3. Formation and Cleavage of P-O Bonds

Hugo Bertschy, Gisèle Baudin and George M. Whitesides

Department of Chemistry, Harvard University, Cambridge, MA 02138

3.1 General remarks

The use of isolated enzymes to form or cleave P-O bonds is an important application of biocatalysts. Restriction endonucleases, (deoxy)ribonucleases, DNA/RNA-ligases, DNA/RNA-polymerases, reverse transcriptases etc. are central to modern molecular biology [1]. Enzyme catalyzed phosphoryl transfer reactions have also found important applications in synthetic organic chemistry. In particular, the development of convenient cofactor regeneration systems has made possible the practical-scale synthesis of carbohydrates, nucleoside phosphates, nucleoside phosphate sugars and other natural products and their analogs. This chapter gives an overview of this field of research covering the literature up to fall 1992.

Hundreds of potentially useful enzymes are available in nature. It is often worthwhile to survey enzymes for applicability in the synthesis of a specific compound, but how to find the best enzyme? Enzymes have been reviewed and classified by many schemes [2],[3],[4]. Enzymes involved in reactions at phosphoryl groups are, unfortunately for the synthetic chemist, spread almost over all

classes. Without a good knowledge of enzymology, it is not easy to find the enzyme classes of interest for a particular transformation. This review links the compound classes and enzyme classification systems in chapter B 3.1.1 to help overcome this barrier.

Most synthetically useful phosphorylating enzymes require nucleoside triphosphates as cofactors. The central importance of cofactor regeneration, and the most used regeneration methods for these cofactors, are discussed in chapter B 3.2.1. The end of chapter 3 includes tabular surveys of the most important applications, classified in compound or structural classes (see B 3.2.2 and 3.3), to facilitate the search for relevant enzymes and procedures.

3.1.1   Enzymes forming or cleaving phosphorous-oxygen bonds

Phosphoesters are ubiquitous in biochemistry and serve several functions [5]. Genetic information is stored in DNA and RNA. In cellular control mechanisms, phosphorylation of proteins is an important mechanism for regulating protein activities [6]. Phosphorylation can activate metabolites or change solubility properties. Enzyme-catalyzed formation and cleavage of P-O bonds are central to the cellular energy balance [7]. Biosynthesis depends heavily on phosphorylated intermediates.

A useful classification for enzymes involved in phosphoryl transfers was introduced by Knowles [8] (see figure 1). This classification, based on enzyme functions and mechanisms, differentiates

((figure 1))

primarily between two groups of enzymes. The first group contains only enzymes that accept phosphoric monoesters as substrates (type A and B). The second group includes all enzymes catalyzing reactions at phosphoryl groups of phosphodiesters (type C - E). Table 1a and 1b link

((table 1a and table 1b))

Knowles' classification and the enzyme classification recommended by the International Union of Biochemistry (IUB; compare chapter A 1.) [2]. The IUB classes give a direct access to the specific enzymes in reference works and to the CA registry numbers necessary for an efficient literature search [2], [4].

Table 1a and 1b list only the most important categories of enzyme classes (EC's). Some enzymes that are involved in reactions at phosphorus are hidden in other classes. For example glyceraldehyde-3-phosphate dehydrogenase, which catalyses the oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate, is classified under EC 1.2.1.12 and 1.2.1.13. Neither the name of the enzyme, nor its IUB-classification, gives information about the phosphorylating step. Identifying enzymes potentially useful in synthesis that have been ambiguously classified is difficult for those outside of biochemistry because no complete reference is available connecting enzymatic activity with synthetic applicability.

A second important point is that many enzyme catalyzed reactions are reversible. Some hydrolytic enzymes can be used in enzyme catalyzed phophorylation reactions. Alkaline phosphatase (EC 3.1.3.1), for example, was used in enzyme-catalyzed phosphorylation of glycerol with inorganic phosphate [9].

In some cases enzymes may catalyze unexpected reactions with unnatural substrates: aminoacyl tRNA synthetases (ARS) were used to synthesize p^1,p^4-di(adenosine 5'-)tetraphosphate (Ap$_4$A; 1), a natural inhibitor of human platelet aggregation [10] (figure 2). Here, in the first step an

((figure 2))

amino acid (AA) reacts reversibly with ATP and ARS and forms an aminoacyl-AMP-ARS complex and PP$_i$; the back reaction of this intermediate with ATP leads to the desired product Ap$_4$A [11],[12],[13].

One of the most important criteria in the evaluation of a new process is the availability of an enzyme [14] (see part C: Tabular Survey of Commercially Available Enzymes). If the enzymes are not commercially available, their isolation and purification can be expensive and time consuming (see chapter A 2.: Production and Isolation of Enzymes). The importance of the product to be synthesized may sometimes justify the additional effort.

Mechanistic aspects of P-O bond formations and cleavages have been reviewed recently [15] and are outside the scope of this work. The use of enzymes catalyzing the formation of P-N bonds -- for example, phosphorylations of amino acids (EC 2.7.3.) -- are discussed only briefly. Enzymes dealing with the formation of aminoacyl tRNA (EC 6.1.1.), acyl-CoA derivatives (6.2.1.) or peptides (6.3.2.) are also not covered, even if cleavages of nucleoside phosphates are involved.

3.1.2 Biological phosphorylating agents

To compare the ability of different compounds to transfer a phosphoryl group, phosphorylation of water was chosen as a standard reaction [17]. The free energy of hydrolysis of a phosphorus compound ($\Delta G_{hydr}°$) is called its phosphorylating potential. Table 2 summarizes the phosphorylating

((table 2))

potentials of the most important biological compounds (figure 3) having phosphoryl donor abilities.

((figure 3))

By far the most important strong biological phosphorylating agent is adenosine 5'-triphosphate (ATP, 8). ATP is ubiquitous and plays a central role as cofactor in anabolic and catabolic processes. Moreover, many enzymes involved in the formation of P-O bonds are ATP dependent. The biologically active form of ATP is, in most cases, the magnesium salt $MgATP^{2-}$ [22]. Other nucleoside triphosphates have similar phosphorylating potentials but they are rarely used as phosphoryl group donors [23],[24]; usually GTP, CTP and UTP act as nucleoside or nucleoside phosphate donors (see 3.2.2.2).

Creatine- and arginine phosphate (7 and 9) play important roles in the storage of phosphorylating potential in vertebrates and invertebrates, respectively [25],[26]. In living cells, these N-phospho-

guanidine derivatives are formed by phosphoryl group transfer from ATP, and in the reverse reaction ADP is the only acceptor for **7** and **9**.

1,3-Diphosphoglycerate (**5**) and phosphoenolpyruvate (**2**) are important phosphorylating agents of ADP in the glycolytic pathway. Polyphosphate [27], phosphoramidate [28] and pyrophosphate [29] are involved in the biochemical phosphorylation of D-glucose, hexoses and L-serine respectively in some organisms. Carbamylphosphate (**4**) and acetylphosphate (**6**) have high phosphorylating potentials (see table 2), but nature uses them mainly as donors of carbamyl [30] or acetyl groups [31]. Only in a few cases do they act as phosphoryl donors [30],[32].

Phosphorylations with low-potential phosphorylating agents are thermodynamically not favorable. In biological systems, these processes are made possible by coupling them to a thermodynamically more favorable process. Examples of weak phosphorylating agents are sugar phosphates such as glucose- and ribose phosphates, which can transfer their phosphate group to other sugars [32] or to nucleosides like riboflavin [33]. Phosphate sugars are formed when polysaccharides are cleaved with a phosphorylase and inorganic phosphate [34].

3.2 Phosphorylation

Chemical phosphorylations usually involve many protection and deprotection steps. Enzymatic phosphorylations can make synthesis more efficient by eliminating many of these steps. In addition,

enzyme-catalyzed introduction of phosphoryl groups can be diastereo- [35] or enantiospecific [36],[37].

One of the major challenges in enzyme-catalyzed phosphorylation reactions is, as mentioned above, the choice of the most convenient enzyme. The other major difficulty is the availability of the coenzymes. Cofactors act as biological phosphoryl donors and in enzyme catalyzed synthesis, they have to be added in stoichiometric amounts or coupled to an efficient regeneration system.

3.2.1 Regeneration of nucleoside triphosphates

In enzyme catalyzed synthesis, adenosine 5'-triphosphate (8) is the cofactor most often used as phosphoryl group donor. Other nucleoside phosphates , UTP, or CTP are used principally as donors of a nucleoside phosphate moiety to form activated intermediates in biological pathways (see 3.2.2.2). For example: UTP precedes the activated form of glucose, UDP-glucose, in the Leloir synthesis of polysaccharides, CTP precedes CDP-choline in the synthesis of phospholipids and CMP-NeuAc in the formation of glycosides of sialic acids (see chapter B 1.3.2: Formation of O-Glycosides). The costs for a mole CTP, GTP or UTP vary from $ 32,000 to 90,000 (as research biochemicals)[38].The high price of these cofactors precludes their large-scale use in stoichiometric quantities and makes cofactor regeneration necessary. Even with ATP, one of the least expensive cofactors used in organic synthesis [39],[38] and available through mole scale synthesis from RNA [40], regeneration remains of central importance. The use of a cofactor regeneration system not only eliminates the need for stoichiometric quantities of cofactor but it can also favorably influence the position of the reaction equilibrium and

prevent the accumulation of cofactor by-products that may inhibit the forward process. Product isolation is simplified as well.

A nucleoside phosphate regeneration system must meet several specifications to be practical. To be economical, a regeneration method must be capable of recycling the cofactor 10^2 - 10^6 times [39]. All materials should be readily available, inexpensive, easily handled, stable under reaction conditions and compatible with the rest of the reaction system. The transfer of phosphate should be thermodynamically and kinetically favorable and it should be regioselective in forming a high-energy P-O bond.

3.2.1.1 Regeneration of ATP from ADP and AMP

At the scale required for synthesis of fine chemicals, the mayor problems of ATP regeneration have been solved [39],[41],[42]. Three strategies have been applied: chemical synthesis; biological methods including whole cells, organelles, and fermentation processes; and cell-free enzymatic catalysis. Chemical methods often lack the necessary specificity and are not compatible with biochemical transformations. Biological and enzymatic systems provide the most efficient ATP regenerating systems [39]. The use of cell-free enzymes requires a greater initial effort or expense than do the biological methods, but are more specific than biological systems and often generate fewer by-products (see ref. [39] and references cited therein).

A) From ADP

Several procedures for the large-scale regeneration of ATP from ADP using isolated enzymes as catalysts are known [43],[39]. These methods have in common the characteristic that phosphoryl groups are transferred from a high-energy phosphoryl donor to ADP (compare 3.1.2). The advantages and disadvantages of these methods are summarized in table 3.

((table 3))

In practice, for most synthetic applications, either acetyl phosphate/acetate kinase or phosphoenol-pyruvate/pyruvate kinase are used to regenerate ATP. Because of the ease of preparing AcP, AcP / AcK is the most economical method for large scale work. Its application is, however, limited to fast phosphorylation reactions where the hydrolysis of AcP is not important. The PEP / pyruvate kinase system is used in instances where the requirement for a strong, stable phosphorylating reagent outweighs the relative inconvenience of preparation of PEP.

Phosphoenolpyruvate / pyruvate kinase

Phosphoenolpyruvate (PEP; **2**) / pyruvate kinase (PK: EC 2.7.1.40) is the most efficient system for the regeneration of ATP from ADP. The phosphorylating agent PEP can be prepared in a mole scale [47]. Starting from crude pyruvic acid, the crystalline monopotassium salt PEP$^-$K$^+$ is synthesized in a three-step procedure. For transformations on a scale < 1 mole, PEP can be prepared from commercially available 3-phosphoglyceric acid in an enzyme catalyzed reaction [64]. This method is

more expensive than the chemical preparation, but is more convenient because it requires less time and produces less organic waste (see 3.2.1.2; figure 5).

Pyruvate kinases are commercially available from multiple sources [38]. The enzyme generally used in ATP regeneration -- from rabbit muscle -- has high specific activity (~500 U per mg of protein), is inexpensive ($ 2 - 4 /1000 U), and is stable when immobilized [65],[66],[67]. This regeneration system can be used in membrane enclosed enzyme catalysis (MEEC technique [68],[69]) as well.

The stability of PEP in solution and its strength as a phosphoryl donor (table 2) make PEP particularly convenient for use in slow and thermodynamically unfavorable phosphorylation reactions. It is also the method of choice for the regeneration of ATP at low concentrations of ADP, since the Michaelis constant for PK is smaller (K_m(MgADP)= 0.1 mM [18]) than for acetate kinase (K_m(MgADP)= 0.4 mM [50]).

The PEP/PK regeneration method has two minor disadvantages. First, the synthesis of PEP [64],[47] requires more effort and expense than does the synthesis of AcP [57]. Second, pyruvate is a strong inhibitor of PK (see table 3). The reactions are therefore carried out in dilute solutions to keep the pyruvate concentration low, and pyruvate is either removed from the reaction solution or PEP is used at high concentrations to minimize the effects of inhibition.

Acetyl phosphate / acetyl kinase

Acetyl phosphate (AcP; **6**) / acetyl kinase (AcK; EC 2.7.2.1) is the most widely used large scale ATP-regeneration system. AcP is modestly stable in aqueous solutions and is a phosphoryl donor of intermediate strength (table 2 and 3). Diammonium acetyl phosphate can be prepared from ketene and anhydrous phosphoric acid [56] or, more easily, from acetic anhydride and anhydrous H_3PO_4 [57]. However, the use of the diammonium salt in ATP regeneration has three disadvantages. First, NH_4^+ reacts with acetyl phosphate in solution. Second, it forms an insoluble precipitate with Mg^{2+} under reaction conditions. Third, its preparation involves several steps that require careful experimental control and that are difficult to carry out at large scale. Preparation of aqueous solutions of acetyl phosphate as its sodium [51] or potassium salt [18] circumvents these drawbacks.

