BIOMOLECULES
SEM-5, CC-12
PART-10, PPT-30

Part-10: Nucleic Acids-I

CONTENTS

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BIOMOLECULES (PART-10, PPT-30)

Nucleic Acids-I

Nucleic Acids: Introduction

Nucleoproteins are one of the classes of conjugated proteins. The nuclei acid part is the prothestic group, and the protein part consists of protamins and histones. These latter compounds are basic and form salt-like compounds, the nucleoproteins, with the nucleic acid. On careful hydrolysis, nucleoproteins are broken down into the nucleic acids and protein. Nucleic acids are naturally occurring chemical compounds that serve as the primary information-carrying molecules in cells and make up the genetic material. They are the molecules that preserve hereditary information and play an important role in directing protein synthesis.

The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are found in abundance in all living things, where they create, encode, and then store information of every living cell of every life-form on Earth.

Structure of the Nucleic Acids

Nucleic acids are colourless solids, all of which contain the following elements: carbon, hydrogen, oxygen, nitrogen and phosphorous. The following chart shows the nature of the products obtained by hydrolysis of nucleic acid under different conditions.

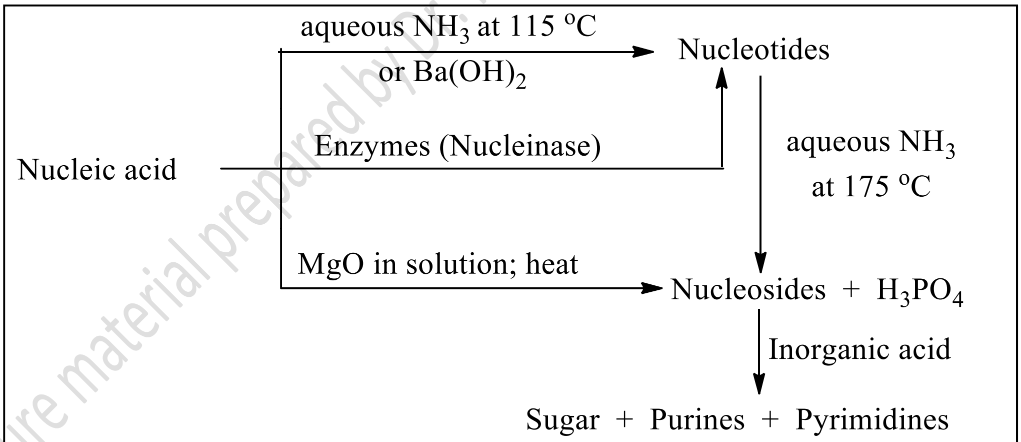


Chart 1: Hydrolysis products of nucleic acid under different conditions

Complete hydrolysis of the purine nucleotides by dilute acid occurs relatively easily, but the pyrimidine nucleotides usually require heating under pressure. On the other hand, hydrolysis of nucleic acids may be carried out by heating with 12(N) hydrochloric acid or with formic acid. Alkaline hydrolysis results in the formation of ribonucleoside 2´- and 3´-phosphates. Enzymic hydrolysis produces nucleoside 3´- and 5´-phosphates, the actual product depending on the nature of the enzyme.

Separation and isolation of the various types of hydrolytic products of nucleic acids are carried out by chromatographic methods and by *countercurrent distribution*.

Countercurrent distribution is a separation process that is founded on the principles of liquid-liquid extraction where a chemical compound is distributed (partitioned) between two immiscible liquid phases (e.g., oil and water) according to its relative solubility in the two phases. The simplest form of liquid-liquid extraction is the partitioning of a mixture of compounds between two immiscible liquid phases in a separatory funnel.

The purine and pyrimidine bases are readily separated and isolated by means of ion-exchange chromatography. Paper chromatography is particularly useful when dealing with small amounts of nucleic acids, and paper electrophoresis is very useful for the separation of small amounts of nucleotides. Column chromatography and countercurrent distribution, etc., have been used to separate and purify polynucleotides.

Sugars Present in Nucleic Acids

Two sugars have been isolated from the hydrolysates of nucleic acids, both are pentoses; D(-)-ribose (I) and 2-deoxy-D(-)-ribose (II) as shown in Figure 1.

