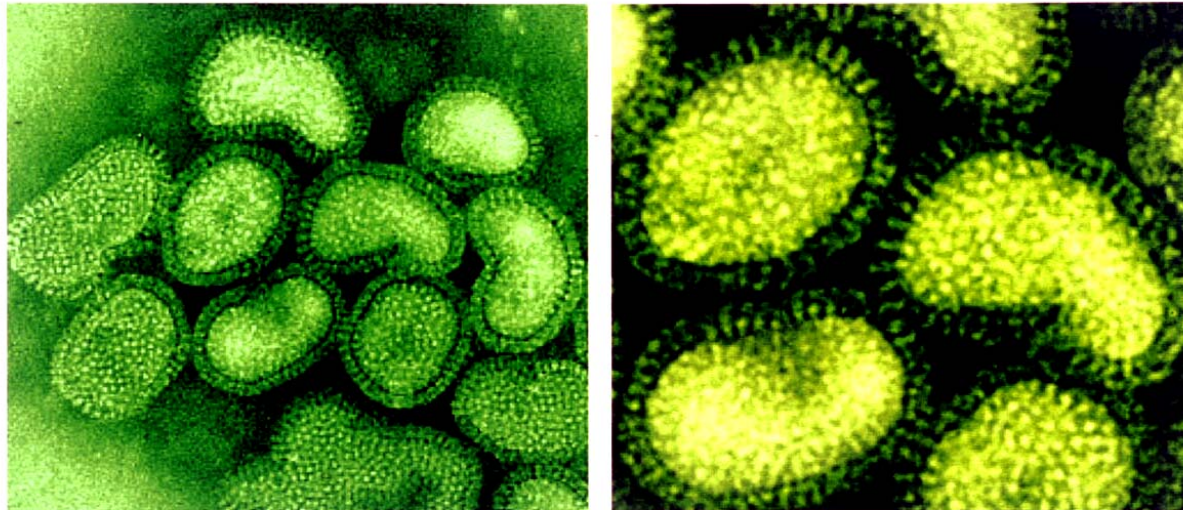


# INFLUENZA VIRUS

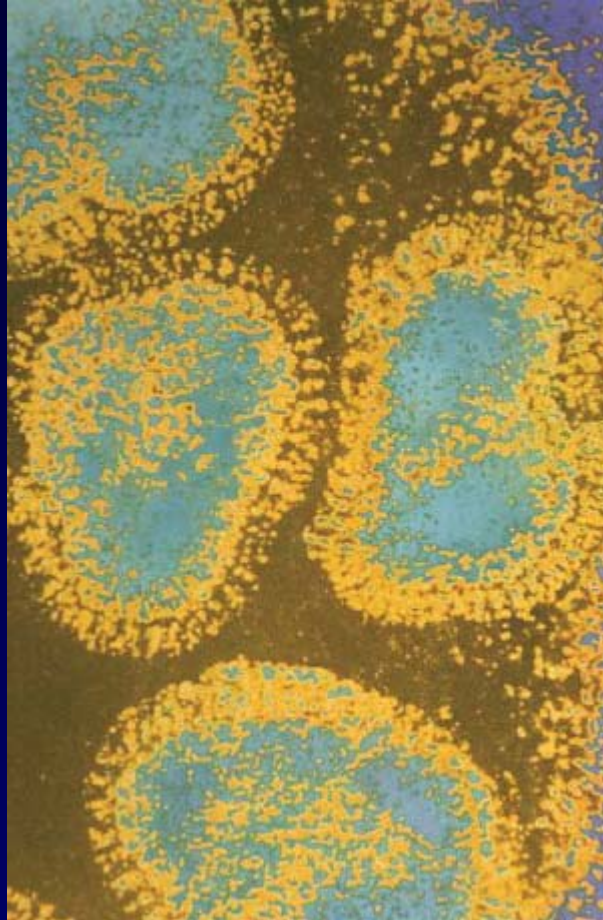
Adapté en partie des exposés de la Chaire Francqui 2003  
"Antiviral drugs and Discoveries in Medicine"  
Prof. E. De Clercq, KU-Leuven  
<http://www.md.ucl.ac.be/chaire-francqui/>

## Influenza virus



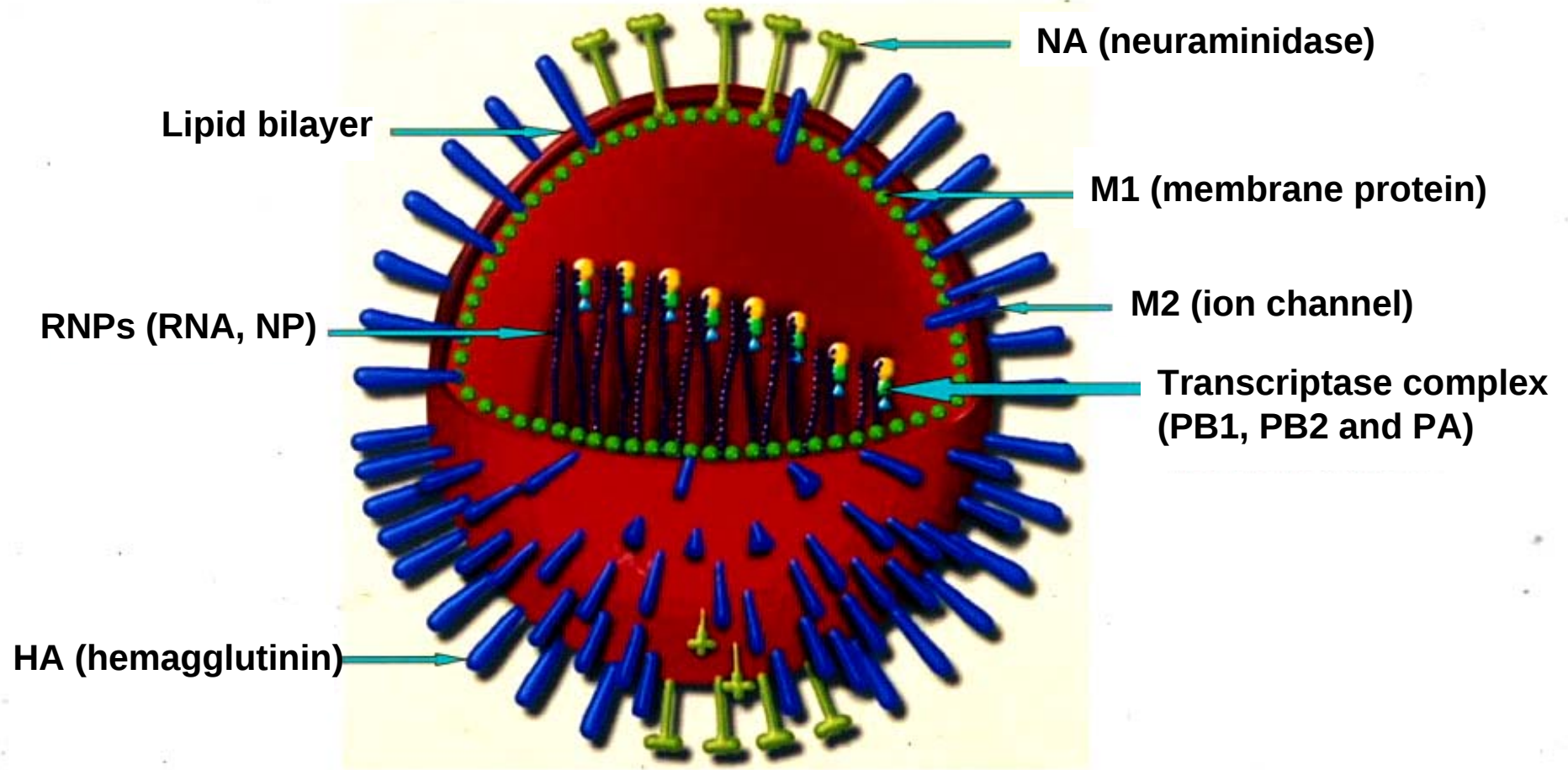
Electron micrographs of purified influenza virions. Hemagglutinin (HA ) and neuraminidase (NA) can be seen on the envelope of viral particles. Ribonucleoproteins (RNPs) are located inside the virions.

