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Intermediate Compounds and Intermediate Reactions in the Synthesis of Oligonucleotides

V.F.Zarytova and D.G.Knorre

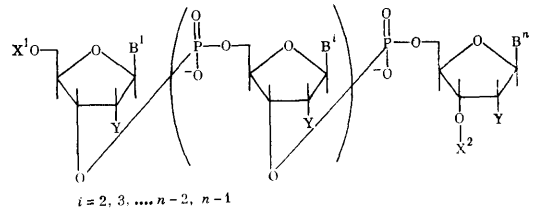
The principal features of the phosphorylation of nucleosides and other alcohols by phosphoric acid monoesters and diesters in the presence of the most widely used condensing agents (arenesulphonyl chlorides, arenesulphonyl azolides, carbodiimides, mixtures of arenesulphonyl chlorides with azoles and pyridines) are described. Data concerning the intermediate compounds and intermediate reactions in the oligonucleotides synthesis as well as model processes, obtained mainly with the aid of ^{31}P NMR, are discussed.
The bibliography includes 67 references.

CONTENTS

I.	Introduction	185
II.	The principal stages of phosphoester condensations	185
III.	The interaction of the P-component with condensing agents	187
IV.	Pyridinium derivatives and azolides of phosphoesters	190
V.	The mechanism of the phosphodiester condensations	192
VI.	The mechanism of the phosphotriester condensations	195

I. INTRODUCTION

Oligonucleotides having the general structure



$i = 2, 3, \ldots, n-2, n-1$

[X^1 and X^2 = H or $OP(O)O^-$, Y = H or OH, and B = nitrogen-containing heterocycles, usually adenine, guanine, cytosine, uracil and thymine] are fragments of the most important class of biopolymers—nucleic acids: DNA when K = H or RNA when Y = OH. Their chemical synthesis is one of the central tasks of bio-organic chemistry. Oligonucleotides are intermediate oligomers in the chemical-enzymic synthesis of genetic material. Their role as a research tool in molecular biology and genetic engineering has been continuously growing.

The main and frequently repeated stage in any method for oligonucleotide synthesis is the formation of new phosphoester (internucleotide) bonds between the monomers or the intermediate oligomers. Until recently, the main advances in the synthesis of oligonucleotides were achieved with the aid of phosphoester condensations, i.e. using the reaction between the hydroxy-group of one component (the OH-component) and the phosphate group of another component (the P-component), which takes place in the presence of condensing agents. Arenesulphonyl chlorides ($ArSO_2Cl$) and arenesulphonyl azolides [tetrazolides ($ArSO_2Tet$ and 3-nitro-1,2,4-triazolides ($ArSO_2NTr$)] have been most widely used as the latter. During the initial period of the establishment of the chemistry of oligonucleotides, dicyclohexylcarbodiimide (DCC) was widely used. Oxidation-reduction condensing agents, mainly a mixture of triphenylphosphine and di(2-pyridyl) disulphide have also been successfully employed.

The first serious advances in the chemical synthesis of oligonucleotides involved the development of the so-called phosphodiester method of synthesis in which the P-component is the phosphomonoester and the condensation product is the phosphodiester. Khorana and coworkers[1,2] achieved by this procedure the first syntheses of genes, using DCC and arenesulphonyl chlorides as the condensing agents. However, at the end of the 1970's there was an abrupt return to the phosphotriester method of synthesis in which the P-component is the diester and the condensation product is the triester. In the latter case the internucleotide phosphate is blocked by some kind of protective group, for example, the *p*-chlorophenyl group. Despite the necessity to remove the blocking groups from the internucleotide phosphate in the last stage, this has resulted in an overall significant simplification and acceleration of the synthetic procedure as a result of the acceleration of the intermediate condensations and the simplification of the purification of the intermediate oligomers. The employment of the phosphotriester method became effective as a result of the appearance of new condensing agents—arenesulphonyl azolides[3-8] and mixtures of arenesulphonyl chlorides with nucleophilic catalysts, namely tetrazole,[6] 3-nitro-1,2,4-triazole,[9,10] dimethylaminopyridine,[11-13] and *N*-methylimidazole.[10,14-19]

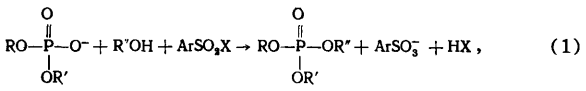
The importance of the phosphoester condensations in the chemistry of nucleotides led to interest in the study of their mechanism. A number of hypotheses concerning the nature of the intermediate species involved in these processes have been put forward by the founders of the chemistry of nucleic acids—Todd, Michelson, and Khorana. Systematic studies became possible following the appearance of the pulsed ^{31}P NMR method, which makes it possible to follow the course of the phosphoester condensations by recording the accumulation of the main and side products as well as certain intermediates.

The aim of the present review is to give an account of the present ideas about the mechanism of the formation of phosphoesters with the aid of condensing agents; these ideas are to a large extent based on studies of the intermediates and intermediate reactions in the oligonucleotide synthesis carried out by the authors of this review and their coworkers.

II. THE PRINCIPAL STAGES OF PHOSPHOESTER CONDENSATIONS

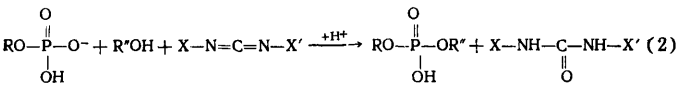
The phosphoester condensations are coupled reactions involving the formation of phosphoesters and transformations of the condensing agents. In the case of arenesulphonyl chlorides and arenesulphonyl azolides such a reaction is the

hydrolysis of the condensing agents and the overall condensation equation can be formulated thus:



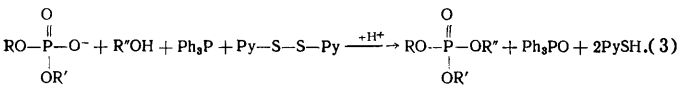
(1)

where X = Cl, the tetrazole residue, or the 3-nitro-1,2,4-triazole residue. In the case of DCC and other disubstituted carbodiimides, the formation of the phosphodiester is accompanied by the hydration of the condensing agent with formation of the corresponding disubstituted urea:



(2)

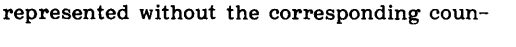
(for DCC, X, X′ = cyclo-C_6H_{11}). In the oxidation-reduction condensation one component of the pair is reduced and the other is oxidised, for example:



(3)

In scheme (1) and (3) R′ = H (for the phosphodiester condensation) or is an organic group (in the phosphotriester condensation). Depending on the solvents employed, the ions can exist as individual species or as ion pairs; here and below they are represented without the corresponding counterions.

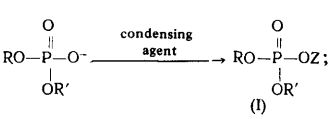
In conformity with the general ideas concerning the mechanisms of the coupled reactions,[20] reactions (1)–(3) should proceed via the formation of intermediates, which arise on interaction of the condensing agent (which behaves as the inductor) with one of the components to be condensed. It has come to be assumed that this interaction takes place with the P-component. The ability of all the condensing agents enumerated above to react with the P-component in the absence of the OH-component supports this view. On the other hand, these condensing agents either do not react at all with the OH-component under the conditions of phosphoester condensations, as happens in the case of DCC, or they are known *a priori* to give rise to side products. Thus arenesulphonyl chlorides and arenesulphonyl azolides sulphonate the OH-component via the reaction



However, hitherto it has not been possible to detect the products of the direct interaction of the condensing agents enumerated above with the P-component in any one instance; only the products of the subsequent reactions have been found.

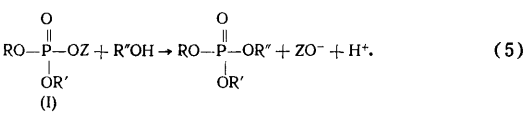
Thus the first problem which arises in the study of the mechanism of phosphoester condensations is that of the nature of the active intermediate species formed directly on interaction of the molecules of the condensing agent and the P-component and also of the mechanism of this interaction (see Section II).

It has been suggested[21] that the species formed are powerful electrophiles capable of phosphorylating the OH groups (Z is a fragment of the condensing agent):



(4)

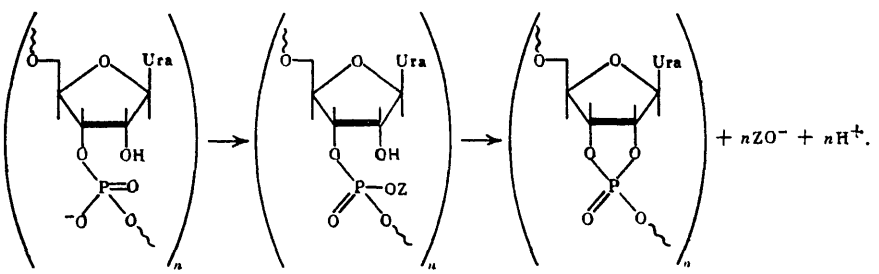
(I)



(5)

(I)

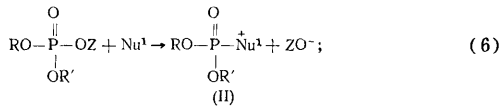
As an example of a process in the course of which the primary products of the interaction of the phosphate with the condensing agent are consumed mainly in the reaction with the OH groups, one may quote the interaction of the condensing agents triisopropylbenzenesulphonyl chloride (TPS) and DCC with the cetavlon salt of polyuridylic acid and dimethylformamide (DMF).[22] In both cases the disappearance of the signals due to the internucleotide phosphate and the appearance of signals of the cyclic phosphotriester groups, which have undergone a significant downfield shift, are observed in the ^{31}P NMR spectra, in conformity with the reaction in accordance with the scheme



The spatial proximity of the phosphate and 2'-hydroxy-groups favours reaction (5), which is in this instance intramolecular.

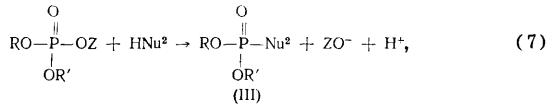
However, under the conditions of the formation of internucleotide bonds, this simple mechanism of the phosphoester condensation does not operate. In this case the reaction of the species (I) with the molecules of the OH-component is intermolecular and the system contains other nucleophiles more powerful than the OH-component.[2]

Among the nucleophiles competing with the OH-component for reaction with compound (I), mention should be made in the first place of pyridine, which has been used until very recently as the solvent,[21] in virtually all phosphoester condensations, and also certain other nitrogen-containing heterocycles, which have come to be employed in recent years to accelerate phosphoester condensations, namely tetrazole, 3-nitro-1,2,4-triazole, *N*-methylimidazole, and 4-*NN*-dimethylaminopyridine (DMAP). It is to be expected that in their presence the reaction proceeds preferentially via the following pathway:



(6)

(II)

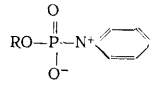


(7)

(III)

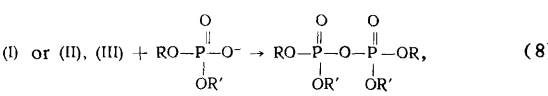
where Nu^1 = pyridine, 4,4-DMAP, or *N*-methylimidazole, HNu^2 = tetrazole or 3-nitro-1,2,4-triazole, R' = H or Ar, and R = protected nucleoside residue.

