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The mechanism of the chemical synthesis of oligonucleotides and its synthetic consequences

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ABSTRACT

The data obtained mainly by pulsed NMR spectroscopy on phosphorus nuclei on the mechanism of the internucleotide phosphodiester (PDE)group formation are summarised. With arylsulphonyl chloride as condensing reagent monomeric nucleotide derivative B (nucleoside metaphosphate or its pyridinium adduct) is the highly reactive intermediate. In the presence of PDE groups in nucleoside or nucleotide component the significantly less reactive derivatives with trisubstituted pyrophosphoryl residues are formed both with arylsulphonyl chloride and dicyclohexylcarbodiimide (DCC). The reactive B form of nucleotide component may be obtained using greater excess of arylsulphonyl chloride with simultaneous convertion of PDE groups to tetrasubstituted pyrophosphates amenable to side reactions. The convertion of PDE groups to easily hydrolysable dicyclohexylurea derivatives by reaction with DCC is proposed to reversible blocking of PDE groups of nucleoside component. The B type derivatives of mononucleotides or oligonucleotides with blocked PDE groups seems to be the best nucleotide components.

INTRODUCTION

At present there exists a number of approaches to the chemical synthesis of nucleic acids. The method proposed by Khorana based on the use of the condensing reagents - dicyclohexylcarbodiimide (DCC) and arylsulphonyl chlorides ($ArSO_2Cl$) is the most widely employed. However there exist some disadvantages of this approach, first of all the formation of the side products including colored substances and some extent of degradation of internucleotide bonds at some stages. The yields are sufficiently high only for rather short oligonucleotides. Therefore, an intensive search of new approaches to oligonucleotide synthesis is continued. It was proposed to use arylsulphonyl imidasolides [1] and arylsulphonyl 1,2,4-

triazolides [2] as condensing reagents. These derivatives do not lead to colored substances and give only small amounts of side products. Some other approaches to the formation of internucleotide bonds were proposed: a method involving oxidation-reduction condensation starting with triphenylphosphine and 2,2'-dipyridyldisulfide [3,4]; a method involving the activation of the hydroxy group of the nucleoside component with potassium butylate [5-8]; a method based on the use of oxyphosphoranes capable to react selectively at first with primary hydroxy group of one component and then with secondary hydroxy group of another component [9-11]. The methods mentioned are very promising and in the future they will probably find wide applications.

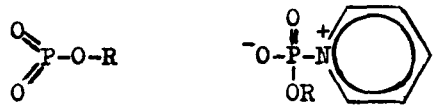
However there is no reason to believe that the classical method based upon condensation which resulted in the first complete synthesis of a gene [12-13] has exhausted its possibilities. Until recently this method was not investigated to the necessary extent, the nature of active phosphorylating intermediates remained unknown , there was no information about the accompanying side process and, therefore, the reason of the decrease of yields with growing chain length was unclear. During the last few years a systematic investigation of the reactions leading to internucleotide bond formation in Khorana's approach was carried out in Novosibirsk in the Institute of Organic Chemistry using pulsed ^{31}P NMR spectroscopy.

The present paper summarizes the data now available regarding the structure and the reactivity of active phosphorylating intermediates derived from the nucleotide component by the action of condensing reagents and the side processes accompanying phosphodiester bond formation. The data obtained are used for the analysis of synthetic schemes, that is for the choice of the optimal approach to the oligonucleotide synthesis through the use of DCC and $ArSO_2Cl$.