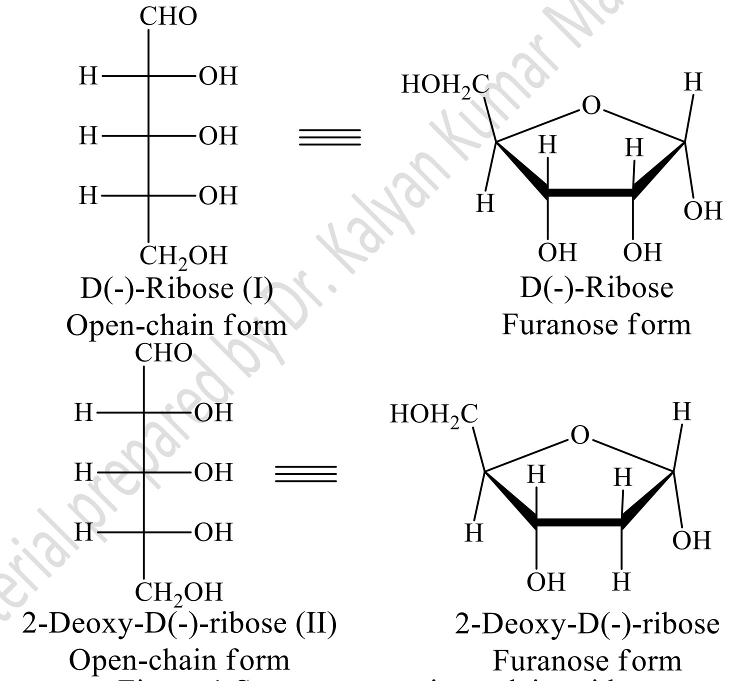


Figure 1:Sugars present in nucleic acids

The sugar (ribose or deoxyribose) is often represented, in a *Haworth projection* in which the C2´–C3´ bond is shown as a heavy line, implying that it is in the front of the page.

The nucleic acids are classified according to the nature of the sugar present. The ribonucleic acids (RNA), and the deoxyribonucleic acids (DNA). Ribonucleoproteins are found mainly in the cyctoplasm of the cell, whereas deoxynucleoproteins are found mainly in the cell nucleus. D(-)-Ribose is the pentose of yeast, liver and pancreas RNAs and 2-Deoxy-D-(-)-ribose occurs in thymus DNA. Nucleic acids also occur in plant and animal viruses.

Bases Present in Nucleic Acids

There are two types of bases which occur in nucleic acids: purine and pyrimidines. The most common purine bases are adenine and guanine. The most common pyrimidine bases are uracil, thymine and cytosine.

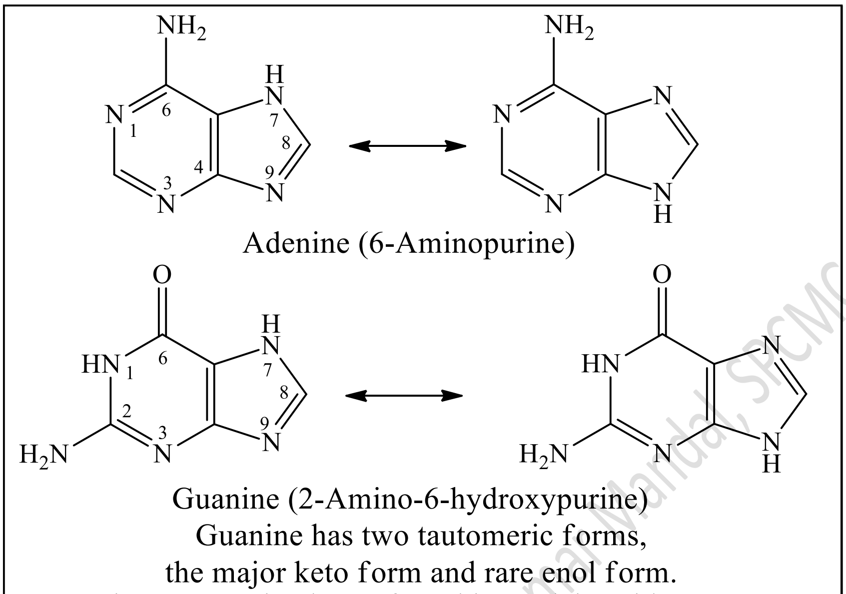


Figure 2: Purine bases found in nucleic acids

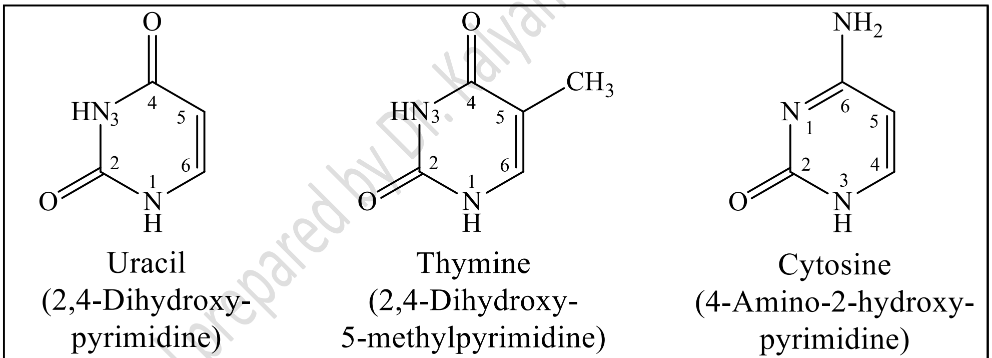


Figure 3: Pyrimidine bases found in nucleic acids

Purine and pyrimidine derivatives play a very important role in the structures of the nucleic acids DNA and RNA, polymers that are responsible for the storing and transmission of genetic information. Both types of nucleic acids (RNA and DNA) contain adenine and guanine. On the other hand, RNAs also contain uracil and cytosine, whereas DNAs contain thymine and cytosine. This distribution of pyrimidines, however, is not rigid, e.g., uracil has been found in certain DNAs. Heterocyclic compounds occur widely in living systems.