# Influenza

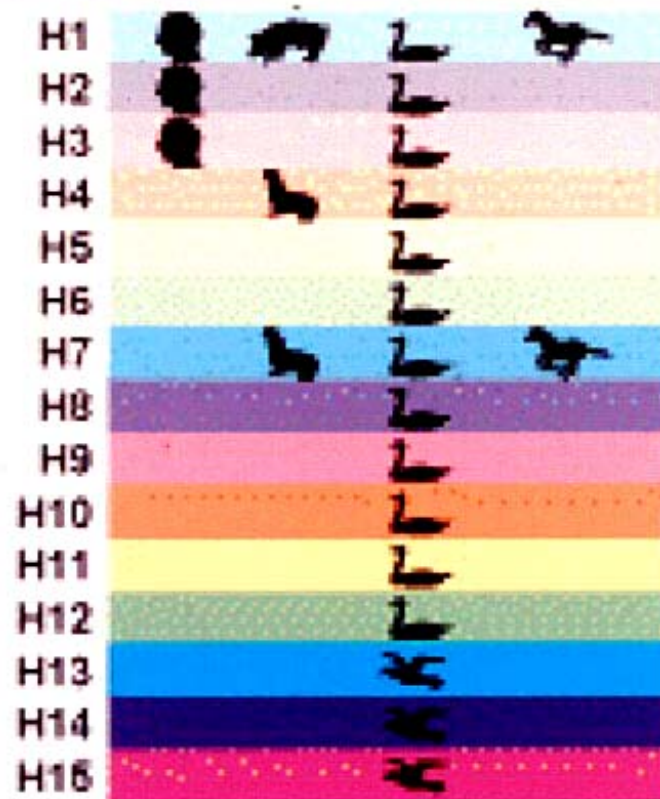


Layne *et al.*, Science 293: 1729 (2001)

