Discipline
GENERAL PHARMACEUTICAL CHEMISTRY
Lesson № 14

DRUGS FOR THE TREATMENT OF HEPATITIS B VIRUS INFECTIONS

QUESTIONS FOR THE LESSON

1. General characteristics of hepatitis B virus.
2. The structure of the hepatitis B virus virion.
3. HBV life cycle. The Golgi apparatus.
4. Treatment of HBV infection. Treatment of HBV infection with drugs:
interferon α-2b
lamivudine, entecavir,
adefovir, adefovir dipivoxil
telbivudine, tenofovir and tenofovir disoproxil GS-9620 and Bay 41-4109,
5. Synthesis and brief description of Lamivudine.
6. Synthesis and brief description of Adefovir.

GENERAL CHARACTERISTICS OF HEPATITIS B VIRUS

Viral hepatitis is a group of infectious liver diseases that are caused by viruses belonging to different taxonomic groups. The causative agents of viral hepatitis differ in:

- ✓ in the form of virions,
- ✓ type of nucleic acid,
- ✓ mechanisms of penetration into the body,
- ✓ ways of infection, pathogenesis of the disease,
- ✓ clinical manifestations,
- ✓ the severity of the current and
- ✓ infection outcomes.
- ✓ in all cases, liver damage is observed.

The study of the hepatitis B virus began with the research of the American scientist B.S. Blamberg (Figure 1), who in 1964 discovered unusual protein virus-like spherical and filamentous (rod-shaped) particles in the blood of Australian aborigines with jaundice, which he named the Australian antigen.

Later, it turned out that the Australian antigen is nothing more than the surface antigen of the hepatitis B virus (HBsAg – Hepatitis B surface antigen), which is formed in excess during the reproduction of the virus in the form of incomplete particles.

Figure 1. B.S. Blamberg

Hepatitis B virus (HBV) is a DNA-containing virus that is part of the family Hepadnaviridae and is a member of the genus Orthohepadnavirus. The only representative of this genus – HBV infects humans.

The family Hepadnaviridae has a second genus, Avihepadnavirus, which viruses infect birds. There are also closely related viruses that infect rodents and primates whose genus has not yet been assigned.

There are 8 genotypes for HBV: A, B, C, D, E, F, G, and H. The differences in the nucleotide sequence of the genomes between the genotypes are about 8 %.

- Genotypes A and D are ubiquitous;
- Genotypes C and B are typical for Southeast Asia and Japan.
- Genotype E is distributed mainly in Africa.
- Genotype F was found among the indigenous populations of South America and Alaska.
- Genotype G is sporadically found in various parts of the world, in particular in the USA and France.

Genotypes E and G are characterized by low variability in the sequence of nucleotides in the genome, compared with other genotypes.

HBV (hepatitis B virus) genotypes may have different biological properties. Recently, more and more importance has been attached to the genotype of the virus in the clinical aspects of the course of viral infection, as well as sensitivity to antiviral drugs. To date, it has been established that infection caused by HBV genotypes B and C correlates with liver damage; and infection caused by HBV genotype A is effectively cured by therapeutic methods using interferon.

The prevalence of chronic hepatitis B depends on the region. The highest prevalence rates (≥ 8%) are found in China, Southeast Asia, and sub-Saharan Africa. In these regions, chronic HBV infection is acquired mainly in early childhood, usually in the perinatal period. In areas of low prevalence, HBV infection is more often acquired in adulthood as a result of unprotected sexual intercourse or intravenous drug use..

It is believed that HBV appeared in the human population about 125,000 years ago. The division into two families Orthohepadnavirus and Avihepadnavirus occurred about 25 thousand years ago. And the division of HBV into genotypes A –

H occurred about 7 thousand years ago.

Hepatitis B infection is a serious global problem of modern healthcare. It is estimated that there are currently over 2 billion worldwide. One person is infected with HBV and more than 350 million people have chronic HBV infection. 780,000 deaths associated with HBV infection are recorded annually..

