

## 1.2 | Principles of Thermodynamics

Thermodynamics relates with energy transformation which occurs in physical and chemical processes. The acquired/released energy is used for the synthesis of various biomolecules to be used in growth and differentiation of the organisms. It also provides an insight into our understanding, how the metabolic processes take place, besides explaining the conditions of various spontaneous reactions. Therefore, in this section we discuss first and second law of thermodynamics, concept of free energy and nature of processes at equilibrium as applicable to chemical and biochemical spontaneity.

### 1.2.1 | First Law of Thermodynamics

The first law of thermodynamics states that the energy is neither created nor destroyed, although it can be transformed from one form to another. This is also known as the law of conservation of energy.

Mathematically, it can be expressed as

$$\Delta U = \Delta H - \Delta W,$$

where  $\Delta U$  is the change in internal energy,  $\Delta H$  is the heat exchanged from the surroundings and  $\Delta W$  is the work done by the system.

### 1.2.2 | Second Law of Thermodynamics

The second law of thermodynamics provides certain criterion for deciding whether a reaction will occur spontaneously or not and states that the total entropy (disorder or randomness of a system) of a system must increase if a process is to occur spontaneously.

In order to understand the laws of thermodynamics, it is necessary to define and understand the fundamental terms used.

### 1.2.3 | Free Energy

Free energy or Gibbs free energy is one of the important determinants of the direction that a spontaneous reaction will take and it indicates the energy yield of a process. If free energy change is negative, it means that the reaction will occur spontaneously and is termed exergonic. A reaction with positive free energy change does not proceed spontaneously and is termed endergonic. Endergonic reaction is thermodynamically not feasible unless the energy is applied. Actual free energy of any process cannot be determined; only the change in free energy ( $\Delta G$ ) can be determined. Delta ( $\Delta$ ) is a symbol of difference between final and initial states. Therefore,  $\Delta G$  of a chemical reaction is the difference in energies of the products and reactants, that is

$$\Delta G_{\text{reaction}} = \Delta G_{\text{product}} - \Delta G_{\text{reactants}}.$$

At constant temperature and pressure, the free energy change ( $\Delta G$ ) of a reaction, and change in entropy is given by the following equation, which combines the two laws of thermodynamics.

$$\Delta G = \Delta H - T\Delta S.$$

In this equation,  $\Delta H$  is the change in enthalpy (heat) that occurs as the reaction proceeds at constant pressure,  $T$  is the absolute temperature at which the reaction occurs and  $\Delta S$  is the change in entropy. The absolute heat and entropy are difficult to measure; it is possible to measure only the changes in these quantities.  $\Delta G$  may be expressed in Joules (J) per mole or kilojoules (kJ) per mole or in calories (cal) per mole or kilocalories (kcal) per mole ( $4.186 \text{ J} = 1 \text{ cal}$ ). According to these units, the free energy yield of a reaction depends on the number of moles of reactants that is involved in the reaction.

### 1.2.4 | Standard Free Energy Change

The chemical reaction has a characteristic standard free energy change ( $\Delta G^\circ$ ). The standard free energy change is a constant for a given reaction and can be calculated from the equilibrium constant of the reaction under standard conditions, that is at a solute concentration of 1.0 M, at temperature of 25 °C (298 K) and at a pressure of 1.0 atmospheric pressure.

The free energy change which corresponds to this standard state is known as standard free energy ( $\Delta G^\circ$ ).  $\Delta G^\circ$  can be calculated from the following equation:

$$\Delta G^\circ = -RT \ln K_{eq} = -2.303 RT \log_{10} K_{eq}$$

Here  $T$  is absolute temperature and  $R$  is a gas constant (1.987 Cal/mol K):

$$\Delta G^\circ = -(1.987) (298) (2.303) \log_{10} K_{eq} = -1363 \log_{10} K_{eq}$$

This relationship which relates  $\Delta G^\circ$  to  $K_{eq}$  is very useful in determining the value of  $\Delta G^\circ$  for a specific reaction. If  $\Delta G^\circ$  is negative, which means the product contains less energy compared with the reactant, then the reaction will form the products under standard conditions. When  $\Delta G^\circ$  is positive, the products will have more free energy than the reactants. Therefore, the reaction will proceed in reverse direction.