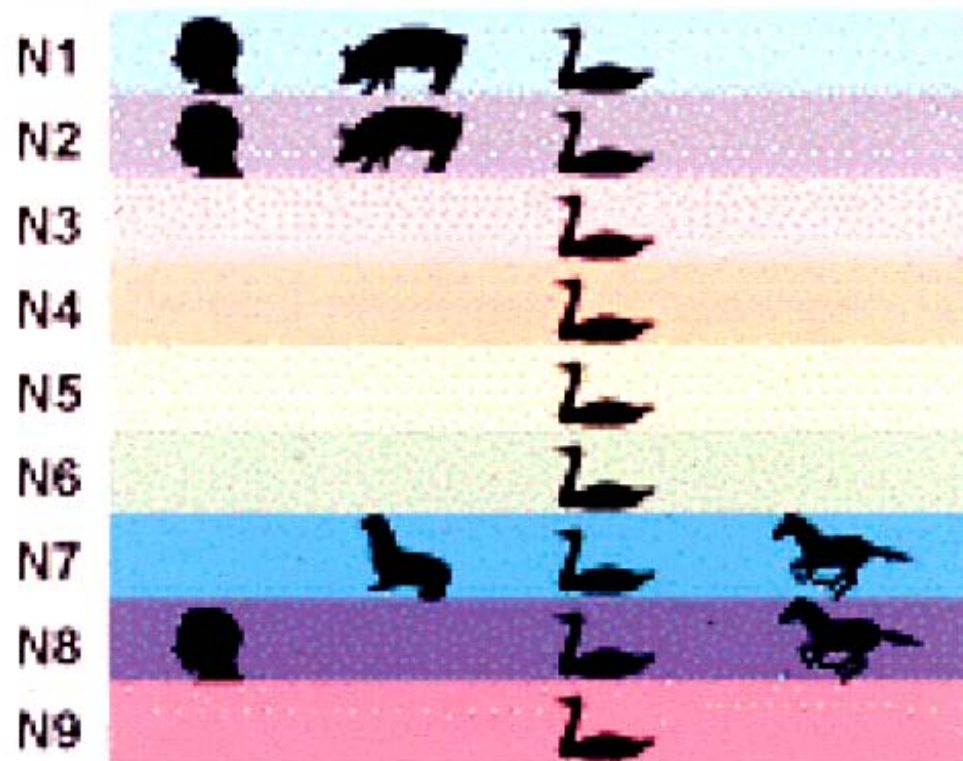
# Diagram of the influenza virus

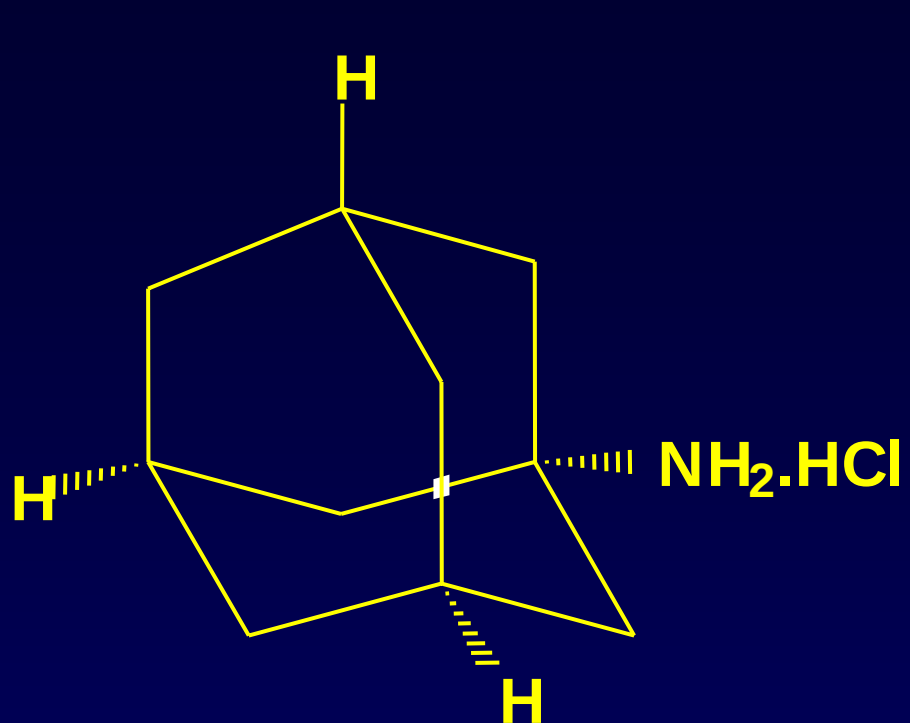


## Distribution of influenza A hemagglutinins in nature

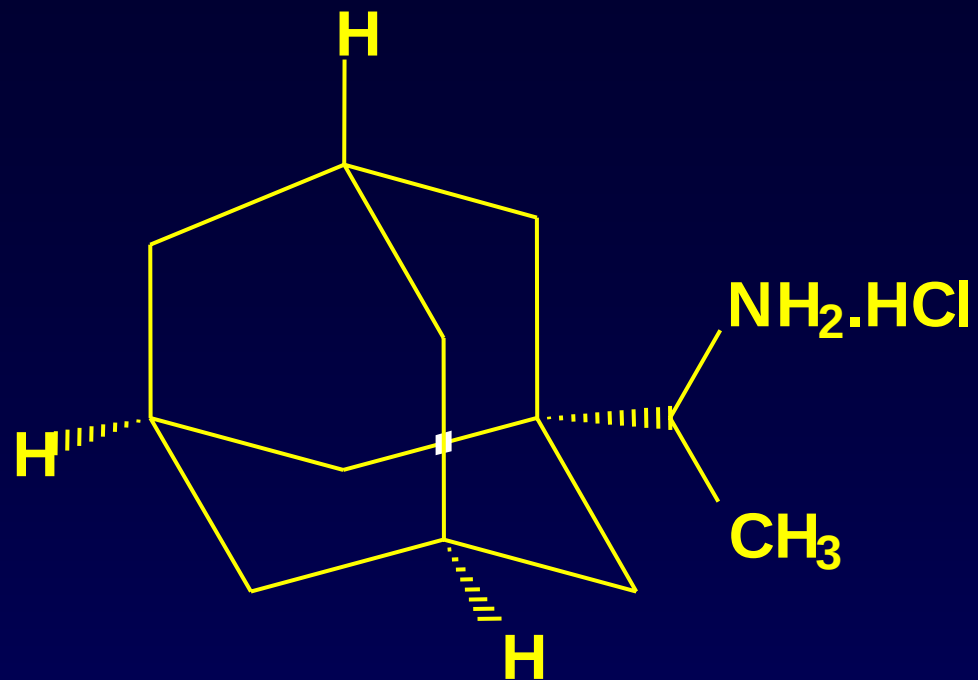


## Distribution of influenza A neuraminidases in nature



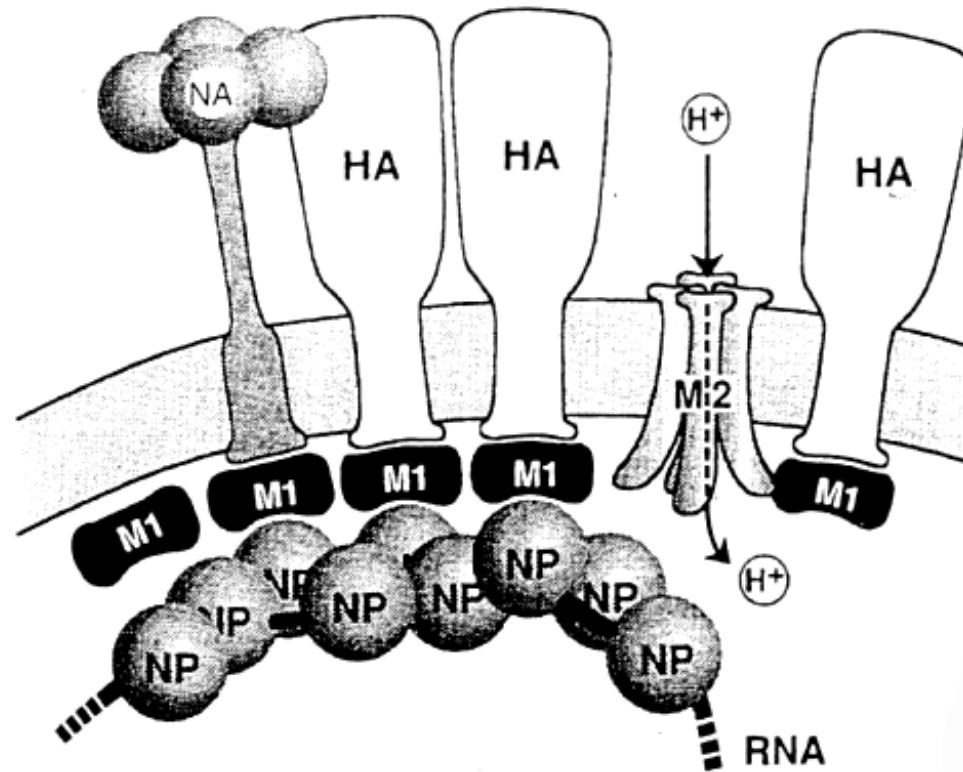


**Amantadine**

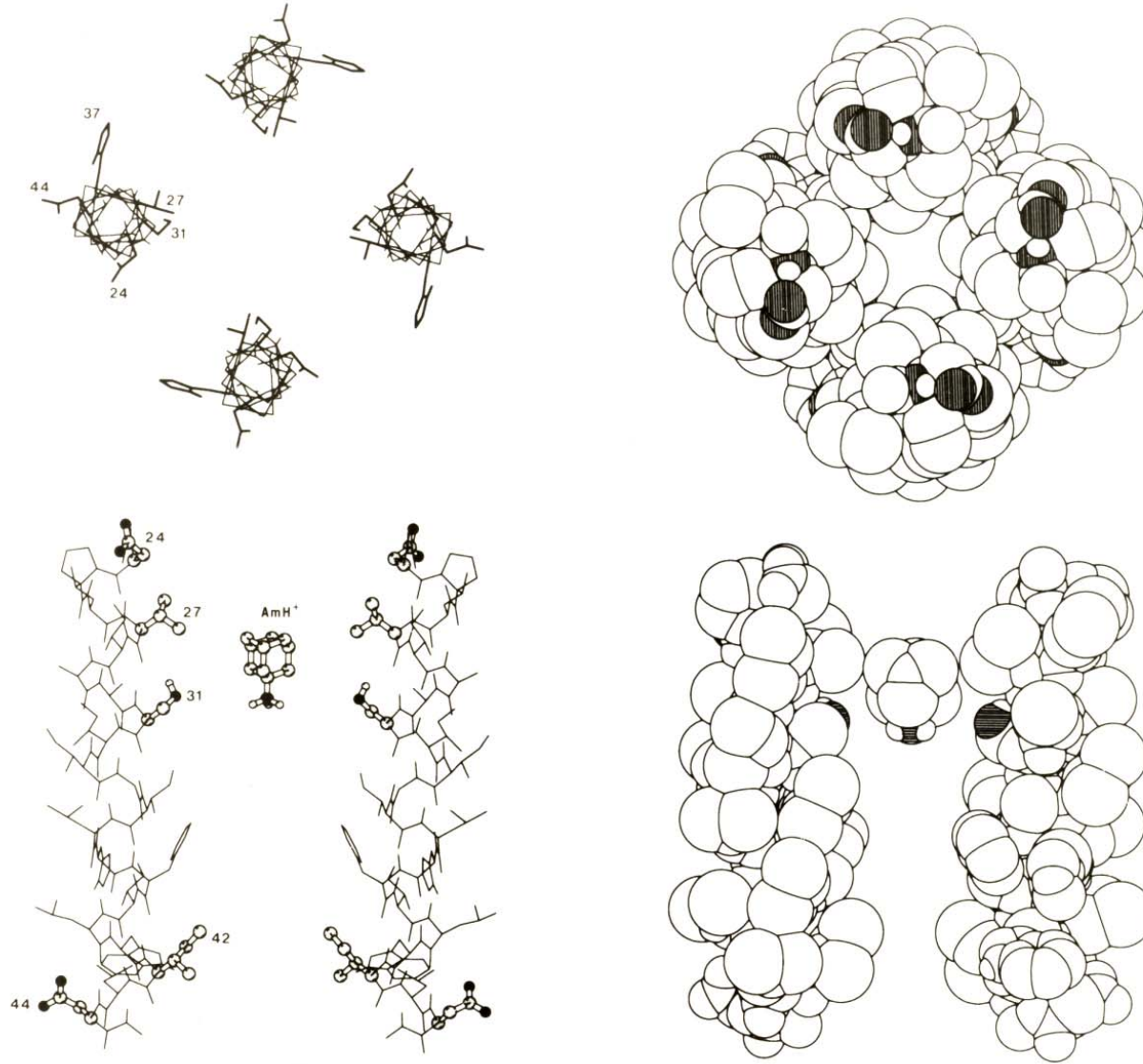


**Rimantadine**

**Amantadine/rimantadine:  
mechanism of action limited to influenza A viruses**

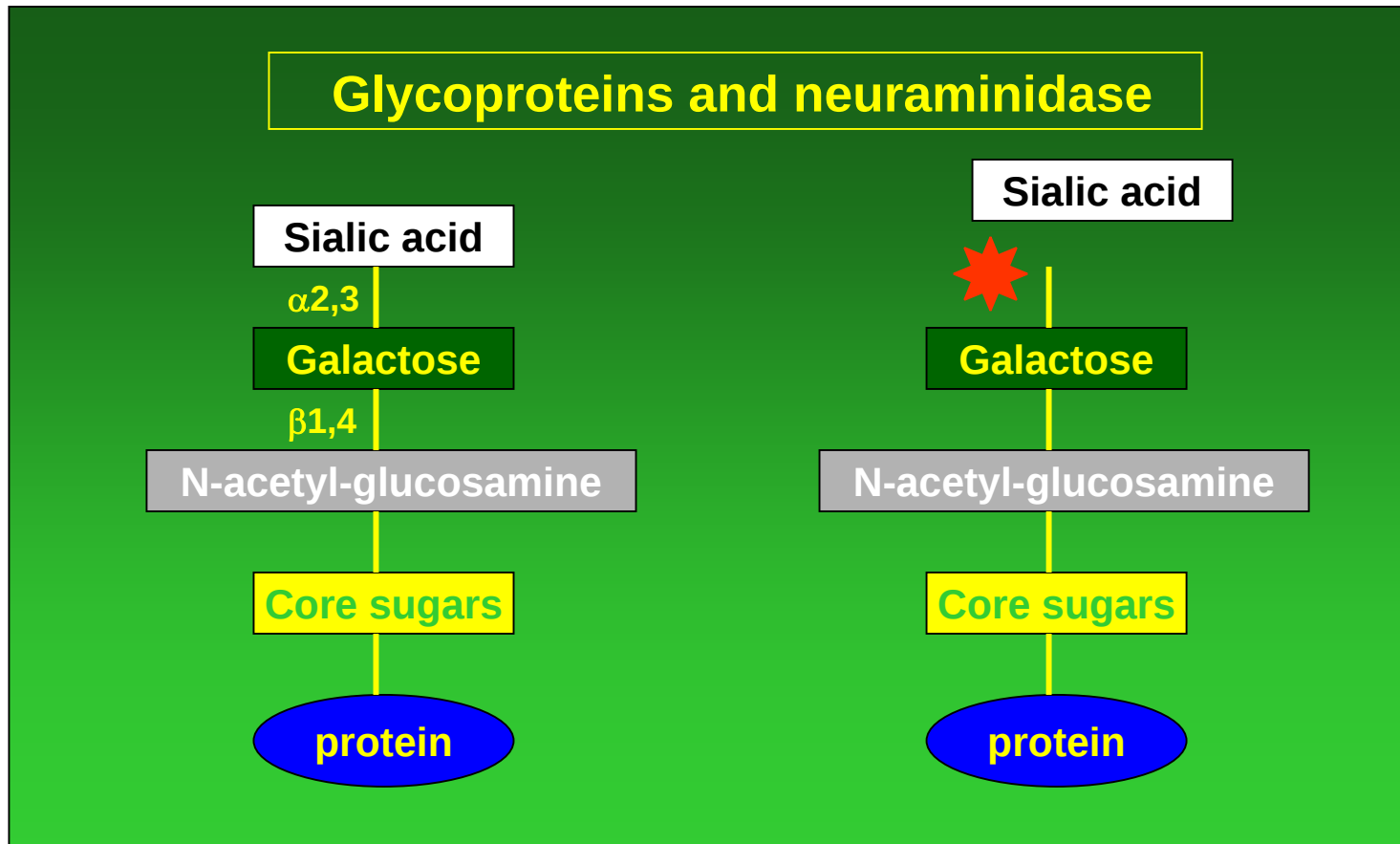


# The tetrameric M2 helix bundle



# Neuraminidase (NA):

Cleaves sialic acid from cell-surface glycoprotein

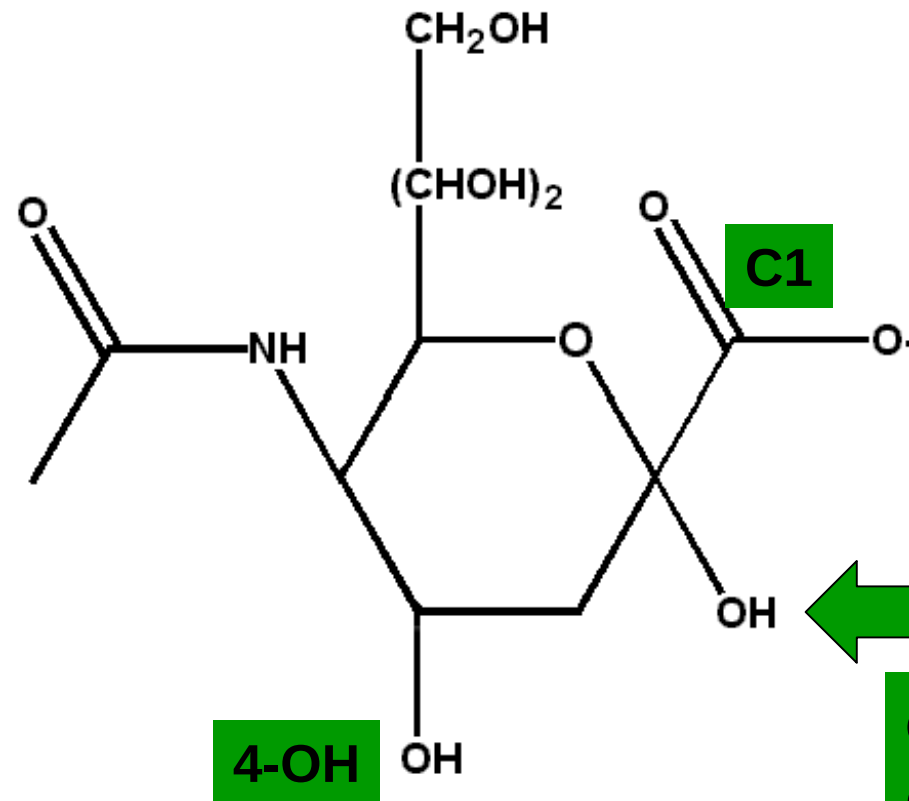


# Influenza virus neuraminidase

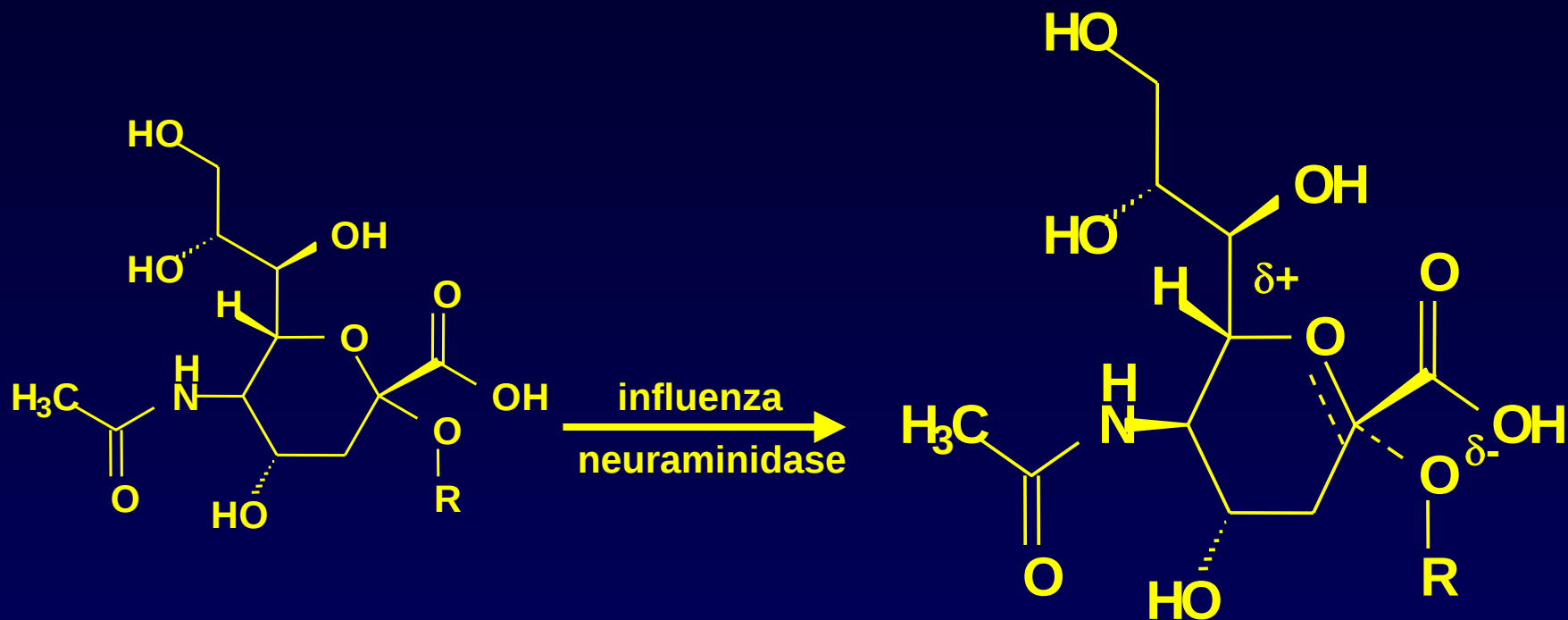
## Functions:

- removes terminal sialic acid residues