Two types of commercially available [38] acetate kinases, from <u>Escherichia coli</u> [58] and from <u>Bacillus stearothermophilus</u> [48], have been used in ATP regeneration. The latter kinase is more expensive but it is preferred for synthetic use because it is thermostable and it is stable to autooxidation [59]. Both enzymes have acceptable specific activities (150 - 300 and 400 - 1200 U respectively per mg protein) and can be stabilized by immobilization [60],[58],[48],[61],[62]. Acetate is a weak inhibitor of AcK, but product inhibition is not a serious problem (see table 3) unless reaction solutions have acetate concentrations greater than 1 M [43]. The relative instability of AcP in solution compared with PEP is the major disadvantage of the AcP / AcK system. The contribution of the enzymes to the total cost of the process is generally low when they are recycled, making the slightly higher cost of AcK compared

with PK a minor disadvantage [39]. Polymer bound ATP was regenerated in a membrane reactor with AcP / AK [63].

Methoxycarbonyl phosphate / acetate kinase

Methoxycarbonyl phosphate (MCP; **3**) was designed to replace AcP as phosphoryl donor [18]. It is comparable to PEP in its high phosphorylating strength (see table 2), but resembles acetyl phosphate in its ease of synthesis. Aqueous solutions of MCP are prepared from aqueous phosphate and methyl chloroformate and used in ATP regeneration without purification. The reaction product after phosphoryl transfer is methyl carbonate, which hydrolyses rapidly to form CO_2 and MeOH. Product isolation is simple and bicarbonate inhibition can be avoided by purging the reaction mixture.

MCP is accepted as a unnatural substrate by AcK (EC 2.7.2.1) and CK (EC 2.7.2.2) but not by PK [18]. The principal disadvantage of methoxycarbonyl phosphate as phosphorylating agent in ATP regeneration is MCP's rapid spontaneous decomposition. The half-life of MCP in aqueous solution is only 0.3 h (25 °C, pH 7) [18]. Because of this short half life, MCP is only used in a few cases where high phosphorylating potentials are required to push the phosphorylation reaction to the product side (see table 4, entry 17).

Others

ATP has been regenerated from ADP with propionyl phosphate / acetate kinase, but propionylphosphate is a poorer substrate than AcP [18].

Carbamyl phosphate (CP; 4) / carbamyl kinase (CK; EC 2.7.2.2) was described as a regeneration method for ATP in 1973 but it has seldom been used [70]. CP is a very strong phosphorylating agent (see table 2) and can easily be prepared from aqueous potassium cyanate and KH_2PO_4 [52]. Rapid decomposition of carbamyl phosphate generates ammonium ions and magnesium ammonium phosphate complex is formed as a gelatinous precipitate. This precipitation lowers activity, both by precipitating the magnesium(II) required for activity of the kinases, and because it occludes enzymes.

ADP phosphorylation with polyphosphate (P_n) and polyphosphate kinase (P_nK; EC 2.7.4.1) has also been demonstrated [44]. The cheap, stable polyphosphates and the stability of P_nK are highly attractive. Unfortunately, P_n has a low phosphorylating potential and P_nK is not readily available. These facts may explain why this regeneration system has not found any practical application.

Another very interesting but little-used regeneration method is based on phosphocreatine (PC; 7) and creatine kinase (CrK; EC 2.7.3.2) [71]. PC is comparable in its phosphorylating potential to AcP, but it is more stable in aqueous solutions (table 3). CrK is inexpensive and fairly stable. The current lack of an efficient and simple laboratory scale synthesis for PC seems to have limited the applications of this method to a few syntheses of sugars [71] and nucleosides [72].

Recently, a promising regeneration system based on a multienzyme system from the glycolytic pathway was described [45] (figure 4). Glucose and inorganic phosphate were used as low-energy

((figure 4))

phosphorylating agents. Eleven enzymes are used to convert glucose to lactate. Two equivalents of inorganic phosphate are consumed and two equivalents of ATP are formed. The overall process of this regeneration system has a favorable free energy (see table 3), which can be useful in the synthesis of compounds with high phosphorylating potentials (see table 4, entry 17). The main backdraw of this method is the complexity of this multienzyme system and the poor stability of some of the enzymes used.

B)   From AMP

In biochemical processes, ATP may be converted to either ADP or AMP. The regeneration of ATP from AMP is slightly more complicated than from ADP. Methods coupling one of the above mentioned regeneration systems and adenylate kinase (AdK; EC 2.7.4.3) have been used extensively in the production of ATP [48],[43]. AdK catalyzes the formation of 2 ADP from ATP and AMP. AcP / AcK and PEP / PK are the methods most often used to convert ADP to ATP (vide infra).

3.2.1.2   Regeneration of other nucleoside triphosphates

The preparation of nucleoside triphosphates (NTP) from nucleoside diphosphates (NDP) follows the same regeneration systems described above (3.2.1.1). AcK and PK have broad substrate specificities and recognize all of the NDPs [48],[73]. The efficient generation of NDP from nucleoside

monophosphates (NMP) has been solved on preparative scale [74]. Adenylate kinase was used in the preparation of cytidine 5'-triphosphate (CTP) and uridine 5'-triphosphate (UTP) from the corresponding nucleoside monophosphates. Nucleoside monophosphate kinase (NMP; EC 2.7.1.4) was used in the synthesis of UTP. Guanosine 5'-triphosphate (GTP) was prepared with guanylate kinase as catalyst, coupled to a conventional ATP regeneration system. Best results were achieved when 3-phosphoglyceric acid served as the ultimate phosphorylating agent, and a multienzyme system was used as transfer catalyst (figure 5).

((figure 5))

3.2.2 Applications

3.2.2.1 Phosphorylations with ATP as a cofactor

The most widely used and best developed enzyme catalyzed phosphorylations are ones that are coupled to ATP regeneration systems. Sugar phosphates, nucleoside phosphates and glycerides are the major classes of compounds prepared with these methods.

Kinases are the enzymes most often used for the phosphorylation of saccharides (table 4, entries 7 - 19). For example, glucose-6-phosphate (11), a useful reagent for the regeneration of nicotinamide cofactors (see chapter B 5.1), was prepared from glucose in a one-step reaction by phosphorylation with ATP [75]. ATP was regenerated with AcP / AcK and the phosphoryl transfer was catalyzed with hexokinase, (HK; EC 2.7.1.1), an enzyme with broad substrate specificity. Both enzymes were

immobilized and reused after product isolation. Alternatively, fluorinated hexopyranose phosphates and glucose phosphate analogs, with sulfur or nitrogen in the ring, were prepared with PEP / PK and HK [76] (table 4, entry 9). The synthesis of 11 starting from polysaccharides or from fructose 1,6-diphosphate [77] was demonstrated, but the former method is less convenient and the latter is more expensive than the procedure starting from D-glucose.

5-phospho-D-ribosyl α-1-pyrophosphate (PRPP; 13) is a key intermediate in the biosynthesis of various nucleotides and other natural products. An interesting application of an ATP / AMP regeneration system is demonstrated in the synthesis of PRPP from D-ribose in a multienzyme reaction [35] (table 4, entries 13 and 14). In the first step, ribose was phosphorylated with ATP using ribokinase (RK; EC 2.7.1.17) as catalyst. In the second step, PRPP-synthetase (EC 2.7.6.1) catalyzed the transfer of a pyrophosphate group from ATP to ribose 5-phosphate (figure 6).

((figure 6))

The preparations of ATP, GTP, CTP and UTP have been discussed in chapter 3.2.1. Kinases are the enzymes most popular for the synthesis and regeneration of nucleoside triphosphates from their mono- and diphosphates. Nucleoside phosphate analogs have been synthesized using the same enzymes. For example, ribavarin triphosphate (RTP; 14), a compound with anti-viral properties, was prepared from ribavarin monophosphate with adenylate kinase (EC 2.7.4.3) and pyruvate kinase (EC 2.7.1.40) as catalysts with PEP as ultimate phosphorylating agent (figure 7) [48]. Here PEP / PK has

((figure 7))

proved to be more useful as regeneration system for ATP than AcP / AcK (see 3.2.1.1) in a typical example of a kinetically unfavored reaction. RMP is one of the rare unnatural substrates accepted by adenylate kinase. Other nucleotide analogs -- for example ATP-α-S and ATP-γ-S -- have also been synthesized using kinases (table 5, entries 4 and 5) [78],[79].

Glycerol kinase (GK; EC 2.7.1.30) catalyzes the enantiospecific phosphorylation of glycerol to form sn-glycerol-3-phosphate, an important intermediate for the synthesis of phospholipids. The enzyme is inexpensive and stable when immobilized. Studies with enzymes from a variety of microbial sources have shown that glycerol kinase accepts a wide range of glycerol analogs as substrates (table 4, entries 3 - 5) [36]. sn-Glycerol-3-phosphate (12) and analogs were synthesized in gram scales, using glycerol kinase as catalyst and PEP / PK or AcP / AcK as ATP regeneration system. The phosphorylation of racemic mixtures produced chiral organic phosphates with enantiomeric excess (ee) > 90 - 95 % and yields of 75 - 95 %. The unphosphorylated enantiomers of the chiral substrates were recovered in yields of 30 - 40 % (80 - 90 % ee). Alkaline phosphatase was used to hydrolyze the phosphorylated enantiomer and to provide enantiomerically pure unphosphorylated material [37]. For example, D-3-Chloropropane-1,2-diol (15) was prepared from a racemic mixture in a two step procedure with a 53 % overall yield (97 % ee) (figure 8).

((figure 8))

Another application of glycerol kinase is the monophosphorylation of dihydroxyacetone [37]. Dihydroxyacetone phosphate (DHAP), an important intermediate in the aldolase catalyzed synthesis of monosaccharides (see chapter B 4.4), was prepared in a 0.4 mole scale using AcP / AcK for the

regeneration of ATP (table 4; entry 6). Guanidine derivatives were phosphorylated with ATP as well. The syntheses of arginine- and creatine phosphate (**7** and **9**), two relatively strong biological phosphorylating agents, are not economical, but they demonstrate the potentials of different ATP regeneration systems (see table 4, entries 20 and 21). Further applications of ATP regeneration systems are given in table 4 and 5.

3.2.2.2 P-O bond formation with other nucleoside triphosphates than ATP

The use of stoichiometric quantities of nucleoside triphosphates or their regeneration from the corresponding mono- or diphosphates have found important applications in the synthesis of activated natural products; for instance, nucleoside phosphate sugars are important biological intermediates in the synthesis of complex carbohydrates, glycoproteins, glycolipids and proteoglycans. All of the eight nucleoside phosphate sugars, used in vivo by mammalian glycosyltransferases in the Leloir pathway, are accessible today by practical enzymatic or chemoenzymatic approaches (see reviews ref. [80] and [81]). The use of these activated monosaccharides in the enzymatic preparation of oligosaccharides and glycoconjugates is discussed in chapter B 1.3.2. The enzymatic synthesis of sucrose is, however, discussed here, to illustrate the efficient in situ generation of UDP-glucose from UDP in a complex multienzyme reaction (figure 9). In a typical example, a nucleoside triphosphate is recycled to

((figure 9))

regenerate a nucleoside phosphate sugar [82]. The synthesis, starting from glucose-1-phosphate and fructose, used sucrose synthetase (EC 2.4.1.13), pyruvate kinase (EC 2.7.1.40) and UDP-glucose

pyrophosphorylase (EC 2.7.7.9). Inorganic pyrophosphatase (EC 2.6.1.1) was used to keep the pyrophosphate concentration in the reaction mixture low and to drive the equilibrium to the product side.

3.2.2.3 Other phosphorylating agents

Agents other than ATP are rare in enzyme catalyzed formation of P-O bonds. Inorganic phosphate, pyrophosphate and polyphosphates were used to prepare phosphorylated monosaccharides, alcohols, polyols [83] and phenols [84]. The yields were poor and the reactions lack specificity (table 4, entries 1, 2 and 4). Glycerol-1-phosphate was prepared from glycerol, for instance, using inorganic phosphate and alkaline phosphatase (EC 3.1.3.1): 75 g of the product was isolated in a 41 % yield [9]. The reaction was regio- but not stereospecific. Phosphorylases were used with isotopically labeled phosphate or with inorganic thiophosphate to prepare (thio)phosphorylated monosaccharides from oligo- or polysaccharides (see table 4, entries 18 and 15). ^{32}P-labeling of phospholipase with [^{32}P]P$_i$ was used to study the biological function of this enzyme complex [85]. p-Nitrophenyl phosphate was used as phosphoryl group donor in the synthesis of allo-uridine, using a phosphotransferase as catalyst (see table 5, entry 6)

3.2.3 Tables containing typical examples ordered according to the classes of compounds

Sugars, nucleosides and their analogs are the classes of compound most often involved in enzyme-catalyzed phosphorylation reactions. Typical carbohydrate phosphorylations are included in table 4, together with the phosphorylation of other non-nucleosidic compounds. Table 5 gives an overview of the enzyme catalyzed phosphorylation reactions of nucleosides and their analogs. A few representative examples of nucleoside sugars are listed, for more detailed information consult the reviews ref. [74], [80], [81].

((table 4 and table 5))

3.3   Cleavage of P-O bonds

In vivo, cleavage of P-O bonds are performed by enzymes such as phosphatases, phosphodiesterases, phosphohydrolases, nucleases, DNases and RNases (see chapter 3.1.1). In vitro, cleavage of a P-O bond is often a trivial synthetic step. Even for an easy step, enzymes attract increasing attention. The enzymatic reactions are preferred when regio- or stereoselectivity is required, and when the substrates are temperature or pH sensitive. Many phosphate analogs have been tested as substrates of enzymes that hydrolyze phosphoryl groups. These analogs are often accepted as substrates for the enzymes, and such reactions could be synthetically valuable. Typical examples are presented in the tables.