Since all reactions do not take place at standard conditions, a relationship has been worked out by which a distinction can be made between  $\Delta G^\circ$  and the actual free energy change ( $\Delta G$ ):

$$\Delta G = \Delta G^\circ + 2.303 RT \log_{10} \frac{(\text{Products})}{(\text{Reactants})}$$

where the ratio of products to reactants is under actual initial concentrations. The actual free energy change depends on the standard free energy change and the ratio of initial concentrations of the products and the reactants.

It is also possible to evaluate  $\Delta G^\circ$  for the situation when concentration of both products and reactants is unity. In this case

$$\Delta G = \Delta G^\circ$$

Therefore,  $\Delta G^\circ$  can be defined as the change in free energy when products and reactants are present in unit concentration. The standard free energy change of a chemical reaction is simply a different mathematical way of expressing its equilibrium constant.

Since biochemical reactions take place near pH 7.0 and often involve  $H^+$  formation or use, pH 7.0 is regarded as the standard pH in biochemical systems. Therefore, the standard free energy change at pH 7.0 in biochemical system is designated by  $\Delta G^{\circ'}$ .

It has been observed that in all spontaneous chemical or physical reactions the free energy of the reacting system  $\Delta G$  is negative, but the standard free energy change  $\Delta G^{\circ'}$  may be positive, negative or zero depending upon the equilibrium constant of the reaction. From the value of  $\Delta G^{\circ'}$  the direction in which and how far a given reaction will go in order to reach equilibrium when it occurs under standard conditions may also be predicted.

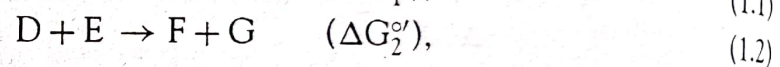
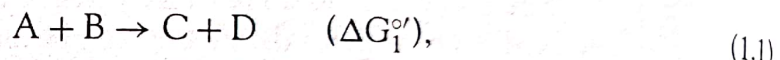
Free energy of nonstandard reactions, in which the concentrations each of reactants and products are not 1.0 M but the pH is 7.0, is known as biochemical actual free energy change  $\Delta G'$  and is related to  $\Delta G^{\circ'}$  in a similar way as  $\Delta G$  is related to  $\Delta G^\circ$ :

$$\Delta G' = \Delta G^{\circ'} + 2.303 RT \log_{10} \frac{(\text{Products})}{(\text{Reactants})}$$

For the determination of the actual free energy change that will occur under non-standard conditions of concentrations as the reaction proceeds to equilibrium, we can substitute the actual initial concentrations of the reactants and the standard values of  $R$ ,

$T$  and  $\Delta G^{\circ'}$  and on solving the equation will give  $\Delta G'$ , the free energy change of the reaction. The value of  $\Delta G'$  will decline as the concentrations of the reactants decline and the concentrations of the products increase. Therefore,  $\Delta G'$  of a chemical reaction will be negative and will slowly progress toward zero. However,  $\Delta G^{\circ'}$  is a constant. In actual sense, there can only be one free energy change for any reaction, no matter how it is represented ( $\Delta G$  or  $\Delta G'$ ).

Most of the metabolic pathways comprise both endergonic as well as exergonic reactions. In these cases the concept of coupling reactions enables us to understand the energetics of such metabolic reactions, since it applies both to the synthesis of energy compounds and to their utilization in synthesis of biomolecules. The concept of coupling may be clear from the following reactions, each reaction has its own equilibrium constant and will also have its standard free energy  $\Delta G_1^{\circ'}$  and  $\Delta G_2^{\circ'}$ :



On summing up the two reactions we obtain reaction (1.3):



Reaction (1.3) will have its own equilibrium constants as well as standard free energy  $\Delta G_3^{\circ'}$ . We can obtain the standard free energy change for the overall reaction by adding the standard free energy changes for all the individual reactions. This relationship is very useful in calculating the standard free energy change of the reactions for which equilibrium constant is not known and also of sequential reactions:

$$\Delta G^{\circ'}_{\text{overall}} = \Delta G_1^{\circ'} + \Delta G_2^{\circ'} + \Delta G_3^{\circ'}$$

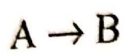
The free energy changes of a reaction vary with the concentration of the reactants as well as the temperature. Free energy is produced during catabolism and stored in energy-rich compounds. This stored energy is subsequently used for the endergonic-reaction characteristics of anabolism.