1. The reactive derivatives of the nucleotide component

In the course of the previous investigations using pulsed ^{31}P NMR spectroscopy it was shown [14-16] that the steps preceeding the formation of the reactive phosphorylating deri-

vative of mononucleotide proceed in accordance with the scheme proposed by Weimann and Khorana [17], namely via consecutive transformation of mononucleotide to substituted pyrophosphate and the subsequent transformation of pyrophosphate to trisubstituted triphosphate. Both processes are the reactions of the second order with the rate constants at 5° respectively 1.1×10^{-2} and 0.7×10^{-2} l/moles [18]. Trisubstituted triphosphate reacts with TPS with rate constant 0.1×10^{-2} l/moles converting to the final product - the reactive phosphorylating derivative with the chemical shift in ^{31}P NMR spectrum δ=5.1ppm containing one P atom (compound B). This derivative does not contain arylsulphonyl residue for it may be produced using cross-linked polystyrene sulphonyl chloride and may be separated from the polymer [19]. Therefore, from all structures discussed in the literature [17,20-23] only two are in agreement with experimental data namely nucleoside metaphosphate [20] and its pyridinium derivative [21]



(B)

Fig. 1 represents the spectra of the derivatives obtained with pdT-Ac, mMTr-dTp and p-nitro phenylphosphate ($pPhNO_2$) obtained without heteronuclear spin-spin decoupling $^{31}P-\{^1H\}$ It is seen that ^{31}P interacts with two protons of 5'-CH_2-group of deoxyribose in the case of pdT-Ac, with one proton of 3'-CH-group in the case of mMTr-dTp. The signal is unsplit in the case of $pPhNO_2$. That means that no spin-spin interaction is seen expected for P-N=C-H system of the pyridinium derivative of metaphosphate analogous to the isoelectronic P-C=C-H system with $J_{P-C-C-H}$ 10 Hz [24]. According to this result the metaphosphate structure seems to be more probable for compound B. Some data indicating the existence of free metaphosphate in gas phase and in solution appeared in the last years [25-28]. In the same time the existence of such a powerful electrophilic particle in the presence of pyridine seems dubious. In any case pyridine plays an important role in the appearance of the reactive derivative of mononucleoti-

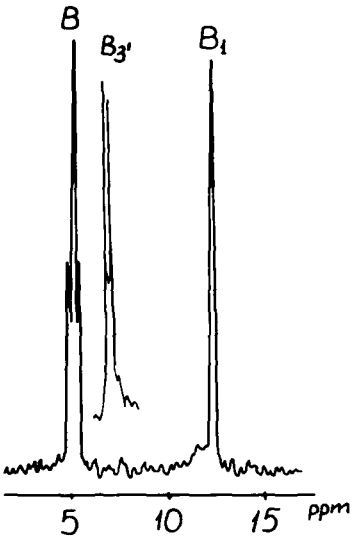


Figure 1

^{31}P NMR spectra of the final products of interaction of 0.15 M pdT-Ac(B),0.15M mMTr-dTp(B_3') and 0.15 M p-nitrophenylphosphate (B_1) with 0.45 M TPS in pyridine recorded without heteronuclear spin-spin decoupling $^{31}P-\{^1H\}$.

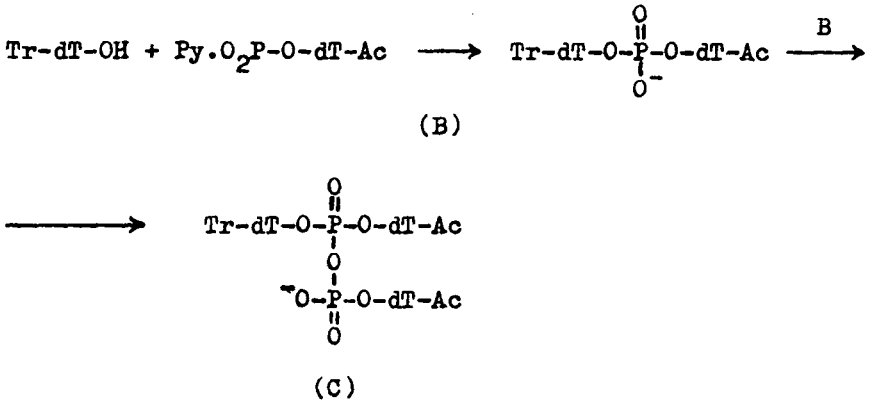
de for this compound may be formed in other solvents in the presence of not less than 30% of pyridine. Therefore, one may suggest that the absence of spin-spin splitting of ^{31}P signal at H atom in P-N=C-H system may be due to high frequency of the exchange of pyridine molecules in the pyridinium derivative exceeding significantly 10 Hz.

