BPS 2110
Assignment 5 Answers

1. Describe the general structures of viruses.
   Protein capsid surrounding genetic material. Some capsids also contain viral proteins and enzymes. Enveloped viruses are surrounded by a membrane containing viral proteins.

2. What are the general difficulties associated with developing antiviral drugs? What kind of features in viruses make the best drug targets?
   Difficulties:
   - Each virus requires a different drug
   - Diagnosis – many viruses produce similar symptoms
   - Resistance – viruses quickly evolve to become drug resistant
   - Most viral proteins bind to host protein – difficult to prevent with small molecule drugs
   - Most viral enzymes used to make nucleic acids – same substrates as host enzymes makes it difficult to have selective inhibitors

   Best targets
   - Viral enzyme, ideally one not involved in nucleic acid replication

3. Describe the stages of a typical viral life cycle. Which of these phases is likely (or unlikely) to be targeted with drugs and why?
   - Adsorption and penetration – virus fuses with cell membrane and enters cell.
     Unlikely to target with drugs – involves protein interactions
   - Uncoating of viral nucleic acid – viral genetic info enters cytoplasm
     Unlikely to target with drugs – involves protein interactions
   - Synthesis of regulatory protein – viral genes are used to make viral proteins which alter the function of the cell
     Unlikely to target with drugs – involves protein interactions
   - Synthesis of viral genetic molecules
     Possible to target with drugs – most antivirals target this phase
   - Assembly of viral particles – viral proteins self-assemble around viral RNA or DNA
     Unlikely to target with drugs – involves protein interactions. Can be done if viral enzymes are used
   - Release from cell – can be lytic (cell destroyed) or budding (cell survives)
     Unlikely to target with drugs – involves protein interactions. Can be done if viral enzymes are used

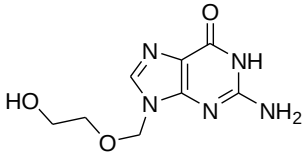
4. Describe how each of the following general antiviral strategies work.
   a. Non-natural bases in nucleoside analogs.
      Replace a nucleoside base with a non-natural base. Requires a viral polymerase to work. Viral polymerase accepts the drug as a substrate and

incorporates it into viral RNA. This RNA is unreadable by the cell ribosome, so viral protein does not get made.

b. Chain terminators in nucleoside analogs.
Replace the 3'OH in nucleoside with a non-nucleophilic isostere. Requires a viral polymerase to work. Viral polymerase accepts the drug as a substrate and incorporates it into viral nucleic acid. Nucleic acid chain now has a non-nucleophilic group at the 3' position and the enzyme cannot add another nucleotide. Enzyme stops, or make a shortened nucleic acid.

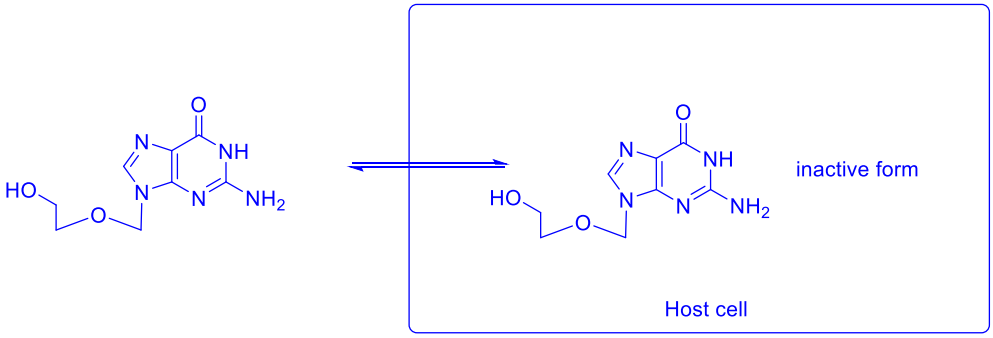
c. Non-natural sugars in nucleoside analogs.
Replace the sugar in nucleoside with a non-natural sugar (usually without a 3'OH group). Requires a viral polymerase to work. Viral polymerase accepts the drug as a substrate and incorporates it into viral nucleic acid. Nucleic acid chain now has an improper group at the 3' position and the enzyme cannot add another nucleotide. Enzyme stops, or makes a shortened nucleic acid.