- promotes release of virus particles from the cells
- destroys cellular receptors recognized by hemagglutinin
- prevents virus aggregation at the cell surface
- prevents viral inactivation by respiratory mucus

# *Sialic acid...*



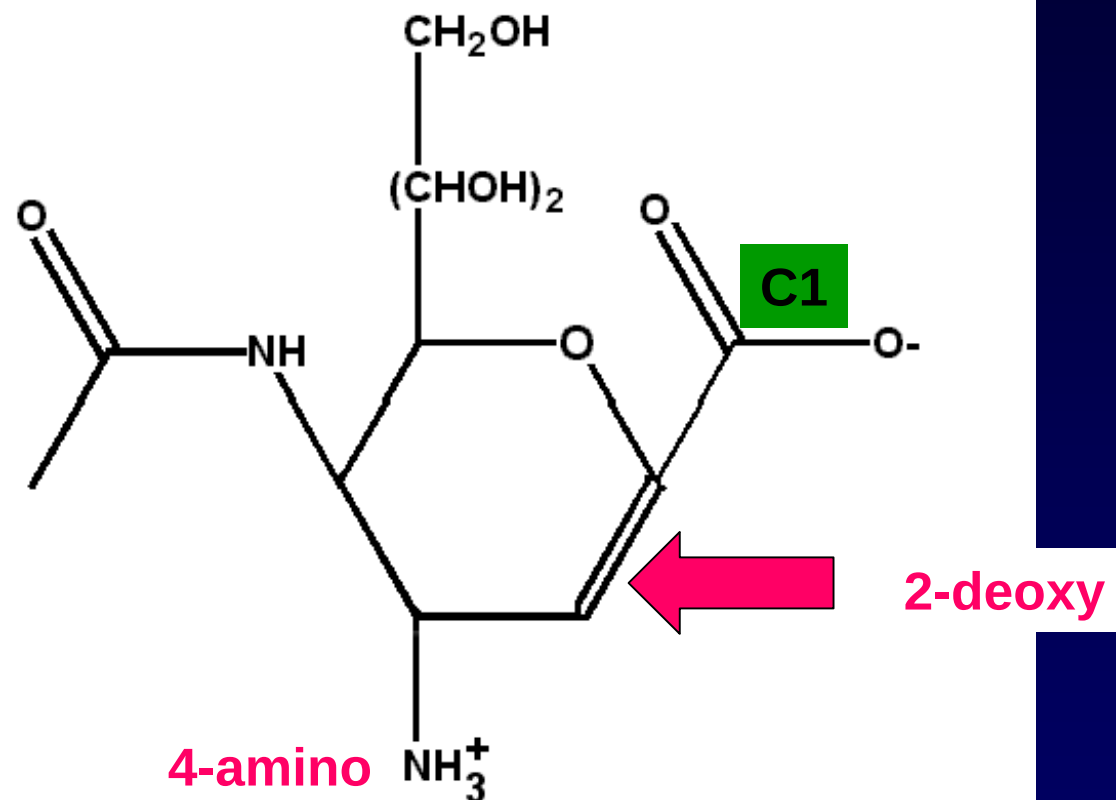
osidic linkage with  
galactose (2 → 3)



**Sialyl  $\alpha$ -glycoside**  
**R = glycoprotein**

**Transition state**

# *First inhibitor of neuraminidase... (1969)*



**2,4-dideoxy-2,3-didehydro-4-amino-D-N-acetylneuraminic acid**

*Meindl et al., Hoppe-Seyler's Z. Physiol. Chem., 350:1088-1092, 1969*

## *From 1969 to 1993...*

- 2,3-dideoxy-2,3-didehydro-4-amino-D-N-acetylneuraminic acid  
 $K_i \sim 0.01 \text{ mM}$  vs  $K_m$  for sialic acid  $\sim 1 \text{ mM}$