Thus the data obtained don't permit to discriminate between metaphosphate and pyridinium derivative structures and this derivative will be called further simply compound B and designated as Py.O_2POR (metaphosphate stabilized in some up-to-now unknown way by pyridine).

Compound B is a strong electrophile easily reacting with the excess of amines, phosphomonoesters and water forming quantitatively nucleosidephosphoamidates, disubstituted pyrophosphates and starting mononucleotide correspondingly.

Compound B reacts with hydroxy group of a nucleoside component forming dinucleosidephosphate. However phosphodiester group appeared being rather strong nucleophile reacts immediately with the next B molecule and the final product of the reaction is trisubstituted pyrophosphate (C)

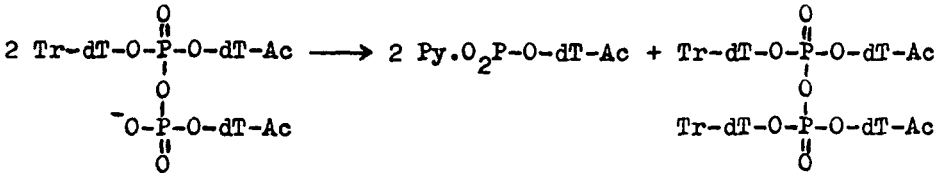
Compound C is rather poor phosphorylating reagent and reacts with hydroxy groups of nucleoside component slowly



(B)

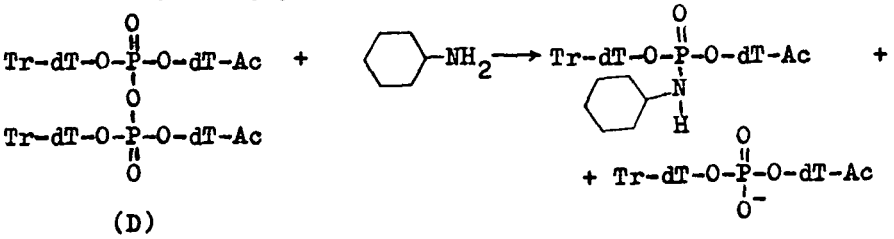
(C)

and unquantitatively. The reactions of this compound with nucleophiles proceed at ionised phosphoryl group thus eliminating the anion of stronger acid [29,30]. The excess of $ArSO_2Cl$ converts slowly the compound C to more reactive compound B with simultaneous formation of tetrasubstituted pyrophosphate [31]



(C) (B) (D)

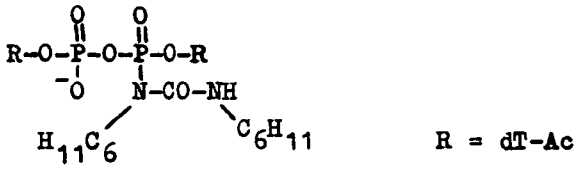
Tetrasubstituted pyrophosphates are known to be reactive phosphorylating derivatives. When treated with amine, alcohol or water they form phosphodiesteramide + phosphodiester, phosphotriester + phosphodiester or two molecules of phosphodiester correspondingly, for example



(D)

With DCC as condensing reagent the early steps of conversion of mononucleotide don't differ from those with $ArSO_2Cl$, that is mononucleotide is consecutively converted to substi-

tuted pyrophosphate, triphosphate and tetraphosphate. Compound B is formed in small amounts. N-(P^1,P^2-bisnucleoside)-pyrophosphoryl-dicyclohexylurea is accumulated in some amounts [32].