5. The antiviral drug Acyclovir shows an unusual pattern of bioavailability that produces a very low incidence of side effects. Describe in detail how the drug is distributed in the body and in virally infected cells. It may be helpful to use a diagram for part of your answer.

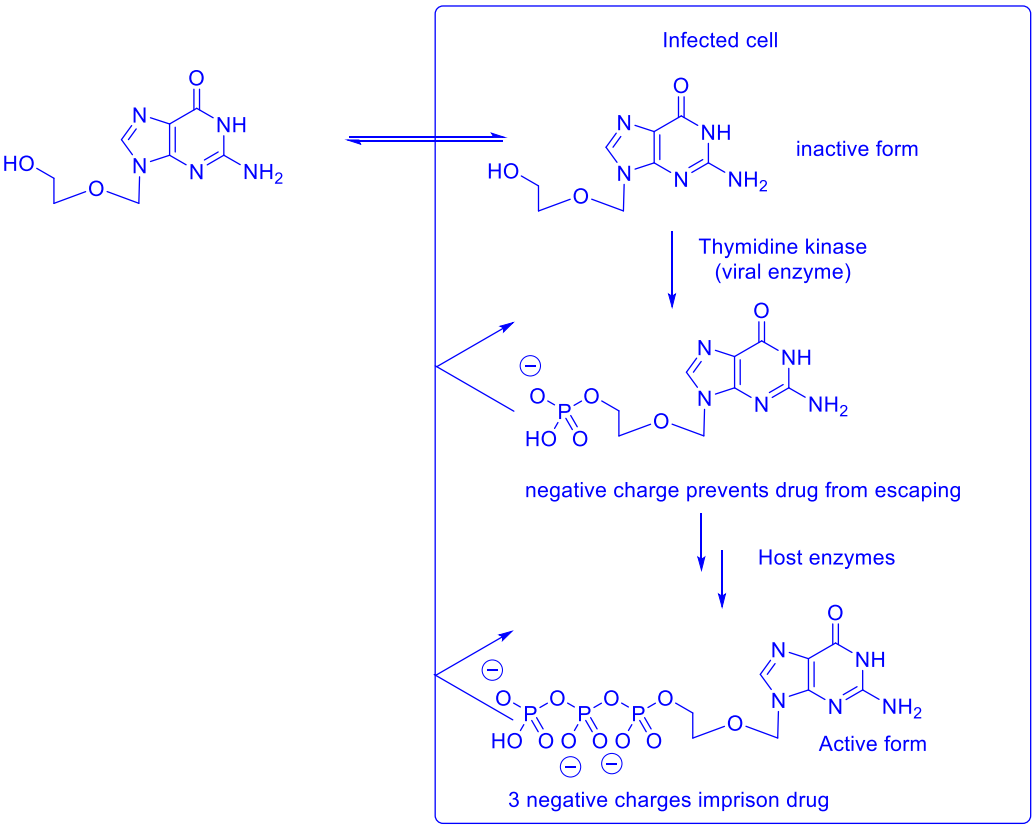


Acyclovir

Drug is not phosphorylated by host enzymes. is able to freely diffuse in and out of host cells. becuase it is not phospharylated, the drug in in the inactive form, and cannot interact with polymerases.



Drug is phosphorylated by viral enzyme. once phosphorylated, host enzymes convert the mono-phosphate into a triphosphate. this carries 3 negative charges which prevent the triphosphate from passing through non-polar membranes and out of the cell. the drug accumulates in cells (LeChatelier's principle) as the triphosphate which is now "active" and can inhibit viral polymerase.