In the solid state and in ribose and deoxyribose nucleosides derived from these bases, adenine exists in the amino form, cytosine and guanine in the keto-amino form and uracil in the diketo form. X-ray analysis of the various bases has shown that all are planar. Combination of a base (either a purine or pyrimidine) with a sugar (ribose or deoxyribose) gives rise to a nucleoside, e.g., adenosine (ribose + adenine), guanosine (ribose + guanine), cytidine (ribose + cytosine), uridine (ribose + uracil), thymidine (deoxyribose + thymine). Combination of a nucleoside with phosphoric acid produces a nucleotide, i.e., nucleotides are nucleoside phosphates, e.g., adenylic, guanylic, cytidylic and uridylic acids.

Nucleotides and Nucleosides

Nucleic acids are linear polymers of monomeric units called nucleotides. Therefore, mild degradations of nucleic acids yield monomeric units, the nucleotides. A general formula for a nucleotide and the specific structure of one called adenylic acid (adenosine 5´-phosphate) from RNA are shown in Figure 4. Complete hydrolysis of a nucleotide furnishes:

1. A heterocyclic base from either the purine or pyrimidine family.
2. A five-carbon monosaccharide that is either D-ribose or 2-deoxy-D-ribose.
3. A phosphate ion.

A ribonucleoside is a compound formed between the furanose form of D-ribose and a heterocyclic base. The stereochemistry of the bond between the base and the ribose is most commonly *β*.

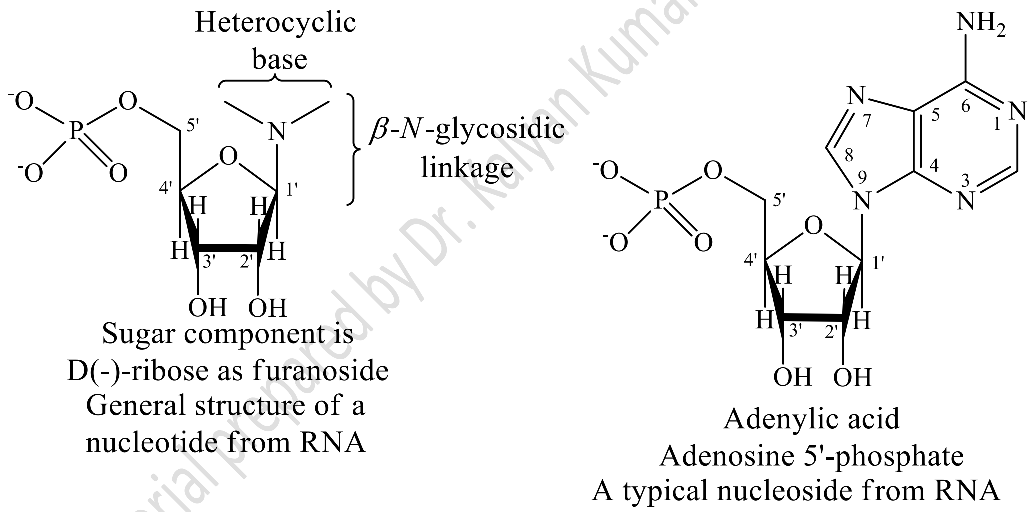


Figure 4: (A) General structure of a nucleotide obtained from RNA; (B) Adenylic acid, a typical nucleotide

In nucleotides obtained from RNA, the sugar component is 2´-D(-)-ribose. The phosphate group of the nucleotide is shown attached at C-5´. It may instead be attached at C-3´. A deoxyribonucleoside is a similar derivative of D-2-deoxyribose and a heterocyclic base. A general formula for a nucleotide and the specific structure of one called 2´-deoxyadenosine 5´-phosphate from DNA are shown in Figure 5.

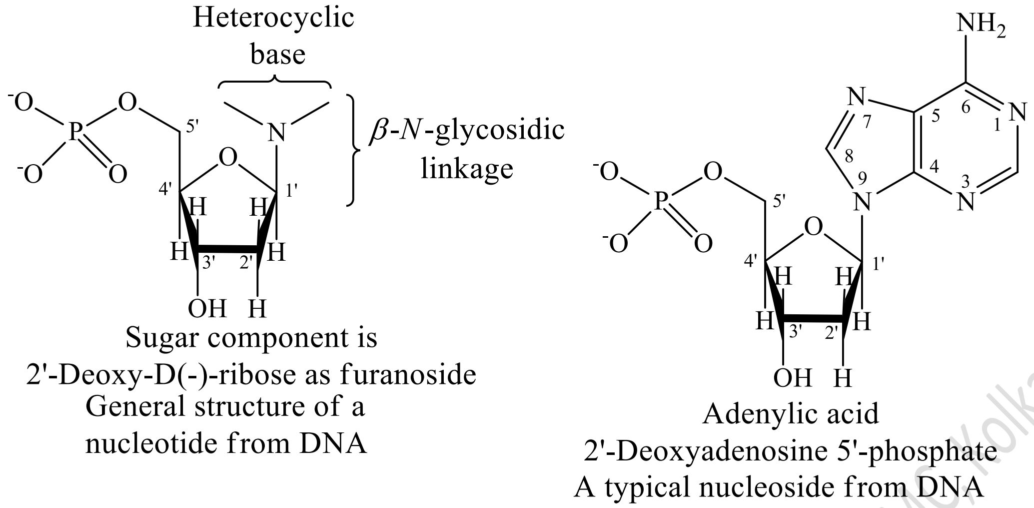


Figure 5: (A) General structure of a nucleotide obtained from DNA; (B) Adenylic acid, a typical nucleotide

