-10 μM

Notes

- 2-Deoxy-2,3-dehydro-*N*-acetylneuraminic acid "Neu5Ac2en" to achieve the same transition state of sialic acid…. Planar trigonal at C2
- Transition-state inhibitor
- Loss of one H-bond interaction (No OH at C-2)
- No energy penalty involved in distorting from chair conformation
- No selectivity, inhibits also bacterial & mammalian sialidases.
- Inactive *in vivo*

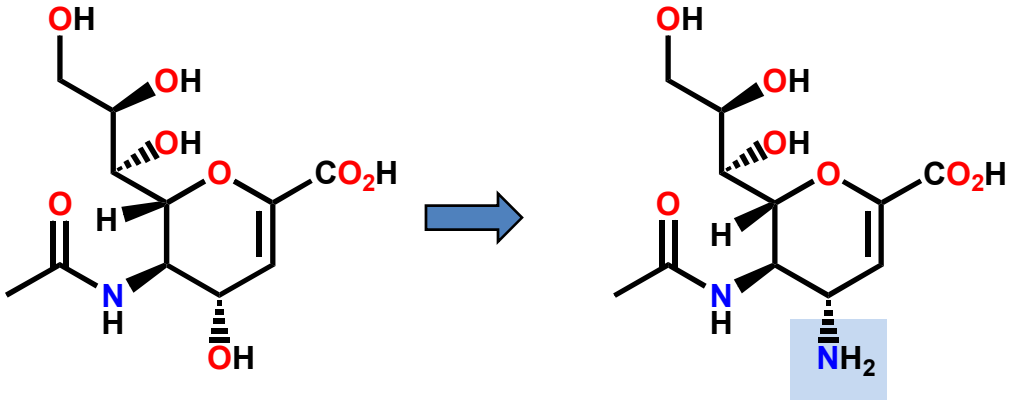
7. Neuraminidase inhibitors - zanamivir

Molecular modelling

- Lattice of grid points set up within model active site
- Probe atoms placed at each grid point
- Interactions with active site calculated and tabulated
- Carboxylate oxygen and ammonium nitrogen used to probe for ionic interactions
- Hydroxyl oxygen used to probe for hydrogen bonds
- Methyl carbon used to probe for vdw interactions
- Multi-atom probes also used
- Potential ionic interaction found for region round 4-OH of sialic acid
- Modeling studies on possible analogues carried out
- Synthesis and testing

7. Neuraminidase inhibitors - zanamivir

Analogues



Neu5Ac2en
K_i(M) 4x10^{-6}; IC_{50} 5-10 μM

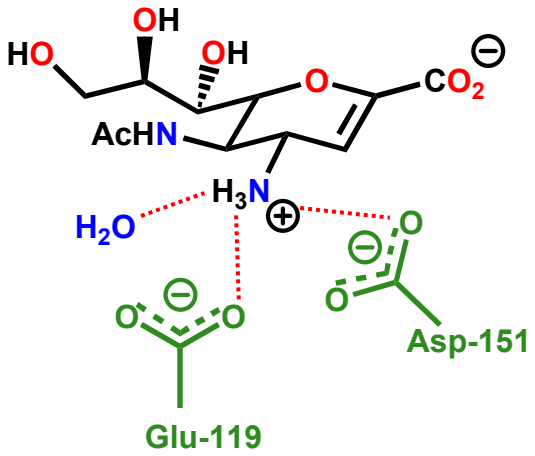
4-Amino-Neu5Ac2en
K_i(M) 4x10^{-8}

Notes

- 4-OH is replaced with a basic amine substituent
- Substituent can protonate and form ionic interactions with the active site
- 4-Amino-Neu5Ac2en is selective and active *in vivo*