3.3.1. Hydrolysis of Phosphate and Pyrophosphate Monoesters

Both acid and alkaline phosphatases have been used to cleave aliphatic and aromatic phosphate monoesters. Table 6 shows typical examples ordered according to the substrate class. This table

((table 6))

includes an example where the enzymatic reaction was run with a sensitive substrate (entry 1), and examples where regio- or a stereoselectivity was required (entries 2 and 5, respectively).

Polyprenyl phosphates and pyrophosphates have been hydrolyzed by acid and alkaline phosphatases (table 6, entry 1). For this hydrolysis, classical chemical methods are inadequate as the reaction products decompose under acid conditions [109].

A regioselective dephosphorylation was used in the synthesis of 2'-carboxy-D-arabinitol 1-phosphate (table 6, entry 2), a natural inhibitor of ribulose 1,5-bisphosphate carboxylase. Either acid or alkaline phosphatases can be used for the selective hydrolysis of the 1-phosphoryl group of 2'-carboxyl-D-arabinitol 1,5-bisphosphate. With acid phosphatase, the conversion was essentially quantitative yielding exclusively the 1-phosphate derivative (cleavage of the 5-phosphoryl group). On the other hand, hydrolysis with alkaline phosphatase gave a 4 : 1 mixture of the 1- and 5-phosphate derivatives.

Many natural and unnatural monosaccharides have been prepared by aldolase catalyzed condensation. The synthesized sugars were often dephosphorylated in situ by an acid phosphatase

(table 6, entry 3). These reactions illustrate multienzyme synthesis. In this case, no isolation of the phosphate intermediate is required: both enzymatic reactions are run in the same pot after adjustment of the pH value.

One of the best examples of an enzymatic dephosphorylation for a synthetic purpose is shown in the entry 5 of table 6. A 5'-ribonucleotide phosphohydrolase was used in the synthesis of (-)-aristeromycin, a carbocyclic analog of adenosine. The (-)-enantiomer of aristeromycin shows some cytostatic and antiviral activity, while the (+)-enantiomer is inactive. The racemate (±)-5'-phosphorylated aristeromycin was resolved by selective hydrolysis of the (-)-enantiomer with the hydrolase. The (-)-alcohol and the (+)-5'-phosphate derivative were separated easily on a silica gel column. Hydrolysis of the (+)-enantiomer with calf intestinal phosphatase yielded pure (+)-alcohol.

Phosphorylated p-nitrophenol was hydrolyzed with an alkaline phosphatase [129]. This hydrolysis was also performed in a two-phase system with an acid phosphatase [125].

The naphtol derivative, table 6, entry 9, is dephosphorylated by an alkaline phosphatase. The resulting naphtol decomposes with chemiluminescent emission and can be used in bioassays to generate a chemiluminescence signal proportional to the concentration of an alkaline phosphatase label.

Inorganic pyrophosphate may be considered as a particular case of a phosphate monoester. The enzymatic decomposition of pyrophosphate by inorganic pyrophosphatase (table 6, entry 10) can be used to drive a multienzyme synthesis (see [35]).

3.3.2. Hydrolysis of S- and N-Substituted Phosphate Monoester Analogs

Enzymatic hydrolysis of oligonucleotide-analogs containing modified phosphoryl moieties have

been examined extensively to study their resistance to the enzymatic hydrolysis.

Thiophosphates (table 7) were subjected to hydrolysis with both acid and alkaline phosphatases.

((table 7))

Most authors claimed that these compounds are substrates for alkaline phosphatases, but the reaction

rate is much lower than with the corresponding phosphates [128], [126]. Neumann [129], however,

reported that these same S-substituted analogs are resistant to alkaline phosphatases but hydrolyzed by

acid phosphatases.

Only the alkaline phosphatases have been used with phosphorothioates (table 8). The presence of

sulfur between the phosphoryl moiety and the residue does affect the enzymatic reaction with alkaline

phosphatases.

((table 8))

Imidodiphosphates are also potential substrates for phosphoryl hydrolyzing enzymes (see table 8,

entry 7). They have been used less often than the S-substituted phosphate analogs.

Another goal of these studies involving analogs with modified phosphoryl groups or isotopically

labeled nucleotides was mechanistic elucidation of the stereochemical course of the reaction [15], [132],

[133].

3.3.3. Hydrolysis of Phosphate and Phosphonate Diesters

3.3.3.1. Nucleic Acids and their Analogs

Endo- and exonucleases have been used successfully with nucleic acids and their analogs for organic synthetic purposes. For example, ATP was synthesized from AMP for use in cofactor recycling (table 9, entry 1). The AMP was obtained from yeast RNA by cleavage with the nuclease P_1

((table 9))

yielding a mixture of nucleoside monophosphates [101]. In another report [73], nucleoside diphosphates were obtained by hydrolysis of RNA with nuclease P_1 and a polynucleotide phosphorylase (the diphosphates are preferred because the diphosphates were more easily transformed to the nucleoside triphosphates than the monophosphates).

Similarly, dATP was synthesized from dAMP, obtained by cleaving herring sperm DNA with DNase I and nuclease P_1 (table 9, entry 2). Selective phosphorylation was obtained with adenylate kinase in the presence of pyruvate kinase and phosphoenol pyruvate.

Synthetic oligonucleotide analogs are interesting in applications in which they suppress translation of mRNAs by hybridization (antisense technology). A good antisense agent would be resistant to nucleases, and able to maintain its biological activity for substantial periods in living organisms [135]. Oligonucleotide analogs modified at the phosphodiester linkage with a phosphorothioate group are the

subject of numerous papers (see table 9). Other oligonucleotide analogs have been tested as substrates for endo- and exonucleases. The natural substrates were modified at either the base residues (table 9, entry 4) or at the sugar moieties (table 9, entries 5, 6, and 7).

The tetraphosphate Ap_4A and its analogs are other examples of a cleavage of a phosphodiester (table 9, entry 8).

3.3.3.2. Other Phosphate and Phosphonate Diesters

Enzymes have often been used as mild catalysts to hydrolyze phosphate and phosphonate diesters.

Cyclic phosphate diesters can be hydrolyzed selectively with RNases and phosphodiesterases to give the corresponding phosphate monoesters (table 10, entries 1 and 2).

((table 10))

Phosphodiesterases have been used to deprotect phosphonate diesters (table 10, entries 3-5). This method is especially useful for sensitive compounds (see table 10, entry 6 : a P-O bond could be cleaved selectively in the presence of a P-N bond).

3.3.4   Other P-O Bond Cleavages

Phosphate and phosphonate esters can also be cleaved enzymatically to give products different from those obtained by enzymatic hydrolysis.

The formal migration of a phosphoryl group between the C_6 and the C_1 of glucose is catalyzed by phosphoglucomutase. Mechanistic studies were performed with the thiophosphate analog of glucose 6-phosphate 16. In the presence of phosphoglucomutase, this analog yields 6-thioglucose 1-phosphate 17, albeit at a slower rate than the natural substrate (figure 10, [96]).

((figure 10))

Aminolysis of phosphonate diester derivatives have been used to form organophosphorus analogs of peptides (18) with phosphatases and phosphodiesterases ([151], [152]).

The equilibrium between phosphoenolpyruvate and phosphonopyruvate (19, figure 10) is catalyzed by a phosphomutase. The mechanism of the transformation of a phosphoryl into a phosphonoyl group has been studied with labeled and S-substituted analogs of the natural substrate ([153], [154], [155], [156], [157], [158]).

Numerous analogs of carbohydrate polymers (i.e., amylose, glycogen) have been prepared from modified monosaccharide 1-phosphates with phosphorylase (figure 11 shows the natural substrates) ([159], [160], [161], [162]).

((figure 11))

Abbreviations

AcK: acetate kinase; AcP: acetyl phosphate; AdK: adenylate kinase; Ap_nA: p^1,p^n-di(adenosine 5'-) n-phosphate; ARS: aminoacyl tRNA synthetase; ATP, ADP, AMP: adenosine 5'-tri-, di-, monophosphate; ATP-α-S: (S_p)-adenosine 5'-O-(1-thiotriphosphate); ATP-γ-S: adenosine 5'-O-(3-thiotriphosphate);CK: carbamyl kinase; CP: carbamyl phosphate; CrK: creatine kinase; CTP, CDP, CMP: cytidine 5'-tri-, di-, monophosphate; dATP, dAMP: deoxyadenosine 5'-tri-, monophosphate; DNA: deoxyribonucleic acid; ΔG: change in free energy; GK: glycerol kinase; GTP, GDP, GMP: guanosine 5'-tri-, di-, monophosphate; HK: hexokinase; IUB: International Union of Biochemistry; MCP: methoxycarbonyl phosphate; NTP, NDP, NMP: nucleoside 5'-tri-, di-, monophosphate; PC: phosphocreatine; PEP: phosphoenol pyruvate; P_i: orthophosphate; PK: pyruvate kinase; P_n: polyphosphate; P_nK: polyphosphate kinase; PP_i: pyrophosphate; PRPP: 5-phospho-D-ribosyl α-1-pyrophosphate; RNA: ribonucleic acid; tRNA: transfer RNA; RK: ribokinase; RTP, RMP: ribavarin tri-, monophosphate; U: one unit: the amount of enzyme that catalyzes the formation of 1 μmol / minute; UTP, UDP, UMP: uridine 5'-tri-, di-, monophosphate.

Acknowledgements

References

(1) J. Darnell, H. Loddish, D. Baltimore, Molecular Cell Biology, 2d ed., Scientific American Books Inc., New York, 1990.

(2) Nomenclature Committee of the International Union of Biochemistry, Enzyme Nomenclature, Academic Press, Orlando, 1984.

(3) P. D. Boyer, E. G. Krebs, D. S. Sigman, The Enzymes 3d ed., Academic Press, New York, 1970-1992, Vol I - XX.

(4) D. Schomburg, M. Salzmann, (GFB-Gesellschaft für Biotechnologische Forschung), Enzyme Handbook, Springer-Verlag, Berlin, D, 1990.

(5) A. Fersht, Enzyme Structure and Mechanism, 2. ed., W.H.Freeman and Co., New York, 1985, p. 235.

(6) P. D. Boyer, E. G. Krebs, Control by Phosphorylation (The Enzymes) 3d ed. 1986, Vol. XVII and XVIII.

(7) E. C. Ball, Energy Metabolism, Addison-Wesley, Reading, MA, USA, 1973.

(8) J. R. Knowles, Ann. Rev. Biochem. 1980, 49, 877-919.

(9) A. Pradines, A. Klaebe, J. Perie, F. Paul, P. Monsan, Enzyme Microb. Technol. 1991, 13, 19-23.

(10) H. Flodgaard, H. Klenow, Biochem. J. 1982, 208, 737-724.

(11) H. Nakajima, I. Tomioka, S. Kitabatake, M. Dombou, K. Tomita, <u>Agric. Biol. Chem.</u> 1989, <u>53</u>, 615-623.

(12) S. Kitabatake, M. Dombou, I. Tomioka, H. Nakajima, <u>Biochem. Biophys. Res. Commun.</u> 1987, <u>146</u>, 173-178.

(13) H. Nakajima, H. Kondo, R. Tsurutani, M. Dombou, I. Tomioka, K. Tomita, <u>ACS Symp. Ser.</u> 1991, <u>466</u>, 111-120.

(14) G. M. Whitesides, C. H. Wong, <u>Angew. Chem. Int. Ed. Engl.</u> 1985, <u>24</u>, 617-638.

(15) J. A. Gerlt in <u>The Enzymes</u> (Ed.: D. S. Sigman), Vol. XX, Academic Press Inc., San Diego, 1992, p. 95-139.

(16) C. Walsh, <u>Enzymatic Reaction Mechanism</u>, W.H. Freeman and Co., New York, 1979, p. 213.

(17) W. P. Jencks in <u>Handbook of Biochemistry</u> (Ed.: H. A. Sober), The Chemical Rubber Co., Cleveland, 1970, p. J-185.

(18) R. J. Kazlauskas, G. M. Whitesides, <u>J. Org. Chem.</u> 1985, <u>50</u>, 1069-1076.

(19) R. A. Alberty, <u>J. Biol. Chem.</u> 1968, <u>243</u>, 1337-1343.

(20) K. Shikama, K.-I. Nakamura, <u>Arch. Biochem. Biophys.</u> 1973, <u>157</u>, 457-463.

(21) G. Rosing, E. C. Slater, <u>Biochim. Biophys. Acta</u> 1972, <u>267</u>, 275-290.

(22) A. L. Lehninger, Biochemie, 2d ed., VCH, Weinheim, D, 1985.

(23) D. Kessel, J. Biol. Chem. 1968, 243, 4739-4744.

(24) R. A. Hiles, L. V. Henderson, J. Biol. Chem. 1972, 247, 646-651.

(25) D. C. Watts in The Enzymes (Ed.: P. D. Boyer), Vol. VIII, Academic Press, New York, 1973, p. 383-455.

(26) J. F. Morrison in The Enzymes (Ed.: P. D. Boyer), Vol. VIII, Academic Press, New York, 1973, p. 457-486.

(27) M. Szymona, W. Ostrowski, Biochim. Biophys. Acta 1964, 85, 283-295.

(28) R. A. Smith, M. C. Theisen in Carbohydrate Metabolism (Ed.: W. A. Wood), Vol. IX, Academic Press, New York, 1966, p. 403-407.