In nucleotides obtained from DNA, the sugar component is 2´-deoxy-D(-)-ribose. The phosphate group of the nucleotide is shown attached at C-5´. It may instead be attached at C-3´. In DNA and RNA a phosphodiester linkage joins C-5´ of one nucleotide to C-3´ of another. The heterocyclic base is always attached through a *β*-*N*-glycosidic linkage at C-1´.

The central portion of the nucleotide is the monosaccharide, and it is always present as a five-membered ring, that is, as a furanoside. The heterocyclic base of a nucleotide is attached through an *N*-glycosidic linkage to C-1´ of the D(-)-ribose or 2-deoxy D(-)-ribose unit, and this linkage is always *β*. The phosphate group of a nucleotide is present as a phosphate ester and may be attached at C-5´ or C-3´. In nucleotides, the carbon atoms of the monosaccharide portion are designated with primed numbers, i.e., 1´, 2´, 3´, etc.

Removal of the phosphate group of a nucleotide converts it to a compound known as a nucleoside. The nucleosides that can be obtained from DNA all contain 2-deoxy-D(-)-ribose as their sugar component and one of four heterocyclic bases: adenine (A), guanine (G), thymine (T) or cytosine (C) (Figure 6).

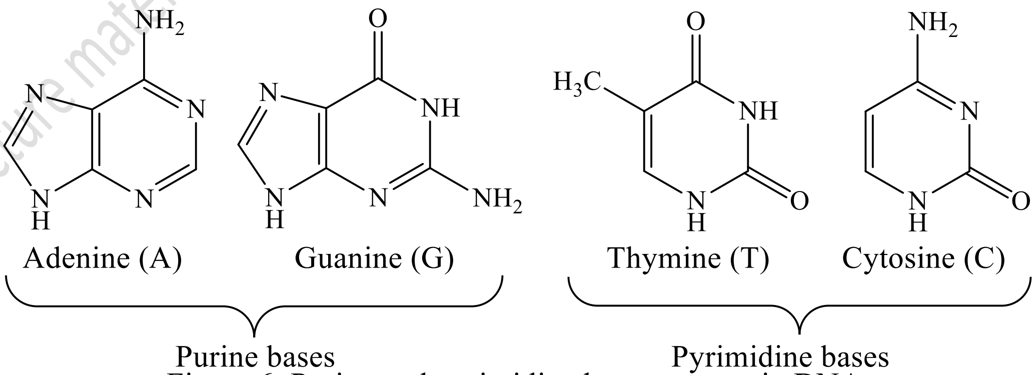


Figure 6: Purine and pyrimidine bases present in DNA

The nucleosides obtained from RNA contain D(-)-ribose as their sugar component and adenine (A), guanine (G), uracil (U) or cytosine (C) as their heterocyclic base.

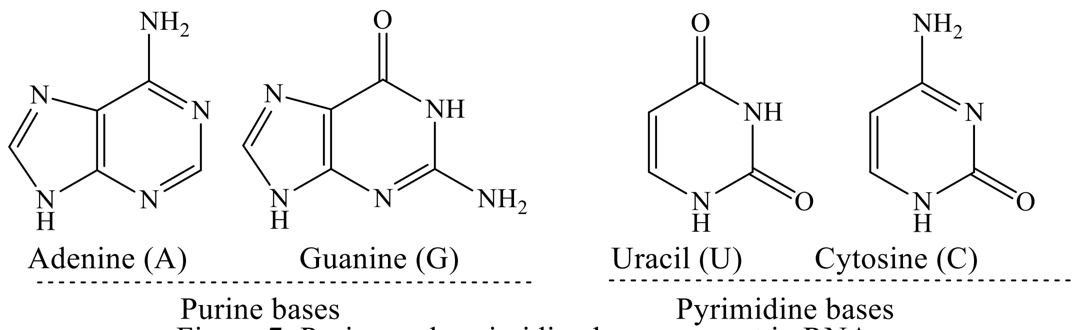


Figure 7: Purine and pyrimidine bases present in RNA

Uracil replaces thymine in an RNA nucleoside (or nucleotide). Some nucleosides obtained from specialized forms of RNA may also contain other, but similar, purines and pyrimidine bases. The heterocyclic bases obtained from nucleosides are capable of existing in more than one tautomeric form. The forms that have shown here are the predominant forms that the bases assume when they are present in nucleic acids.