7. Neuraminidase inhibitors - zanamivir

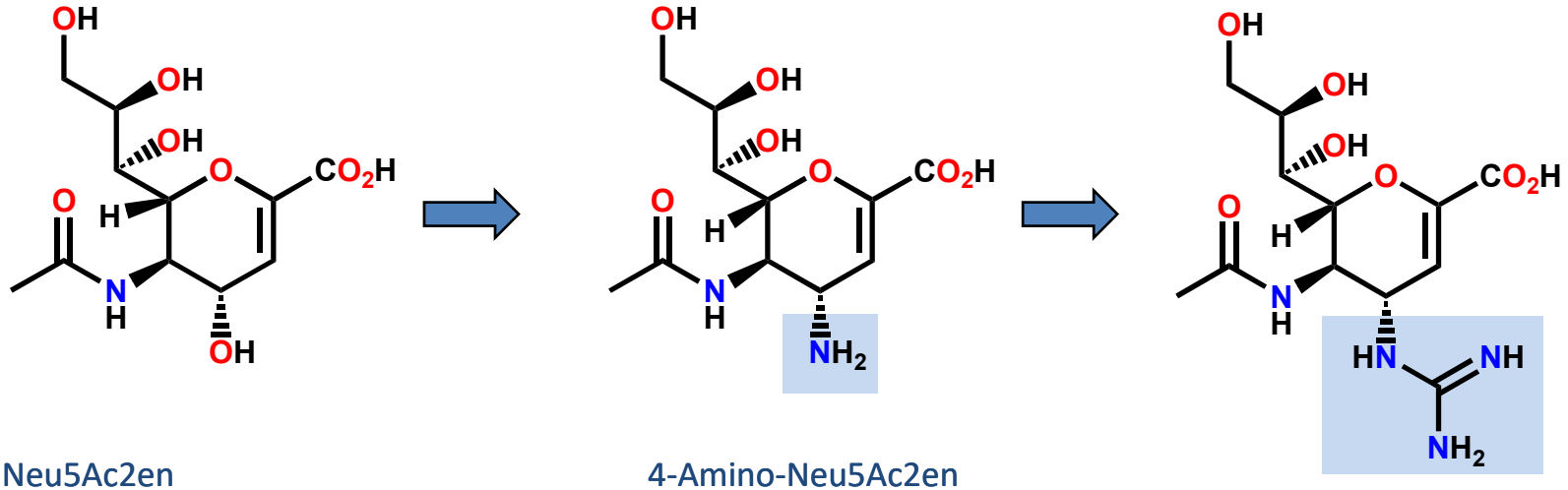
Binding interactions for 4-amino analogue