Chronic HBV infection is associated with significant morbidity and mortality and is the cause of acute and chronic hepatitis, fibrosis, cirrhosis of the liver and hepatocellular carcinoma. It has been established that if people who are chronically infected with HBV are not treated, approximately 15-25% of patients develop liver cirrhosis and hepatocellular carcinoma after 10 years. The clinical course of chronic HBV infection is characterized by a variety of symptoms and may change over time. People infected with HBV in early childhood tend to have an asymptomatic course of the disease that can last for several decades. HBV was one of the first viruses for which a link between chronic hepatitis and hepatocellular carcinoma (colloquially liver cancer is the most common primary malignant liver tumor) in humans was proven.

The exact mechanisms involved in virus-mediated pathology are not fully known. However, there is evidence that suppression of HBV DNA replication can delay and even reverse liver fibrotic disease and reduce the incidence of hepatocellular carcinoma. For this reason, a focus was made on vaccination to prevent the spread of HBV, and on drug therapy to minimize the progression of the disease to cirrhosis and hepatocellular carcinoma in patients with chronic HBV infection.

THE STRUCTURE OF THE VIRION

HBV (Hepatitis B virus) is an enveloped virus, its virion has a spherical shape with a diameter of 42 nm. The outer lipid envelope of the virion contains glycoproteins – surface antigens L, S and M (L-, S- and M-HBsAg — from Hepatitis B surface Antigen). Inside the virion is an icosahedron capsid measuring 28 nm. The capsid consists of the HBcAg monomer protein (from Hepatitis B core Antigen). Inside the capsid is a viral polymerase and viral DNA (Figure 2).

The genome (a collection of hereditary material) HBV is represented by a single copy of double-stranded ring-shaped DNA.

- A short DNA strand is an incomplete plus-strand.
- A long strand of DNA is a negative strand.

While the minus strand contains a complete copy of the viral genome, the plus DNA strand is incomplete and accounts for approximately 60% of the length of the minus strand. The long chain is connected to DNA polymerase, which completes the plus chain to a full-fledged structure. DNA polymerase promotes the synthesis of new DNA strands on the matrix of both DNA and RNA. The total volume of the genome is about 3200 nucleotides (for a full-length chain) and 1700-2800 nucleotides (for a short chain). There are 4 genes and encodes 7 proteins: glycoproteins L-, S- and M-HBsAg, protein-monomer HBcAg, polymerase, HBx protein-regulator of gene expression. (Gene expression is the process by which inherited information from a gene is transformed into a functional product, RNA or protein.) The genes partially overlap, which is caused by the small size of the genome.