Glutamic acid

ADP  $\Delta G^\circ = -3.7 \text{ kcal}$

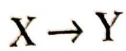
## Free Energy Changes are Additive

Let us first examine how a numerical value for the free energy change is calculated. Consider the following sets of reactions in which the ratio of reactants to products is displaced from equilibrium:



$$[B]/[A] = Q_a$$

where  $K_{eq} = K_a$



$$[Y]/[X] = Q_x$$

where  $K_{eq} = K_x$

then  $\Delta G_{A-B} = \Delta G_{X-Y}$

Since  $K_a/Q_a = K_x/Q_x$ , the value of free energy change can be calculated:

$$\Delta G = -RT \ln \frac{K_{eq}}{Q} \quad \text{or} \quad = -5,706 \log \frac{K_{eq}}{Q} \text{ J mol}^{-1} \text{ at } 25^\circ\text{C}$$

where

$\Delta G$  = Gibb's free energy change

$R$  = gas constant (1.987 calories/mole/degree)

$T$  = absolute temperature

$Q$  = product/reactants

Free energy changes are additive, which can be studied from a series of sequential reactions. Previously, we have introduced a term, standard free energy change ( $G^\circ$ ), which is useful in calculating quantitative values

$$\Delta G^\circ = -RT \ln K_{eq}$$

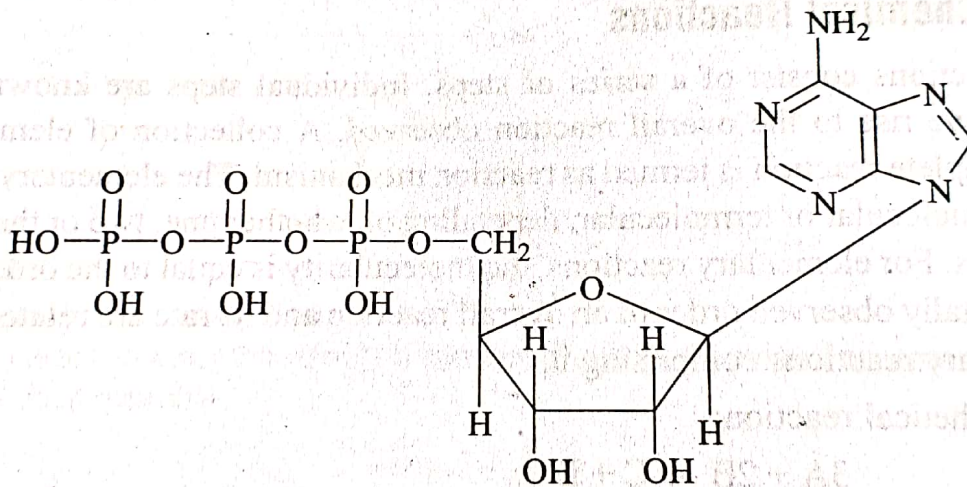
for generating energy under standard conditions. Now consider a reaction

## 13.6 HIGH ENERGY COMPOUNDS

There are different forms of energy that are interconvertible, but the ultimate source of energy for the living systems is the sun without which life would be impossible. Living organisms accumulate free energy from their environment and store it in the chemical bonds of complex biomolecules. Green plants and photosynthetic bacteria use solar energy to carry out photosynthetic reactions. In animals, fungi, non-photosynthetic protista and most microorganisms, the usable energy is derived from food stuffs obtained from plant sources only.

### Adenosine Triphosphate

All living organisms utilize ATP as the source of energy to drive a large number of endergonic reactions. It was proposed by Lipmann in 1940 that ATP is the agent that links all energy-requiring and energy-yielding processes of the cell. When fuel molecules are degraded, bond energy is released and recovered by coupling with ADP to yield ATP through phosphorylation. Living organisms are isothermal in nature and all chemical reactions proceed without appreciable rise in temperature. ATP is a high energy or energy rich compound which yields a large negative free energy when hydrolyzed at physiological pH.