Identified by crystal structure

7. Neuraminidase inhibitors - zanamivir

Analogues



Neu5Ac2en
K_i(M) 4x10^{-6}; IC_{50} 5-10 μM

4-Amino-Neu5Ac2en
K_i(M) 4x10^{-8}

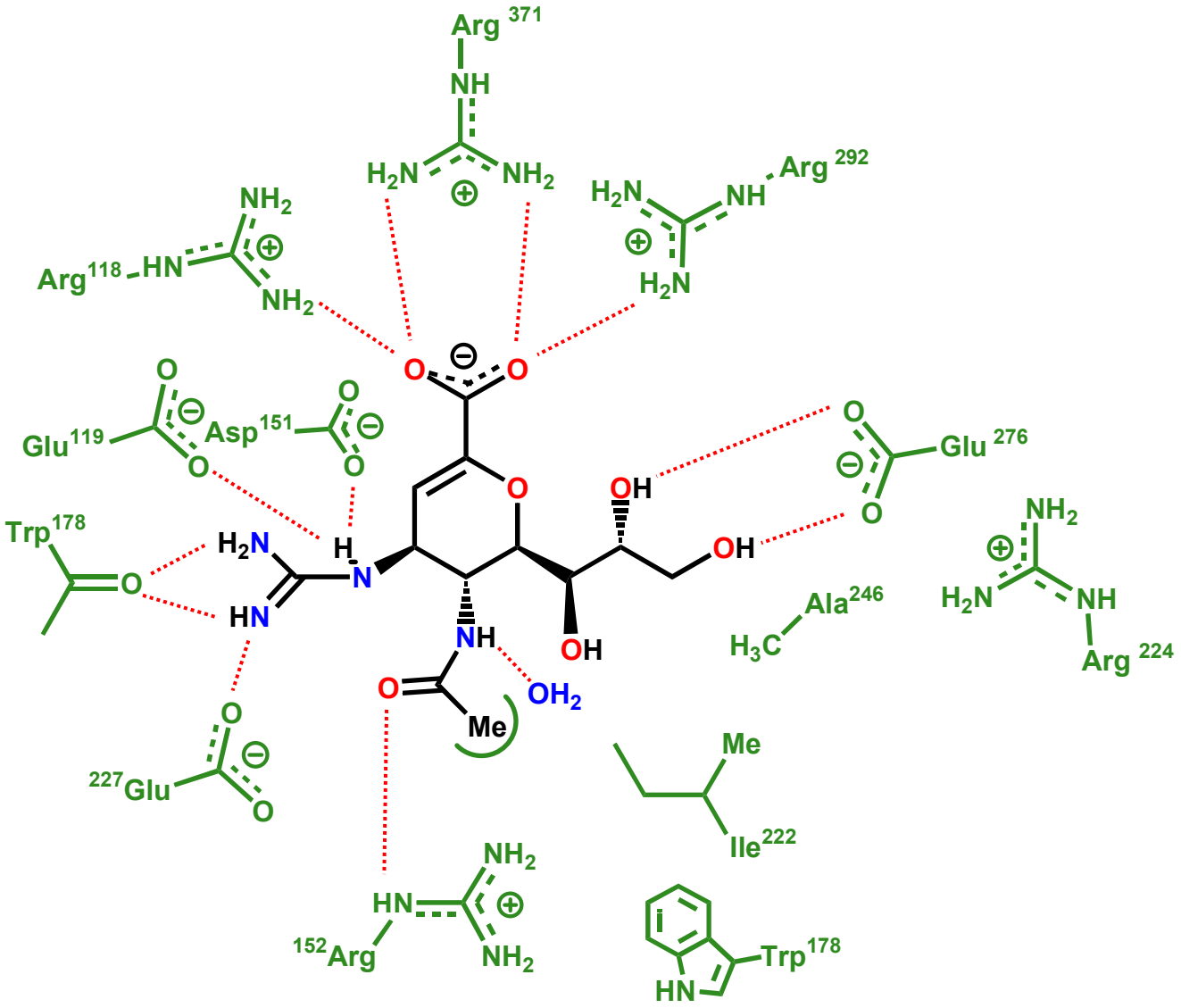
Zanamivir (Relenza)
K_i(M) 3x10^{-11}

Notes

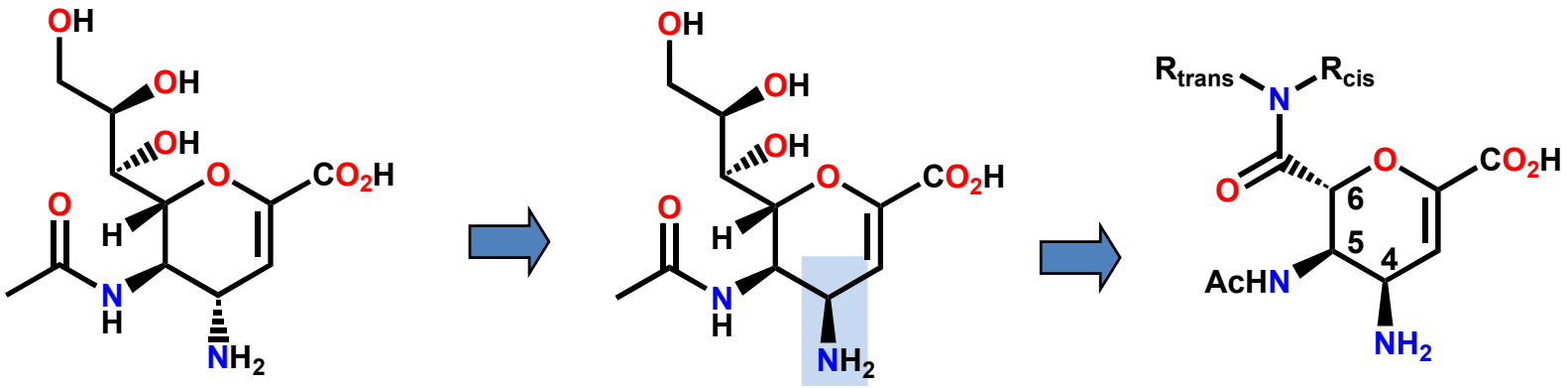
- Guanidinium group forms more H-bonds and vdw interactions
- 100-fold increase in activity
- Approved in 1999 & marketed by Glaxo Wellcome & Biota.
- Poor bioavailability - high polarity
- Administered by inhalation