(29) L. M. Cagen, H. C. Friedmann, J. Biol. Chem. 1972, 247, 3382-3392.

(30) L. Raijaman, M. E. Jones in The Enzymes (Ed.: P. D. Boyer), Vol. IX, Academic Press, New York, 1973, p. 97-119.

(31) E. R. Stadtman in Methods Enzymol. (Ed.: S. P. Colowick and N. O. Kaplan), Vol. I, Academic Press Inc., New York, 1955, p. 596-599.

(32) R. L. Anderson, M. Y. Kamel in Carbohydrate Metabolism (Ed.: W. A. Wood), Vol. IX, Academic Press, New York, 1966, p. 392-396.

(33)   H. Katagiri, H. Yamada, K. Imai, J. Biochem. 1959, 46, 1119-1126.

(34)   D. J. Graves, J. H. Wang in The Enzymes (Ed.: P. D. Boyer), Vol. VII, Academic Press Inc.,
       New York, 1972, p. 435-482.

(35)   A. Gross, O. Abril, J. M. Lewis, S. Geresh, G. M. Whitesides, J. Am. Chem. Soc. 1983, 105,
       7428-7435.

(36)   D. C. Crans, G. M. Whitesides, J. Am. Chem. Soc. 1985, 107, 7008-7018.

(37)   D. C. Crans, G. M. Whitesides, J. Am. Chem. Soc. 1985, 107, 7019-7026.

(38)   Sigma Chemical Co., St Louis, MO, USA, 1992.

(39)   H. K. Chenault, E. S. Simon, G. M. Whitesides, Biotechnol. Genet. Eng. Rev. 1988, 6, 221-270.

(40)   S. L. Haynie, G. M. Whitesides, Appl. Biochem. Biotech. 1990, 23, 205-220.

(41)   G. M. Whitesides, C. H. Wong, Aldrichimica Acta 1983, 16, 27-34.

(42)   G. M. Whitesides, C. H. Wong, A. Pollak, ACS Symp. Ser. 1982, 185, 205-218.

(43)   D. C. Crans, R. J. Kazlauskas, B. L. Hirschbein, C. H. Wong, O. Abril, G. M. Whitesides in
       Methods Enzymol. (Ed.: K. Mosbach), Vol. 136, Academic Press Inc., New York, 1987, p. 263-
       80.

(44) R. C. Hoffman Jr., P. L. Wyman, L. E. Smith, C. L. Nolt, J. L. Conley, J. M. Hevel, J. P. Warren, G. A. Reiner, A. M. Owen Jr., Biotechnol. Appl. Biochem. 1988, 10, 107-117.

(45) L. L. Wei, W. J. Goux, Bioorg. Chem. 1992, 20, 62-66.

(46) M. Marshall, P. P. Cohen, J. Biol. Chem. 1966, 241, 4197-4208.

(47) B. L. Hirschbein, F. P. Mazenod, G. M. Whitesides, J. Org. Chem. 1982, 47, 3765-3766.

(48) M. J. Kim, G. M. Whitesides, Appl. Biochem. Biotech. 1988, 16, 95-108.

(49) S. A. Kuby, L. Noda, H. A. Lardy, J. Biol. Chem. 1954, 210, 65-82.

(50) C. A. Janson, W. W. Cleland, J. Biol. Chem. 1974, 249, 2567-2571.

(51) D. C. Crans, G. M. Whitesides, J. Org. Synth. 1983, 26, 3130-3132.

(52) L. Spector, M. E. Jones, F. Lipmann in Methods Enzymol. (Ed.: S. P. Colowick and N. O. Kaplan), Vol. III, Academic Press Inc., New York, 1957, p. 653-655.

(53) F. De Marchi, C. Verra, Ind. Chim. Belge. (CA70(12): 50437p) 1967, 32 (spec. No.), 174-175.

(54) L. Butler, Biotechnol. Bioeng. 1977, 19, 591-593.

(55) Y.-S. Shih, G. M. Whitesides, J. Org. Chem. 1977, 42, 4165-4166.

(56) G. M. Whitesides, M. Siegel, P. Garrett, J. Org. Chem. 1975, 40, 2516-2519.

(57) J. M. Lewis, S. L. Haynie, G. M. Whitesides, J. Org. Chem. 1979, 44, 864-865.

(58) G. M. Whitesides, A. Lamotte, O. Adalsteinsson, C. K. Colton in Methods Enzymol. (Ed.: K. Mosbach), Vol. XLIV, Academic Press, New York, 1976, p. 887-897.

(59) H. Nakajima, K. Suzuki, K. Imahori, J. Biochem. 1978, 84, 193-203.

(60) G. M. Whitesides, A. L. Lamotte, Ö. Adalsteinsson, R. F. Baddour, A. C. Chmurny, C. K. Colton, A. Pollak, J. Mol. Cat. 1979, 6, 177-198.

(61) H. Kondo, J. Appl. Biochem. 1984, 6, 29-38.

(62) H. Nakajima, J. Appl. Biochem. 1984, 6, 19-28.

(63) W. Berke, H. J. Schütz, C. Wandrey, M. Morr, G. Denda, M. R. Kula, Biotechnol. Bioeng. 1988, 32, 130-139.

(64) E. S. Simon, S. Grabowski, G. M. Whitesides, J. Am. Chem. Soc. 1989, 111, 8920-8921.

(65) J. Campbell, T. Ming Swi Chang, Biochimi. Biophys. Acta 1975, 397, 101-109.

(66) A. Pollak, H. Blumenfeld, M. Wax, R. L. Baughn, G. M. Whitesides, J. Am. Chem. Soc. 1980, 102, 6324-6336.

(67) C. Slegers, S. De Laet, R. H. Lambrecht, C. Block, Enz. Microbiol. Technol. 1986, 8, 153-156.

(68) M. D. Bednarski, H. K. Chenault, E. S. Simon, G. M. Whitesides, J. Am. Chem. Soc. 1987, 109, 1283-1285.

(69) M. D. Bednarski, D. C. Crans, R. DiCosimo, E. S. Simon, P. D. Stein, G. M. Whitesides, Tetrahedron Lett. 1988, 29, 427-430.

(70) D. L. Marshall, Biotechnol. Bioeng. 1973, 15, 447-453.

(71) I. Sakata, H. Kitano, N. Ise, J. Appl. Biochem. 1981, 3, 518-525.

(72) W. J. Goux, USA pat. WO 9205270 A2, 1992 (CA117(15):149473y).

(73) C. H. Wong, S. L. Haynie, G. M. Whitesides, J. Am. Chem. Soc. 1983, 105, 115-117.

(74) E. S. Simon, S. Grabowski, G. M. Whitesides, J. Org. Chem. 1990, 55, 1834-1841.

(75) A. Pollak, R. L. Baughn, G. M. Whitesides, J. Am. Chem. Soc. 1977, 99, 2366-2367.

(76) C. H. Wong, D. G. Drueckhammer, J. Org. Chem. 1985, 50, 5912-5913.

(77) C. H. Wong, G. M. Whitesides, J. Am. Chem. Soc. 1981, 103, 4890-4899.

(78) J. R. Moran, G. M. Whitesides, J. Org. Chem. 1984, 49, 704-706.

(79) O. Abril, D. C. Crans, G. M. Whitesides, J. Org. Chem. 1984, 49, 1360-1364.

(80) J. E. Heidlas, K. W. Williams, G. M. Whitesides, Acc. Chem. Res. 1992, 25, 307-314.

(81) H. Waldmann, Nachr. Chem. Tech. Lab. 1992, 40, 828-834.

(82) S. L. Haynie, G. M. Whitesides, Appl. Biochem. Biotech. 1990, 23, 155-170.

(83) A. Paradines, A. Klaébé, J. Périé, F. Paul, P. Monsan, Tetrahedron 1988, 44 (20), 6373-6386.

(84) E. A. Rubtsova, O. M. Poltorak, E. S. Chukhrai, Zh. Fiz. Khim. 1990, 64, 3127-3130 (CA114(21):206707s).

(85) J. Meisenhelder, T. Hunter in Methods Enzymol. (Ed.: E. A. Dennis), Vol. 197, Academic Press Inc., New York, 1991, p. 288-305.

(86) S. David, C. Auge, C. Gautheron, Adv. Carbohydr. Chem. Biochem. 1992, 49, 175-237.

(87) V. M. Rios-Mercadillo, G. M. Whitesides, J. Am. Chem. Soc. 1979, 101, 5828.

(88) W. B. Knight, W. W. Cleland, Biochemistry 1989, 28, 5728-34.

(89) V. Larreta-Garde, D. Thomas, Biotechnol. Bioeng. 1987, 29, 79-84.

(90) M. D. Percival, S. G. Withers, Biochemistry 1992, 31, 498-505.

(91) R. Stiller, J. Thiem, Liebigs Ann. Chem. 1992, 2, 467-471.

(92) C.-H. Wong, S. D. McCurry, G. Whitesides M., J. Am. Chem. Soc. 1980, 102, 7938-7939.

(93) R. L. Sabina, E. W. Holmes, M. A. Becker, Science 1984, 223, 1193-1195.

(94) W. E. Ladner, G. M. Whitesides, J. Org. Chem. 1985, 50, 1076-1079.

(95) R. R. Drake Jr., R. K. Evans, M. J. Wolf, E. H. Boyd, J. Biol. Chem. 1989, 264, 11928-11933.

(96) W. B. Knight, D. S. Sem, K. Smith, H. M. Miziorko, A. R. Rendina, W. W. Cleland, Biochemistry 1991, 30, 4970-4977.

(97) A. N. Singh, J. S. Newborn, F. M. Raushel, Bioorg. Chem. 1988, 16, 206-214.

(98) H. Waldmann, D. Gygax, M. D. Bednarski, W. R. Shangraw, G. M. Whitesides, Carbohydr. Res. 1986, 157, c4-c7.

(99) D. S. Sem, W. W. Cleland, Biochemistry 1991, 30, 4978-4987.

(100) J. Bolte, G. M. Whitesides, Bioorg. Chem. 1984, 12, 170-175.

(101) H. J. Leuchs, J. M. Lewis, V. M. Rios-Mercadillo, G. M. Whitesides, J. Am. Chem. Soc. 1979, 101, 5829.

(102) P. A. Frey, Stud. Org. Chem. (Amsterdam) 1985, 20, 465-480.

(103) A. Billich, U. Stockhove, H. Witzel, Nucleic Acids Res. 1983, 11, 7611-7624.

(104) R. Stolarski, J. M. Ciesla, D. Shugar, Z. Naturforsch., C: Biosci 1990, 45, 293-299.

(105) B. V. L. Potter, F. Eckstein, J. Biol. Chem. 1984, 259, 14243-14248.

(106) R. R. Breaker, G. R. Gough, P. T. Gilham, Nucleic Acids Res. 1990, 18, 3085-3086.

(107) A. Guranowski, G. Just, E. Holler, H. Jakubowski, Biochemistry 1988, 27, 2959-2964.

(108) D. R. Walt, M. A. Findeis, V. M. Rios-Mercadillo, J. Auge, G. M. Withesides, J. Am. Chem. Soc. 1984, 106, 234-237.

(109) H. G. Davies, R. H. Green, D. R. Kelly, S. M. Roberts, Biotransformations in Preparative Organic Chemistry, Academic Press, London, 1989, p. 75-86.

(110) H. Fujii, T. Koyama, K. Ogura, Biochim. Biophys. Acta 1982, 712, 716-718.

(111) J. F. Wedgwood, J. L. Strominger, J. Biol. Chem. 1980, 255, 1120-1123.

(112) G. S. Adrian, R. W. Keenan, Biochim. Biophys. Acta 1979, 575, 431-438.

(113) G. R. Daleo, R. P. Lezica, FEBS Lett. 1977, 74, 247-250.

(114) E. Willoughby, Y. Highasi, J. L. Strominger, J. Biol. Chem. 1972, 247, 5113-5115.

(115) T. Koyama, H. Inoue, S. Ohnuma, K. Ogura, Tetrahedron Lett. 1990, 31, 4189-4190.

(116) S. Ohnuma, T. Koyama, K. Ogura, Tetrahedron Lett. 1991, 32, 241-242.

(117) S. Gutteridge, G. S. Reddy, G. Lorimer, Biochem. J. 1989, 260, 711-716.

(118) M. D. Bednarski, E. S. Simon, N. Bischofberger, W.-D. Fessner, M.-J. Kim, W. Lees, T. Saito, H. Waldmann, G. M. Withesides, J. Am. Chem. Soc. 1989, 111, 627-635.

(119) K. K.-C. Liu, T. Kajimoto, L. Chen, Z. Zhong, Y. Ichikawa, C.-H. Wong, J. Org. Chem. 1991, 56, 6280-6289.

(120) S. T. Allen, G. R. Heintzelman, E. J. Toone, J. Org. Chem. 1992, 57, 426-427.

(121) A. Straub, F. Effenberger, P. Fischer, J. Org. Chem. 1990, 55, 3926-3932.

(122) E. J. Toone, G. M. Whitesides, ACS Symp. Ser. 1991, 466, 1-22.

(123) T. Baasov, A. Jakob, J. Am. Chem. Soc. 1990, 112, 4972-4974.

(124) P. Herdewijn, J. Balzarini, E. De Clercq, H. Vanderhaeghe, J. Med. Chem. 1985, 28, 1385-1386.

(125) M. Cantarella, L. Cantarella, F. Alfani, Enzyme Microb. Technol. 1991, 13, 547-553.

(126) R. Breslow, I. Katz, J. Am. Chem. Soc. 1968, 90, 7376-7377.

(127) B. Edwards, A. Sparks, J. C. Voyta, R. Strong, O. Murphy, I. Bronstein, J. Org. Chem. 1990, 55, 6225-6229.