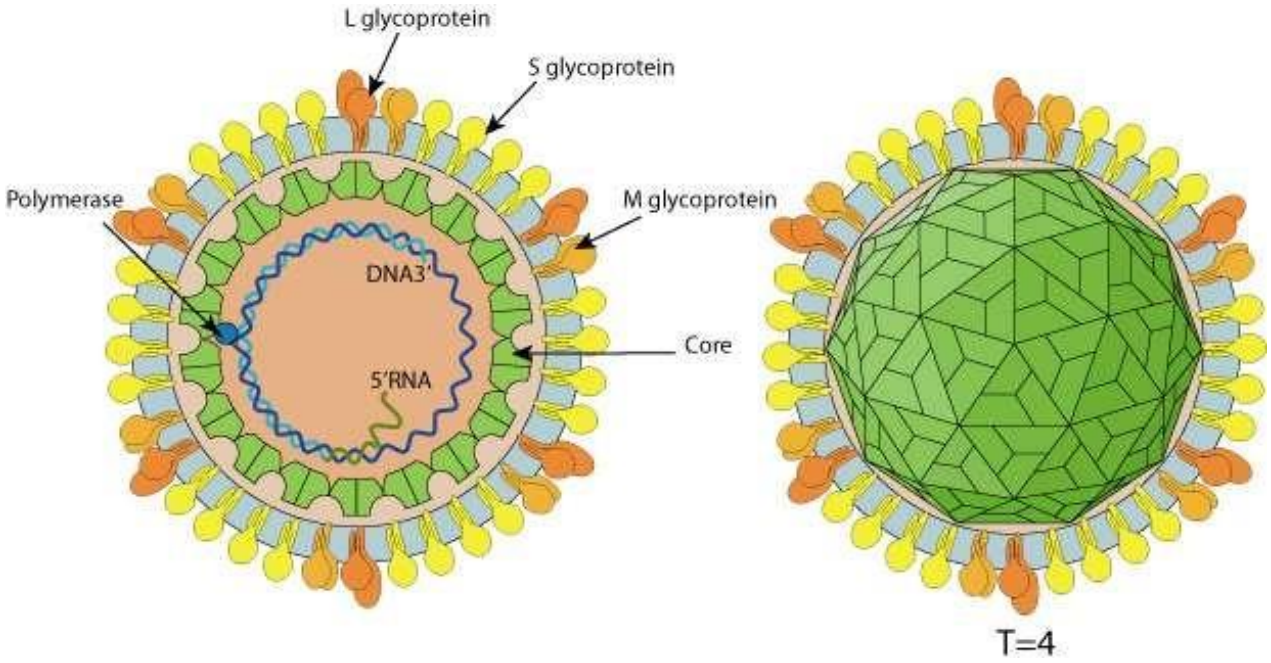


Figure 2. Structure of the hepatitis B virus virion

HBV LIFE CYCLE

HBV is a hepatotropic virus because hepatocytes are the only cells that support HBV replication.

The virion binds to the receptor on the surface of the hepatocyte, NTCP – Na+, a taurocholate co-transporting polypeptide. It is a glycoprotein and participates in the metabolism of bile acids. Entry into the cell is carried out through clathrin-dependent endocytosis. In this case, an endosome is formed, inside which there is a virion attached to a cellular receptor.

After an increase in acidity, the viral and cell membranes fuse inside the endosome, mediated by the work of viral glycoproteins. As a result of this process, the capsid enters the cytoplasm along with the genomic DNA..

- *Hepatotropic viruses in liver cells* (hepatocytes) fulfill their main biological mission – they multiply
- *Clathrin* is an intracellular protein, the main component of the membrane of the fringed vesicles
- *Clathrin-dependent endocytosis* is the entry of fragments of the cytoplasmic membrane, along with all its contents, into a cell in the form of bubbles coated externally with a lattice of polymerized clathrin..

Since HBV synthesizes new copies of genomic DNA through the stage of RNA formation using cellular enzymes, the genomic DNA is transported inside the capsid into the cell nucleus. Upon reaching a pore in the nucleus, the capsid dissociates and the genomic DNA completes the missing sections and turns into covalently closed circular DNA, which serves as a template for transcription of four viral mRNAs (matrix). The largest mRNA, which is larger than the entire viral genome, is used as a template for creating new copies of the genome using viral RNA-dependent DNA polymerase.

On the basis of viral mRNAs, precursor polypeptides are synthesized on ribosomes, which are processed in EPR and the Golgi apparatus. (The endoplasmic reticulum of the EPR, the plasma membrane and the Golgi apparatus form a single membrane system of the cell, within which the processes of protein and lipid metabolism take place using directed and regulated intracellular membrane transport). After assembly, the mature virion is released by secretion from the Golgi apparatus..