ATP is a nucleoside triphosphate belonging to the family of energy rich compounds. Let us consider the structure of this compound in some detail. It contains an adenine base and a 5-carbon

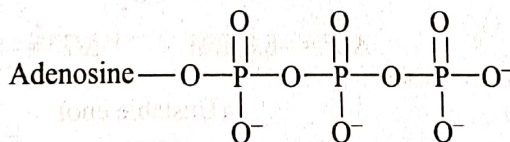
D-ribose sugar, to which three phosphate groups are attached. The first phosphate group is attached at the 5-carbon position of the ribose molecule through an ester linkage, whereas the second and third phosphate groups are linked linearly in anhydride linkages. ATP is an energy rich compound, which is highly charged and gets completely ionized at pH 7.0. The phosphate groups can be removed by hydrolysis in several ways with liberation of free energy. However, the free energy of hydrolysis of ATP is not constant in a cell, it varies from cell to cell depending upon pH and  $Mg^{2+}$  ion concentration.

Hydrolysis of ATP and the corresponding standard free energy changes are given in Table 13.2. Table 13.2 Hydrolysis of ATP and standard free energy changes with corresponding equilibrium constants at 25°C and pH 7.0

Reaction	$\Delta G^\circ$ Joules/mole	$K_{eq}$
$ATP + H_2O \rightarrow ADP + Pi$	-36,800	$2.8 \times 10^6 M$
$ADP + H_2O \rightarrow AMP + Pi$	-36,000	$2.0 \times 10^6 M$
$ATP + H_2O \rightarrow AMP + PPi$	-40,600	$1.3 \times 10^7 M$
$AMP + H_2O \rightarrow A + Pi$	-12,600	$1.6 \times 10^2 M$
$PPi + H_2O \rightarrow 2 Pi$	-31,800	$3.7 \times 10^5 M$

Standard free energy change associated with the hydrolysis of terminal phosphate is -7.3 kcal/mol. When the second phosphate is hydrolyzed, another -7.3 kcal of energy is released. These phosphate groups were linked through anhydride bonds which trap a large amount of energy. When the last phosphate group is hydrolyzed, the energy released has a smaller value, i.e., about -2.0 kcal. The smaller values are associated with ester bonds. Therefore, the terminal and second phosphate bonds in ATP are high-energy bonds denoted by ~P symbol (-P~P~P).

There are several classes of energy-rich compounds which are used in biological systems (Table 13.3). Now we might ask the question as to what factors contribute to the large negative free energy change? In case of ATP, the energy-rich bonds are present in the pyrophosphate component which gets fully ionized at pH 7.0.



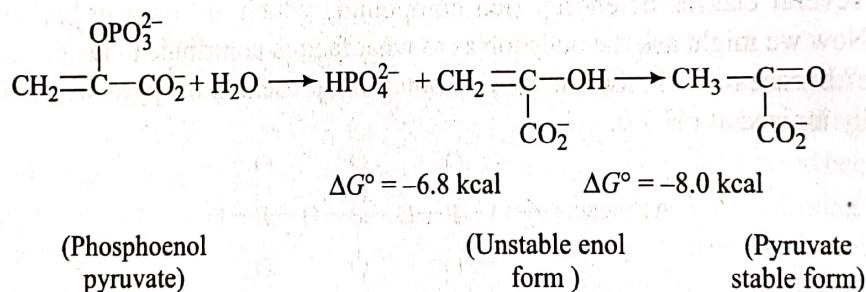
An important feature is that at pH 7.0 ATP has 3.8 negative charges that are closely placed. Negative charges repel each other and as a consequence terminal phosphate group is removed. This ensures elimination of some electrical stress so that  $ADP^{3-}$  and  $HPO_4^{2-}$  ions are formed. Because of charge repulsion, these ion products do not recombine to form ATP. The second feature is that  $ADP^{3-}$  and  $HPO_4^{2-}$  are stabilized as they are resonance hybrids. The negatively charged electrons around P and O atoms of the terminal phosphate bond of ATP compete with each other and are drawn closer to the electronegative oxygen atom. Because of this resonance, all negatively charged electrons in the terminal pyrophosphate bond of intact ATP molecule do not attain a low energy level, as happens when  $ADP^{3-}$  and  $HPO_4^{2-}$  are separate from each other.

### Enol Phosphates

Phosphoenol pyruvate is another energy rich compound which has a large free energy of hydrolysis (-14.8 kcal) at pH 7.0. It is an intermediate in carbohydrate metabolism, used for regenerating ATP from ADP by phosphorylation.