I

The final reaction mixture consists from nearly equal amounts of all afore-mentioned compounds exept pyrophosphate and starting mononucleotide (Fig.2). No signals with δ = 20-25 ppm expected for cyclic trinucleoside trimetaphosphate are encountered in ^{31}P NMR spectrum. Therefore, no indications were found on the formation of this derivative suggested to be active phosphorylating intermediate in oligonucleotide synthesis by Weimann and Khorana [17].

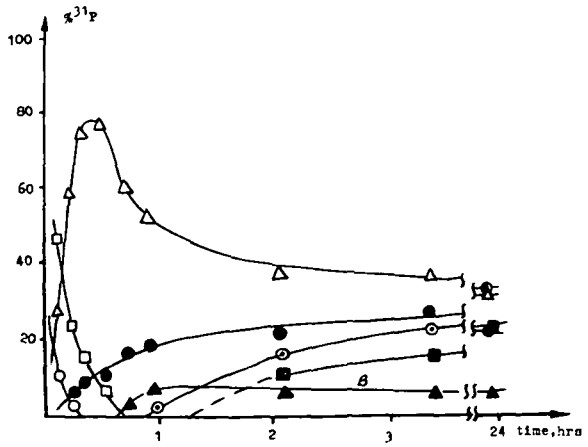


Figure 2. Kinetics curves of the interaction of 0.15M pdT-Ac with 1.5M DCC. o -pdT-Ac; □ -pyrophosphate O-(pdT-Ac)$_2$; △ - trinucleoside triphosphate; ● - tetranucleoside tetraphosphate; ⊙ - DCU - derivative of pyrophosphate (I); ▲ - compound B (Py.O$_2$PO-dT-Ac); ■ - unindentified signal.

Fig.3 represents the kinetic curves of the reaction of mononucleotide with nucleoside in the presence of DCC. It is seen that the early steps of conversion are the same as in the absence of nucleoside. However rapid conversion of polyphosphates is observed and in 20 min the major compound of the reaction mixture is trisubstituted pyrophosphate C.

The data obtained don't permit to suggest any reasonable reaction scheme of the formation of I and C. The process is complicated and probably proceeds via several routes. Trisubstituted pyrophosphate is the final product with the excess of mononucleotide. With the excess of nucleoside (as is the case in fig.3) compound C is an intermediate. The main final product is dicyclohexylurea (DCU) derivative of dinucleoside phosphate II [32]

When dinucleotides (or longer oligonucleotides) are used as phosphorylating component phosphomonoester and phosphodiester groups both are present in the same molecule. There-

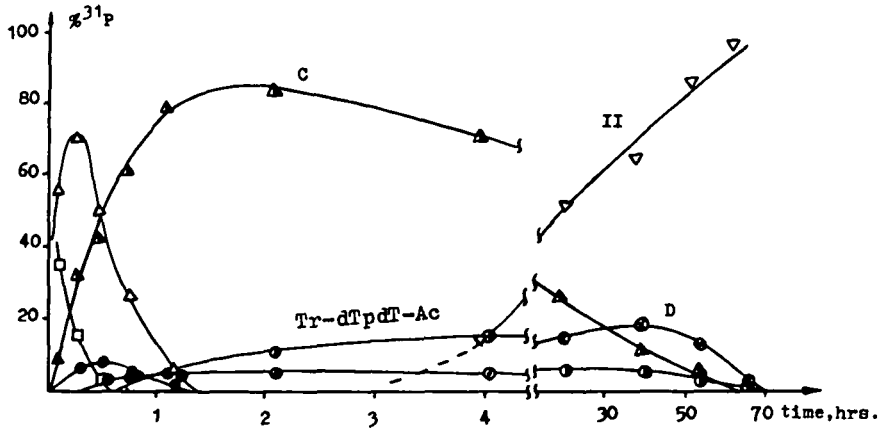
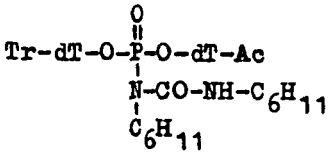


Figure 3

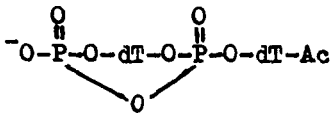
Kinetics curves of the interaction of 0.15M pdT-Ac, 0.3M Tr-dT with 1.5M DCC. □ - pyrophosphate O-(pdT-Ac)$_2$; △ - trinucleoside triphosphate; ◎ - DCU - derivative of pyrophosphate (I).