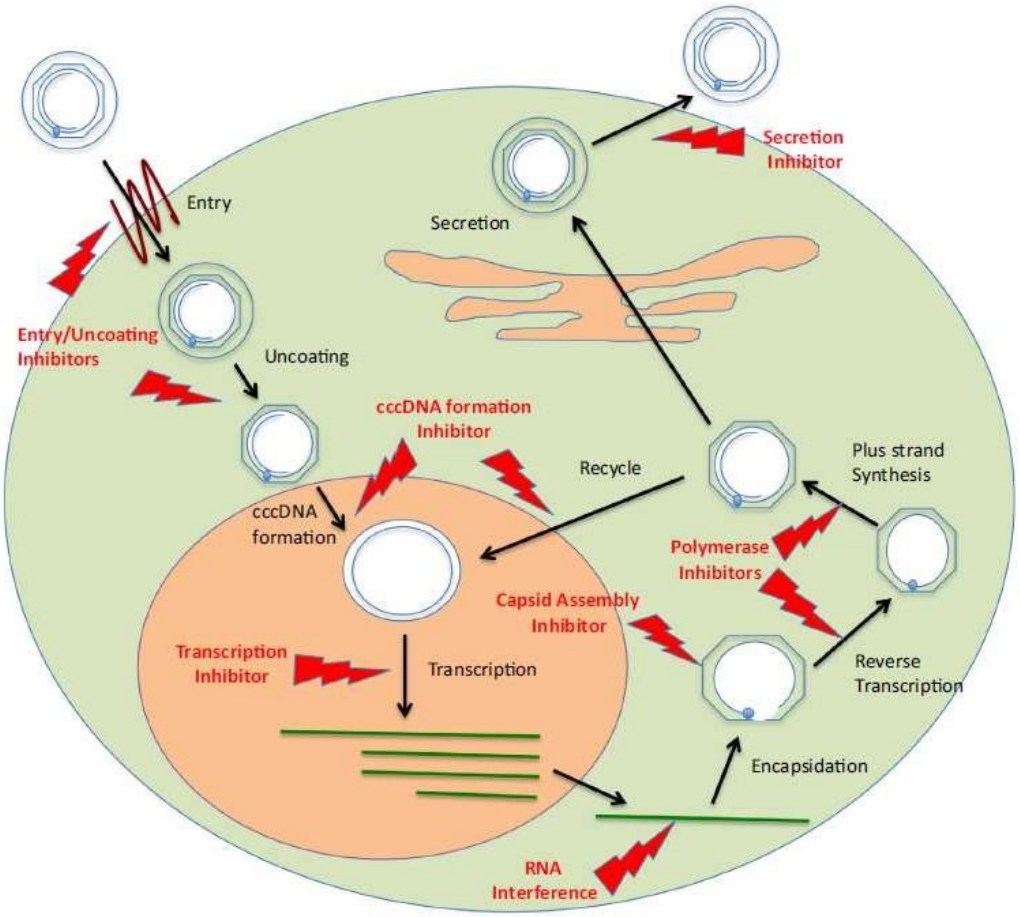


Figure 3. Golgi apparatus.

HBV INFECTION THERAPY

Several recent advances in the study of chronic HBV infection have led to a deeper understanding of the nature of this condition. The introduction of a sensitive polymerase chain reaction to detect HBV DNA has shown that many patients who were previously thought to have an inactive form of chronic HBV infection actually have detectable levels of HBV replication. In this regard, significant changes have been made in approaches to the treatment of chronic HBV infection. As a result, drug intervention begins in the early asymptomatic stages of the disease, which significantly reduces the risk of liver cirrhosis and hepatocellular carcinoma.

Vaccines directed against HBV small envelope protein HBsAg (HBsAg – Hepatitis B surface antigen) are quite effective in preventing HBV infection. Large-scale studies on universal infant vaccination have shown that vaccination can significantly reduce the incidence of both chronic HBV infection and hepatocellular carcinoma in children. However, the use of the vaccine has a number of side effects that limit its use.

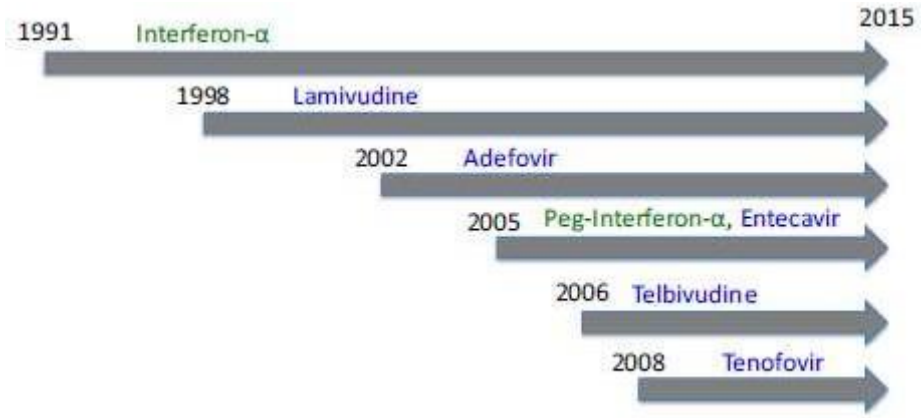
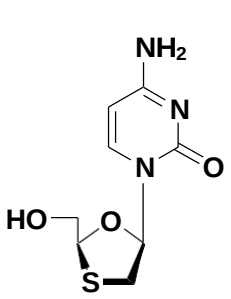