(128) P. Mushak, J. E. Coleman, Biochemistry 1972, 11, 201-205.

(129) H. Neumann, J. Biol. Chem. 1968, 243, 4671-4676.

(130) K. D. Sikkema, M. H. O'Leary, Biochemistry 1988, 27, 1342-1347.

(131) R. G. Yount, D. Babcock, W. Ballantyne, D. Ojala, Biochemistry 1971, 10, 2484-2489.

(132) J. B. Jones, Tetrahedron 1986, 42, 3351-3403.

(133) S. Mehdi, J. A. Gerlt, Biochemistry 1984, 23, 4844-4852.

(134) A. W. Nicholson, K. R. Niebling, P. L. McOsker, H. D. Robertson, Nucleic Acids Res. 1988, 16, 1577-1591.

(135) K. Shinozuka, T. Morita, Y. Hirota, H. Sawai, Chem. Lett. 1991, 1941-1944.

(136) K. Kariko, S. W. Li, R. W. J. Sobol, L. Suhadolnik, N. L. Reichenbach, R. J. Suhadolnik, R. Charubala, W. Pfleiderer, Nucleosides Nucleotides 1987, 6, 173-184.

(137) P. M. J. Burgers, F. Eckstein, Biochemistry 1979, 18, 592-596.

(138) M. Koizumi, E. Ohtsuka, Biochemistry 1991, 30, 5145-5150.

(139) H. P. Vosberg, F. Eckstein, J. Biol. Chem. 1982, 257, 6595-6599.

(140) S. Spitzer, F. Eckstein, Nucleic Acids Res. 1988, 16, 11691-704.

(141) J. W. Bodnar, W. Zempsky, D. Warder, C. Bergson, D. C. Ward, J. Biol. Chem. 1983, 258, 15206-15213.

(142) M. J. Damha, P. A. Giannaris, P. Marfey, L. S. Reid, Tetrahedron Lett. 1991, 32, 2573-2576.

(143) K. Augustyns, A. Van Aerschot, A. Van Schepdael, C. Urbanke, P. Herdewijn, Nucleic Acids Res. 1991, 19, 2587-2593.

(144) A. Holy in Phosphorus Chem. Directed Biol., Lect. Int. Symp., Meeting Date 1979, (Ed.: S. J. Wojcieck), Pergamon Press, Oxford, 1980, 53-64.

(145) D. Lazewska, A. Guranowski, Nucleic Acids Res. 1990, 18, 6083-6088.

(146) A. Holy, Collect. Czech. Chem. Commun. 1970, 35, 3686-3711.

(147) A. Holy, G. S. Ivanova, Nucleic Acids Res. 1974, 1, 19-34.

(148) I. A. Nachev, Tetrahedron 1988, 44, 1511-1522.

(149) I. A. Nachev, Phosphorus Sulfur 1988, 37, 133-141.

(150) I. A. Nachev, Synthesis 1987, 1079-1084.

(151) I. Nachev, Tetrahedron 1991, 47, 1239-1248.

(152) I. Nachev, Bull. Chem. Soc. Jpn. 1988, 61, 3711-3715.

(153) H. Nakashita, A. Shimazu, H. Seto, Agric. Biol. Chem. 1991, 55, 2825-2829.

(154) P. M. Cullis, R. Misra, J. Am. Chem. Soc. 1991, 113, 9679-9680.

(155) M. S. McQueney, S. L. Lee, E. Bowman, P. S. Mariano, M. D. Dunaway, J. Am. Chem. Soc. 1989, 111, 6885-6887.

(156) S. Freeman, H. M. Seidel, C. H. Schwalbe, J. R. Knowles, J. Am. Chem. Soc. 1989, 111, 9233-9234.

(157) M. S. McQueney, S. L. Lee, W. H. Swartz, H. L. Ammon, P. S. Mariano, D. Dunaway-Mariano, J. Org. Chem. 1991, 56, 7121-7130.

(158) E. Bowman, M. McQueney, R. J. Barry, D. Dunaway-Mariano, J. Am. Chem. Soc. 1988, 110, 5575-5576.

(159) C. Niemann, R. Nuck, B. Pfannemüller, W. Saenger, Carbohydr. Res. 1990, 197, 187-196.

(160) S. G. Withers, Carbohydr. Res. 1990, 197, 61-73.

(161) J. Zemek, S. Kucar, J. Zamocky, Eur. J. Biochem. 1978, 89, 291-295.

(162) M. Kitaoka, T. Sasaki, H. Taniguchi, Agric. Biol. Chem. 1991, 55, 1431-1432.

table 1a: Enzymes accepting phosphoric monoesters as substrates

Enzyme type[a]	Functional class[b]	Function[c]	IUB classes with titles, containing such types of enzymes[d]
A	Phosphomutases	Phosphoryl group transfer, for which the acceptor is another functional group on the donor molecule.	2.7.5. Phosphomutases 5.4.2. Intramolecular phosphotransferases
A	Phosphorylases	Formation of a P-O bond under phosphorolytic cleavage of a C-Heteroatom bond.	2.4.1. Hexosyltransferases 2.4.2. Pentosyltransferases
A	Nucleotidases	Phosphoryltransfer from a nucleotide to water as an acceptor molecule. (Nucleotides are cleaved hydrolytically.)	3.1.3. Phosphoric ester hydrolases (3.1.4. Phosphoric diester hydrolases)
A / B	Phosphatases	Phosphoryl group transfer from a phosphoric monoester to water as an acceptor molecule. (Phosphoric monoesters are cleaved hydrolytically.)	3.1.3. Phosphoric ester hydrolases 3.6.1. Hydrolases acting on acid anhydrides in phosphorous-containing anhydrides
A / B	Phosphokinases and Phosphotransferases	Phosphoryl group transfer: Nucleoside triphosphate is the donor and some other molecules than H_2O are the acceptors. Compounds different than nucleoside triphosphates are the donor and some other molecules than H_2O are the acceptors.	2.7.1. Phosphotransferases with an alcohol group as acceptor 2.7.2. Phosphotransferases with a carboxyl group as acceptor 2.7.4. Phosphotransferases with a phosphate group as acceptor
B	ATPases	Phosphatases which are responsible for the coupling of ATP cleavage to other metabolic processes.	3.6.1.3 ATPases

a) See figure 1; b) functional classes based on ref. [8]; c) see ref. [8] and [4]; d) see ref. [2].

table 1b: Enzymes accepting phosphoric diesters as substrates

Enzyme type[a]	Functional class[b]	Function[c]	IUB classes with titles, containing such types of enzymes[d]
C	Pyrophosphokinases	Pyrophosphate group transfer from ATP to an acceptor molecule other than water.	2.7.6. Diphosphotransferases
D	Nucleotidyl transferases	Transfer of nucleotidyl moieties	2.7.7. Nucleotidyltransferases
D	Nucleotidyl cyclases	Nucleoside triphosphate cyclisation under formation of pyrophosphate	4.6.1. Phosphorus oxygen lyases
E	Triphosphohydrolases	Triphosphate transfer from a nucleoside triphosphate to water as an acceptor molecule.	3.1.5. Triphosphoric monoester hydrolases
E	Polynucleotide synthetases	Responsible for the linkage of two poly- or oligo-nucleotide moieties to form polynucleotide chains	6.5.1. Ligases forming phosphoric ester bonds
E	Phospholipases	Hydrolytic cleavage of phosphoglycerides (essentialy phospholipase C and D)	3.1.4. Phosphoric diester hydrolases
E	Nucleases	Phosphonucleotide transfer from a polynucleotide to water as an acceptor molecule. (Polynucleotides are cleaved hydrolytically.)	3.1.4. Phosphoric diester hydrolases 3.1.. Endo- and exonucleases
E	Phosphodiesterases	Phosphomonoester transfer from a phosphodiester other than polynucleotide to water as an acceptor molecule. (Phosphodiesters are cleaved hydrolytically.)	2.7.8. Transferases for other substituted phosphate groups 3.1.4. Phosphoric diester hydrolases

a) See figure 1; b) functional classes based on ref. [8]; c) see ref. [8] and [4]; d) see ref. [2].

table 3:   Properties of ATP-regeneration systems

		PEP / PK	AcP /AcK	MCP / AcK	CP / CK	CrP / CrK	P_n / P_nK	P_i / glycolysis
	costs [$ / 1000U], (source)[38]	2.5 (rabbit muscle)	378 (B. stearo-thermophilus)	378 (B. stearo-thermophilus)	10 (Strepto-coccus faecalis)	2.6 (rabbit muscle)	isolated from E. coli [44]	66 (from diff. sources)[a]
	product inhibition / K_i [mM][b]	pyruvate 10, C	acetate 400, NC	HCO_3^- 500, NC	[c]	creatine[25] 6 - 40[d], NC	-	-
Enzyme properties	spec. activity [U/mg protein][38]	300-500	400-1200	≥ 140[b]	400-900	150-250	150-250[44]	8[h]
	stability	+++e,[47]	+++e,[48]	+++f,b	+[30]	+++f,[49]	+++e,[44]	+a,l
	K_m(ADP)[f] [mM]	0.1[b]	0.4[50]	-	0.05[c]	0.05[25]	0.17[44]	-
	K_m(P-donor) [mM]	0.07[b]	0.4[b]	1.6[b]	0.1[c]	5f,b	0.003[44]	-
	ease of preparation / availability	+[47]	+++[51]	++[b]	+++[52]	+[53]	++++[54]	++++[38]
P-donors properties	ΔG(P-transfer)[g] [kcal ; kJ/mole]	- 5.5 ; - 23.0	- 3.0 ; - 12.6	- 5.1 ; - 21.3	- 5.0 ; - 20.9	- 3.0 ; - 12.6	≤ - 0.7 ; - 2.9[i]	- 16 ; - 67.5[k]
	half life for hydrolysis (25°C, pH 7)[b]	$\sim10^3$	21	0.3	2.2	$\sim10^2$ [l]	-	-
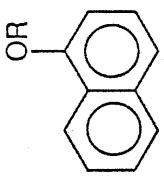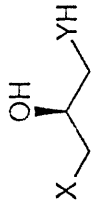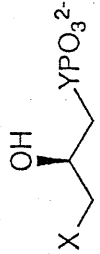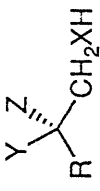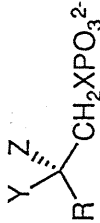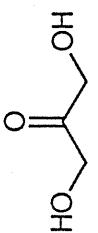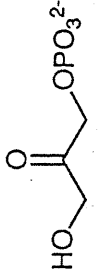
table 3:
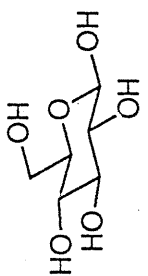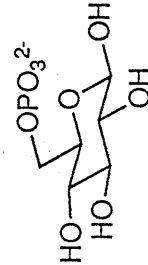
a) calculated from 1000 U of each enzyme; see ref. [45] and [38]; b) see ref. [18], C = competitive, NC = non-competitive; c) carbamate kinase kinetics is complex, inhibition plays an important role [46]. d) K_i depends strongly on the anions present in solution [25]; e) immobilized enzyme(s); f) value(s) for the free enzyme(s); g) calculation based on the values from table 2. (ΔG(P-transfer) = $\Delta G^{\circ}{}_{hydr}$(P-donor) - $\Delta G^{\circ}{}_{hydr}$(ATP); $\Delta G^{\circ}{}_{hydr}$(ATP) = - 7.3 kcal/mole ; 30.5 kJ/mole); h) calculation based on the sum of all enzymes present; i) based on table 2, values for PP_i; k) the driving force of this process is the transformation of glucose to 2 equivalents of lactate (ΔG = -197 kJ/mole) [22]; l) calculated from data in ref. [55].
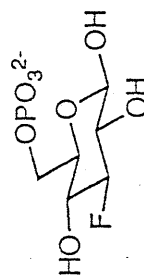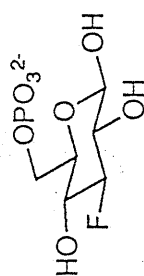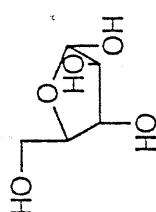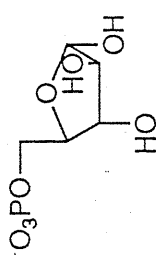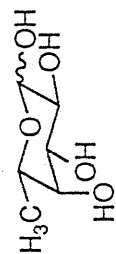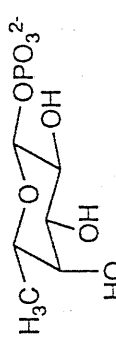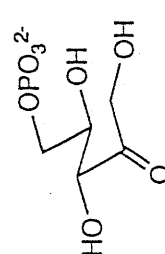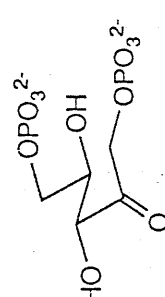
table 4   P-O bond formation at non-nucleoside compounds
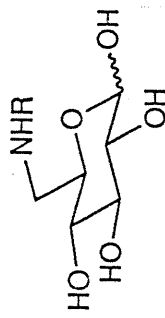
Entry	Starting Material	Product	Enzyme	P-Source	Cofactor Regeneration	Reference

Aliphatic and Aromatic Alcohols

Entry	Starting Material	Product	Enzyme	P-Source	Cofactor Regeneration	Reference
1	Monosaccharides, alcohols, polyols	Phosphorylated products	Alkaline Phosphatase (EC 3.1.3.1)	PP_i	none	[83]
2	OR (naphthalene structure) R = H	R = PO_3^{2-} (45%)	Alkaline Phosphatase (EC 3.1.3.1)	P_i		[84]