Compounds (II) and (III) should be fairly powerful phosphorylating agents, the former by virtue of the positive charge on the nitrogen atom and the latter as a result of the fairly high electron-accepting capacities of the tetrazole and nitrotriazole rings. The possible role of phosphorylpyridinium derivatives having the structure



as intermediates in phosphoester condensations has been pointed out by Michelson.[21] The second question of major importance for the understanding of the mechanisms of phosphoester condensations is therefore that of the role of compounds of types (II) and (III) and the formation of new phosphoester linkages, in first place of their reactivity in relation to alcohols. The data available on this question will be examined below (in Section III).

Together with the heterocyclic nucleophiles mentioned above, during the initial phase of the process the reaction mixture contains the P-component which has not reacted and which is also a nucleophile, as a rule stronger than the OH-component. The interaction of the molecules of the P- component with compounds (I)–(III) leads to their consumption with formation of pyrophosphate:

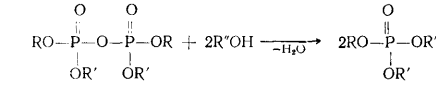


(8)

preventing thereby the formation of new phosphoester linkages. In the phosphotriester condensation this entails the formation of the pyrophosphotetraester, which lacks nucleophilic properties. Vigorous phosphorylation of the OH-component is possible only after the complete consumption of the P-component via reaction (8).

In the phosphodiester condensation the situation is significantly complicated as not only the initial P-component but also the pyrophosphodiester formed from it via reaction (8) and even the required reaction product (the phosphodiester) are nucleophiles. For the effective phosphorylation of the OH-component, it is necessary that both the P-component itself and all its nucleophilic derivatives should be fully exhausted. These reactions are described in Section (IV) in connection with the consideration of the general mechanism of the phosphodiester condensation.

As can be seen from the simplest example of reaction (8), in the first phase of the process the active intermediates of types (II) and (III) are consumed in the first place via their reactions with the P-component. In the second phase of the process, where new internucleotide bonds are mainly formed, the species (II) and (III) are regenerated from pyro- and poly-phosphates. This requires the involvement of nitrogen-containing heterocycles, which do not compete with the OH-component for compounds (II) and (III), since the interaction with these compounds is an exchange process which does not lead to the consumption of these species. Furthermore, the condensing agent can also participate in this process. For example, it follows from the stoichiometric equation of the reaction of pyrophosphodiester or pyrophosphotetraester with the OH-component,

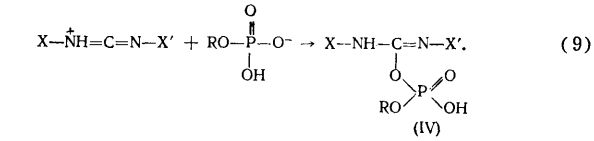


that the completion of the reaction requires the binding of the water molecule, i.e. the involvement of the condensing agent is essential.

The mechanisms of the regeneration of compounds (II) and (III) are also significantly different in phosphodiester and phosphotriester condensations; they will be considered separately in Sections (IV) and (V).

III. THE INTERACTION OF THE P-COMPONENT WITH CONDENSING AGENTS

According to Khorana,[23–25] in the case of disubstituted carbodiimides, the first condensing agents widely introduced into practical oligonucleotide chemistry, the primary product of the interaction of the P-component with the condensing agent is the corresponding *O*-phosphorylisourea (IV). Since phosphorylation in the presence of carbodiimides is strongly suppressed by basic amines, it has been suggested that the reaction proceeds between the protonated form of the carbodiimide and the phosphoester anion.[21,23–26]

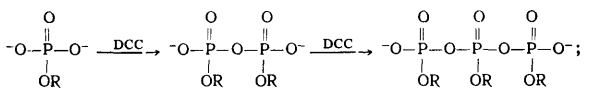


(9)

(IV)

However, compound (IV) could be neither isolated nor even detected in the reaction mixture. The primary product which can be isolated from the reaction mixture is the pyrophosphate. The subsequent reaction in the case of phosphomonoesters leads to the accumulation of trisubstituted triphosphate.[26] This result was subsequently used in other investigations[27–29] in which the reaction between 3'-acetylthymidine 5'-phosphate and DCC in pyridine was followed by

means of ^{31}P NMR, because the chemical shifts of the compounds formed, and in the case of the triphosphate also the structure of the spectrum, differ significantly and the behaviour of each component of the reaction mixture can be followed separately (Figs. 1 and 2):



R = 3'-acetyl-5'-thymidinyl.

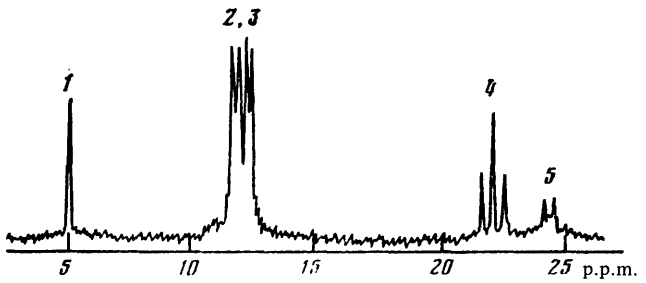


Figure 1. ^{31}P NMR spectrum of the reaction mixture obtained in the interaction of 0.15 M pT(Ac) with 1.5 M DCC in pyridine after 1 h from the start of the reaction: *1*) signal of the pyridinium derivative of pT(Ac); *2*) and *3*) signals of the terminal phosphorus atoms of the trinucleoside triphosphate and the tetranucleoside tetraphosphate; *4*) signals of the central phosphorus atom of the trinucleoside triphosphate; *5*) signals of the central phosphorus atoms of the tetranucleoside tetraphosphate.[37]

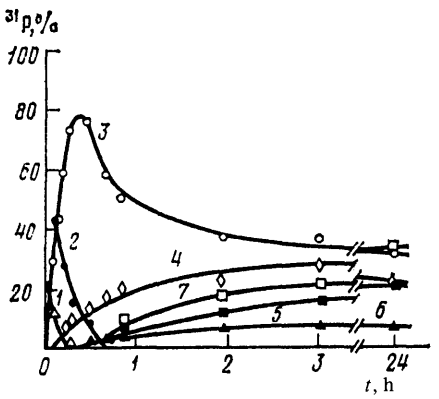
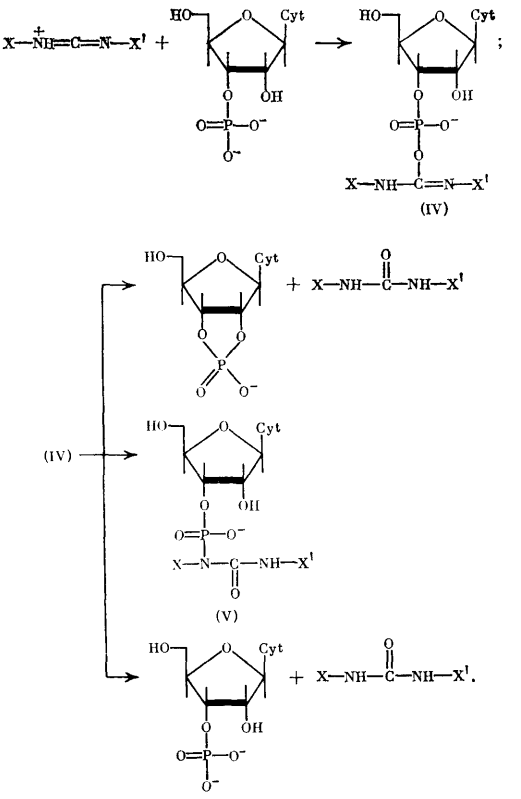


Figure 2. Kinetic curves for the interaction of 0.15 M Pt(Ac) with 1.5 M DCC in pyridine: *1*) initial mononucleotide; *2*) dinucleoside pyrophosphate; *3*) trinucleoside triphosphate; *4*) tetranucleoside tetraphosphate; *5*) *N*-pyrophosphorylurea; *6*) pyridinium derivative of pT(Ac); *7*) unidentified product.[37]

The kinetic test of mechanism (9) was carried out[30] in relation to the reaction of cytidine 2'(3')-monophosphate (CMP) with a water-soluble carbodiimide, namely *N*-cyclohexyl-*N*'-β-(*4-methylmorpholino*)*ethylcarbodiimide toluene-p-sulphonate* (henceforth abbreviated to CME-carbodiimide) in aqueous solution. In this case the reaction product is mainly cytidine cyclic 2',3'-phosphate (cCMP), which is apparently formed directly from the active intermediate (IV) as a result of the spatial proximity of the activated phosphate and the 2'-hydroxy-group. The formation of pyrophosphate is not observed in this instance. The accumulation of a certain amount of *N*-cytidylyl-ME-urea (V), which can be regarded as the product of the isomerisation of *O*-cytidylyl-CME-isourea (IV), was recorded.[30] It is significant that the rate of consumption of CME-carbodiimide is much higher than the rate of accumulation of the CMP reaction products. This shows that the reaction of CME-carbodiimide with CMP involves the formation of an intermediate, which, together with transformation into cCMP and compound (V), can hydrolyse with reformation of the initial CMP. In the light of the Khorana mechanism, the complete process mechanism can be represented as follows:

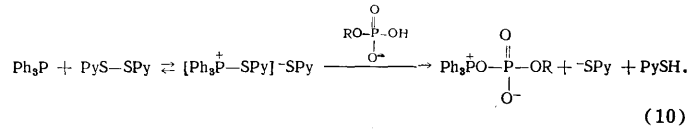


CME-carbodiimide is consumed via a first-order reaction with respect to both components, the effective reaction rate constant depending on the pH and the rate of reaction being proportional to the product of the concentrations of the protonated form of CME–carbodiimide and the double ionised form of CMP.[30]

In the reaction of CME–carbodiimide in aqueous solution with nucleoside 5'-phosphates at low concentrations of the latter, the hydration of carbodiimide is the main reaction pathway, in contrast to the reaction of nucleoside 2'(3')-CMP.[31] It has been shown[32] that, in the interaction of adenosine 5'-monophosphate, labelled with ^{18}O in the phosphate group, with DNA in pyridine in the presence of a large excess of $H_2^{16}O$, the labelled oxygen passes almost quantitatively to the dicyclohexylurea formed. This is also definite evidence in support of the intermediate formation of a compound of type (IV).