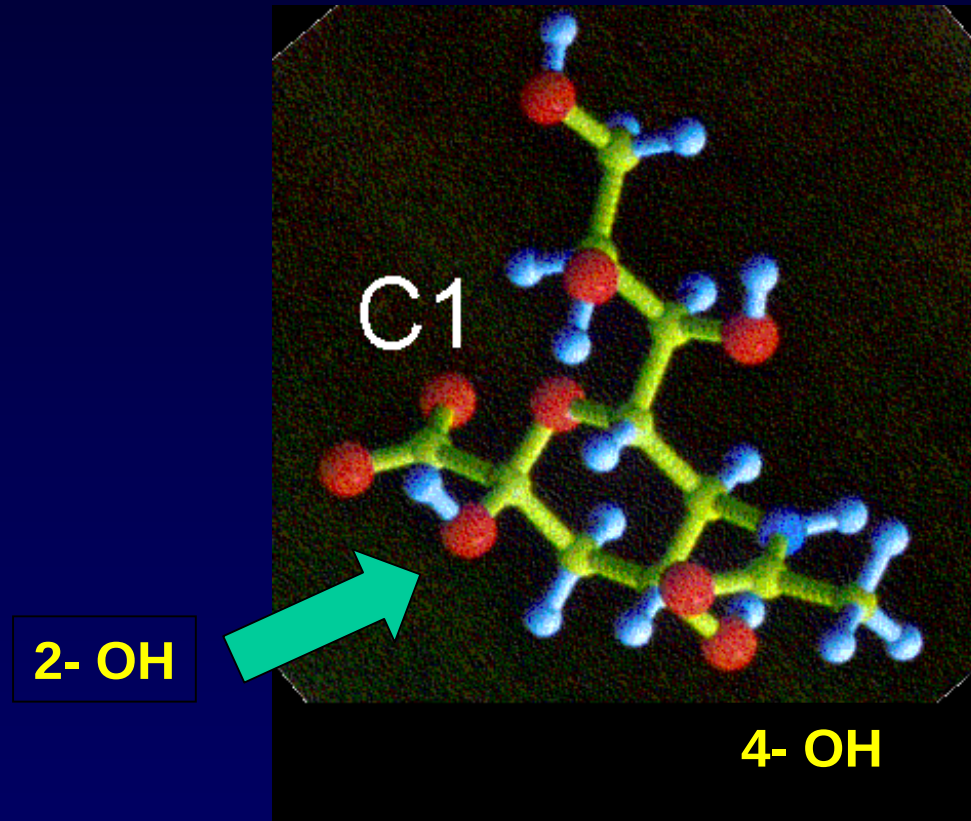
 does not work...

- 1983: structure of neuraminidase at 2.9 Å resolution  
several residues at catalytic site are constant  
antigenic sites are highly variable...

*(Colman et al., Nature 303:41-44, 1983)*

 Can you visualize the catalytic site ?

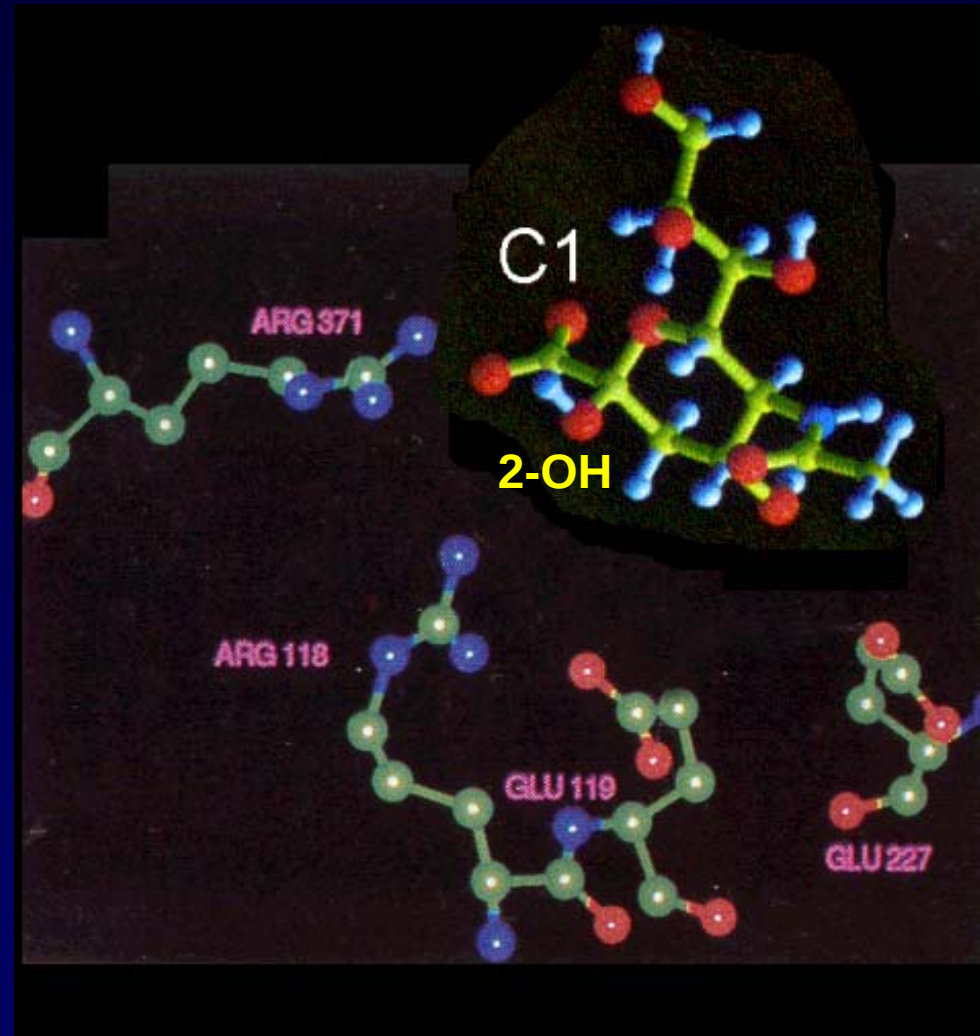
# From sialic acid to zanamivir... (1)



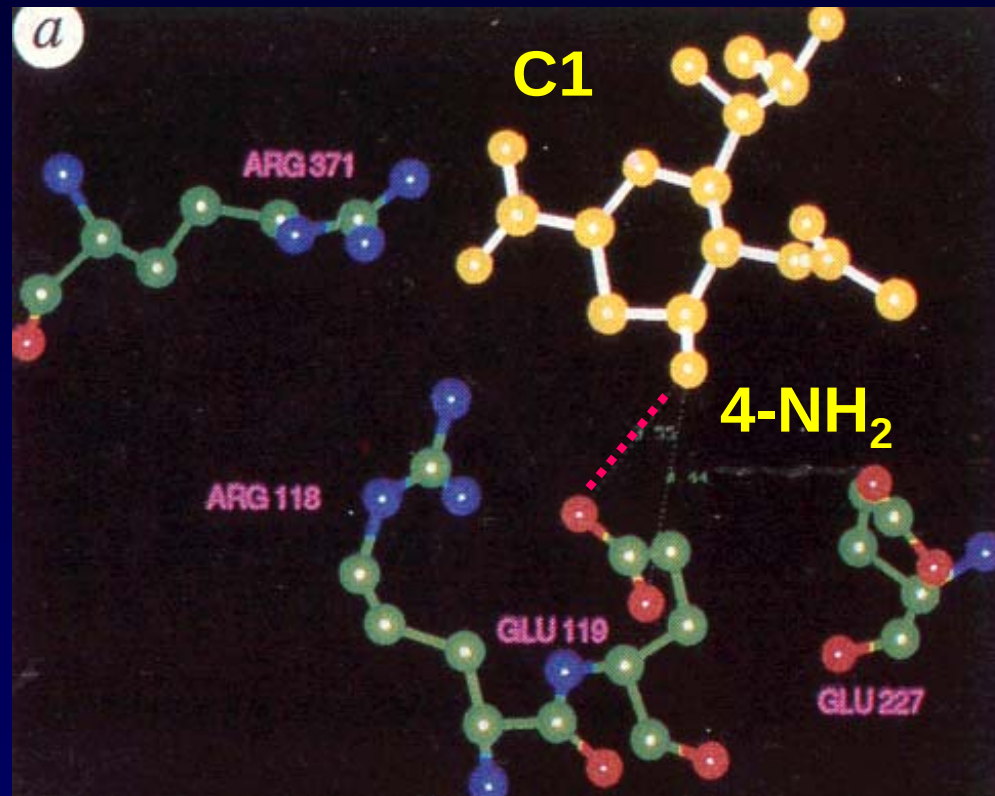
acide sialique ou N-acétyl-neuraminique

# Dfrom sialic acid to zanamivir... (2)

sialic acid  
binds through  
its C1  
carboxylate to  
Arg 371



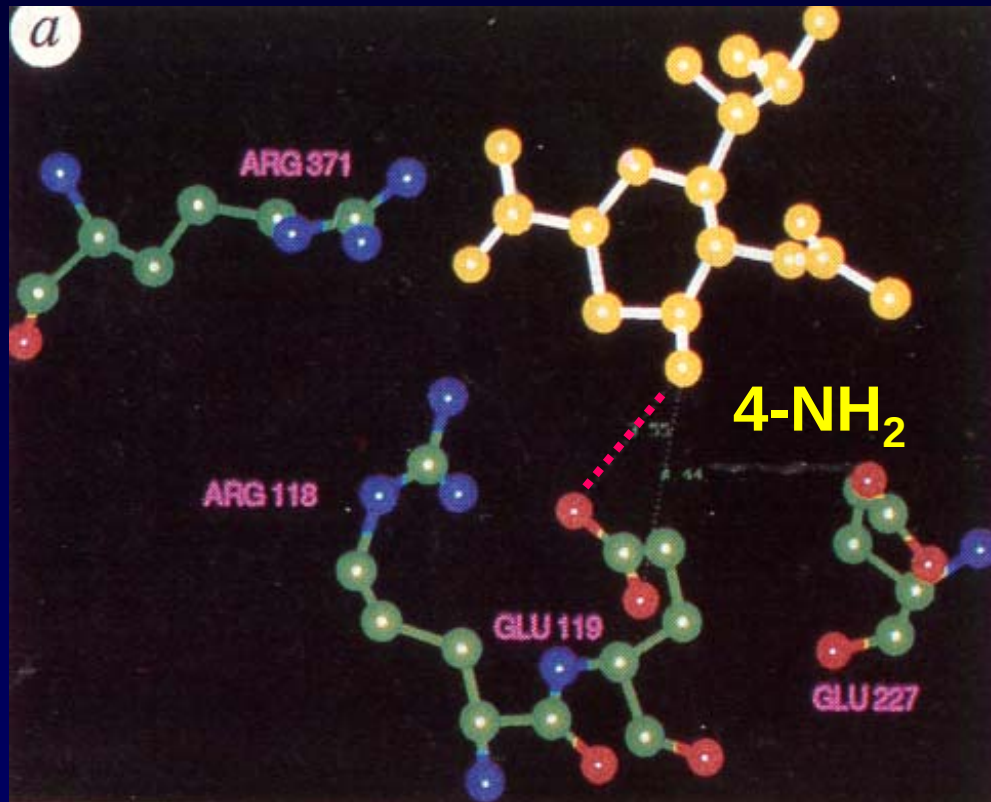
# From sialic acid to zanamivir... (3)