Glycerol, Glycerol Analogs, Dihydroxyacetone

Entry	Starting Material	Product	Enzyme	P-Source	Cofactor Regeneration	Reference
3	OH / X—YH (rac) X = Cl, SH, OCH₃, CH₂OH, Br, CH₂CH₃, OH; Y = O or NH	OH / X—YPO_3^{2-} + non phosphorylated enantiomer (29 - 95 %)	Glycerol Kinase (EC 2.7.1.30)	ATP	PEP/PK or AcP/AcK	[37]; see also [43], [8], [83], [87], [9]

2

Table (continued)

#	Substrate / Product	Enzyme	Cofactor	Regeneration	Ref.
4	$X = OH$; $Y = O$ — (rac)-glycerolphosphate	Alkaline Phosphatase (EC 3.1.3.1)	P_i, PP_i		[83], [9]
5	$X = O$, NH, H; $Y = OH$, H, CH_2OH, F, SH, NH_2; $Z = H$, CH_3, CH_2CH_3; $Y = Z = O$. (45 – 96%)	GK (EC 2.7.1.30)	ATP	PEP/PK	[36]; see also [88]
6	DHAP (83%)	GK (EC 2.7.1.30)	ATP	PEP/PK or AcP/AcK	[43]; see also [79]

Monosaccharides
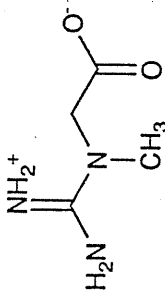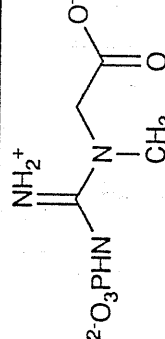
#	Substrate / Product	Enzyme	Cofactor	Regeneration	Ref.
7	11 (65%)	Hexokinase (EC 2.7.1.1)	ATP	AcP/Ac	[75]
8	ibid → 11 (80%)	γ-Glutamyl-Cysteine Synthetase (EC 6.3.2.2)	ATP	AcP/AcK	[63]; See also [13], [89], [77]

#	Substrate	Product	Enzyme			Reference
9	(glucose analog) (and other glucose analogs)	(86%)	Hexokinase (EC 2.7.1.1)	ATP	PEP/PK	[76]; See also [90]
10	D-arabinose	(63%)	Hexokinase (EC 2.7.1.1)	ATP	PEP/PK	[69]
11	(deoxy sugar)	(80%)	Fucose Kinase (EC 2.7.1.52)	ATP	PEP/PK	[91]
12	ribulose 5-phosphate	ribulose 1,5-bisphosphate	Phosphoribulokinase (EC 2.7.1.19)	ATP	PEP/PK (82%) or AcP/AcK (66%)	[35], [92]

3|

4

	Substrate		Product	Enzyme			Ref.
13	D-ribose	→	D-ribose 5-phosphate (74%)	Ribokinase (EC 2.7.1.17)	ATP	PEP/PK	[35]
14	D-ribose 5-phosphate	→	PRPP (75%)	PRPP Synthase (EC 2.7.6.1)	ATP	AcP/AcK	[35]; see also [93], [94]
15	Glycogen	→	[^{32}P]glucose 1-phosphate	Phosphorylase a (EC 2.4.1.1)	[^{32}P]P$_i$	none	[95]
16		→	OPO$_3^{2-}$	Fructokinase (EC 2.7.1.11) and Phosphogluco-isomerase (EC 5.3.1.9)	ATP	none	[96]
17	Galactose	→	Galactose 1-thiophosphate (25%)	Galactokinase (EC 2.7.1.6)	ATP(γS)	none	[97]

#	Substrate	Product	Enzyme	Phosphoryl donor	Regeneration system	Ref.
18	Sucrose	Glucose 1-thiophosphate (55%)	Sucrose Phosphorylase (EC 2.4.1.7)	Na_3SPO_3	none	[97]; see also [98]
19	R = H	R = PO_3^{2-} (18%)	Hexokinase (EC 2.7.1.1)	ATP	PEP/PK	[99]

Guanidine derivatives
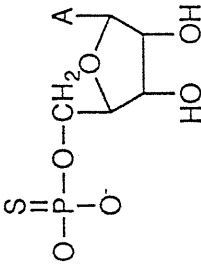
#	Substrate	Product	Enzyme	Phosphoryl donor	Regeneration system	Ref.
20	creatine	phosphocreatine	Creatine Kinase (EC 2.7.3.2)	ATP	AcP/AcK (24%)	[55]
					MCP/AcK (55%)	[18]
					Glucose, P_i + Mutienzyme system	[45]
21	L-Arginine	Phospho-arginine	Arginine Kinase (EC 2.7.3.3)	ATP	PEP/PK (67%) or AcP/AcK (31%)	[100]

6

Enzyme

22	Phospholipase C	Phosphorylated Phospholipase	P_i	[85]
		Acid Phosphatase (EC 3.1.3.2)		

6

Enzyme

| 22 | Phospholipase C | Phosphorylated Phospholipase | Acid Phosphatase
(EC 3.1.3.2) | P_i | [85] |

table 5   P-O bond formation at nucleosides

Nucleotides and Analogs

Entry	Starting Material	Product	Enzyme	P-Source	Cofactor Regeneration	Reference
1	dAMP (DNA)	dATP (67%)	Adenylate Kinase (EC 2.7.4.3)	ATP	PEP/PK	[94]
2	NMP (RNA)	ATP, GTP, CTP, UTP	NMP-Kinase (EC 2.7.4.4)	ATP	AcP/AcK	[40]; see also [101], [74]
3	RMP	RTP (**14**) (93%)	Adenylate Kinase (EC 2.7.4.3)	ATP	PEP/PK	[48]
4	(AMPS)	ATP-α-S (53%)	AdK (EC 2.7.4.3) / PK (EC 2.7.1.40)	ATP	PEP/PK	[78]; see also [102]
5	ADP	ATP-γ-S (80%)	Phosphoglycerate-Kinase (EC 2.7.2.3)	1-(thiophospho)-3-phosphoglycerate	Dihydroxyacetone, Na2HSPO3, Multienzyme System	[79]

6	Phosphotransferase (Malt Sprouts)	p-Nitrophenyl phosphate	[103]; see also [104]
			none

$R= PO_3^{2-}$

(31%)

$R= H$ (allo-uridine)

Oligonucleotides and Analogs

7	Single Stranded DNA + Mixture of Deoxynucleoside 5'-O-(1-thiotriphosphate) phosphorothioates	DNA Polymerase I (EC 2.7.7.7) and T4 DNA Ligase (EC 6.5.1.1)	[105]
8		Polynucleotide Phosphorylase (EC 2.7.7.8)	[106]

(~90%)

A = Adenine

#	Substrate	Enzyme	Cofactor	Regeneration	Ref.
9	ATP	Ap4A (98%)	Leucyl t-RNA Synthetase (EC 6.1.1.4)	ATP	AcP/AcK AdK	[12]; see also [11], [107], [13]

Nucleoside Phosphate Sugars
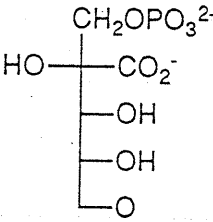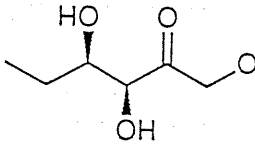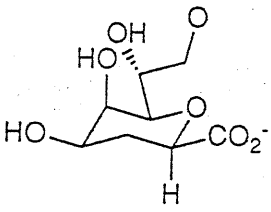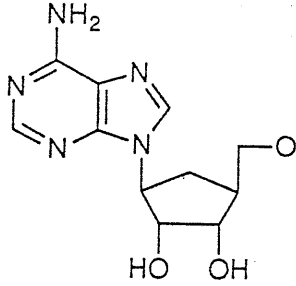
#	Substrate	Product	Enzyme	Cofactor	Regeneration	Ref.
10	UTP + Glucose 1-Phosphate	UDP-Glucose (95%)	UDP-Glucose Pyrophosphorylase (EC 2.7.7.9)	ATP	AcP/AcK AdK	[73]; see also [86], [95]
11	Galactose 1-thiophosphate and UDP-glucose	Uridine-5'-O-(2-thiodiphosphogalactose) (UDP(βS)-Galactose) (50%)	Galactose-1-Phosphate Uridyl Transferase (EC 2.7.7.12)			[97]

NAD(P)+

#	Substrate	Product	Enzyme	Cofactor	Regeneration	Ref.
12	ATP + Nicotinamide Mononucleotide	NAD+ (>90%)	NAD Pyrophosphorylase (EC 2.7.7.1)	ATP	AcP/AcK AdK	[108]
13	NAD+	NADP+ (quant)	NAD Kinase (EC 2.7.1.23)	ATP	AcP/AcK	[108]

table 6:   Hydrolysis of Phosphate and Pyrophosphate Monoester

$$R\text{-}O\text{-}PO_3^{2-} \longrightarrow R\text{-}OH$$

$$R\text{-}O\text{-}P(O_2^{2-})\text{-}O\text{-}PO_3^{2-} \longrightarrow R\text{-}OH$$

Entry	R-O	Enzyme	Reference
1	Polyprenol (phosphates and pyrophosphates)	Acid Phosphatase (EC 3.1.3.2) or Alkaline Phosphatase (EC 3.1.3.1)	[110], [111], [112], [113], [114], [115], [116]
2		Acid Phosphatase (EC 3.1.3.2) or Alkaline Phosphatase (EC 3.1.3.1)	[117]
3		Acid Phosphatase (EC 3.1.3.2)	[118]; see also [119], [120], [121], [122]
4		KDO 8-Phosphate Phosphatase	[123]
5		5'-Ribonucleotide phosphohydrolase (EC 3.1.3.5)	[124]
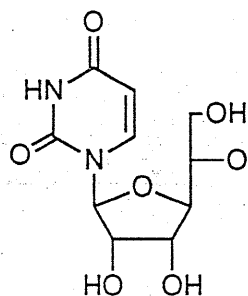
6		Alkaline Phosphatase (EC 3.1.3.1) or Acid Phosphatase (EC 3.1.3.2)	[103]
7		Alkaline Phosphatase (EC 3.1.3.1)	[104]
8		Alkaline Phosphatase (EC 3.1.3.1) or Acid Phosphatase (2-Phases System) (EC 3.1.3.2)	[125], [126]
9	in 3 or 6	Alkaline Phosphatase (EC 3.1.3.1)	[127]
10		Inorganic Pyrophosphatase (EC 3.6.1.1)	[35]
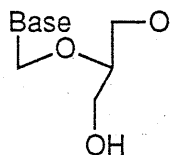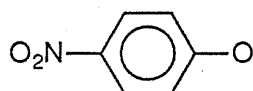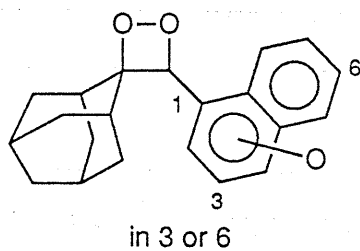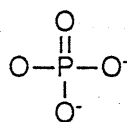
Table 7:   Hydrolysis of Thiophosphates
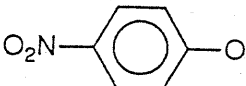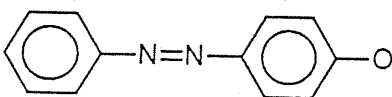
$R\text{-}O\text{-}PSO_2{}^{2-} \longrightarrow R\text{-}OH$

Entry	R-O	Enzyme	Reference
1	$H_3C{-}O$	Alkaline Phosphatase (EC 3.1.3.1)	[128], [129]
2	$O_2N{-}\langle\text{ring}\rangle{-}O$	Alkaline Phosphatase (EC 3.1.3.1) or Acid Phosphatase (EC 3.1.3.2)	[126], [129]
3	$\langle\text{ring}\rangle{-}N{=}N{-}\langle\text{ring}\rangle{-}O$	Alkaline Phosphatase (EC 3.1.3.1)	[128]

table 8: Hydrolysis of Phosphorothioates and Imidodiphosphates
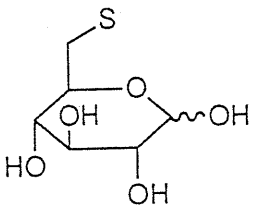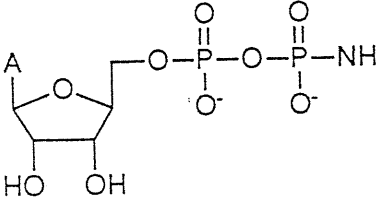
$$R\text{-}S\text{-}PO_3^{2-} \longrightarrow R\text{-}SH$$
$$R\text{-}NH\text{-}PO_3^{2-} \longrightarrow R\text{-}NH_2$$