Table 13.3 Standard free energy of hydrolysis of some energy rich biological compounds

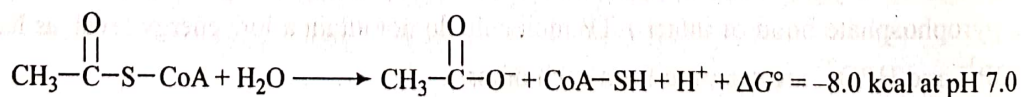
Compound	$\Delta G^\circ$ at pH 7.0 (kcal/mol)
Phosphoenol pyruvate	-14.8
Cyclic AMP	-12.0
1, 3-Diphosphoglyceric acid	-11.9
Acetyl phosphate	-10.5
Acetoacetyl CoA	-10.3
Phosphocreatine	-10.0
S-Adenosylmethionine	-8.0
Pyrophosphate	-7.7
Acetyl CoA	-8.6
ATP to AMP+PPi	-7.3
ATP to ADP+Pi	-7.6
UDP-glucose	-6.6
Sucrose	-5.0
Glucose-1-phosphate	-3.8
Fructose-6-phosphate	-3.3
Glucose-6-phosphate	-3.0
Lactose	-2.4
3-Phosphoglyceric acid	-2.2
Sn-Glycerol-3-phosphate	-2.2



Phosphoenol pyruvate is a phosphorylating agent that can be used to synthesize organic phosphates. Hydrolysis of enol phosphate results in the formation of unstable enolic form of pyruvate, which undergoes isomerization to generate stable keto form.

### Thiol Esters

Thiol esters come under the category of energy rich compounds which are largely used to generate ATP. Acetyl coenzyme A is one such compound which yields approximately -8.0 kcal/mol upon hydrolysis.

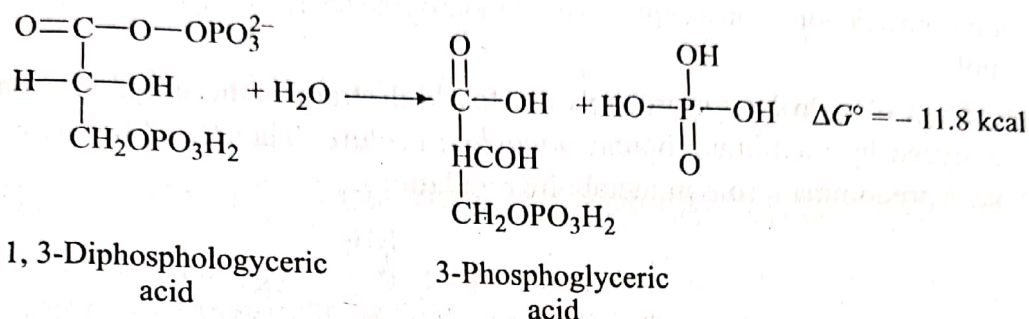


Thiol esters, unlike oxygen esters, do not show resonance character, and because of this the interaction between pi electrons of sulphur atom and the carbonyl group creates a driving force for hydrolysis and subsequent release of a large negative free energy. Sulphur atom does not release its electrons for double bond formation, but it may act as electron acceptor.