6. What are the three general types of influenza virus? A, B and C
   a. Which ones cause serious disease and which do not?
      A and B cause human disease, type C does not cause serious disease
   b. Why are some forms more dangerous than others?
      Forms with very high mutation rate difficult for the immune system to clear

   c. Why is it necessary to be vaccinated against influenza each year?

Virus has a very high mutation rate, and there is a new circulating virus type each year. Once you have immunity to each virus type you will not get sick because of it, but because there is a new type every year, new immunity is necessary.

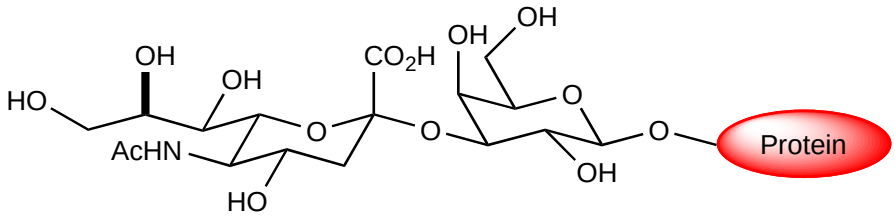
7. In 2009 the H1N1 virus caused serious concern.
   a. What does the designation H1N1 mean?
      H1 is the hemagglutinin type
      N1 is the neuraminidase type
      The numbers indicate how different each virus sub-type is

   b. Why is this system used to classify influenza viruses?
      hemagglutinin and neuraminidase are found on the outside of the virus, on the envelope. As such they are easily detected using antibodies. By testing the reaction of virus particles to antibodies corresponding to each protein type, the virus can be classified.

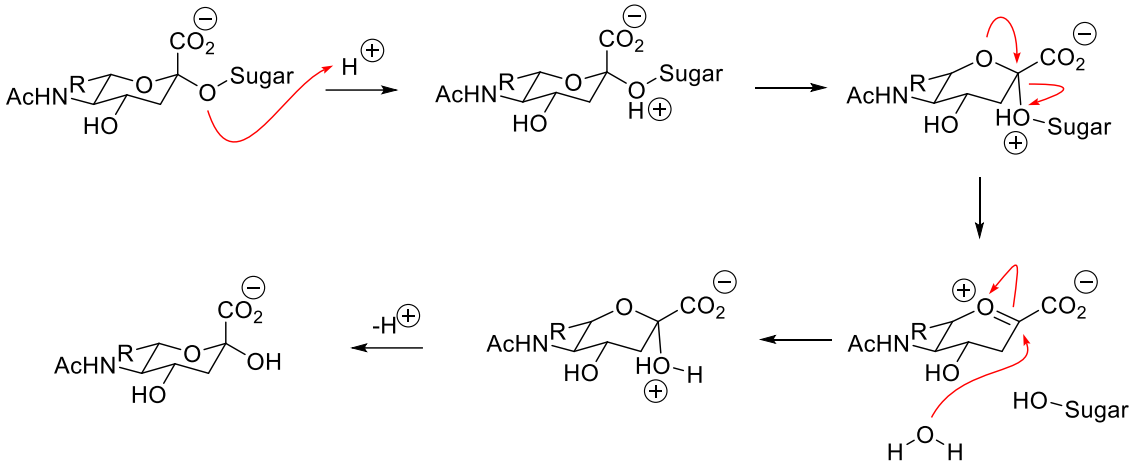
   c. Describe the general function of each of the components you identified in part a.
      Hemagglutinin is a viral membrane protein that sticks to the sugar sialic acid. Sialic acid is found on human membrane proteins that the virus uses to gain entry into the cell.
      Neuraminidase is a viral membrane enzyme that removes sialic acid from other proteins. Removal of sialic acid from the human proteins on the virus envelope is necessary to prevent virus particles from sticking to other virus particles.

8. The enzyme neuraminidase is an important part of the influenza virus.
   a. What is the function of this enzyme?
      Remove sialic acid from the sugars attached to human membrane proteins

   b. Provide the structure of its main substrate.