Figure 4. Reduction of morbidity after vaccination.

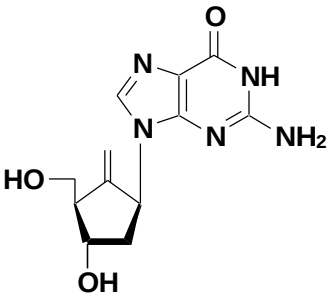
In 1991, a new protocol for the treatment of chronic HBV infection was proposed, which was based on the use of interferon α-2b. It is a highly purified recombinant protein with a molecular weight of 19,300 daltons (the molecular weight "Dalton" is used for the masses of molecules, atoms = $1{,}6605 \cdot 10^{-24}$ g). Interferon α-2b has an antiviral effect due to its interaction with specific membrane receptors and the induction of RNA and, ultimately, protein synthesis. (It has an effect that prevents the proliferation of tumor or precancerous cells).

When treated with interferon α-2b. In a significant number of patients, a decrease in the level of HBsAg (HBsAg – Hepatitis B surface antigen) in the blood was achieved after a 16-week course of subcutaneous injections. However, interferon treatment has been associated with a wide range of side effects, such as flu-like symptoms, fatigue, loss of appetite, and weight loss.

In 1998, the first nucleoside analog lamivudine was approved for the treatment of chronic HBV infection. Clinical studies have shown the presence of histological cell improvements and suppression of HBV DNA replication. However, the virus was found to be resistant to lamivudine in 70% of patients 5 years after the start of therapy.

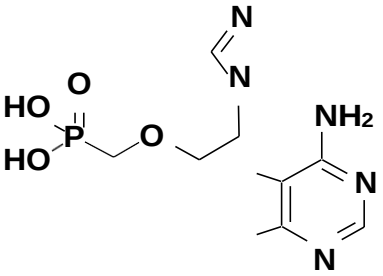


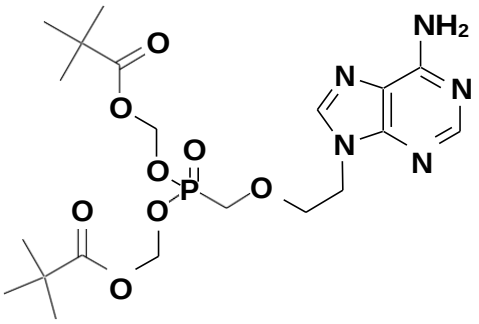
Lamivudine



Entecavir

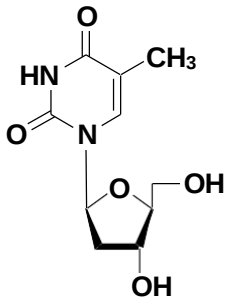
In 2002, adefovir was approved for the treatment of HBsAg-positive and HBsAg-negative patients with chronic HBV infection. The virus's resistance to adefovir is developing at a slower rate than to lamivudine. After five years of therapy, the presence of resistance was noted in 29% of patients with chronic HBV infection ламивудин. Через пять лет терапии наличие резистенции отмечено у 29% пациентов с с хронической ВГВ инфекцией.