7. Neuraminidase inhibitors - zanamivir

Binding interactions for zanamivir



8. Neuraminidase inhibitors: 6-carboxamides



4-Amino-Neu5Ac2en
K_i(M) 4×10^{-8}

4-Epi-amino-Neu5Ac2en
K_i(M) 3×10^{-7}

6-Carboxamides

Notes

- Change of stereochemistry at C-4 places amino group in small binding region
- Zanamivir is so polar due to the glycerol side chain, so removal of glycerol chain lowers polarity but loses binding interactions
- Carboxamide side chain retains activity
- Tertiary amides show selectivity for A form of the enzyme over B
- R_{trans} is variable, R_{cis} should be ethyl or *n*-propyl

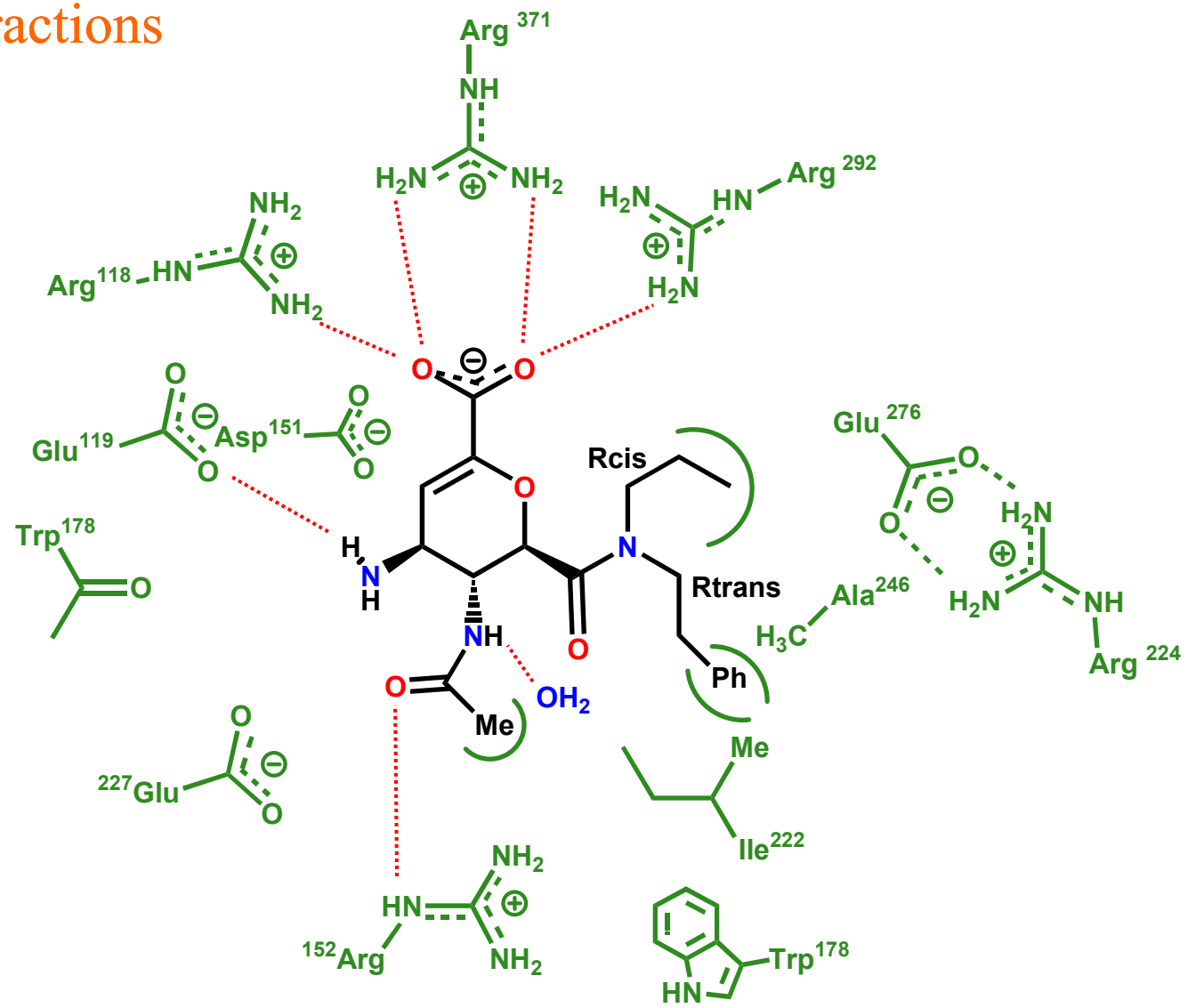
8. Neuraminidase inhibitors: 6-carboxamides