Nucleosides and Nucleotides Found in RNA

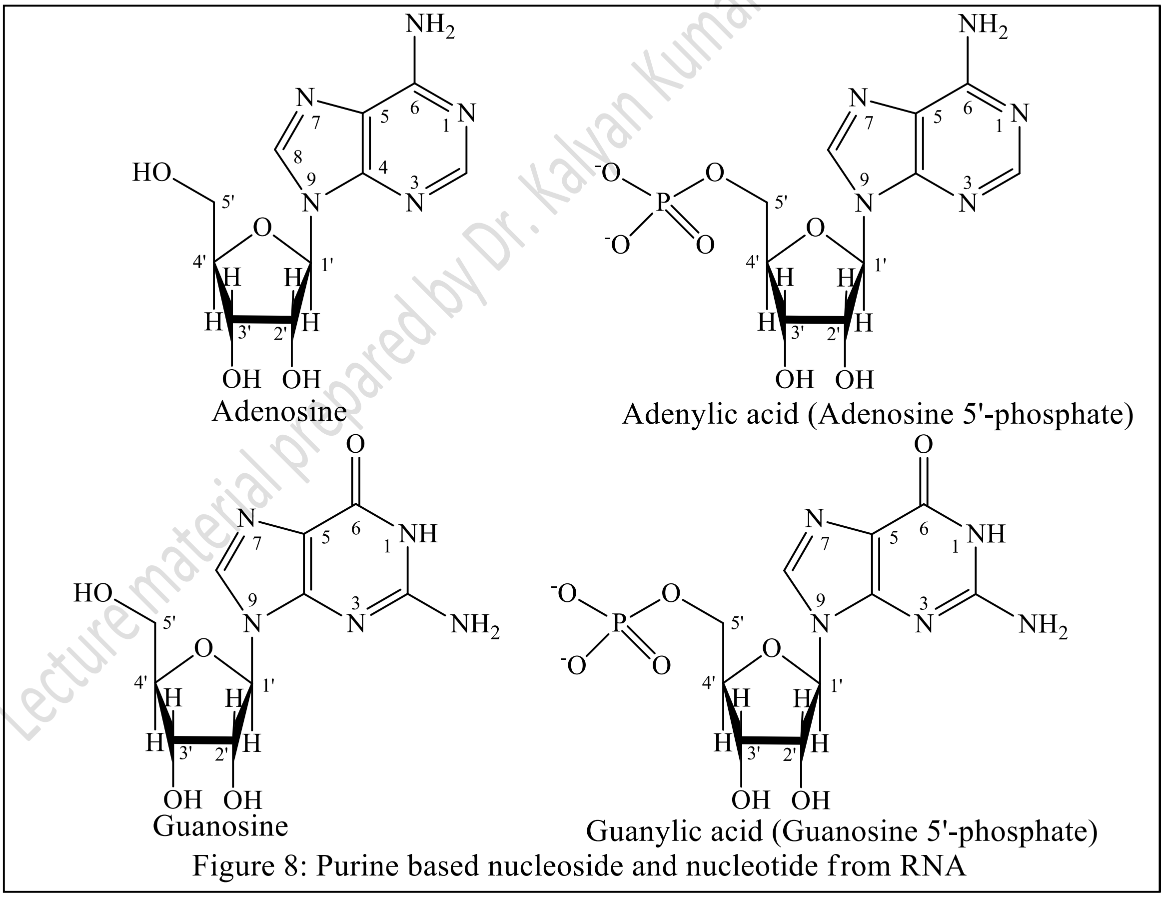
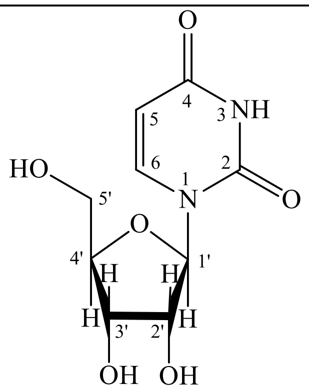
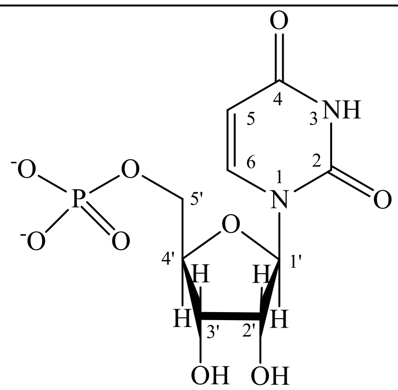


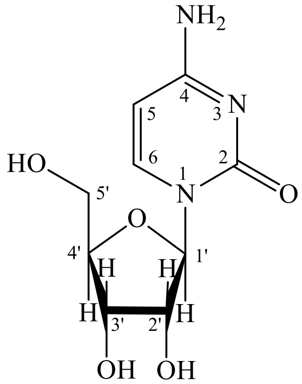
Figure 8: Purine based nucleoside and nucleotide from RNA