Thus all the available data agree with the mechanism proposed by Khorana and coworkers,[23–26] i.e. with the primary formation of *O*-phosphorylisourea.

The following mechanism of the formation of active phosphorylating species has been proposed for condensation in the presence of the triphenylphosphine–dipyridyl disulphide pair:[33,34]



(10)

The primary product detected by ^{31}P NMR in the case of 3′-*O*-acetylthymidine 5′-phosphate is, as in the case of carbodiimides, the corresponding pyrophosphate, which is subsequently converted into the triphosphate.[35] When dipyridyl disulphide is mixed with triphenylphosphine, no changes are observed in the ^{31}P NMR spectrum of the reaction mixture, which implies that, even if the process does proceed in accordance with scheme (10), the equilibrium in the first stage is strongly displaced to the left. The process has not been studied in detail.

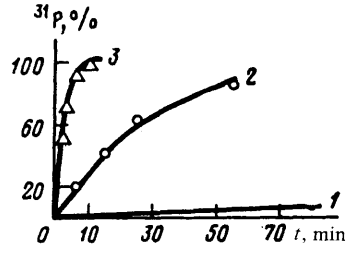
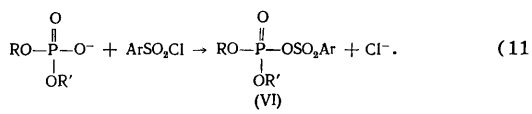


Figure 3. Kinetic curves for the formation of a tetrasubstituted pyrophosphate in the reaction of 0.1 M (ClC_6H_4). .pT(Lev)(triethylammonium salt) and 0.2 M TPS in chloroform at 30 °C in the presence of various additives: *1*) 0.4 M triethylamine; *2*) 0.4 M pyridine; *3*) 0.4 M *N*-methylimidazole.[18]

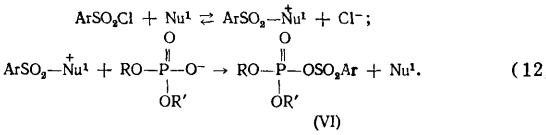
The first phase of the phosphoester condensation has been studied most thoroughly for arenesulphonyl chlorides and arenesulphonyl azolides. In both phosphodiester and phosphotriester condensations, the product of the interaction of the initial P-component with the condensing agent is the corresponding pyrophosphate.[17,27–29,36–43] In the reaction of phosphomonoesters with $ArSO_2Cl$, the pyrophosphodiester formed reacts to produce a trisubstituted triphosphate. The subsequent reactions of the latter will be considered in the next Section. The kinetics of the primary reaction with TPS have been investigated in the reaction involving 3′-*O*-acetylthymidine 5′-phosphate [pT(Ac)] and P^1P^2-di(3′-*O*-acetyl-5′-thymidinyl) pyrophosphate in pyridine. In both cases the reaction is of first order with respect to TPS and the P-component, which proves the involvement of only one molecule of the latter in the rate-limiting stage. This is in full agreement with the hypothesis of Corby, Todd, and Khorana,[44–46] according to which the primary product of the reaction of arenesulphonyl chloride with phosphates is a mixed anhydride of the phosphate and the arenesulphonic acid formed in accordance with the reaction



(11)

(VI)

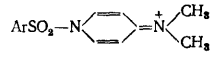
However, reaction (11) is complex and requires the participation of a nucleophilic catalyst.[18] It has been shown for the reaction of the p-chlorophenyl ester of 3′-*O*-levulinylthymidine 5′ phosphate [(ClC_6H_4)PT(Lev)] with TPS in a 7 : 1 chloroform–acetonitrile mixture that the formation of pyrophosphate is not detected at 30 °C in the course of 2 h, while in pyridine the process is completed in several minutes. Fig. 3 shows that the reaction is appreciably accelerated after the addition of a small excess (relative to TPS) of pyridine and to an even greater extent after the addition of *N*-methylimidazole; the addition of similar amounts of triethylamine leads merely to an insignificant accumulation of pyrophosphate. The nucleophilicity of these three bases varies in precisely the same sequence. Thus nucleophilic catalysis plays an appreciable role in the formation of the mixed anhydride (VI). The reaction apparently proceeds in accordance with the following mechanism:



(12)

(VI)

In conformity with this hypothesis, the application of the infrared spectroscopic method to a mixture of TPS or tosyl chloride with dimethylaminopyridine, which is also widely used as a nucleophilic catalyst, in methylene chloride revealed the formation of the arenesulphonyldimethylaminopyridinium derivative, which is apparently stabilised owing to the formation of a quinonoid structure:[47]



In the case of pyridine and *N*-methylimidazole such a derivative could not be detected, most probably because equilibrium (12) is greatly displaced to the left. Thus arenesulphonyl chlorides proved to be insufficiently powerful electrophiles to interact directly with the weakly nucleophilic anion of the phosphodiester, at least in solvents with a low dielectric constant.

The interaction apparently becomes possible with more nucleophilic phosphomonoesters in more polar solvents. Thus pT(Ac) reacts with TPS in DMF. However, the addition of pyridine significantly accelerates the reaction.[48]

The detected product of the interaction of arenesulphonyl azolides (the tetrazolide and 3-nitro-1,2,4-triazolide) with the P-component is also the corresponding pyrophosphate;[17,40–43] by analogy with the reaction of arenesulphonyl chlorides, one can suppose that the primary product is the same mixed anhydride (VI). In methylene chloride the interaction of (ClC_6H_4). .pT(Lev) with toluene-*p*-sulphonyl tetrazolide is slow but is significantly accelerated after the addition of pyridine.[43] Presumably in this instance the phosphodiester is sulphonated by the arenesulphonylpyridinium derivative. The formation of the pyrophosphate in pyridine on reaction of TsTet with (ClC_6H_4)pT(Ac) is significantly accelerated after the addition of toluenesulphonic acid and is almost fully blocked after the addition of an excess of triethylamine.[41] These effects agree well with the stoichiometric equation for the formation of the

arenesulphonylpyridinium derivative:

$$ArSO_2Tet + Nu^1 + PyH^+ \rightleftarrows ArSO_2\text{—}\overset{+}{N}u^1 + TetH + Py. \qquad (13)$$

Equilibrium (13) should be displaced to the left following the addition of a strongly basic amine and to the right after the addition of an acid.

The formation of the mixed anhydride (VI) is the rate-limiting stage in the formation of pyrophosphate. Kinetic measurements apparently do not make it possible to reach a definite conclusion concerning the mechanism of the subsequent transformation of compound (VI) into the pyrophosphate. It can take place either as a result of the direct interaction of compound (VI) with a second molecule of the P-component [Eqn.(14)][13,43] or via the preliminary formation of a phosphorylpyridinium or phosphorylazolium derivative [Eqns.(15) and (16)], as has been postulated in a number of investigations:[6,9,10,16]

$$RO\text{—}\overset{\overset{O}{\|}}{\underset{\underset{OR'}{|}}{P}}\text{—}O^- + RO\text{—}\underset{\underset{OR'}{|}}{\overset{\overset{O}{\|}}{P}}\text{—}O\text{—}SO_2Ar \rightarrow RO\text{—}\underset{\underset{OR'}{|}}{\overset{\overset{O}{\|}}{P}}\text{—}O\text{—}\underset{\underset{OR'}{|}}{\overset{\overset{O}{\|}}{P}}\text{—}OR + ArSO_3^-; \qquad (14)$$

(VI)

$$RO\text{—}\overset{\overset{O}{\|}}{\underset{\underset{OR'}{|}}{P}}\text{—}O\text{—}SO_2Ar + Nu^1 \rightleftarrows RO\text{—}\overset{\overset{O}{\|}}{\underset{\underset{OR'}{|}}{\overset{+}{P}}}\text{—}\overset{+}{N}u^1 + ArSO_3^-; \qquad (15)$$

$$RO\text{—}\overset{\overset{O}{\|}}{\underset{\underset{OR'}{|}}{\overset{+}{P}}}\text{—}\overset{+}{N}u^1 + RO\text{—}\underset{\underset{OR'}{|}}{\overset{\overset{O}{\|}}{P}}\text{—}O^- \rightleftarrows RO\text{—}\underset{\underset{OR'}{|}}{\overset{\overset{O}{\|}}{P}}\text{—}O\text{—}\underset{\underset{OR'}{|}}{\overset{\overset{O}{\|}}{P}}\text{—}OR + Nu^1. \qquad (16)$$

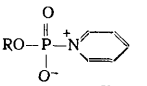
The sharp acceleration of the formation of the mixed anhydrides (VI) in the presence of pyridine and *N*-methylimidazole was interpreted above as a result of the significantly faster reaction (12) compared with the direct reaction (11). This means that the amines indicated are significantly more active than the phosphoester anions and attack the tetrahedral sulphur atom in $ArSO_2Cl$. It is logical to suppose that qualitatively the same relative rates obtain in the attack by the same nucleophiles on the tetrahedral phosphorus atom in compound (VI), i.e. reaction (15) predominates significantly over reaction (14) and the main pathway to the formation of the pyrophosphate is via stages (15) and (16).

In the case of phosphomonoesters the formation of the pyrophosphate and its subsequent conversion into the trisubstituted triphosphate and longer polyphosphates takes place on treatment with $ArSO_2Cl$ in DMF in the absence of nucleophilic catalysts. However, the addition of pyridine accelerates the process significantly, i.e. in the reaction with phosphomonoesters arenesulphonylpyridinium is also a stronger sulphonating agent than $ArSO_2Cl$.

IV. PYRIDINIUM DERIVATIVES AND AZOLIDES OF PHOSPHOESTERS

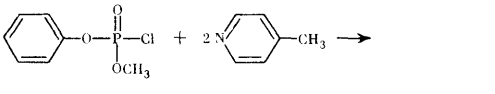
The most detailed information about the role of derivatives of type (II) as active intermediates phosphorylating the hydroxy-groups of the OH-component have been obtained in the study of the phosphodiester condensation in pyridine.