Binding interactions

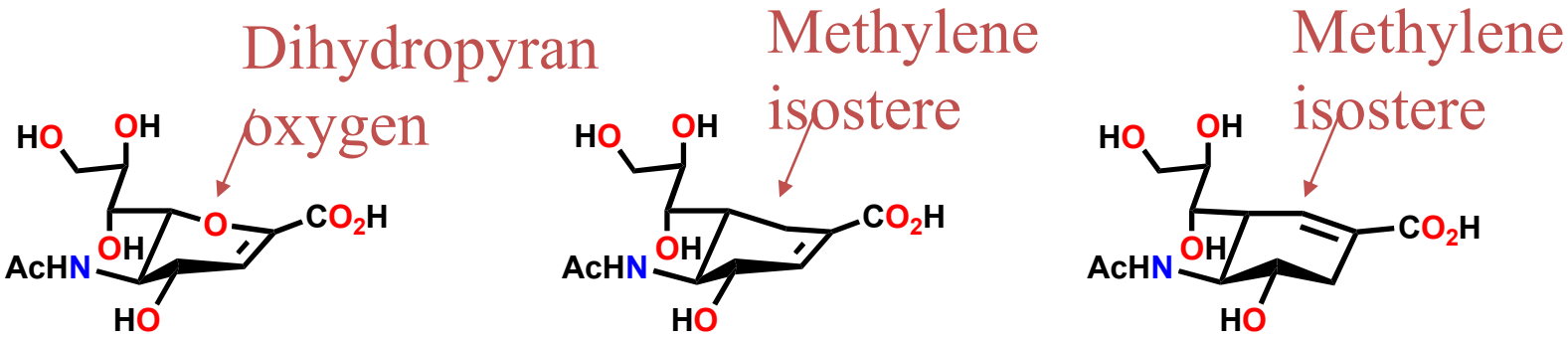
- Dihydropyran moiety binds like zanamivir
- Carboxylate ion, 4-amino group and 5-acetamido group are important binding groups
- No interaction with Glu-276 (unlike zanamivir)
- Glu-276 forms a salt bridge with Arg-224
- Reveals a small lipophilic pocket for R_{cis}
- R_{trans} fits an extended lipophilic cleft on the enzyme surface
- Formation of a salt bridge in the A form requires little distortion of the protein backbone
- Not so for the B form - implies an energy penalty

8. Neuraminidase inhibitors: 6-carboxamides

Binding interactions



9. Neuraminidase inhibitors - carbocyclics



Neu5Ac2en

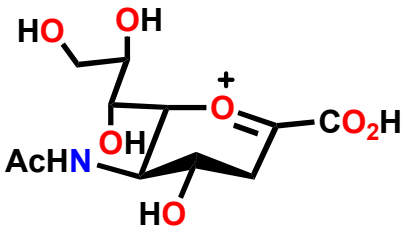
Structure I
IC_{50} 850 μM

Structure II
IC_{50} 20 μM

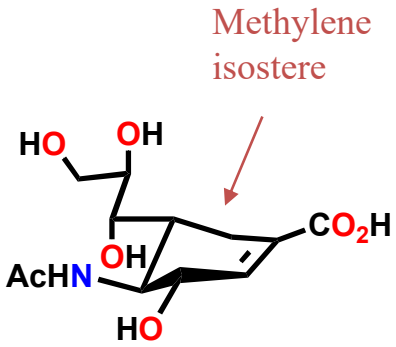
Notes

- Dihydropyran oxygen has no important binding role
- Replace with a methylene isostere - carbocyclic analogues
- Removes a polar oxygen
- Decreases polarity
- Aim is to increase oral bioavailability

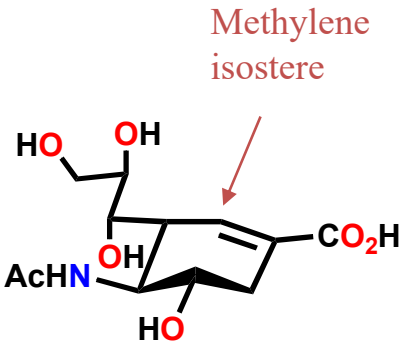
9. Neuraminidase inhibitors - carbocyclics