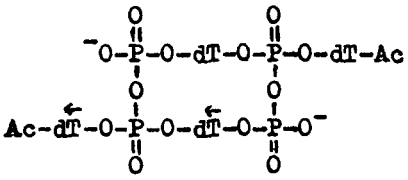
II

fore a complicated mixture of dinucleotide derivatives is formed by treatment of dinucleotide with condensing reagents [33-35].

With a moderate excess of TPS or DCC cyclic pyrophosphates III and IV are formed as the main components with the signals in ^{31}P NMR spectrum in the range 11-13 ppm typical of the structure of the C type (IV) and in the range 14-17ppm typical of cyclic pyrophosphate (III) [35]



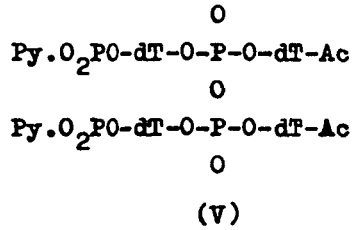
(III)



(IV)

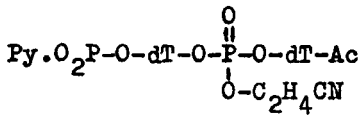
($\overleftarrow{T}$ means thymidine residue with 3'-hydroxy group to the right and 5'-hydroxy group to the left).

With greater excess (5-7 eqv.) of triisopropylbenzene sulphonyl chloride (TPS) the signal with δ = 5.1 ppm appears in the ^{31}P NMR spectrum of the reaction mixture of TPS with pTpT-Ac typical of the compound of the B type; simultaneously appears the signal with δ = 14 ppm typical of tetrasubstituted phosphate. That means that the reaction takes place similar to the described above conversion of C to the mixture of B and D with the excess of $ArSO_2Cl$. The product of the reaction most probably has the structure V



(V)

In the same time it was shown [36] that transformations of dinucleotide with internucleotide phosphate esterified with β-cyanoethyl alcohol proceed similar to those of mononucleotide compound VI being the final product



(VI)

The ^{31}P NMR spectrum of the final reaction mixture (fig.4) is represented mainly by the singlet of esterified internucleotide phosphate and the singlet with δ = 5 ppm typical of the B form derivative of phosphomonoester.

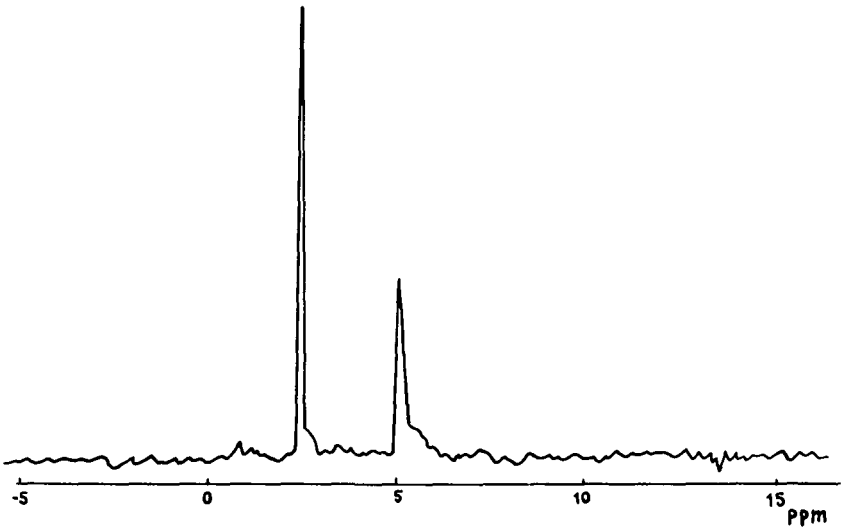


Figure 4

^{31}P NMR spectrum of the final product of interaction of 0.1M pdTp(CE)dT-Ac with 0.4M TPS, 24 hours.