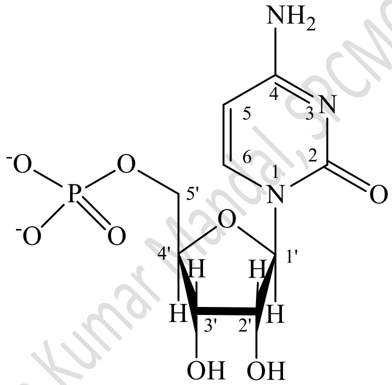
Uridine



Uridylic acid
Uridine 5'-phosphate



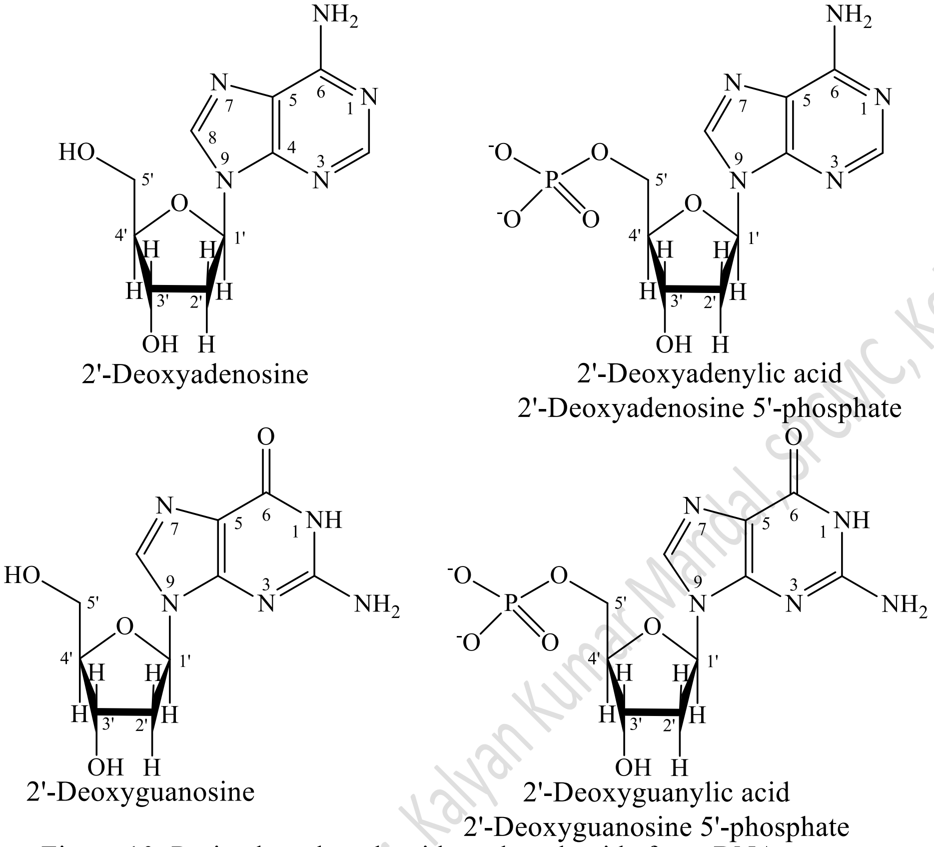
Cytidine



Cytidylic acid
Cytidine 5'-phosphate

Figure 9: Pyrimidine based nucleoside and nucleotide from RNA

Nucleosides and Nucleotides Found in DNA



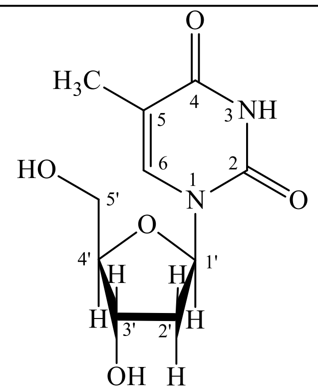
2'-Deoxyadenosine

2'-Deoxyadenylic acid
2'-Deoxyadenosine 5'-phosphate

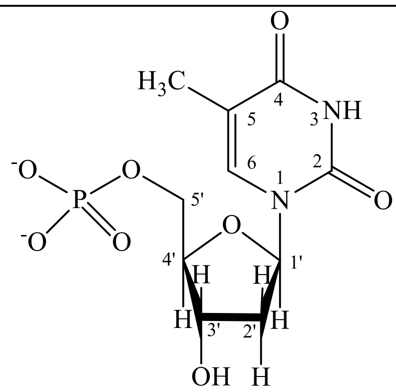
2'-Deoxyguanosine

2'-Deoxyguanylic acid
2'-Deoxyguanosine 5'-phosphate

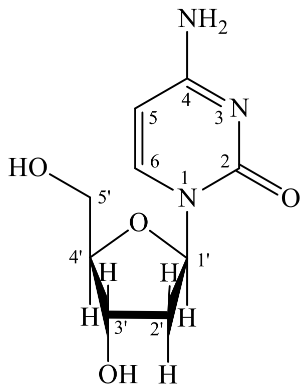
Figure 10: Purine based nucleoside and nucleotide from DNA