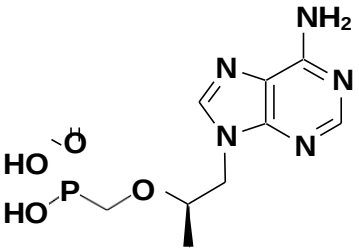


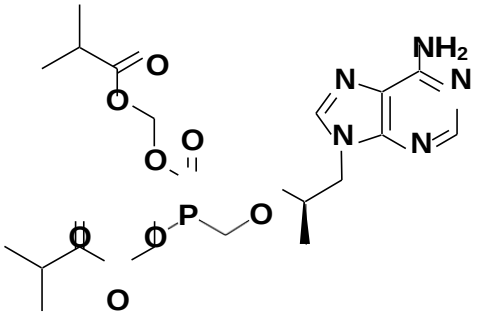


Adefovir **Adefovir dipivoxil**

Entecavir was approved in 2005, followed by telbivudine in 2006 and tenofovir in 2008.



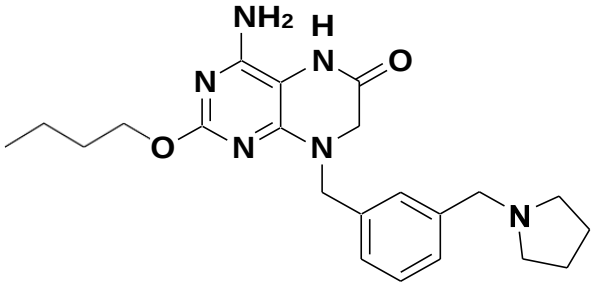




Telbivudine **Tenofovir** **Tenofovir disoproxil**

Gilead Sci. is developing a new HBV replication inhibitor, GS-9620 (a pyrrolopyrimidine compound). The mechanism of its action is that the compound binds to the TLR-7 receptor, which is a pathogen recognition receptor. This receptor is expressed in dendritic cells and B lymphocytes. The GS-9620 compound activates these cells, which recognize viral RNA and blocks HBV replication.

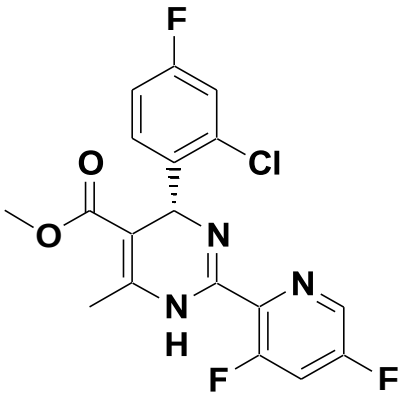
The compound is undergoing the final stage of clinical trials The compound is undergoing the final stage of clinical trials.



GS-9620

Another candidate drug is the compound Bay 41-4109 (a member of the heteroaryl dihydropyrimidine family), which is currently.

It is undergoing clinical trials. The mechanism of action of this compound is that it inhibits HBV replication and destabilizes the assembly of the viral capsid, thereby preventing the maturation of viral progeny.



Bay 41-4109

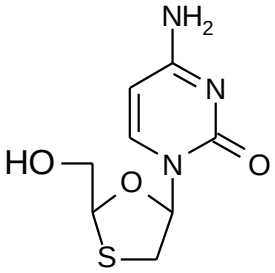
Bay 41-4109 is obtained by the three-component synthesis of Bijinelli.

Currently, there are many new drugs at different stages of development that affect different stages of the HBV virus life cycle. These include:

- Inhibitors of HBV binding to the NTCP receptor (cell entry inhibitor)
- inhibitors of HCV DNA (replication),
- Nucleocapsid assembly inhibitors,
- Genome editing technologies (TALEN, CRISPR/Cas, etc.) and RNA interference.

In addition to direct antiviral drugs, approaches are being developed to enhance the innate and adaptive immune response.