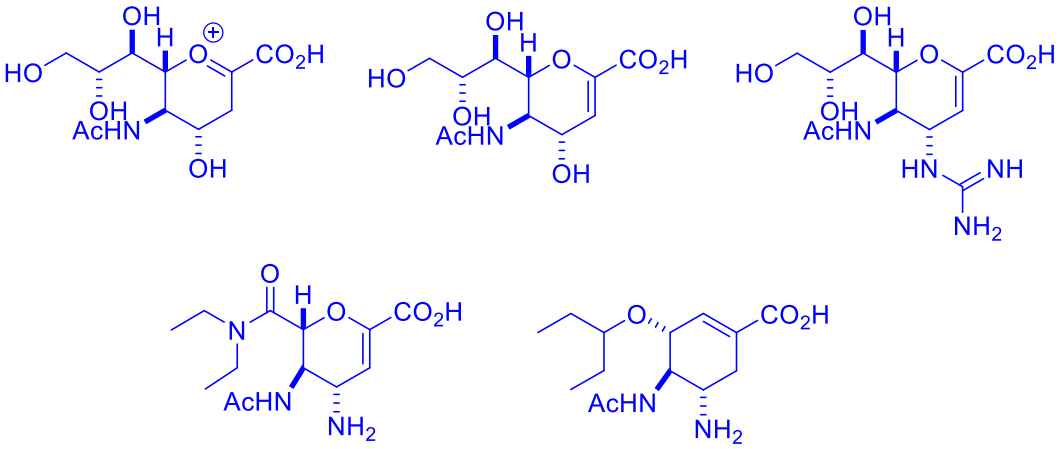
   c. Provide the mechanism by which the enzyme operates.



d. What is the general strategy that is used to design inhibitors of this enzyme?
Rational drug design. Make a drug that resembles the transition state of the reaction that the enzyme catalyzes.

e. What general type of inhibitor is best used to block enzyme function using the above method?
Make a transition state analogue (mimic)

f. Provide an example of such an inhibitor.



9. The drug Tamiflu was introduced in 1999 as the first designed drug for influenza.
   a. What are the benefits of this drug?
   Reduces the course of the disease by 1 day

   b. What are the disadvantages of this drug?

- Must be administered within first 24 to 48 hours
- Very expensive
- Limited supply
- Has many side effects

c. Why is the drug very expensive?
Difficult to manufacture – requires complex chemistry
Raw material required is in limited supply

10. Describe how the method of electronic chain termination works.
Place a strong electron-withdrawing group close to the 3'OH of a nucleoside. The EWG reduces the nucleophilicity of the OH enough to prevent chain extension.

11. AIDS drugs are generally ineffective when administered by themselves, but combinations of drugs are successful. Explain this apparent contradiction.
When administered as single drugs, the virus becomes resistant very quickly because of its high mutation rate. Drugs administered in combination make resistance less likely because the virus has to evolve too many changes at once.

12. The AIDS virus has two enzyme domains that are used to replicate its genetic information.
    a. What are these two enzymes?
    - reverse transcriptase
    - RNAse H

    b. What characteristics of these enzymes contribute to HIV's exceptionally high mutation rate?

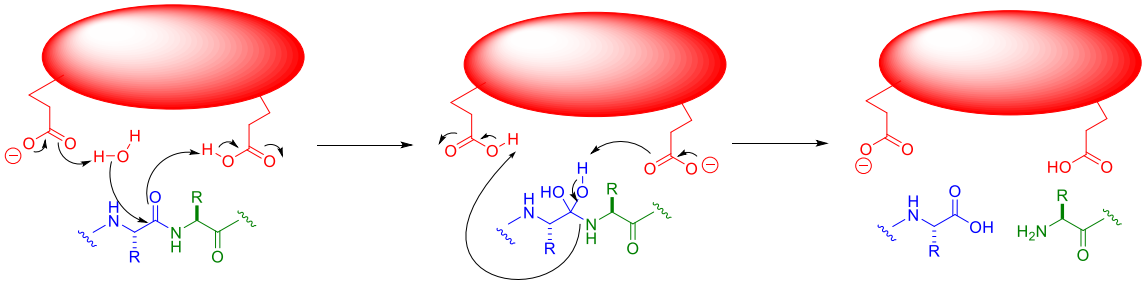
- Reverse transcriptase is a sloppy enzyme that makes lots of mistakes (approx 1 per every 9000 bases)
- RNAse H destroys the viral RNA template as DNA strand is being made. Therefore no error correction (proofreading) is possible