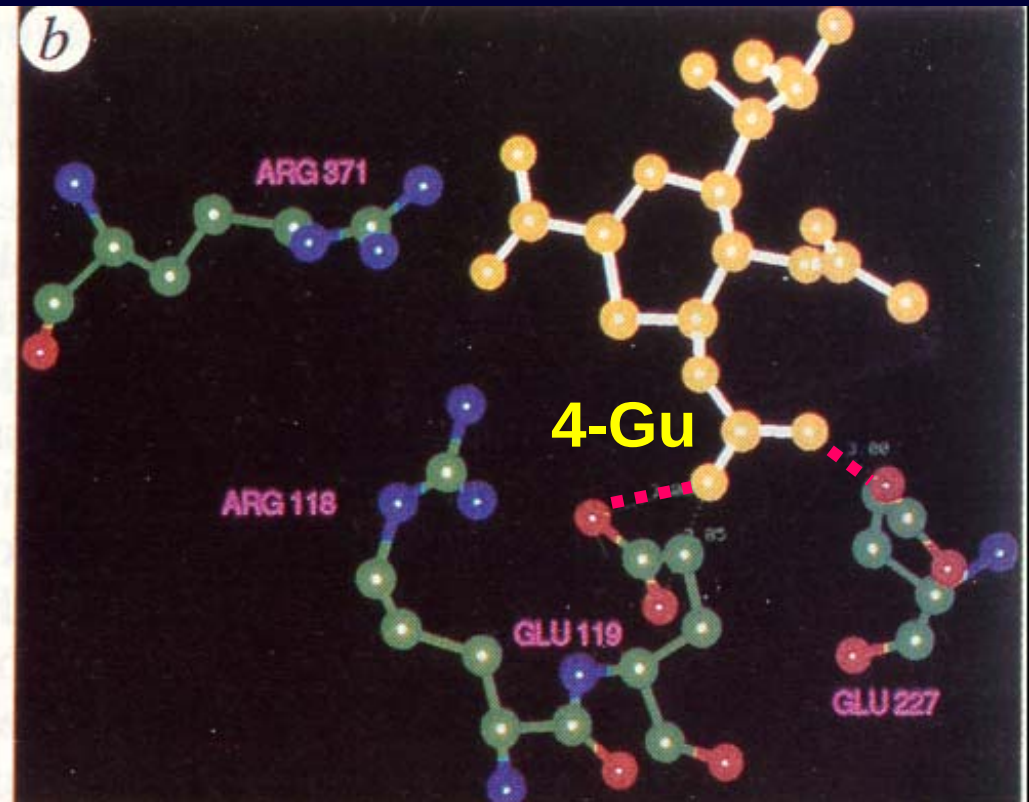
4-deoxy-4-amino ...

*résidues 119 et 227 are highly conserved...*

# From sialic acid to zanamivir... (4)



4-deoxy-4-amino ...

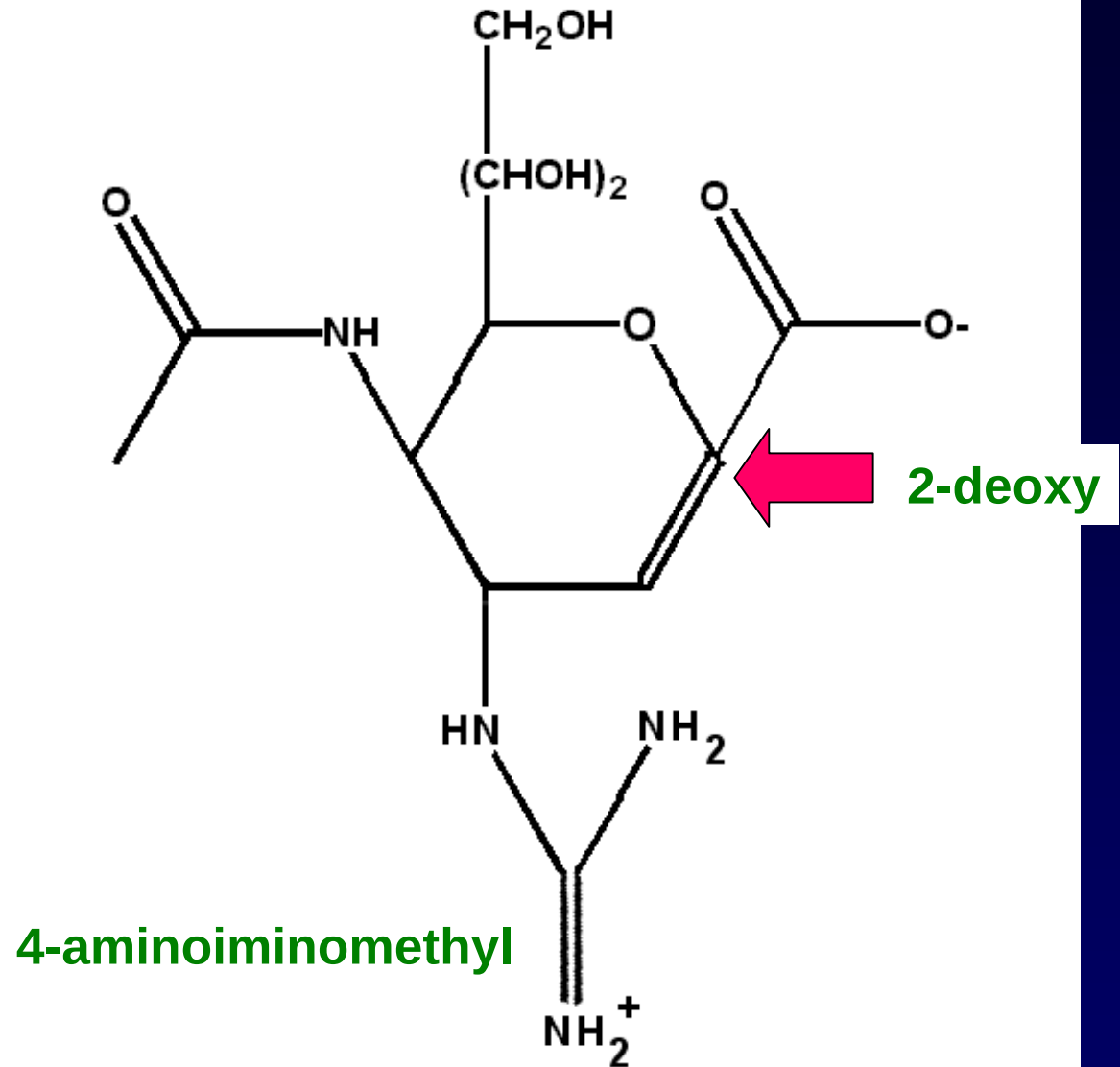


4-deoxy-4guanidino...  
*both residues 119 and 227 are now  
involved and 199 is much closer....*

# *Zanamivir...* (1993)

**2,4-dideoxy-2,3-  
didehydro-4-guanidino-  
D-N-acetylneuraminic  
acid**

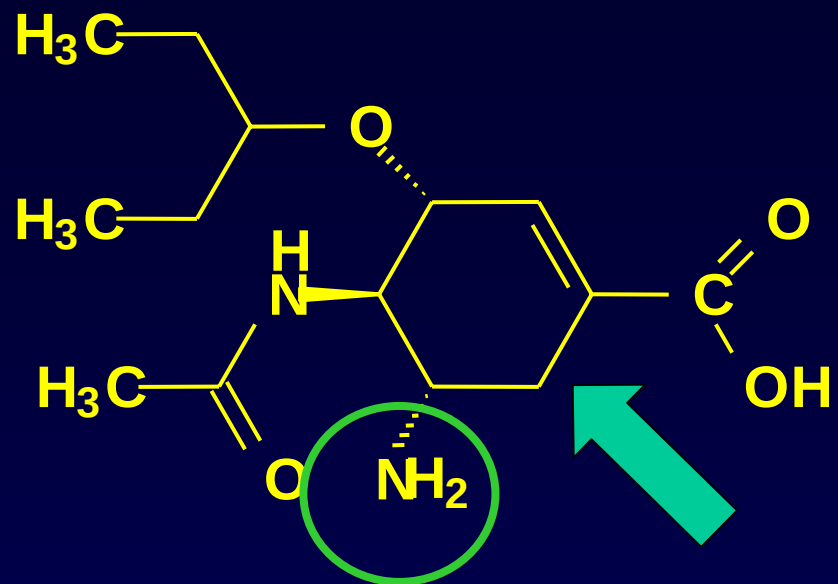
*von Itzstein et al.,  
Nature 363: 418-423, 1993*