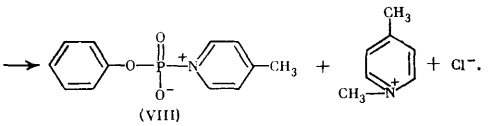
On treatment of phosphomonoesters (nucleoside 5'-phosphates, phenyl phosphate, nitrophenyl phosphate, etc.) with an excess of TPS[27-29] or with a cross-linked poly(styrenesulphonyl chloride) in pyridine,[49] ^{31}P NMR spectra revealed a virtually quantitative conversion of the esters into derivatives whose spectrum consisted of a singlet displaced upfield by ~5 p.p.m. compared with the initial ester. Detailed data for the chemical shifts and structure of the ^{31}P NMR spectra of phosphate esters and their derivatives have been published.[50] Nucleoside 5'-phosphates undergo the same reaction on treatment with an excess of a mixture of triphenylphosphine and dipyridyl disulphide in pyridine.[35,51,52] The comparatively slight change in the chemical shift on formation of these derivatives indicates the retention of the tetrahedral configuration of the phosphorus atom. The necessity for the presence of pyridine to ensure their formation suggests that they are phosphorylpyridinium derivatives having the structure



(VII)

Direct proof of this structure has been found for the derivative of phenyl phosphoric acid (VIII); which had been obtained by the following reaction for this purpose:[52,53]





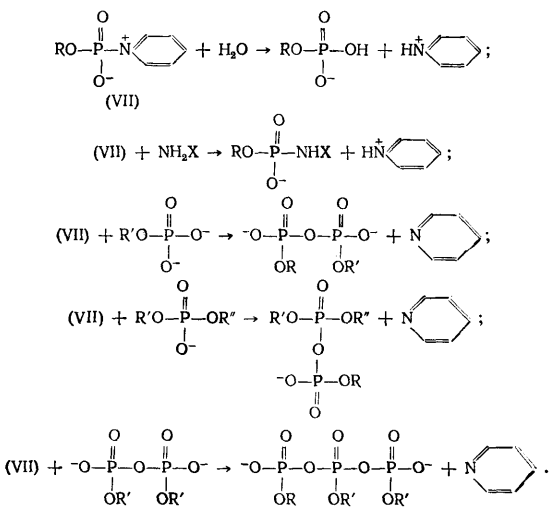
(VIII)

The splitting of both the ^{31}P signal by the α-protons of the picolinium derivative and of the ^1H signal of the α-protons by the phosphorus nucleus ($^3J_{PNCH} = 5.5$ Hz) has been observed in the NMR spectra at −50 °C. This demonstrates the presence of a chemical bond between the phosphorus atoms and the γ-picoline residue. The chemical shifts for both the α-protons and the protons of the γ-methyl group are displaced downfield relative to picoline itself, which is due to the appearance of a positive charge near the picoline fragment. With increase of temperature, exchange is observed between free picoline and the derivative, which leads to the merging of the signals of both the α- and γ-methyl protons (Fig.4) in the spectra of free picoline and compound (VIII) and also to the disappearance of the splitting of the ^{31}P NMR signal by α-protons†.

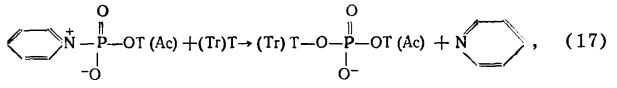
At room temperature compound (VII) reacts vigorously with water, amines, phosphomonoesters, phosphodiesters, and substituted pyrophosphates, all the reactions reaching completion after less than 1–2 min (the time necessary to record the ^{31}P NMR spectrum.

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†The absence of such splitting under the conditions of the phosphoester condensations at room temperature in the presence of an excess of pyridine made it impossible in the first studies of the formation and reactions of compound (VII) to give preference to structure (VII) proposed by Michelson[21] and, in conformity with the hypothesis of Todd[45] and Khorana,[26,54] this compound was regarded as the corresponding metaphosphate $ROP\overset{O}{\underset{O}{\diagdown\!\!\diagup}}$.



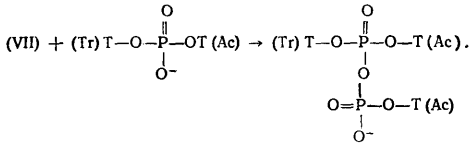
The reaction with alcohols takes place somewhat more slowly. In this instance it is possible to obtain with the aid of ^{31}P NMR the kinetic curve for the consumption of compound (VII) and to determine the rate constant. For the reaction



(17)

it amounts to 7.3×10^{-3} litre mol^{-1} at 30 °C and to 0.8×10^{-3} litre mol s^{-1} at 2 °C.[51] The rate constant depends significantly on the nature of the OH-component, which shows that the process proceeds via an associative mechanism. Thus the rate constant for the reaction of the same derivative at 2 °C with ethylenecyanohydrin is 11.3×10^{-3} litre mol^{-1} s^{-1}.[51]

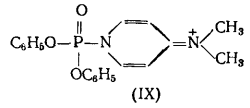
In the reaction of compound (VII) with alcohols the ^{31}P NMR spectra reveal the accumulation not of the phosphodiester but of the pyrophosphotriester—the product of the interaction of the phosphodiester formed with yet another molecule of compound (VII):



Pyrophosphotriesters have two non-equivalent phosphorus atoms, one of which is asymmetric; the ^{31}P NMR spectrum exhibits a complex signal, which consists of the superposition of the signals of two *AB* spin systems of two diastereoisomers[55,56] (Fig. 5).

It has been shown for the *P*-tetrazolide of pT(Ac) that the tetrazolides of phosphomonoesters are less reactive by at least two orders of magnitude on the analogous derivative of type (VII).[13]

The question of the reactivity of phosphodiester derivatives of types (II) and (III) has been investigated[11,12,40–43] on model systems—derivatives of diphenyl phosphoric acid obtained by treating diphenyl phosphorochloridate by the corresponding heterocycles. The 4-*NN*-dimethylaminopyridinium derivative was chosen as an example of a compound of type (II). When $(PhO)_2POCl$ is treated with DMAP in methylene chloride, the signal of the initial compound disappears from the ^{31}P NMR spectrum and another signal appears, which is split at −40 °C by the α-protons of the dimethylaminopyridinium fragment.[11] The signals of H_α, H_β, and CH_3 in the ^1H NMR spectrum are displaced downfield relative to the corresponding DMAP signals owing to the appearance of a positive charge in the DMAP fragment. This made it possible to identify the compounds formed as diphenyl phosphoryldimethylaminopyridinium derivatives, which apparently have the quinonoid structure



(IX)

Compound (IX) is extremely reactive with respect to alcohols. Thus the addition of ethylenecyanohydrin results in an almost instantaneous transformation into 2-cyanoethyl diphenyl phosphate.[11]

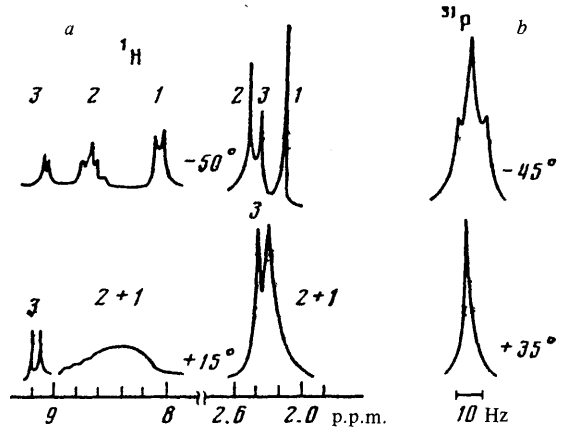


Figure 4. *a*) The structure of the ^1H NMR signals of the α-protons and γ-methyl groups as a function of temperature; *1*) of γ-picoline; *2*) of compound (VIII); *3*) of *N*-methyl-γ-picolinium; *b*) the structure of the ^{31}P NMR signals of compound (VIII) as a function of temperature. The spectra were recorded 1.5 h after the start of the reaction of phenyl methyl phosphorochloridate with 2 equivalents of γ-picoline in methylene chloride at room temperature.[52]

It must be emphasised that the analogous derivatives of phosphomonoesters are significantly less reactive with respect to alcohols than the pyridinium derivatives.[11–13] The greater stability of derivatives of DMAP is associated both with its higher basicity compared with pyridine and with the possibility of additional stabilisation via the formation of a quinonoid structure. The sharp increase of reactivity on passing from dimethylaminopyridinium derivatives of phosphomonoesters to derivatives of phosphodiesters is due to the fact that the former are zwitter-ions, while the latter carry an uncompensated positive charge.

Diphenyl phosphotetrazolide has been chosen as an example of compounds of type (III). When diphenyl phosphorochloridate is treated with tetrazole in dioxan in the presence of triethylamine, the ^{31}P NMR spectrum, measured after the precipitation of triethylammonium chloride, reveals a signal at 21.5 p.p.m., which differs significantly from that of the initial substance (5.5 p.p.m.).[41] The position of the signal makes it possible to identify the compound formed as diphenyl

phosphotetrazolide.[50] The addition of (Tr)T as the OH-component to the reaction mixture leads to the conversion of the tetrazolide of diphenylphosphoric acid into the corresponding phosphotriester (Tr)TOP(O)$(OPh)_2$. In the absence of bases the reaction is fairly slow. The data presented in Fig.(6) show that the reaction is significantly accelerated after the addition of pyridine and to an even greater extent after the addition of γ-collidine, which is a stronger base. The addition of triethylamine leads to an instantaneous conversion of the tetrazolide of the phosphodiester into the phosphotriester.[41,57]

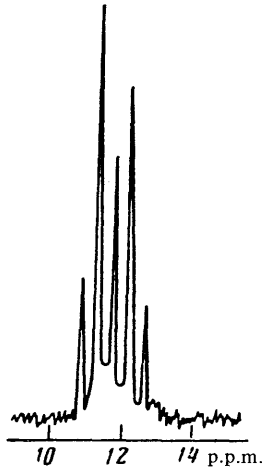


Figure 5. ^{31}P NMR spectrum of the pyrophosphotriester obtained on interaction of 0.0075 M (Tr)T with 0.15 M pyridinium derivative (VII) after 80 min from the start of the reaction.[29]

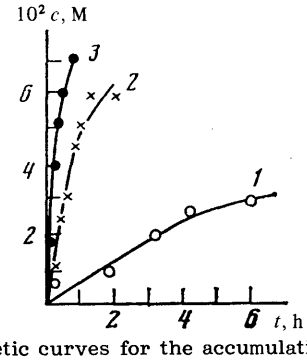


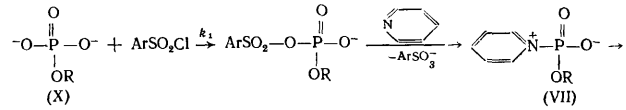
Figure 6. Kinetic curves for the accumulation of the phosphotriester (Tr)TOP(O)$(OPh)_2$ in the interaction of 0.08 M tetrazolide of diphenyl phosphoric acid with 0.01 M (Tr)T in dioxan at 30 °C: *1*) without additives; *2*) in the presence of 0.04 M pyridine; *3*) in the presence of 0.04 M γ-collidine.[57]

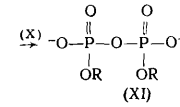
Thus species of types (II) and (III) are extremely reactive with respect to alcohols and there are grounds therefore for regarding them as the direct precursors of new phosphoester groups in phosphoester condensations.