    c. What drug strategy is used in the clinic to counteract this situation (Name and acronym)?
    HAART
    Highly Active Antiretroviral Therapy

    d. Explain how the above drug strategy works.
    Administer several drugs all at same time. Drugs should be targeted against different viral enzymes. Chances of the virus becoming resistant to several different drugs all at the same time is very small. Even if virus emerges that is resistant to one drug, it will likely be killed by the others.

    e. What key aspect of anti-HIV drug structure has made possible therapies with dramatically reduced side effects?
    Certain pseudoenantiomer nucleosides are substrates for host kinases, and the resulting nucleotides are substrates for HIV reverse transcriptase. This results in chain termination of the viral DNA. Host polymerases will not accept pseudoenantiomer nucleotides as substrates, and so these drugs are unable to chain terminate host nucleic acid synthesis.

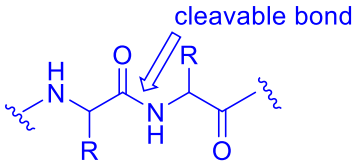
13. The HIV virus also expresses a specialized type of enzyme called an aspartyl protease.
    a. Provide a detailed mechanism to explain how an aspartyl protease works.

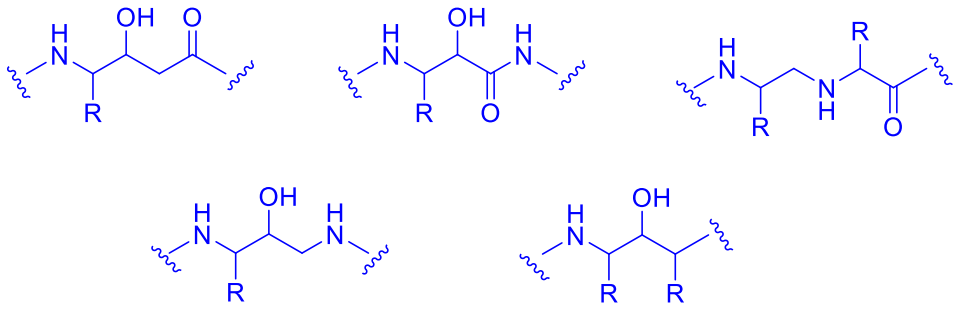


    b. What general type of inhibitor works best for this type of enzyme?
    Transition state analog (mimic)

    c. What kind of isostere is a key part of these drugs?
    Non-cleavable amide bond isostere

    d. Give an example of one of these isosteres.





14. HIV is a member of a class of viruses called retroviruses.
    a. What is meant by the term retrovirus?
    Virus carries its genetic information in the form of RNA. The RNA is translated into double stranded DNA inside the cell.
    b. What is the key enzyme found in retroviruses that give rise to this behavior?
    Reverse transcriptase

    c. What general drug strategy is used to inhibit this enzyme?
    Rational drug design using chain terminators and non-natural sugar nucleoside analogues.

15. The HIV drug 3-TC is an unusual HIV drug in that it has a very "clean" side effect profile.
    a. What is the key structural element that gives rise to this "clean" profile?
       It is a pseuodoenantiomer of natural nucleosides

    b. Why does this feature make the drug so selective?
       The pseuodoenantiomer is accepted by HIV reverse transcriptase and becomes incorporated into viral nucleic acid. The drug acts like a chain terminator and stops nucleic acid replication. Because it is a pseuodoenantiomer it will not be accepted as a substrate by host polymerases. Because it does not inhibit any host enzymes or nucleic acid production, it does not produce a lot of side effects.