Reaction intermediate



Structure I
IC_{50} 850 μM

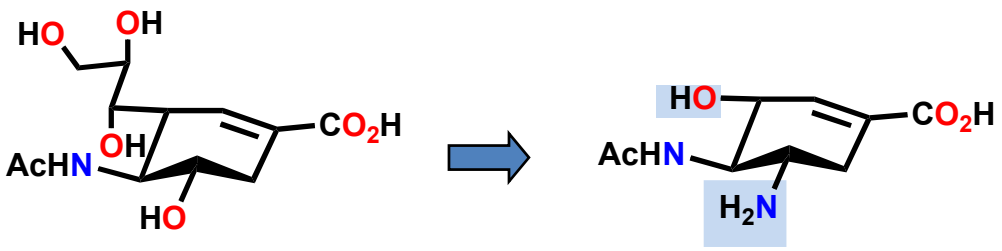


Structure II
IC_{50} 20 μM

Notes

- Structure II is 40x more potent than structure I
- Conformation of the ring is important to potency
- Structure II is closer to the conformation of the reaction intermediate

Drug design to oseltamivir



Structure II
IC_{50} 20 μM

Structure III
IC_{50} 6.3 μM

Notes

- Hydroxyl group is replaced with 4-amino (better binding interactions)
- Glycerol side chain is removed - reduces polarity
- Hydroxyl group is introduced in place of the side chain

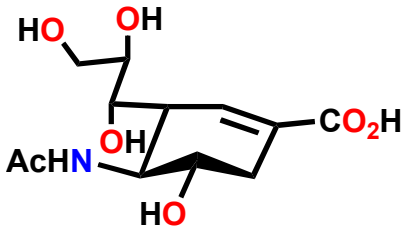
  - electron-withdrawing group

  - reduces the electron density of the double bond

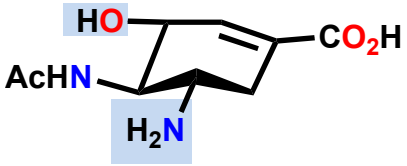
  - oxonium double bond in the transition state is electron deficient but the double bond in the carbocyclic structures is electron rich.