Entry	R-S or R-NH	Enzyme	Reference
1	$H_2N\text{—}CH_2\text{-}CH_2\text{·}S$	Alkaline Phosphatase (EC 3.1.3.1)	[129]
2	$H_3C\text{—}\overset{\displaystyle O}{\overset{\|}{C}}\text{—}NH\text{-}CH_2\text{-}CH_2\text{·}S$	Alkaline Phosphatase(EC 3.1.3.1)	[129]
3	$OOC\text{—}CH_2\text{-}CH_2\text{·}S$	Alkaline Phosphatase (EC 3.1.3.1)	[129]
4	CO_2^- with S (structure)	Alkaline Phosphatase (EC 3.1.3.1)	[130]
5	CO_2^- with S (structure)	Alkaline Phosphatase (EC 3.1.3.1) or Pyruvate Kinase (EC 2.7.1.40)	[130]
6	(sugar structure with S)	Alkaline Phosphatase (EC 3.1.3.1)	[96]
7	(nucleotide structure, A base, $\text{O}\text{—}P\text{—}O\text{—}P\text{—}NH$)	Alkaline Phosphatase (EC 3.1.3.1)	[131]

table 9:   Hydrolysis of Nucleic Acids, Nucleosides and their Analogs

Entry	Starting Material	Product	Enzyme	Reference
1	RNA	Nucleoside Monophosphates or Nucleoside Diphosphates	Nuclease P_1 (EC 3.1.30.1) or Nuclease P_1 (EC 3.1.30.1) and Polynucleotide Phosphorylase (EC 2.7.7.8)	[101], [73]
2	denatured DNA	Deoxy Nucleoside Monophosphates	DNase I (EC 3.1.21.1) Nuclease P_1 (EC 3.1.30.1)	[94]
3	Phosphorothioate Substituted Nucleic Acids		Endo- (EC 3.1.30.1) and Exonucleases (EC 3.1.4.1) (EC 3.1.16.1)	[134], [135], [136], [137], [105], [138], [139], [140]
4	Nucleotide Analogs Containing Modified Bases		Restriction Endonucleases	[141]
5	Nucleic Acid Analogs Containing L-Ribose		Exonucleases	[142]
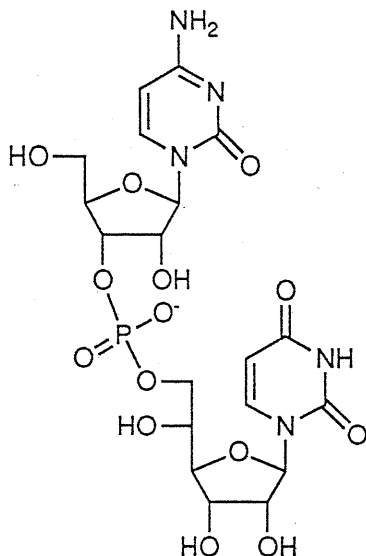
6		cytidine + allo-uridine 6'phosphate	RNase A (EC 3.1.27.5) RNase T₂(EC 3.1.27.1) Nuclease S₁ (EC 3.1.30.1)	[103]
7	Nucleotide Analogs Containing Acyclic Sugar Analogs		Nucleases Phosphodiesterases (EC 3.1.16.1)	[143], [144]
8	Thiophosphate Analogs of AppppA		Ap₄A Hydrolases (EC 3.6.1.17) (EC 3.6.1.41) Ap₄A Phosphorylase (EC 2.7.7.53)	[145], [107]
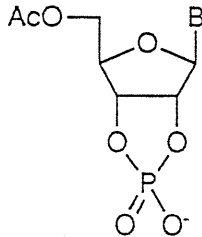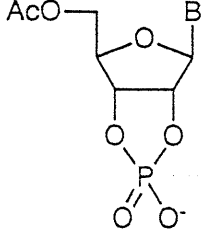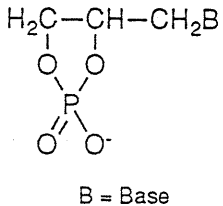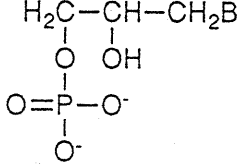
table 10:   Hydrolysis of Phosphate and Phosphonate Diesters

$$R\text{-}O\text{-}PO_3R'_2 \longrightarrow R\text{-}O\text{-}PO_3HR'$$

$$R\text{-}CR'_2\text{-}PO_3R''_2 \longrightarrow R\text{-}CR'_2\text{-}PO_3H_2 \text{ or } R\text{-}CR'_2\text{-}PO_3HR''$$

Entry	Starting Material	Product	Enzyme	Reference
1	(structure: AcO—sugar ring with B, cyclic phosphate $O=P{-}O^-$)	(structure: AcO—sugar ring with B, cyclic phosphate $O=P{-}O^-$)	RNase T$_1$ (EC 3.1.27.3) and RNase T$_2$ (EC 3.1.27.1)	[146]
2	(structure: H_2C—CH—CH$_2$B, cyclic $O{-}O$, $O=P{-}O^-$) B = Base	(structure: H_2C—CH—CH$_2$B, O OH, $O=P{-}O^-$ with O^-)	RNases or Phosphodiesterase	[147]
3	$(EtO)_2HC{-}CH{=}CH{-}CH_2{-}\overset{O}{\underset{OR'}{P}}{-}OR$ R = R' = Et	R = R' = H	Phosphodiesterase I (EC 3.1.4.1)	[148], [149]
4	TFA—Ala·Asp NH·CHCOOEt | CH CH | CH$_2$PO$_3$R$_2$ R = Et	R = H	Phosphodiesterase I (EC 3.1.4.1)	[148]
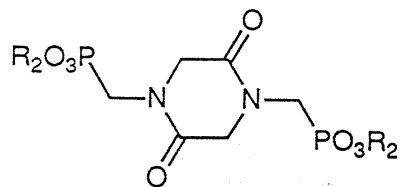
5		R = H	Phosphodiesterase I	[150]
			(EC 3.1.4.1)	

R = Et
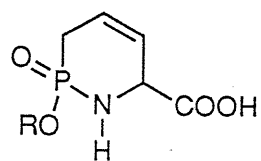
6		R = H	Phosphodiesterase I	[148]
			(EC 3.1.4.1)	

R = Et

figure 1:   Classes of enzymes involved in reaction at phosphorous. A and B represent enzyme types that handle phosphoric monoesters and related compounds (*O may be an oxygen of a hydroxyl, carboxyl, or phosphoryl group, or the nitrogen of a guanidine group. For simplicity, displacements at the γ-phosphoryl groups of nucleosides triphosphates were classified with these reactions.). C, D and E represent the enzymes that catalyze transformations of phosphoric diesters (displacements at α or β phosphorous groups of nucleoside triphosphates and transfer of pyrophosphates were classified with the reactions of phosphoric diesters.).

figure 2:   Enzymatic Synthesis of p^1,p^4-di(adenosine 5'-)tetraphosphate (Ap4A; **1**) with aminoacyl tRNA synthetases (ARS). AA can be leucine, for example, and ARS leucyl t-RNA synthetase [11].

figure 3:   Structures of the most important biological phosphorylating agents. P = phosphate.

figure 4:   ATP regeneration via the glycolytic pathway [45]. Eleven enzymes are used to catalyze the conversion of glucose to lactate.

figure 5: Enzymatic Synthesis of Nucleoside Triphosphates. i) Phosphoglycerate mutase (EC 2.7.5.3); ii) enolase (EC 4.2.1.11); iii) pyruvate kinase (EC 2.7.1.40); iv) adenylate kinase (EC 2.7.4.3, X = A, C, U), guanylate kinase (EC 2.7.4.8, X = G) or nucleoside monophosphate kinase (EC 2.7.4.4, X = U). P = phosphate. See ref. [74].

figure 6: Coupled enzymatic synthesis of PRPP from D-ribose [35]. i) ribokinase; ii) pyruvate kinase; iii) PRPP-synthetase; iv) adenylate kinase.

figure 7: Enzymatic synthesis of ribavarin 5'-triphosphate (RTP; **14**) from ribavarin 5'-monophosphate (RMV) [48]. i) adenylate kinase (EC 2.7.4.3); ii) pyruvate kinase (EC 2.7.1.40).

figure 8: Enzyme catalyzed separation of a racemic mixture of D,L-3-chloropropane-1,2-diol. i) glycerol kinase; ii) alkaline phosphatase.

figure 9:   General scheme for an enzyme-catalyzed synthesis of sucrose with UDP-glucose [82]. i) sucrose synthetase; ii) pyruvate kinase; iii) UDP-glucose pyrophosphorylase; iv) inorganic pyrophosphatase.

figure 10:   P-O bond cleavages with hydrolytic enzymes, not leading to the products of hydrolysis.

figure 11:   Phosphorylase catalyzed formation of polysaccharides and modified polysaccharides. i) phosphorylase.
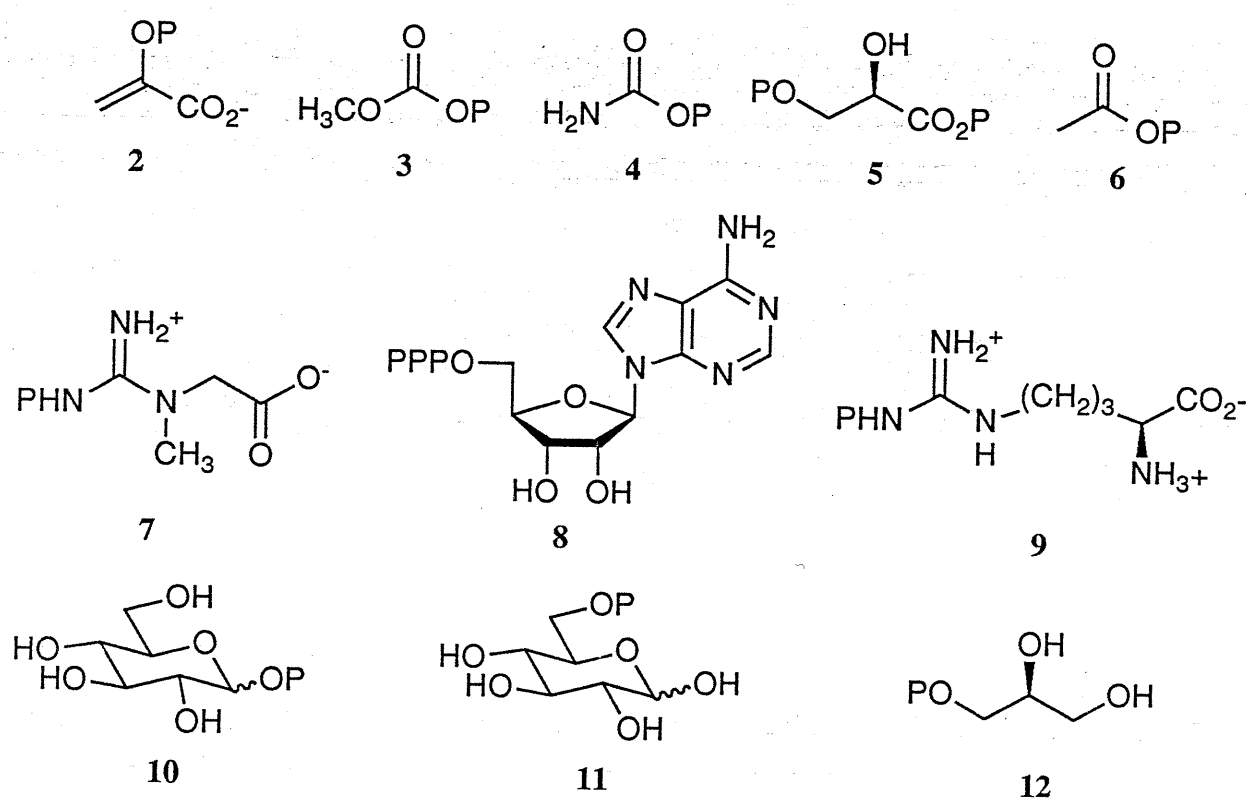
figure 1

AA + ATP $\xrightarrow{\text{ARS}}$ AA — AMP — ARS $\xrightarrow{\text{ATP}}$ Ap$_4$A