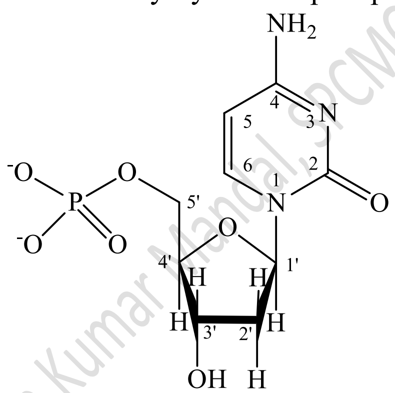
2'-Deoxythymine



2'-Deoxythmidylic acid
2'-Deoxythymine 5'-phosphate



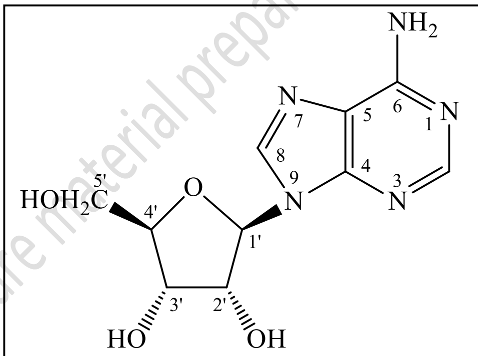
2'-Deoxycytidine



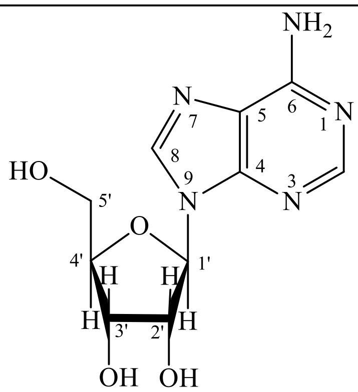
2'-Deoxycytidylic acid
2'-Deoxycytidine 5'-phosphate

Figure 11: Pyrimidine based nucleoside and nucleotide from DNA

Nucleosides in Different Projections



Adenosine
line-and-wedge structure
for the sugar



Adenosine
Haworth projection
for the sugar

Figure 12: Different projection of adenosine

Neither representation, however, conveys the fact that the five-membered ring actually exists as an equilibrium mixture of several rapidly interconverting puckered conformations.

Nucleosides in Different Orientations

In a nucleoside or nucleotide, the base is conventionally shown in either of two ways that differ by a 180° angle of internal rotation about the glycosidic bond - that is, the bond between the base and the sugar (C1´-N9 in adenosine).

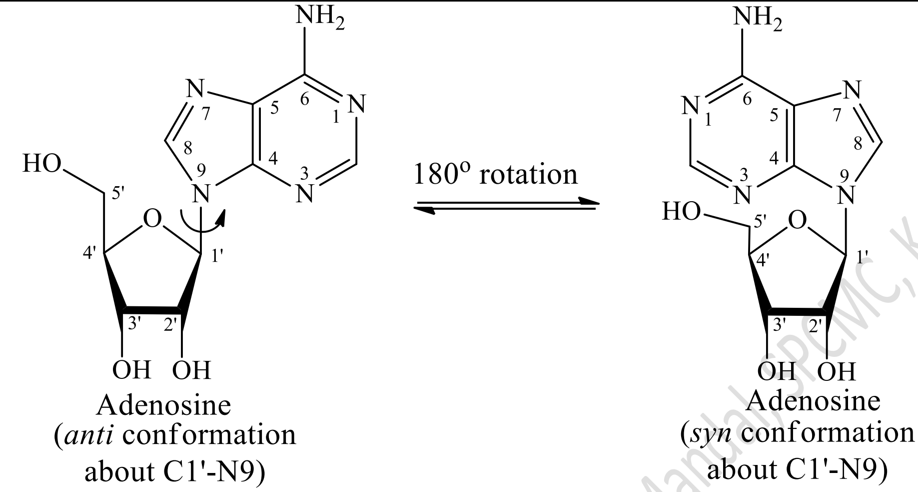


Figure 13: Different orientations of adenosine

Naming of Nucleotides

Nucleotides are named in several ways. Adenylic acid (Figure 8), for example, is usually called AMP, for adenosine monophosphate. The position of the phosphate group is sometimes explicitly noted by use of the names adenosine 5´-monophosphate or 5´-adenylic acid. Uridylic acid is usually called UMP, for uridine monophosphate, although it can also be called uridine 5´-monophosphate or 5´-uridylic acid.

If a nucleotide is present as a diphosphate or triphosphate, the names are adjusted accordingly, such as ADP for adenosine diphosphate or GTP for guanosine triphosphate. Nucleosides and nucleotides are found in places other than as part of the structure of DNA and RNA. For example, adenosine units are part of the structures of two important coenzymes, NADH and coenzyme A. The 5´-triphosphate of adenosine is, of course, the important energy source, ATP.

Adenylic acid is abbreviated as AMP (for adenosine monophosphate). The di- and tri-phosphorylated derivatives are called ADP (adenosine diphosphate) and ATP (adenosine triphosphate), respectively.