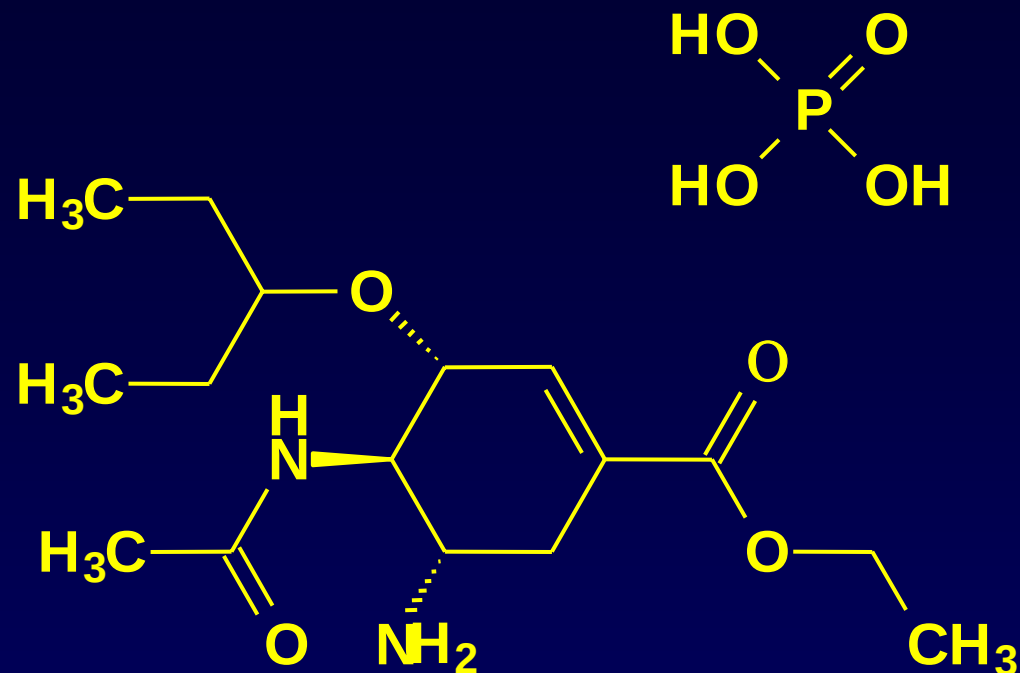
# Zanamivir

---

- active against both influenza A and B
- $IC_{50}$  : 0.21-2.6 ng/ml for influenza neuraminidase
- efficacy demonstrated in mouse and ferret models for influenza (upon topical administration)
- has to be administered by inhalation : 10 mg bid
- therapeutically effective (5 days) : significant reduction in duration of illness
- prophylactically effective (4 weeks) : significant reduction in number of ill subjects
- well tolerated : clinical adverse events not different from placebo
- no evidence for emergence of drug-resistant virus



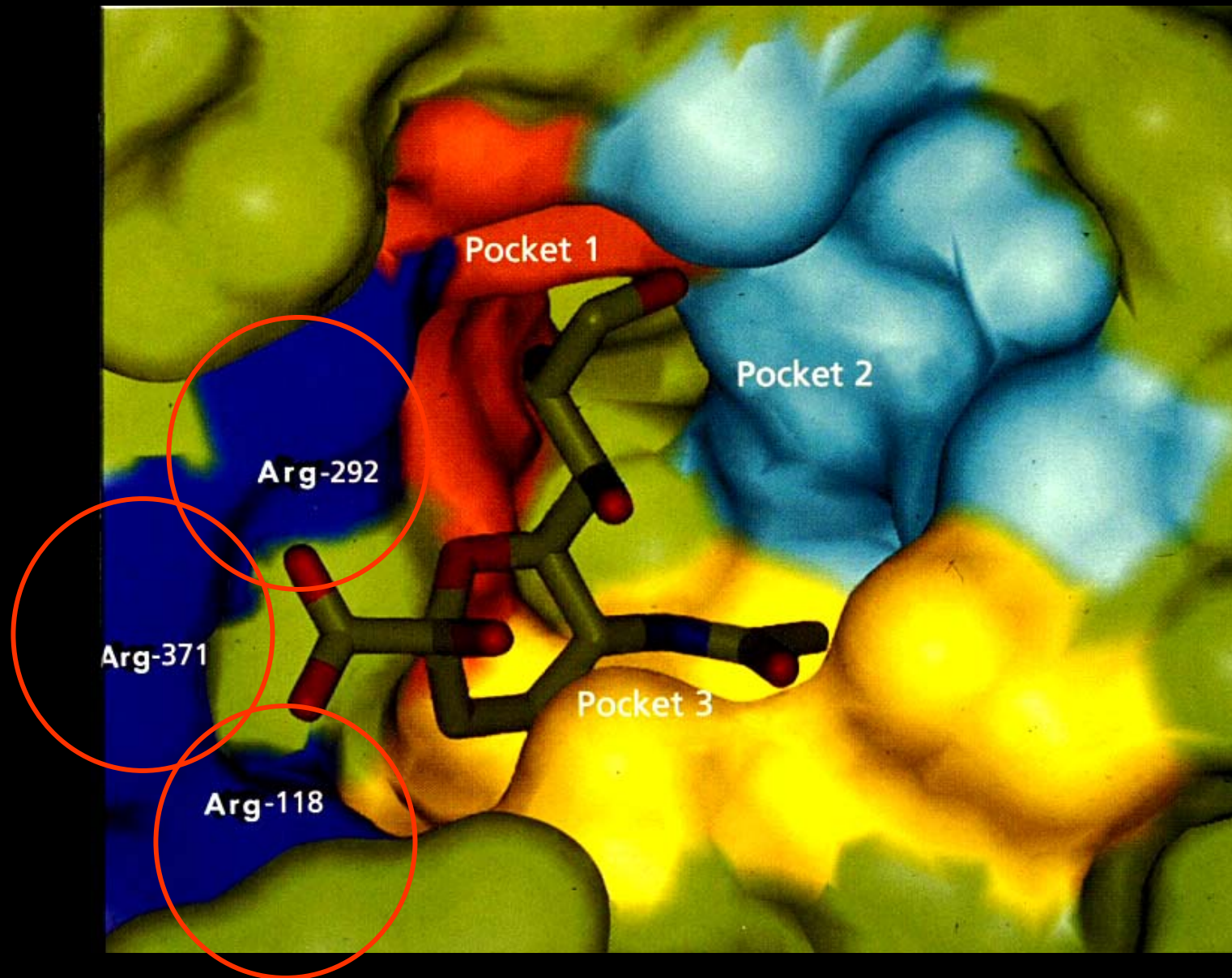
GS4071



Oseltamivir phosphate



# GS4071 bound to influenza neuraminidase



# Oseltamivir

---

- active against both influenza A and B
- $IC_{50}$  : < 1 ng/ml for influenza neuraminidase
- efficacy demonstrated in mouse and ferret models for influenza (upon oral administration)
- can be administered orally : 75 or 150 mg bid
- therapeutically effective (5 days) : significant reduction in duration of illness
- prophylactically effective (6 weeks) : significant reduction in number of ill subjects
- well tolerated : clinical adverse events not different from placebo
- no evidence for emergence of drug-resistant virus

# RESISTANCE MUTATIONS TO NEURAMINIDASE INHIBITORS

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## Neuraminidase

**119 Glu → Gly:**

- specific for zanamivir;
- Glu 119 interacts with guanidinium group of zanamivir

**292 Arg → Lys:**

- found for zanamivir; cross-resistance to oseltamivir
- Arg 292 interacts with carboxylic acid group of zanamivir and oseltamivir

## Hemagglutinin

**Some mutations (i.e. 198 Thr → Ile) diminish affinity of hemagglutinin for its receptor**

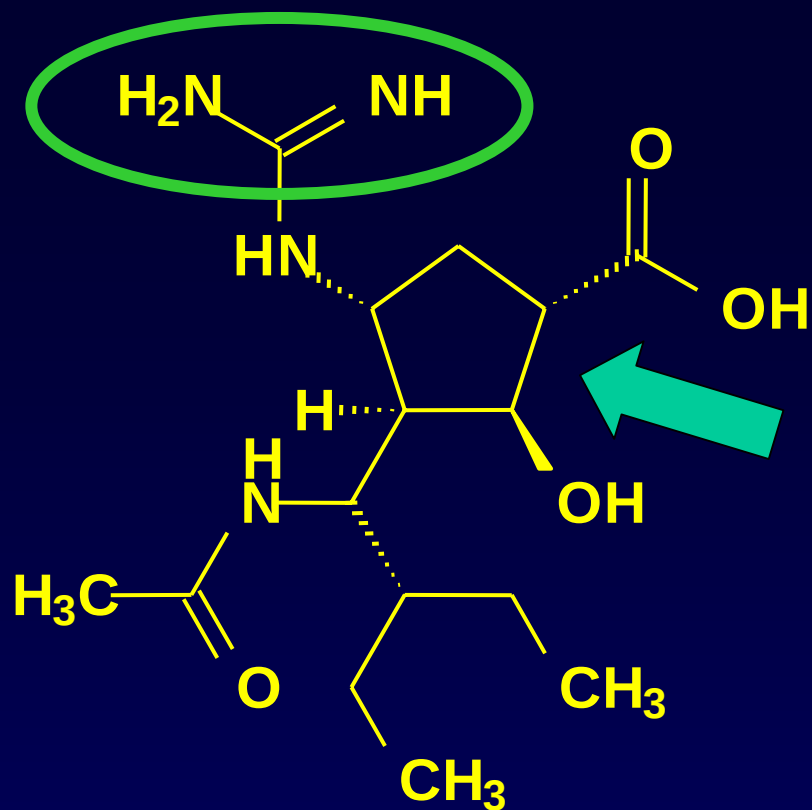
# **Benefits offered by neuraminidase inhibitors**