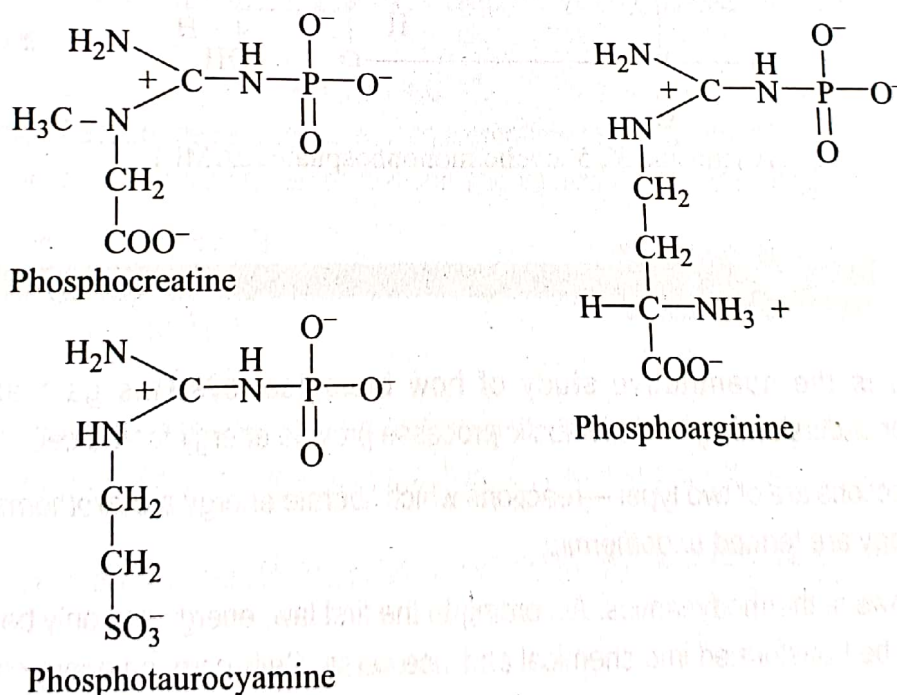
## Acyl Phosphates

Acyl phosphates are energy rich compounds having a large negative free energy. One such compound is 1,3-Diphosphoglyceric acid in which the electrons of the C=O bond are attracted towards the electronegative oxygen, making it more polar. The carbon and the phosphorus atoms have partial positive charges which repel each other and in order to overcome this repulsion, energy is required. When acyl phosphates are hydrolyzed, this energy is released.

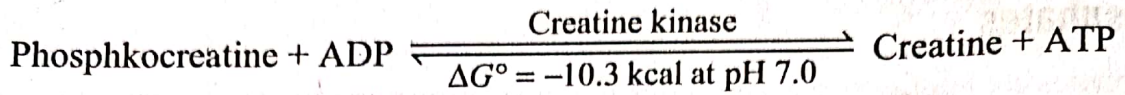


## Phosphagens

Phosphagens or phosphoguanidines are important energy-rich compounds which serve as energy storage molecules in muscles of vertebrates and invertebrates. Some important examples of naturally occurring phosphagens are phosphocreatine, phosphoarginine and phosphotaurocyamine.



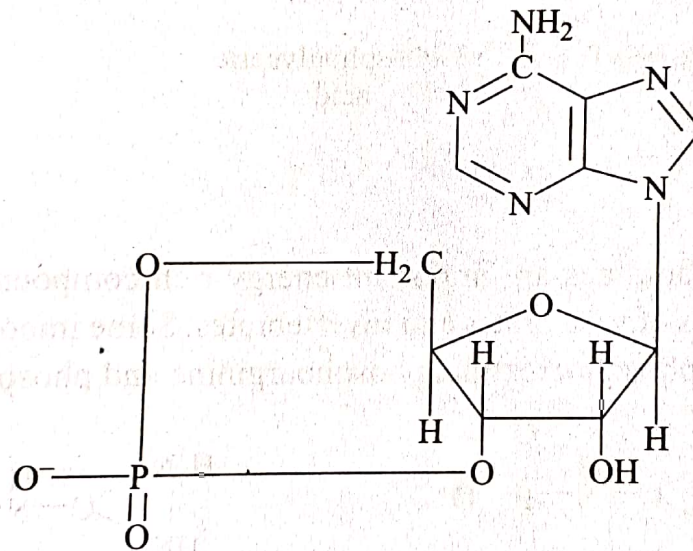
Normally, the concentration of compounds like nucleoside triphosphates and diphosphates is very low since the required amounts are regenerated during chemical reactions. Hence they do not form storage fuels. But in muscles the situation is different. Contraction of muscles occur at the expense of high energy phosphates readily available in the tissue itself. Phosphagens serve this purpose, occurring in hundreds of millimoles per kilogram of muscle tissue. The standard free energy of hydrolysis of such compounds is about 10.0 kcal per mol at normal physiological pH.



### Cyclic AMP

Cyclic adenosine monophosphate (cAMP) is also an energy rich compound which not only functions as a second messenger, but also serves to activate a specific enzyme, protein kinase, that transfers a phosphate group from a donor to an acceptor. The standard free energy of hydrolysis of this compound is  $-12.0 \text{ kcal/mol}$ .

Such a high energy of hydrolysis is probably due to a high strain on the anhydride bond. Synthesis of cAMP is catalyzed by membrane-bound *adenylate cyclase*. Though cAMP is a high energy compound, it has a predominant role in metabolic regulations.



Adenosine 3', 5' cyclic monophosphate (cAMP)