II. Side reactions of nucleotide and nucleoside components

The data obtained in the course of investigation of the internucleotide bond formation revealed a number of by-processes accompanying oligonucleotide synthesis. The main complications occur due to the presence in nucleotide and nucleoside

component of internucleotide phosphate groups with nucleophilicity exceeding that of hydroxy group to be phosphorylated. Therefore all reactive forms of mono- and dinucleotide react more readily with internucleotide phosphate than with hydroxy group [29,33,37].

The addition of compound B to Tr-dTpdT-Ac results in the instant formation of trisubstituted pyrophosphate C. The reaction of B with hydroxy group of Tr-dT proceeds significantly slower [29,34]. Therefore it is reasonable to expect that with longer nucleoside components containing phosphodiester groups the active phosphorylating intermediate should be consumed in the first line by the reaction with these groups thus being converted to significantly less reactive trisubstituted pyrophosphates. This reaction does not lead to any by-products for compounds of the C type either react slowly with hydroxy group of the nucleoside component forming desirable new internucleotide linkage or are hydrolyzed by water in the course of the subsequent treatment of the reaction mixture to starting nucleoside and nucleotide components. But the reaction rate is significantly lowered or otherwise one has to use a great excess of the phosphorylating intermediate taking into account its reaction with all preexisting and newly formed phosphodiester groups.

With the excess of $ArSO_2Cl$ as it was already mentioned the B form of phosphorylating component is regenerated and internucleotide bond formation continues. But simultaneously tetrasubstituted pyrophosphates D are formed. The latter being reactive phosphorylating reagents [21,31] may form phosphotriesters with hydroxy groups of the nucleoside component. E.g. in the excess of NCC_2H_4OH and $p\text{-}NO_2\text{-}C_6H_4OH$ added to tetrasubstituted pyrophosphate the formation of 25-50% of the corresponding triesters takes place. These products are rather dangerous in the oligonucleotide synthesis for they may lead to the breakage of the oligonucleotide chains in the course of subsequent treatments.

Trisubstituted pyrophosphates are formed easily in the case of the activation of phosphomonoesters with DCC. Fig.5 represents the kinetic curves of the reaction of Tr-dTpdT-Ac

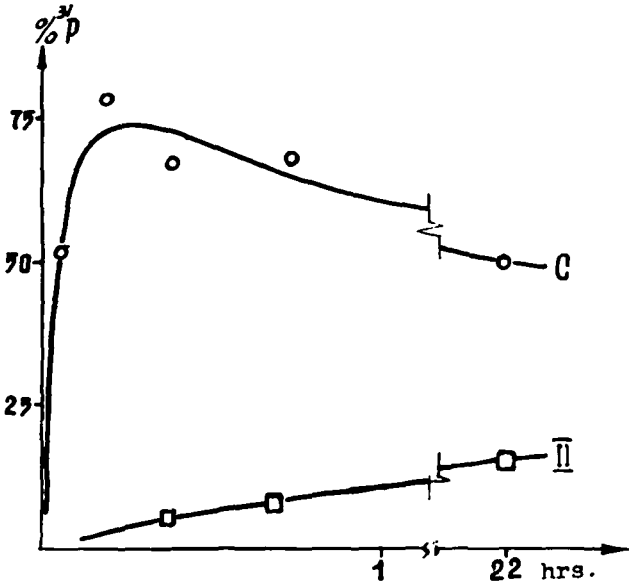
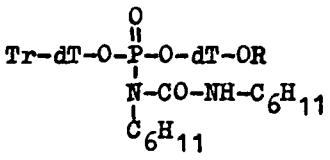