## **Therapeutically:**

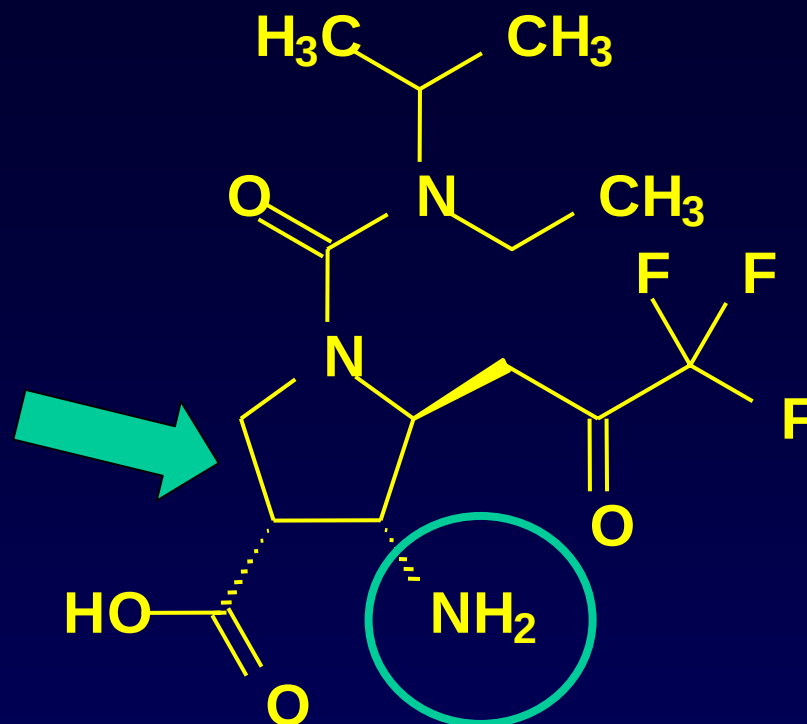
- **Reduction in illness duration by 1-2 days**
- **Reduction in risk-virus transmission to household or healthcare contacts**
- **Reduction in complications (sinusitis, bronchitis)**
- **Reduction in use of antibiotics**

## **Prophylactically:**

- **Seasonal prevention of infection**



**RWJ-270201**



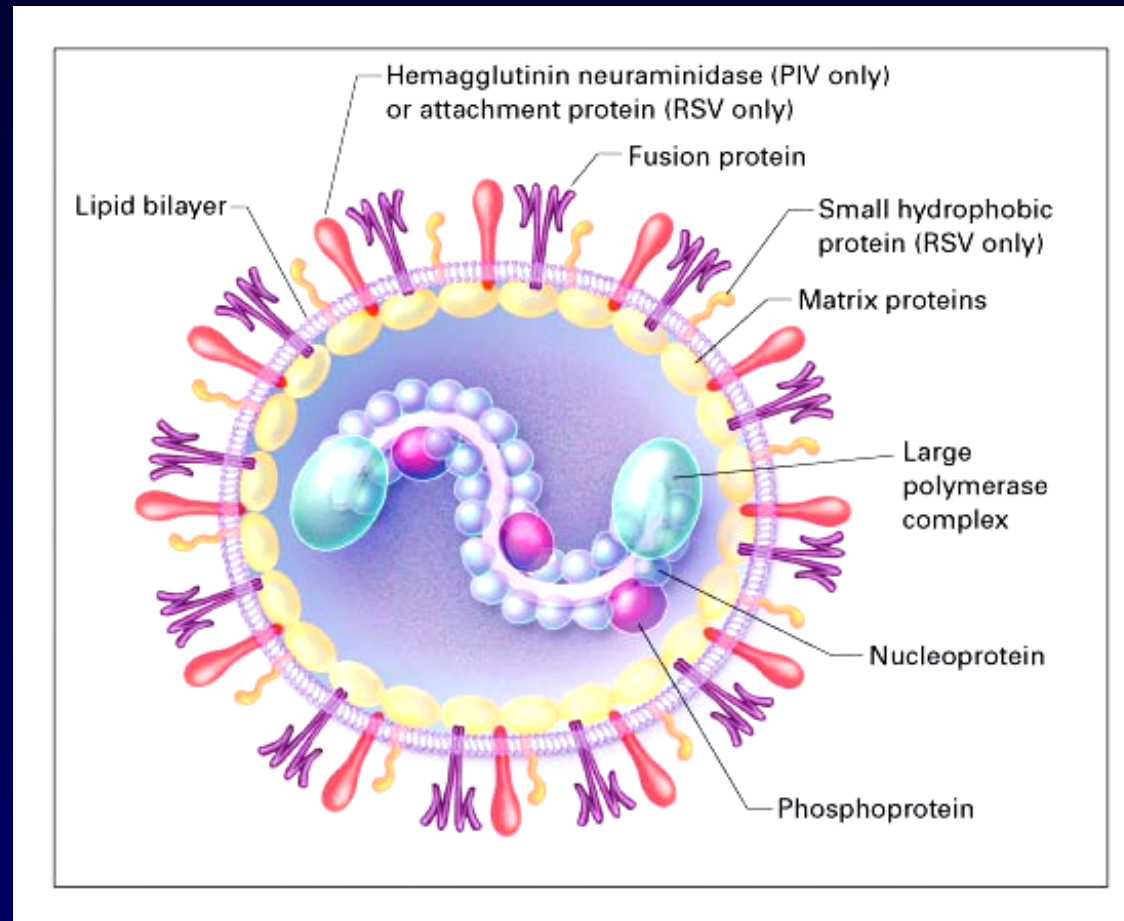
Wang et al., J. Med. Chem. 44: 1192-1201 (2001)

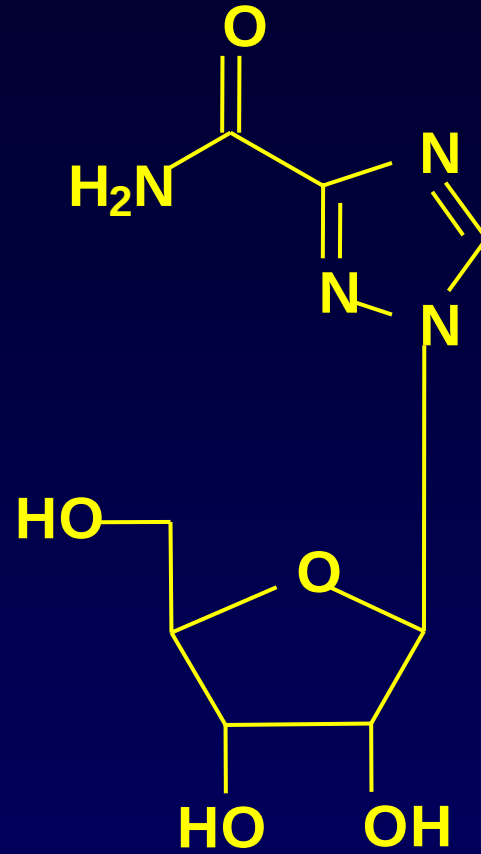
Smee et al., Antimicrob. Agents Chemother. 45: 743-748 (2001)

Sidwell et al., Antimicrob. Agents Chemother. 45: 749-757 (2001)

# RESPIRATORY SYNCYTIAL VIRUS (RSV)

# Respiratory Syncytial Virus (RSV) and Parainfluenza Virus (PIV)





**Ribavirin**

**Virazole®**

# **APPROVED ANTIVIRAL DRUGS FOR THE TREATMENT OF THE MAJOR RESPIRATORY TRACT VIRUS INFECTIONS in 2003**

|                                    |                                                                                                    |
|------------------------------------|----------------------------------------------------------------------------------------------------|
| <b>Adenoviruses</b>                | <b>: none</b>                                                                                      |
| <b>Picornaviruses</b>              |                                                                                                    |
| <b>Entero</b>                      | <b>: none</b>                                                                                      |
| <b>Rhino</b>                       | <b>: none</b>                                                                                      |
| <b>Orthomyxoviruses</b>            |                                                                                                    |
| <b>Influenza</b>                   | <b>: Neuraminidase inhibitors:<br/>                                    zanamivir, oseltamivir</b>  |
|                                    | <b>: Amantadine and rimantadine<br/>                                    (for influenza A only)</b> |
| <b>Paramyxoviruses</b>             |                                                                                                    |
| <b>Parainfluenza</b>               | <b>: none</b>                                                                                      |
| <b>Respiratory syncytial virus</b> | <b>: Ribavirin</b>                                                                                 |
| <b>SARS virus</b>                  | <b>: none</b>                                                                                      |