V. THE MECHANISM OF PHOSPHODIESTER CONDENSATIONS

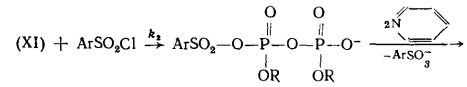
The mechanism of phosphodiester condensations has been investigated in greatest detail in relation to TPS as the condensing agent. The study of the complete system containing the P-component, the OH-component, and the condensing agent was preceded by a detailed investigation of the reaction of the P-component with the condensing agent in the absence of the OH-component. When 3'-*O*-acetylthymidine 5'-phosphate is acted upon by TPS in pyridine, the same sequence of reactions is initially recorded[27-29] as for DCC[58] (Fig.2).

It has been shown for the reaction of pT(Ac) and the pyrophosphate O[pT(Ac)]$_2$ formed from it that the order of the reaction with respect to both components is close to unity,[39,59] i.e. the rate-limiting stage of the process is the interaction of the condensing agent (apparently in the form of its pyridinium derivative) with one molecule of the phosphate or pyrophosphate. The product of the reaction of the pyrophosphate is triphosphate (XII). This indicates the conversion of the mixed anhydride of the pyrophosphate and the arenesulphonic acid into a mononucleotide derivative, which then attacks the pyrophosphate which has not yet reacted. After the consumption of the pyrophosphate, the accumulation of nucleoside 5'-phosphorylpyridinium in the reaction mixture begins. At the same time the accumulation of a certain amount of the tetrasubstituted tetraphosphate (XIII) is observed. Overall, the process kinetics can be described satisfactorily by the following scheme:

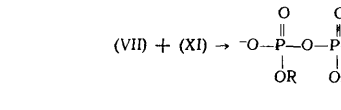


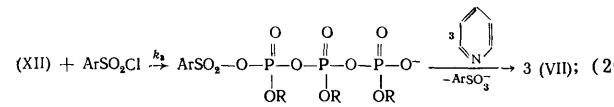


(18)

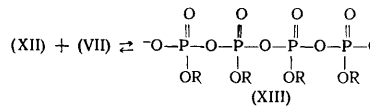


(19)





(20)



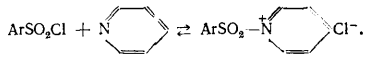
(21)

The Table presents the rate constants for the first three stages of the interaction of PT(Ac) and TPS at 5 °C and the entropies and enthalpies of activation estimated for these processes.[39]

In formulating reactions (18)–(21) and in calculating the rate constants for stages (18)–(20), no account was taken of the observed[48] significant accelerating effect of pyridine on the formation of pyrophosphates and polyphosphates. The species attacking the anions of the phosphomonoester (X), the pyrophosphate (XI), and the triphosphate (XII) is appar-

ently the arenesulphonylpyridinium cation $ArSO_2\overset{+}{N}\langle$. The rate constants listed in the Table are complex and represent the values of the true rate constants for the elementary

reactions of compounds (X)–(XII) with $ArSO_2-\overset{+}{N}$ multiplied by the constant concentration of pyridine and the equilibrium constant for the process

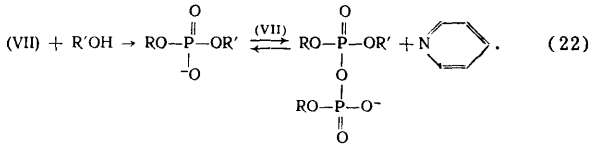


Kinetic parameters of reactions (18)–(20) at 5 °C.[39]

Reaction	$10^3 k$ litre mol^{-1} s^{-1}	$\Delta H^{\neq}$, kcal mol^{-1}	$\Delta S^{\neq}_0$, e.u.
(18)	11	9.8	−31.7
(19)	7	9.9	−32.6
(20)	1.1	9.7	−37.2

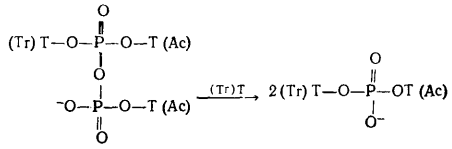
The pyrophosphate (XI) is only slightly less reactive with respect to TPS than the initial phosphomonoester.[59] The triphosphate is less reactive by an order of magnitude[59] and the decrease of the rate constant is largely associated with the decrease of the entropy of activation.

In the complete system the same sequence of transformations of the P-component is initially recorded. As compound (VII) appears in the reaction mixture, the formation of new internucleotide bonds becomes possible. However, as already stated, the phosphodiester formed reacts almost instantaneously with yet another molecule of compound (VII), forming the pyrophosphotriester (XIV):[55,56]

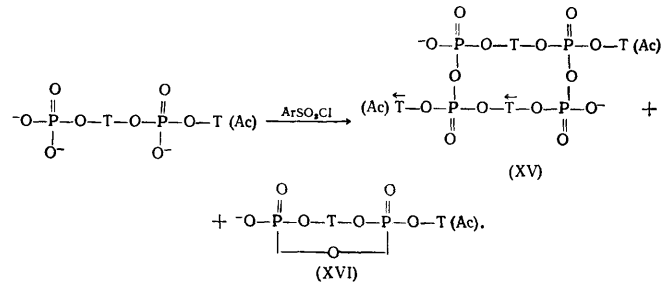


(22)

Pyrophosphotriesters are weak phosphorylating agents, the rate constants for the interaction of which with alcohols in pyridine are lower by two orders of magnitude than for the reactions of compound (VII) with the same alcohols. Thus the rate constant for the reaction



in pyridine at 30 °C is 9×10^{-5} litre mol^{-1} s^{-1}, while that for reaction (17) under the same conditions is 7.3×10^{-3} litre mol^{-1} s^{-1}.[39] Hence, even in the simplest case of the phosphodiester condensation designed to obtain a dinucleoside monophosphate, one of the two molecules of the two active phosphorylating derivative (VII) formed is converted into a significantly less reactive form. The situation is impaired even more when the P-component or the OH-component (or both) contains phosphodiester groups. If the OH-component is not a nucleoside but an oligonucleotide, compound (VII) formed from the P-component reacts in the first place with the phosphodiester groups and only when these have been converted into pyrophosphotriester groups does the reaction with hydroxy-groups proceed to an appreciable extent. Thus a large excess of compound (VII), which increases with increase of the length of the OH-component, is necessary.[38] On the other hand, when the P-component is an oligonucleotide, then, in the presence of a small excess of the condensing agent, pyrophosphotriester groups will form in it instead of the phosphorylpyridinium groups. When the dinucleotide pTpT(Ac) is acted upon by a small excess of TPS, the formation of compounds (XV) and (XVI) is mainly observed.[56–62] These are less reactive [the rate constants for the reactions with (Tr)T at 30 °C in pyridine are 0.6×10^{-4} and 2.8×10^{-4} litre mol^{-1} s^{-1} respectively]:[39]



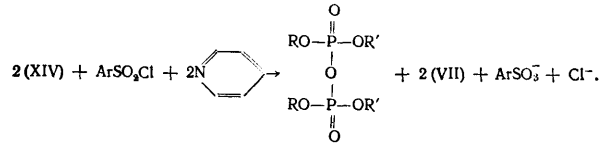
(XV)

(XVI)

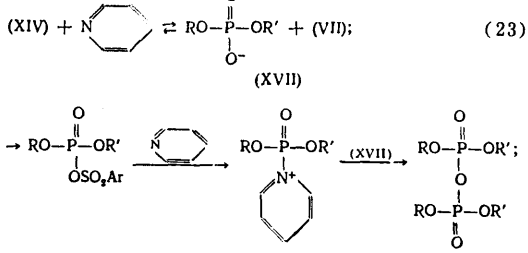
(The arrow above T denotes the direction from the 5′-OH group to the 3′-OH group of thymidine).

Compounds (XV) and (XVI), which have been obtained in DMF in the absence of pyridine, are so resistant to the action of nucleophiles that they can be separated chromatographically in aqueous solution.[62] There are therefore grounds for the assumption that their reaction with the OH-component in pyridine solution is feasible as a result of the nucleophilic catalysis by pyridine,[63] i.e. as a result of some reversibility of the second stage of reaction (22). This is also true of compound (XIV); as can be seen from the rate constants for the reactions of these pyrophosphotriesters with (Tr)T, for this purpose it is sufficient if less than 2% of compound (VII) is present in equilibrium with compounds (XIV), which is outside the limits of the sensitivity of the ^{31}P NMR method.

The highly reactive phosphorylpyridinium form is also regenerated from the pyrophosphotriester on treatment with an excess of the condensing agent.[55] The overall reaction is



Its mechanism can be represented in the following manner:



(23)

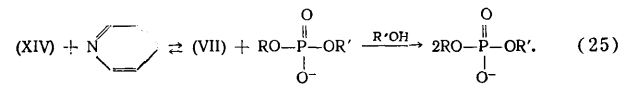
(XVII)

(XVIII)

R=(Tr)T, R′=T(Ac). (24)

It is noteworthy that the rate constant for the reaction of compound (XVII) with TPS, i.e. the rate-limiting stage of reaction (24), is 3.1×10^{-3} litre mol^{-1} s^{-1} at 5 °C, i.e. is only four times smaller than for the reaction with pT(Ac).[39]

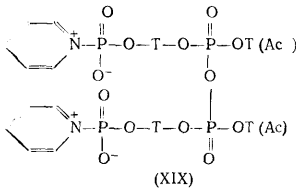
In the presence of a small excess of the condensing agent, when the rate of reaction (24) is low, the dominant pathway leading to the conversion of compound (XIV) into the diester may be the reaction



(25)

Reactions (23) and (24) lead to the conversion of the phosphodiester into the pyrophosphotetraester (XVIII), which can react to some extent with the OH-component, giving rise to the phosphotriester as the side product in the phosphodiester condensation.