PP$_i$ AA + ARS

1

figure 2

figure 3

$+ \; 2\,P_i \; + \; 2\,ADP^{\,3-} \;\rightleftharpoons\; 2\,ATP^{\,4-} \; + \; 2\,H_2O \; + \; 2$
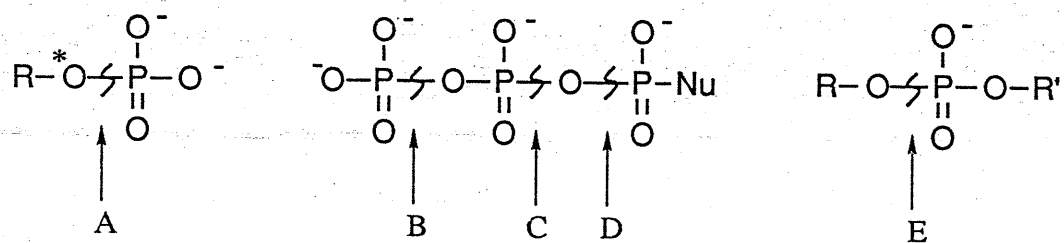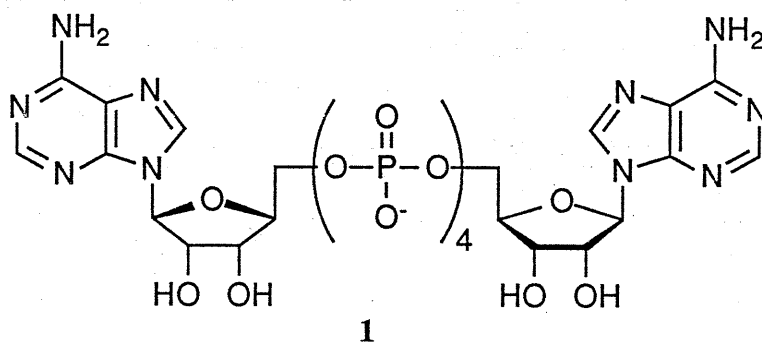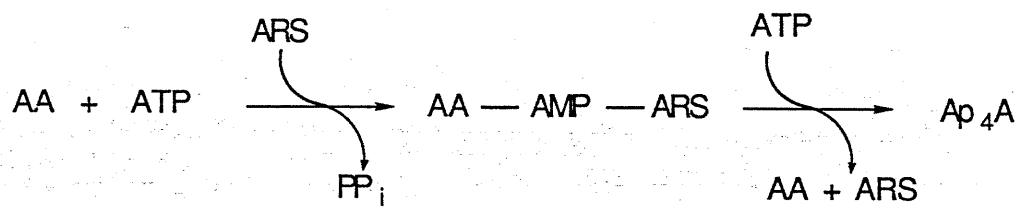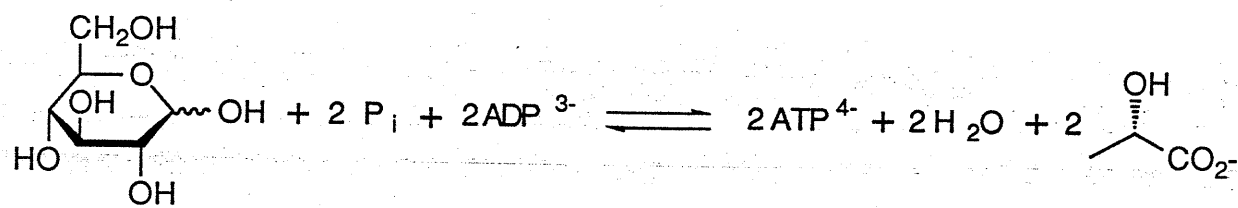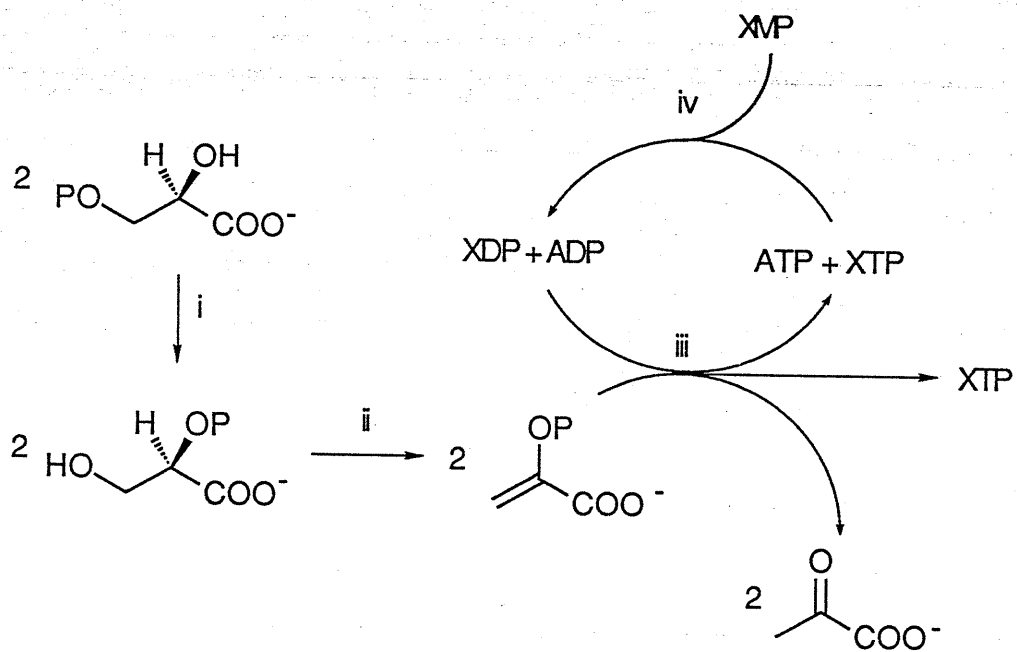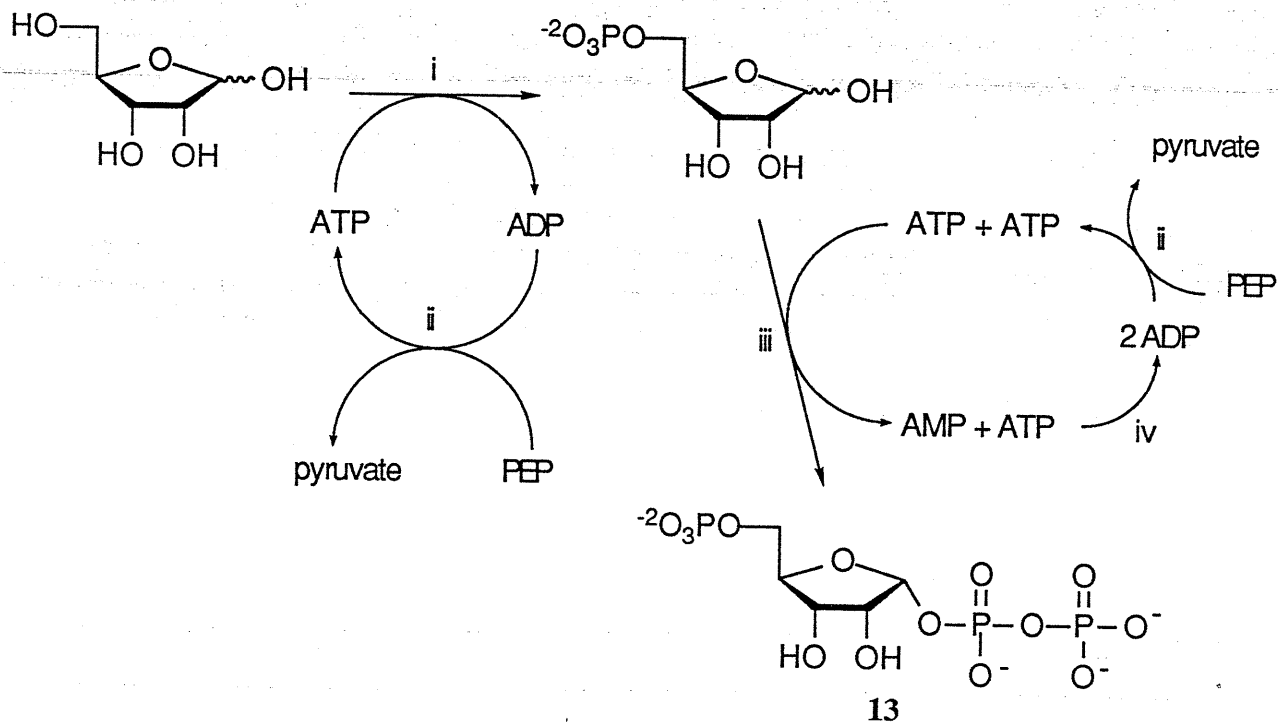
figure 4

figure 5

figure 6

14

figure 7

figure 8

HO

O OH

HO

HO OH

D-fructose

i

OH

HO O

HO

OH

O

HO

O OH

HO

OH

UDP-glucose

UDP

PEP

iv

2P$_i$ ← PP$_i$

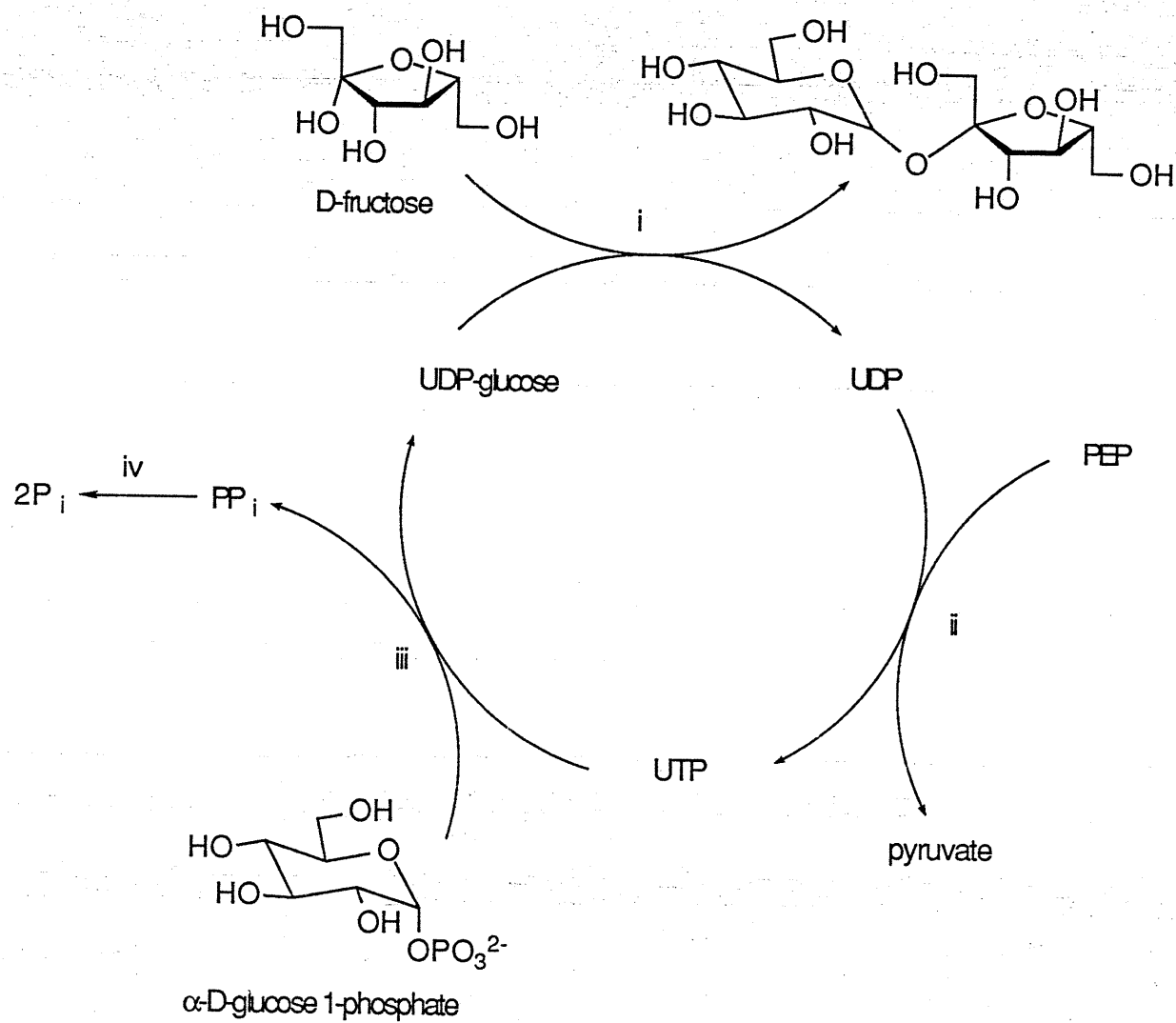iii

ii

UTP

pyruvate

OH

HO

O

HO

OH OPO$_3{}^{2-}$

α-D-glucose 1-phosphate
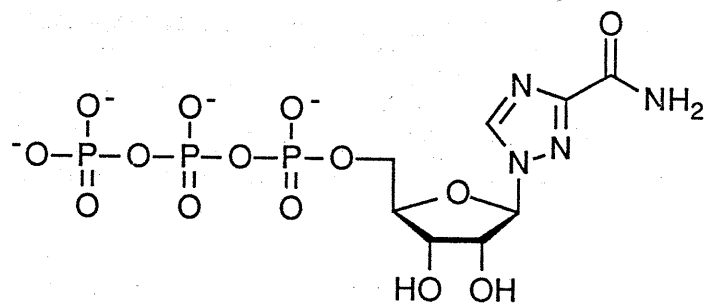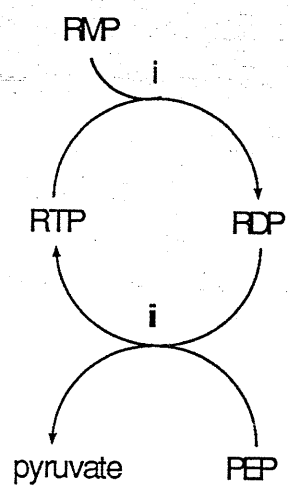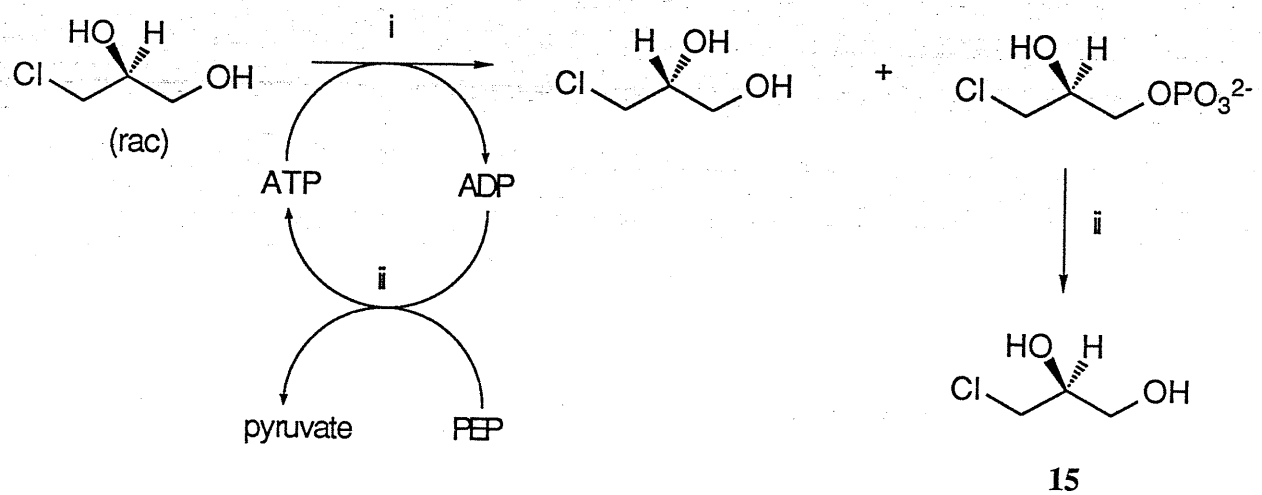
figure 9

figure 10

figure 11