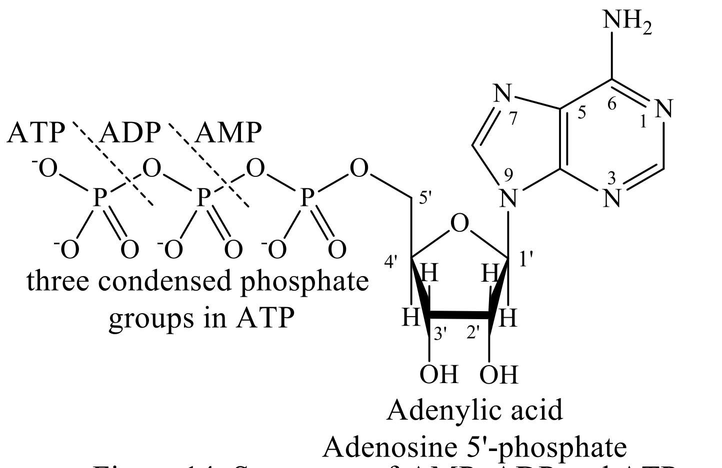


Figure 14: Structures of AMP, ADP and ATP

Nucleosides and nucleotides are found in places other than as part of the structure of DNA and RNA. For example, adenosine units are part of the structures of two important coenzymes, NADH and coenzyme A. The 5´-triphosphate of adenosine is, of course, the important energy source, ATP. The compound called 3´,5´-cyclic adenylic acid (or cyclic AMP) (Figure 15) is an important regulator of hormone activity. Cells synthesize this compound from ATP through the action of an enzyme, *adenylate cyclase*. In the laboratory, 3´,5´-cyclic adenylic acid can be prepared through dehydration of 5´-adenylic acid with dicyclohexylcarbodiimide (DCC).

Cyclic Adenosine Monophosphate

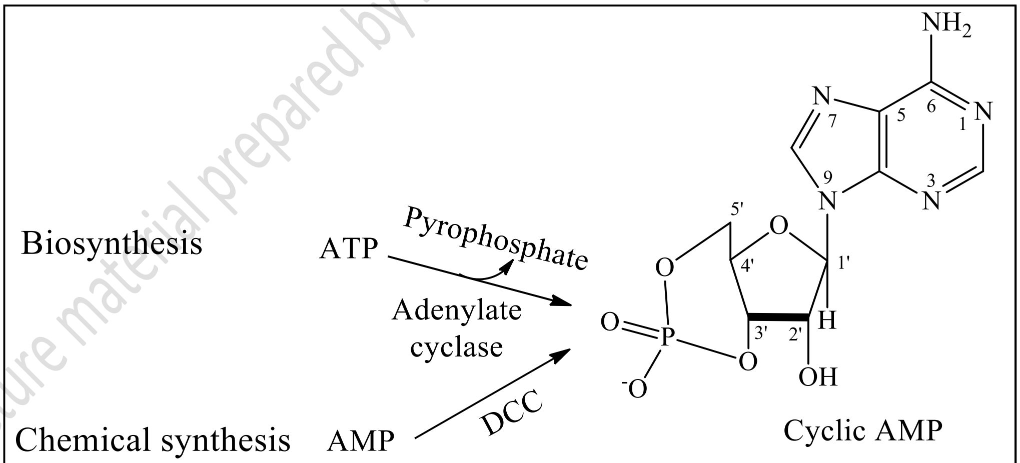


Figure 15: 3',5'-Cyclic adenylic acid (cyclic AMP)

When 3´,5´-cyclic adenylic acid is treated with aqueous sodium hydroxide, the major product that is obtained is 3´-adenylic acid (adenosine 3´-phosphate) rather than 5´-adenylic acid. The reaction is illustrated in Figure 16. The reaction appears to take place through an S_N2 mechanism. Attack occurs preferentially at the primary 5-carbon (C-5) atom rather than at the secondary 3-carbon (C-3) atom due to the difference in steric hindrance.

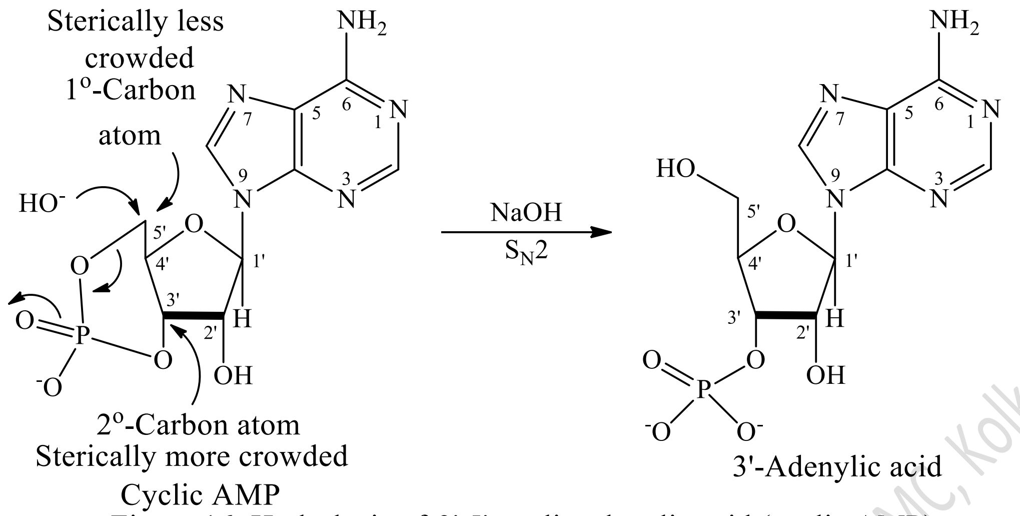


Figure 16: Hydrolysis of 3',5'-cyclic adenylic acid (cyclic AMP)