The pyrophosphotriesters (XV) and (XVI) apparently undergo analogous reactions. In the presence of a sufficient excess of TPS, the dinucleotide pTpT(Ac) is converted into the compound[60-62]



(XIX)

containing the highly reactive phosphorylpyridinium groups [the rate constant for the reaction with (Tr)T at 30 °C is 6.3×10^{-3} litre mol^{-1} s^{-1}].[39]

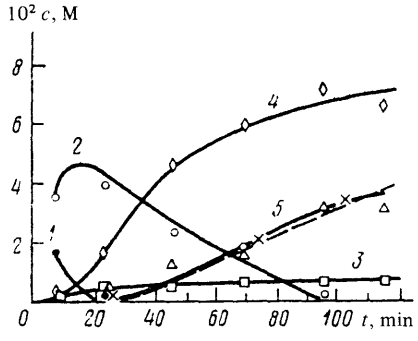
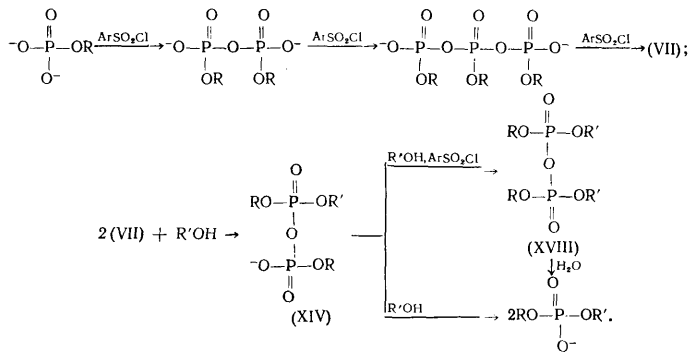


Figure 7. Kinetic curves for the interaction of 0.15 M Pt(Ac) with 0.15 M (Tr)T and 0.42 M TPS in pyridine: *1*) O[pT.(Ac)]$_2$; *2*) trinucleoside triphosphate; *3*) tetranucleoside tetraphosphate; *4*) pyridinium derivative of pT(Ac); *5*) the pyrophosphotriester (XIV).[51]

The reaction of compound (VII) with hydroxy-groups makes the main contribution to the formation of new phosphodiester groups.[52] Fig. 7 presents the kinetic curves for the accumulation of compounds (VII) and (XIV) in the reaction of pT(Ac) with (Tr)T in the presence of TPS at 2 °C. At the same time, the kinetic curve for the accumulation of compound (XIV) as a result of reaction (17), the rate-limiting stage in the formation of compound (XIV), has been calculated from the rate constant presented above for this reaction (dashed curve in Fig. 7). Evidently the calculated and experimental curves agree satisfactorily. Consequently the contribution of the reaction of the OH-component with other reactive species to the formation of phosphodiester is insignificant.

Thus even in the simplest case of the interaction of the P-component containing one phosphate fragment and the OH-component without phosphate groups, the mechanism of the phosphodiester condensations is fairly complex and is described by reactions (18)–(25), the majority of which consist of several elementary stages. The principal stages in the phosphodiester condensation in pyridine, detected by ^{31}P NMR, are presented schematically below:

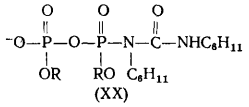


Regardless of the presence of the OH-component, the phosphomonoester undergoes a successive transformation into a substituted pyrophosphate and then into the corresponding triphosphate and the pyridinium derivative (VII). In the presence of the OH-component the derivative (VII) interacts with the latter via reaction (22), forming the trisubstituted pyrophosphate (XIV); the latter is capable of interacting with the OH-component, whereupon the phosphodiester accumulates but at a rate approximately eighty times lower than compound (VII).[39]

In the presence of a sufficient excess of the condensing agent reactions (23) and (24) result in the accumulation of compound (XVIII). However, when the reaction mixture is decomposed with water, this compound readily hydrolyses to the free phosphodiester.[36,58]

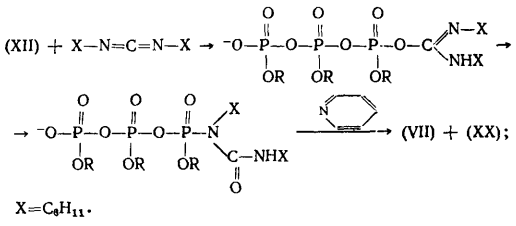
When pT(Ac) reacts in pyridine with a triphenylphosphine–dipyridyl sulphide mixture, the same sequence of transformations of the P-component to pyrophosphate, triphosphate, and finally the derivative (XIV) is observed.[51] The main pathway leading to the formation of phosphodiester group is in this instance also reaction (22).[51]

The reaction of pT(Ac) with DCC in pyridine is more complex. Both in the complete system and in the absence of the OH-component, the same sequence of transformations of the phosphomonoester into the corresponding pyrophosphate and triphosphate is observed. However, even in the absence of the OH-component, the final composition of the reaction mixture is more complex than for TPS and a triphenylphosphine–dipyridyl disulphide mixture. Together with compound (VII), a considerable amount of the triphosphate (XII) and the tetraphosphate (XIII) is retained and certain other derivatives, a considerable fraction of which consists of the ureido-derivative of the pyrophosphates (XX), are also detected:[58]



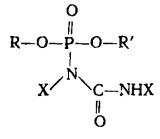
(XX)

This derivative, which should be regarded as the product of the isomerisation of the corresponding *O*-phosphoryldicyclohexylisourea in the light of Khorana's mechanism,[25] begins to accumulate after the complete consumption of the pyrophosphate, i.e. its formation is most probably associated with the reactions of the triphosphate. One of the possible mechanisms of such a reaction consists of this following sequence of stages:



$X = C_6H_{11}$.

When the reaction is carried out in the presence of the OH-component, the formation of compound (VII) is in fact not detected (Fig.8) and the formation of compound (XX) is appreciably suppressed. By virtue of the absence of compound (VII), the free phosphodiester begins to accumulate in the system and is converted into the pyrophosphotetraester only to an insignificant extent. In the final reaction mixture most of the phosphodiester is present in the form of the ureido-derivative:



This derivative is only slowly hydrolysed by water to the corresponding phosphodiester and dicyclohexylurea.

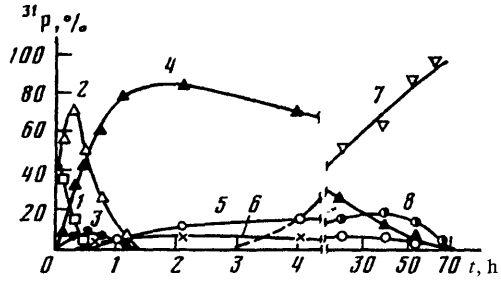
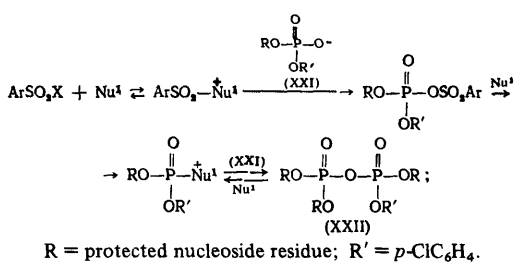


Figure 8. Kinetic curves for the interaction of 0.15 M pT(Ac) with 0.3 M (Tr)T and 1.5 M DCC at 22 °C in pyridine: *1*) the pyrophosphate O[pT(Ac)]$_2$; *2*) trinucleoside triphosphate; *3*) tetranucleoside tetraphosphate; *4*) the pyrophosphotriester (XIV); *5*) (Tr)TpT(Ac); *6*) signal intensity in the region of 5 p.p.m.; *7*) ureido-derivative of dinucleoside phosphate; *8*) the tetrasubstituted pyrophosphate (XVIII).[37]

VI. THE MECHANISM OF THE PHOSPHOTRIESTER CONDENSATIONS

Depending on the nature of the condensing agent, the first phase in the phosphotriester condensation is described by Eqn.(11) or (12) and then by Eqns.(15) and (16), i.e., overall, it can be represented by the equation



R = protected nucleoside residue; R′ = *p*-ClC$_6$H$_4$. (26)

This process is analogous to reaction (18) in the phosphodiester condensation. However, in contrast to this reaction, the pyrophosphate (XXII) does not undergo further transformations under the influence of the condensing agent, i.e. processes analogous to reactions (19)–(21) are absent in the phosphotriester condensation.

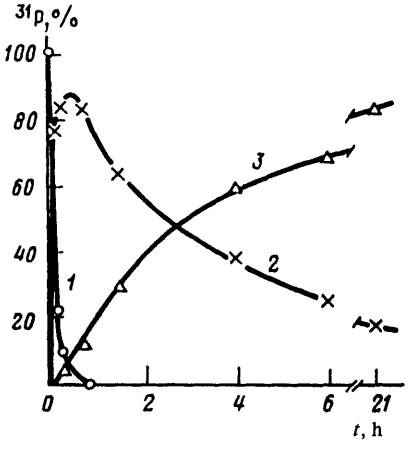


Figure 9. Kinetic curves for the reaction of 0.1 M (Tr)Tp.(C$_6$H$_4$Cl) with 0.1 M T(Ac) and 0.3 M TPS in pyridine at 30 °C: *1*) initial diester; *2*) the tetrasubstituted pyrophosphate (XXII); *3*) the triester (Tr)OTO – P(=O)(OC$_6$H$_4$Cl) – OT(Ac).[42]

The inability of the pyrophosphate (XXII) to react with the condensing agent, except for the reaction analogous to reaction (19), necessitates at the same time the presence of another pathway leading to the transformation of the pyrophosphate into an active derivative of type (II) or (III). It is reasonable to suppose that this takes place analogously to the interaction of the pyrophosphotriester with the OH-component via a reaction analogous to reaction (25):

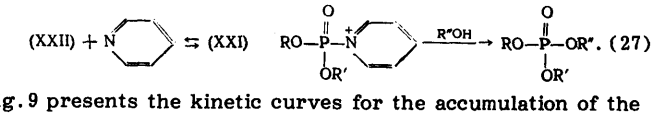


Fig.9 presents the kinetic curves for the accumulation of the pyrophosphotetraester and the phosphotriester and for the consumption of the initial phosphodiester in the reaction of

(Tr)Tp(C_6H_4Cl) with T(Ac) in pyridine in the presence of a three-fold excess of TPS. The curves were obtained with the aid of ^{31}P NMR.[42] It is seen that, whereas the first phase of the reaction, i.e. the accumulation of the pyrophosphate, is completed in less than 1 h, the second phase takes more than 24 h. Such a prolonged contact of the OH-component with the condensing agent leads to undesirable side processes, for example, the sulphonation of the OH-component. There is no reason to doubt the extremely high electrophilicity of the species (XXIII), which carries an uncompensated positive charge. The low rate of the second phase of the reaction in pyridine is apparently associated with the fact that the species (XXIII), formed as an ion pair with the phosphodiester anion, interacts preferentially with the latter, which greatly displaces the equilibrium in the first reaction of process (27) to the left.