  - potential to generate ether analogues

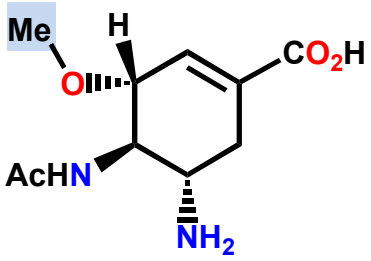
Drug design to oseltamivir



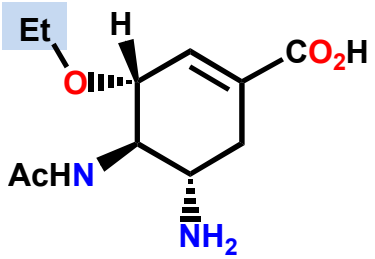
Structure II
IC_{50} 20 nM



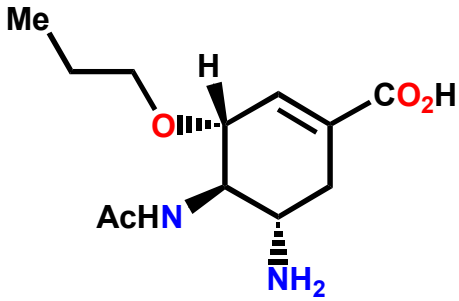
Structure III
IC_{50} 6.3 μM



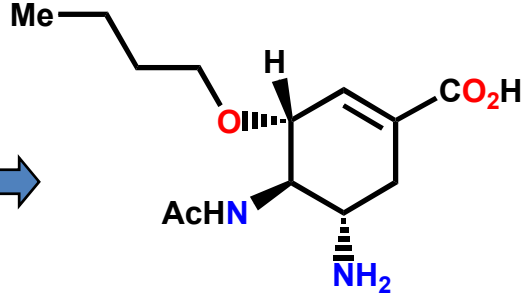
Methyl ether
IC_{50} 3.7 μM



Ethyl ether
IC_{50} 2.0 μM



Propyl ether
IC_{50} 0.18 μM

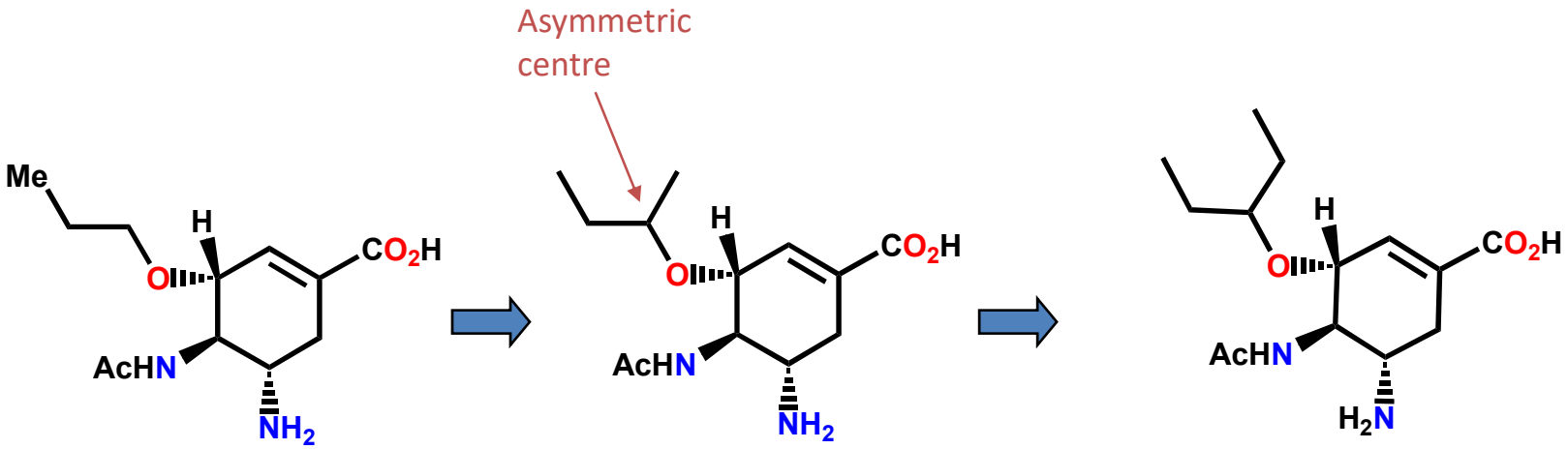


Butyl ether
IC_{50} 0.30 μM

Notes

- Alkyl ether occupies a hydrophobic region
- Increased vdw and hydrophobic interactions result in increased activity

Drug design to oseltamivir



Propyl ether
IC_{50} 0.18 μM

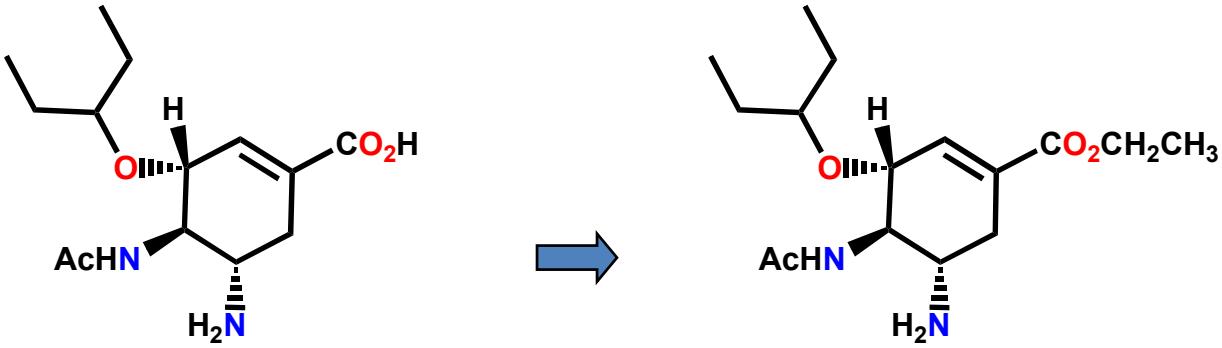
Branched ether
IC_{50} 0.01 μM

GS 4071
IC_{50} 0.001 μM

Notes

- Branching increases binding interactions and activity
- Optimum side chain = pentyloxy group
- Alkoxy side chain interacts with the region normally occupied by the glycerol side chain
- Glu-276 is reorientated (compare with carboxamides)

Drug design to oseltamivir “Tamiflu”



GS 4071
IC_{50} 0.001 μM

Oseltamivir (Tamiflu)

Notes

- Oseltamivir is the ethyl ester prodrug for GS4071
- Approved in 1999 for influenza A and B, the drug is marketed by La Roche
- Oral administration
- Converted to GS4071 by esterases in gastrointestinal tract