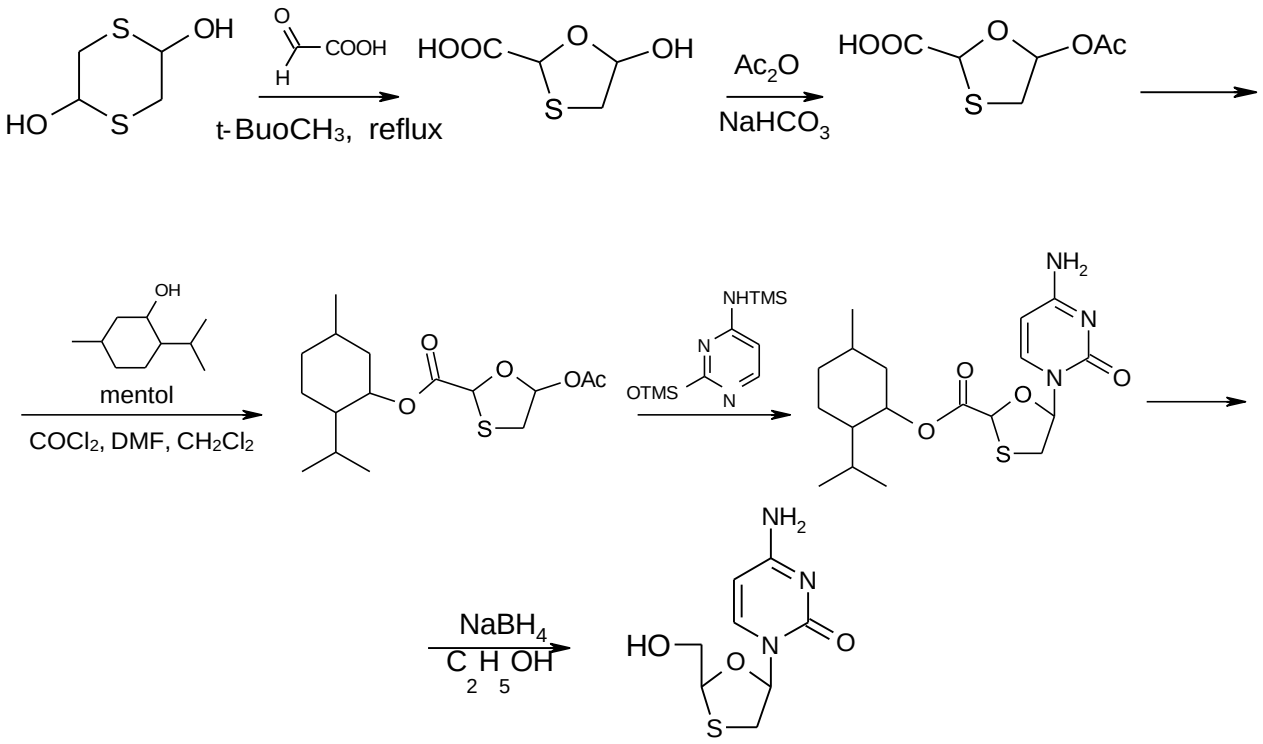
SYNTHESIS OF LAMIVUDINE



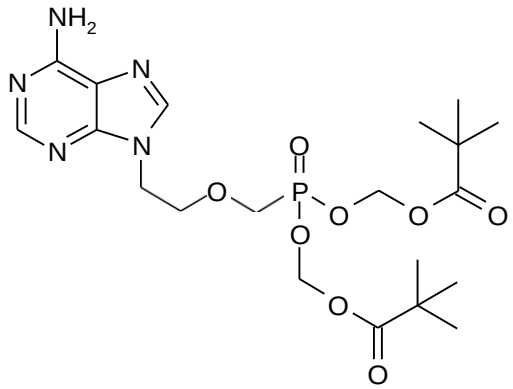
Lamivudine

Lamivudine has activity against hepatitis B virus, therefore it is useful in the treatment of HIV-infected people with hepatitis B. It improves the seroconversion of hepatitis B e-antigen, and also improves the histological condition of the liver. Prolonged use of the drug, however, leads to mutation and subsequent development of hepatitis B virus resistance. Despite this

lamivudine is still widely used due to its good tolerability.