Figure 5
The kinetic curves of the interaction of 0.1M Tr-dTpdT-Ac with 0.1MpdT-Ac, 1.5M DCC in pyridine.

with pdT-Ac and DCC. In this case there is no hydroxy group to be phosphorylated and the main product is the compound C. The reaction rate exceeds significantly that in the reaction mixture of Tr-dT + pdT-Ac + DCC with reaction of hydroxy group being rate determining step (of fig.3). Therefore, one may conclude that with DCC as well as with $ArSO_2Cl$ the reactive derivative is consumed in the first line by internucleotide phosphodiester groups.

It was found that phosphodiester group may react also with DCC forming dicyclohexylurea derivatives (DCU - derivatives)



II R = $COCH_3$

VII R = H

These compounds have ^{31}P NMR spectrum with two singlet signals with δ = -4.8 and δ = -5.1 ppm typical for

phosphoric acid derivatives with P-N bond [32,38,39] existing as two diastereoisomers [40,41] (fig.6).

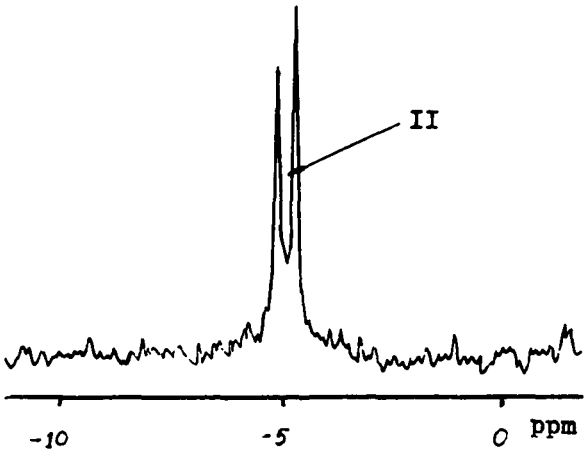


Figure 6

^{31}P NMR spectrum of the compound II, obtained by interaction of 0.1M Tr-dTpdT-Ac with 1M DCC.

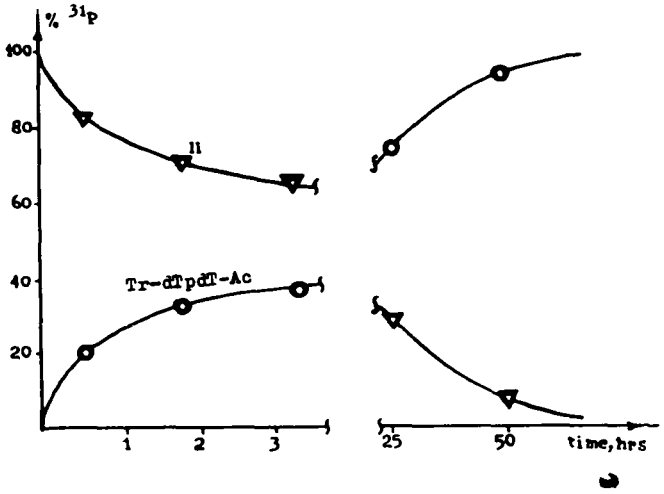
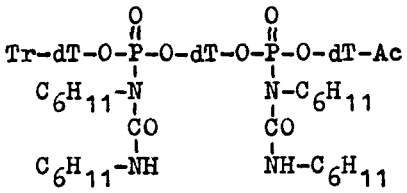


Figure 7

The kinetic curves of the hydrolysis of the compound II (0.1M) with water (10M) in pyridine.

It was shown that DCU-derivative II is stable in the presence of TPS (condensing reagent), triisopropylbenzenesulphonic acid (by-product of the reaction), compound B (electrophilic reagent), aliphatic amines (nucleophilic reagents), that is DCU-derivatives are quite stable in the conditions of oligonucleotide synthesis. The addition of water to the pyridine solution of II results in a quantitative formation of Tr-dTpdT-Ac (fig.7).