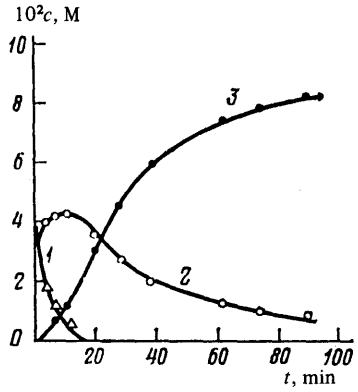
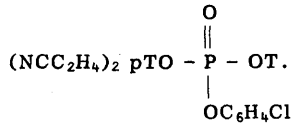


Figure 10. Kinetic curves for the interaction of 0.1 M (ClC_6H_4)pT(Ac) with 0.1 M (NCC_2H_4)$_2$pT, 0.3 M TPS, and 0.3 M tetrazole at 20 °C in pyridine: *1*) (ClC_6H_4)pT(Lev); *2*) the pyrophosphotetraester; *3*) the phosphotriester



[17]

In conformity with the foregoing, the effective catalysts of the second phase of the phosphotriester condensation proved to be compounds of two types. Firstly, these are compounds of the "pyridine" type, namely DMAP[12,13,17] and *N*-methylimidazole.[17,18] Being significantly stronger nucleophiles than pyridine, these compounds apparently give rise to species of type (II), which are significantly more stable than compounds (XXIII). Secondly, tetrazole[17,41,43] and 3-nitro-1,2,4-triazole[17] proved to be catalysts of this kind. They give rise to uncharged phosphodiester derivatives of type (III). As an illustration, Fig.10 presents kinetic curves for the reaction of (ClC_6H_4)pT(Lev) with (NCC_2H_4)$_2$pT in pyridine in the presence of a threefold excess of an equimolar mixture of TPS and tetrazole.[17] The reaction takes place to an extent greater than 70% in 1 h. As in the eactions of TPS in pyridine the absence of other catalytic additives, more than 90% of the pyrophosphate accumulates in the first phase of the reaction and the latter is then converted into the phosphotriester. In the light of these data, a sharp acceleration of the phosphotriester condensation following the replacement of arenesulphonyl chlorides by arenesulphonyl tetrazolides[4,5] can be accounted for most probably by the fact that tetrazole, catalysing the second phase of the process, is produced in the first phase. Similar kinetic curves, involving the formation of up to 90% of the pyrophosphate in the first phase, have been obtained also for all the other catalysts enumerated.[17]

In order to establish the role of pyridine in the stage involving the formation of the phosphotriester from the pyrophosphate, the latter was obtained in the form of a solution in methylene chloride from (ClC_6H_4)pT(Ac) in the presence of TPS and (Tr)T as the OH-component. In the absence of pyridine, the reaction stops at this point; the addition of pyridine or γ-picoline leads to the formation of the dinucleoside phosphate. On the other hand, the addition of benzo-1,4-diazabicyclo[2.2.2]octene, whose $pK_a = 5.6$ and is close to that of pyridine, does not lead to the synthesis of the phosphotriester, which shows that in the given stage pyridine plays primarily the role of a nucleophilic catalyst.[43,56]

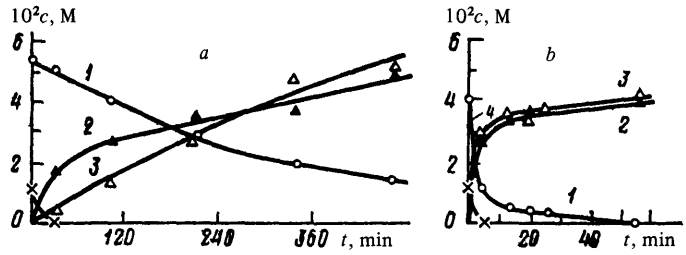
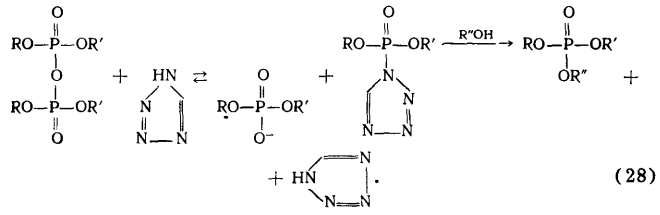


Figure 11. Kinetic curves for the reactions at 30 °C in pyridine; *a*) reaction of 0.05 M tetraphenyl pyrophosphate and 0.01 M diphenyl phosphorochloridate with 0.11 M (Tr)T; *b*) reaction of 0.04 M tetraphenyl pyrophosphate and 0.01 M diphenyl phosphorochloridate with 0.12 M (Tr)T in the presence of 0.25 M tetrazole; *1*) tetraphenyl pyrophosphate; *2*) the phosphotriester (Tr)TOP(O)$(OPh)_2$; *3*) diphenyl phosphoric acid; *4*) diphenyl phosphorochloridate.[41]

The catalytic role of tetrazole has been demonstrated in relation to the model reaction of tetraphenyl pyrophosphate with (Tr)T.[41] In order to rule out the possible influence of the condensing agent, tetraphenyl pyrophosphate was obtained by the reaction of diphenyl phosphoric acid with diphenyl phosphorochloridate. A small excess of the latter ensured the absence of undesirable traces of moisture from the system. After the addition of (Tr)T, diphenyl phosphorochloridate disappears in the course of several minutes and a gradual conversion of the pyrophosphate into diphenyl phosphate and the phosphotriester (Tr)Tp$(OC_6H_5)_2$ is observed (Fig.11*a*). The addition of tetrazole simultaneously with (Tr)T leads to the acceleration of the process by a large factor (Fig.11*b*); the reaction is completed in less than half an hour instead of several hours. If triethylamine is added simultaneously with tetrazole, the reaction is completed faster than it is possible to record the ^{31}P NMR spectrum, i.e. in less than 1–2 min. The diphenyl phosphotetrazolide signal is not recorded in either case, in agreement with the rapid interaction of this compound with the OH-component noted in

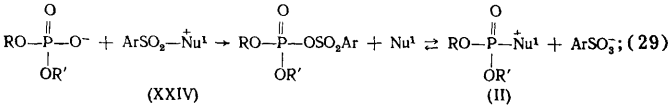
Section (III). Overall, the transformation of the pyrophosphate into the phosphotriester in the presence of tetrazole can be represented by the following scheme:



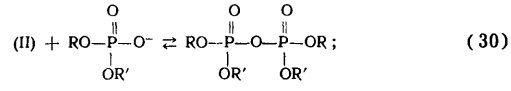
(28)

The equilibrium in the first stage of reaction (28) is greatly displaced to the left, because the addition of tetrazole to a pyrophosphate solution does not result in the appearance of a signal due to the tetrazolide of the phosphodiester in the ^{31}P NMR spectrum. The presence of a strong base should shift the equilibrium to the right (the proton is bound), which may account for the sharp acceleration of the interaction of tetraphenyl pyrophosphate with (Tr)T in the presence of tetrazole following the addition of triethylamine. However, the second stage also contributes to the acceleration of the process and this stage is likewise accelerated by triethylamine, as stated in Section III.

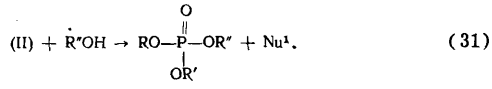
Taking into account the foregoing, including the fact that the $ArSO_2\overset{+}{N}\diagdown\!\!\diagup$ derivative of the condensing agent and not the condensing agent itself interacts with the initial phosphodiester, the overall mechanism of the phosphotriester condensation in the presence of TPS and pyridine or an analogous catalyst can be formulated as follows:



(29)



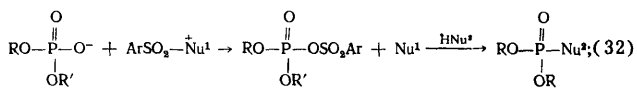
(30)



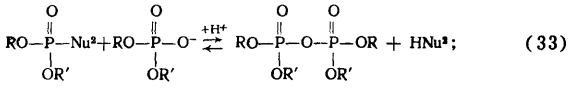
(31)

In the first phase of the reaction, the species (II) react preferentially via pathway (30), which leads to the accumulation of the pyrophosphotetraester. As the initial phosphodiester is consumed, the process is switched to pathway (31) to an increasing extent. Presumably in the second phase of the reaction, where reaction (30) is reversed as a result of the sharp decrease of the concentration of the phosphodiester, the main bulk of the product is formed via reactions (29) and (31).

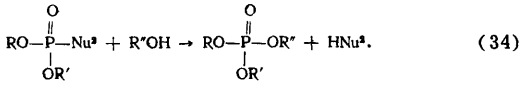
An analogous scheme can also be formulated for catalysis by tetrazole or nitrotriazole:



(32)



(33)



(34)

Since it is sufficient to have catalysts of the "pyridine" type for the occurence of the first phase of the reaction, including the formation of the derivative (XXIV), in phosphotriester condensations pyridine can be fully replaced by *N*-methylimidazole, while acetonitrile, dioxan, and many halogeno-hydrocarbons can be used as solvents.[10,15-18]

In contrast to this, tetrazole cannot convert the arenesulphonyl chloride into an active derivative of type (XXIV). In order that the reaction of the arenesulphonyl chloride with tetrazole, leading to the formation of the arenesulphonyl tetrazolide, should occur, a strong base must be present; in pyridine such interaction does not take place under the conditions of the phosphotriester condensation.[6] At the same time, as stated in Section (II), strongly basic amines prevent the formation of the mixed anhydride (VI) from arenesulphonyl tetrazolides and the phosphodiester. In catalysis of the phosphotriester condensation by tetrazole and nitrotriazole, pyridine, which is essential for the formation of compound (XXIV), must therefore be used as the solvent. Furthermore, pyridine is involved as a base in the reverse reaction (33) and reaction (34).[43]

It is noteworthy that, whereas the pyrophosphotetraester behaves as a typical intermediate in terms of the character of the kinetic curves (Figs.9 and 20), it is strictly speaking not an intermediate. Reactions (30) and (33) are not intermediate processes but constitute reversible side reactions. Their suppression can significantly accelerate the process. The question whether the pyrophosphotriester can be formed when the phosphotriester condensations are carried out on insoluble polymeric carriers, where the P-component is fixed on the carrier while the OH-component is in solution,[19,64-67] remains open.