SYNTHESIS OF ADEFOVIR
ADEFOVIR DIPIVOXIL



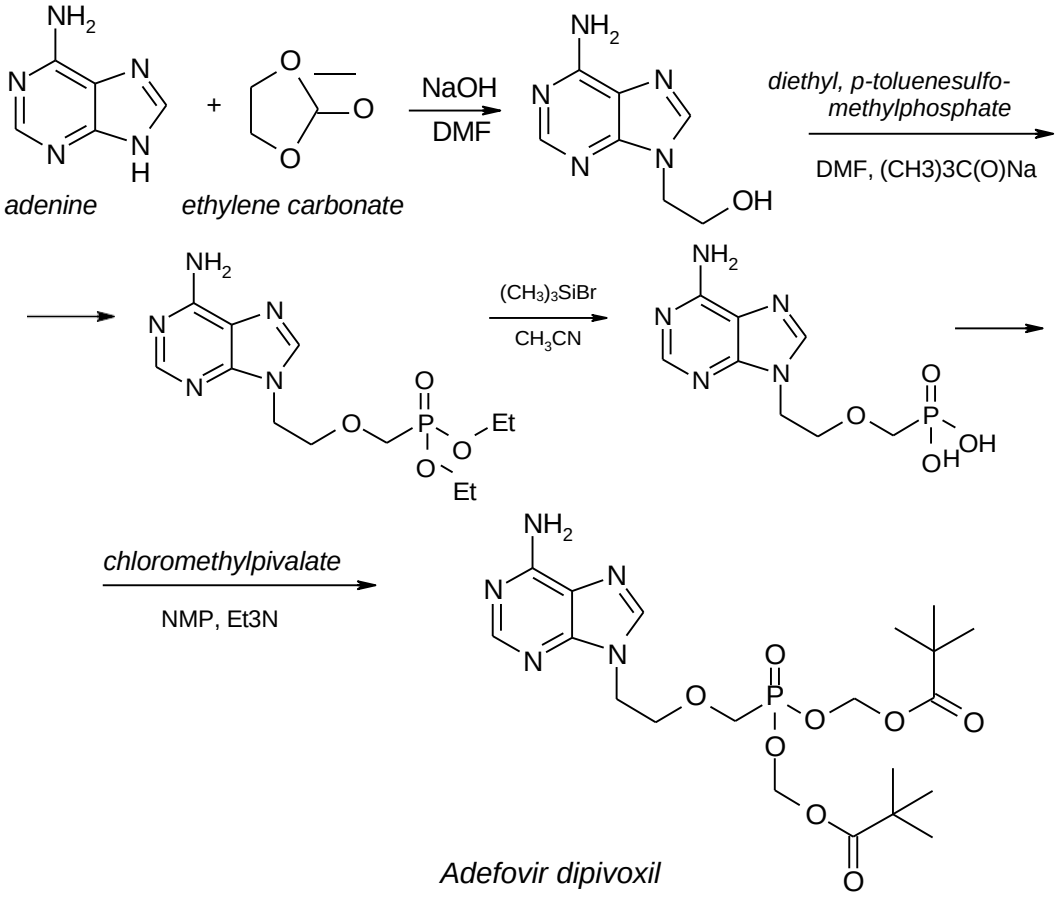
Adefovir

Adefovir is a prescription medication used to treat hepatitis B virus (HBV). It is distributed under the trade names Preveon and Hepsera. It is an oral nucleotide analogue of a reverse transcriptase inhibitor..

Adefovir was invented at the Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic by chemist Antonin Goli. The drug was developed by Gilead Sciences for the treatment of HIV under the brand name Preveon.

However, in November 1999, a panel of experts advised the FDA (FDA) not to approve the drug due to concerns about the severity and frequency of renal toxicity at a dosage of 60 or 120 mg. The FDA followed this advice, refusing to approve adefovir as a treatment for HIV..

Gilead Sciences discontinued its development for the treatment of HIV infection in December 1999, but continued to work with it as a drug for the treatment of hepatitis B (HBV), in which it proved effective at a much lower dose: 10 mg. Approval from the FDA for use in the treatment of hepatitis B in the United States was received on September 20, 2002, after which adefovir is sold under the brand name Hepsera.



The synthesis of tenofovir is similar.