REFERENCES

1. H.G.Khorana, K.L.Agarwal, H.Büchi, M.N.Caruthers, N.K.Gupta, K.Kleppe, A.Kumar, E.Ohtsuka, U.L.Raj-Bhandary, J.H.van de Sande, V.Sgaramella, T.Terao, H.Weber, and T.Yamada, J.Mol.Biol., 1972, **209**.
2. H.Khorana, K.L.Agarwal, P.Besmer, H.Büchi, M.H.Caruthers, P.J.Cashion, M.Fridkin, E.Jay, K.Kleppe, R.Kleppe, A.Kumar, P.C.Loewen, R.C.Miller, K.Minamoto, A.Panet, U.L.Raj-Bhandary, B.Ramamoorthy, T.Sekiva, T.Takeya, and J.H.van de Sande, J.Biol.Chem., 1976, **251**, 565.
3. K.Itakura, N.Katagiri, and S.A.Narang, Canad.J. Chem., 1974, **52**, 3689.
4. J.Stawinski, T.Hozumi, and S.A.Narang, Canad.J. Chem., 1976, **54**, 670.
5. J.Stawinski, T.Hozumi, S.A.Narang, C.P.Bahl, and R.Wu, Nucl.Acids Res., 1977, **4**, 353.
6. A.Seth and E.A.Jay, Nucl.Acids Res., 1980, **8**, 5445.
7. C.B.Reese, R.C.Titmas, and L.Yau, Tetrahedron Letters, 1978, **19**, 2727.
8. C.B.Reese and A.Ubasawa, Tetrahedron Letters, 1980, **21**, 2265.
9. R.Charubala and W.Pfleiderer, Nucl.Acids Res., Symposium Series, 1981, 161.
10. L.A.Kayushin, Yu.A.Berlin, and M.N.Kolosov, Bioorg.Khim., 1982, **8**, 660.
11. V.F.Zarytova, E.M.Ivanova, D.G.Knorre, A.V.Lebedev, A.I.Rezvukhin, and E.V.Yarmolinskaya, Dokl.Akad.Nauk SSSR, 1979, **248**, 1124.
12. V.F.Zarytova, E.M.Ivanova, D.G.Knorre, and H.Vorbrüggen, Dokl.Akad.Nauk SSSR, 1980, **255**, 1128.

13. D.G.Knorre and V.F.Zarytova, in "Phosphorus Chemistry Directed Towards Biology", Pergamon Press, New York, 1980, p.13.
14. V.A.Efimov, S.V.Reverdatto, and O.G.Chakhmakhcheva, Bioorg.Khim., 1982, **8**, 231.
15. V.A.Efimov, S.V.Reverdatto, and O.G.Chakhmakhcheva, Tetrahedron Letters, 1982, 961.
16. V.A.Efimov, S.V.Reverdatto, and O.G.Chakhmakhcheva, Nucl.Acids Res., 1982, **10**, 5675.
17. E.M.Ivanova, L.M.Khalimskaya, V.P.Romanenko, and V.F.Zarytova, Tetrahedron Letters, 1982, **23**, 5447.
18. V.F.Zarytova, E.M.Ivanova, and V.P.Romanenko, Dokl.Akad.Nauk SSSR, 1982, **265**, 378.
19. V.V.Gorn and V.F.Zarytova, Bioorg.Khim., 1983, **9**, 1511.
20. N.M.Emanuel' and D.G.Knorre, "Kurs Khimicheskoi Kinetiki" (Textbook of Chemical Kinetics), Izd. Vysshaya Shkola, Moscow, 1974.
21. A.M.Michelson, "The Chemistry of Nucleosides and Nucleotides" (Translated into Russian), Izd.Mir, Moscow, 1962.
22. V.F.Zarytova, V.K.Rait, and T.F.Chernikova, Bioorg.Khim., 1977, **3**, 1626.
23. H.G.Khorana and A.R.Todd, J.Chem.Soc., 1953, 2257.
24. C.Dekker and H.G.Khorana, J.Amer.Chem.Soc., 1954, **76**, 3522.
25. M.Smith, J.G.Moffatt, and H.G.Khorana, J.Amer. Chem.Soc., 1958, **80**, 6204.
26. G.Weimann and H.G.Khorana, J.Amer.Chem.Soc., 1962, **84**, 6223.
27. V.F.Zarytova, D.G.Knorre, A.V.Lebedev, A.S.Levina, and A.I.Rezvukhin, Dokl.Akad.Nauk SSSR, 1973, **212**, 630.
28. V.F.Zarytova, D.G.Knorre, A.V.Lebedev, and A.I. Rezvukhin, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser. Khim.Nauk, 1974, 1(2), 85.
29. D.G.Knorre, A.V.Lebedev, A.S.Levina, A.I.Rezvukhin, and V.F.Zarytova, Tetrahedron, 1974, 3073.
30. G.T.Babkina, D.G.Knorre, and L.G.Oleinich, Izv. Sibir.Otd.Akad.Nauk SSSR, Ser.Khim. Nauk, 1972, 1(2), 120.
31. D.G.Knorre, V.A.Kurbatov, G.S.Mushinskaya, and E.G.Saikovich, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser. Khim.Nauk, 1967, 3(7), 137.
32. B.I.Braslavskii, V.F.Zarytova, D.G.Knorre, I.K.Korobeinicheva, and V.A.Kurbatov, Bioorg.Khim., 1977, **3**, 759.
33. T.Mukaiyama and M.Hashimoto, Bull.Chem.Soc.Japan, 1971, **44**, 196.
34. T.Mukaiyama, Phosphorus and Sulphur, 1976, **1**, 371.
35. D.G.Knorre, G.F.Mishenina, V.V.Samukov, and T.N.Shubina, Dokl.Akad Nauk SSSR, 1977, **236**, 613.
36. V.F.Zarytova, D.G.Knorre, A.V.Lebedev, A.S.Levina, and A.I.Rezvukhin, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser.Khim.Nauk, 1974, 3(7), 126.
37. V.F.Zarytova, E.M.Ivanova, and A.V.Lebedev, Bioorg.Khim., 1976, **2**, 189.
38. D.G.Knorre and V.F.Zarytova, Nucl.Acids Res., 1976, **3**, 2709.
39. D.G.Knorre, V.F.Zarytova, A.V.Lebedev, L.M.Khalimskaya, and E.A.Sheshegova, Nucl.Acids Res., 1978, **5**, 1253.
40. V.F.Zarytova and E.A.Sheshegova, Bioorg.Khim., 1978, **4**, 901.
41. V.F.Zarytova, L.M.Khalimskaya, and E.V.Yarmolinskaya, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser.Khim. Nauk, 1980, 6(14), 78.
42. L.I.Drozdova, V.F.Zarytova, and L.M.Khalimskaya, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser.Khim.Nauk, 1981, 1(2), 125.
43. V.F.Zarytova, G.V.Shishkin, and L.M.Khalimskaya, Bioorg.Khim., 1981, 7, 900.
44. N.S.Corby, G.W.Kenner, and A.R.Todd, J.Chem. Soc., 1952, 1124.
45. A.R.Todd, Proc.Nat.Acad.Sci.USA, 1959, **45**, 1389.
46. T.M.Jacob and H.G.Khorana, J.Amer.Chem.Soc., 1964, **86**, 1630.
47. T.V.Mal'tstva, E.M.Ivanova, I.K.Korobeinicheva, and V.F.Zarytova, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser. Khim.Nauk, 1982, 3(7), 120.
48. V.F.Zarytova and L.M.Khalimskaya, Izv.Sibir.Otd. Akad.Nauk SSSR, Ser.Khim.Nauk, 1982, 6(14), 119.
49. S.I.Turkin, V.K.Potapov, Z.A.Shabarova, V.F.Zarytova, and D.G.Knorre, Bioorg.Khim., 1975, **1**, 1430.
50. A.V.Lebedev and A.I.Rezvukhin, Bioorg.Khim., 1983, **9**, 149.
51. V.F.Zarytova, D.G.Knorre, V.P.Starostin, and L.M.Khalimskaya, Izv.Sibir.Otd.Akad.Nauk SSSR, Ser.Khim.Nauk, 1979, 1(2), 88.
52. V.F.Zarytova, D.M.Graifer, E.M.Ivanova, D.G.Knorre, A.V.Lebedev, and A.I.Rezvukhin, Nucl.Acids Res.Spec.Publ., 1978(4), 209.
53. D.M.Graifer, V.F.Zarytova, E.M.Ivanova, A.V.Lebedev, and A.I.Rezvukhin, Dokl.Akad.Nauk SSSR, 1978, **242**, 616.
54. G.Weimann and H.G.Khorana, Chem.Ind., 1962, 271.
55. V.F.Zarytova, D.G.Knorre, A.V.Lebedev, A.S.Levina, and A.I.Rezvukhin, Izv.Sibir.Otd. Akad.Nauk SSSR, Ser.Khim.Nauk, 1974, 3(7), 121.
56. V.F.Zarytova, D.G.Knorre, A.V.Lebedev, A.S.Levina, and A.I.Rezvukhin, Izv.Sibir.Otd. Akad.Nauk SSSR, Ser.Khim.Nauk, 1974, 2(4), 139.
57. V.F.Zarytova and D.G.Knorre, Nucl.Acids Res., 1984, **12**, 2091.
58. V.F.Zarytova, E.M.Ivanova, and A.V.Lebedev, Bioorg.Khim., 1976, **2**, 189.
59. V.F.Zarytova, D.G.Knorre, and L.M.Khalimskaya, Zhur.Fiz.Khim., 1975, **49**(10), 2504 [Russ.J.Phys. Chem., 1975(10)].
60. A.G.Badashkevich, V.F.Zarytova, D.G.Knorre, A.V.Lebedev, and T.N.Shubina, Dokl.Akad.Nauk SSSR, 1975, **222**, 97.
61. D.G.Knorre, A.V.Lebedev, and V.F.Zarytova, Nucl. Acids Res., 1976, **3**, 1401.
62. V.F.Zarytova and A.V.Lebedev, Bioorg.Khim., 1977, **3**, 1211.
63. A.V.Lebedev and V.F.Zarytova, "XI International Symposium on Chemistry of Natural Products", Varna, 1978, p.283.
64. V.K.Potapov, G.A.Potemkin, V.V.Gorn, V.F.Zarytova, Yu.G.Sredin, Z.A.Shabarova, and D.G.Knorre, Dokl.Akad.Nauk SSSR, 1982, **263**, 1386.
65. N.V.Amirkhanov, V.P.Kumarev, M.I.Rivkin, and V.I.Rybakov, Bioorg.Khim., 1982, **8**, 126.
66. A.N.Sinyakov, A.I.Lomakin, V.F.Yamshchikov, and S.G.Popov, Bioorg.Khim., 1982, **8**, 490.
67. R.Belagaje and C.Brush, Nucl.Acids Res., 1982, 10, 6295.

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