VIII

Similar compounds are formed with longer oligonucleotides e.g. with Tr-dTpdTpdT-Ac. Rather complicated signal at $\delta \approx$ -5 ppm is seen in the ^{31}P NMR spectrum of the reaction mixture of Tr-dTpdTpdT-Ac (fig.8) which may be ascribed to P-atoms of compound VIII existing as a mixture of four diastereoisomers.

Similar reaction may proceed with other carbodiimides. A.S.Levina demonstrated the formation of similar compounds in the reaction of Tr-dTpdT-Ac with N-cyclohexyl-N-β-ethyl-

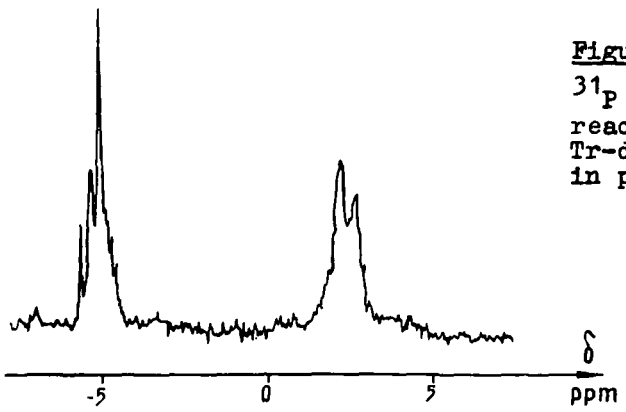
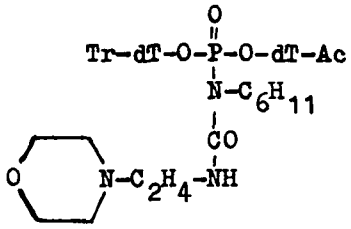


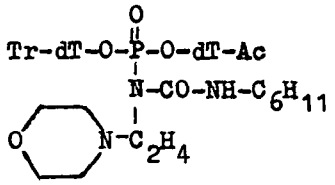
Figure 8

^{31}P NMR spectrum of the reaction mixture of 0.05M Tr-dTpdTpdT-Ac and 1M DCC, in pyridine.

morpholyl-carbodiimide, probably the mixture of IXa and IXb



(IXa)



(IXb)

Therefore we may summarize the following side processes with participation of phosphodiester groups of nucleoside component:

1) The formation of trisubstituted pyrophosphates (C);

2) The conversion of the C type compounds to tetrasubstituted phosphates (D) in the presence of the excess of $ArSO_2Cl$ that is with arylsulphonyl chlorides used as condensing reagents;

3) The formation of the DCU-derivatives (II, VII, VIII) with DCC used as condensing reagent.

The existence of phosphodiester groups in nucleotide component as it was shown in the first part of the report results in:

1) The formation of intra- and intermolecular cyclic pyrophosphates (III,IV) poorly reactive in the oligonucleotide synthesis with moderate excess of condensing reagent;

2) Unescapable formation of the D type fragments simultaneously with the reactive B form of phosphomonoester group with $ArSO_2Cl$ taken in sufficient excess.

III. Synthetic consequences

As it was already mentioned the reactive phosphorylating derivative B being attached to phosphodiester residues become significantly less reactive in the internucleotide linkage formation. Therefore when longer oligonucleotides are to be synthesized one has to use significant excess of phosphorylating component. With nucleoside component with n phosphodiester groups one has to use not less than (n + 2)-fold excess of the activated nucleotide component to take into account the reaction with n phosphodiester groups of nucleoside com-

ponent, one newly formed group and hydroxy group to be phosphorylated group. It was demonstrated that it is necessary to use two-fold excess of activated pdT with CE_2pdT as nucleoside component and three-fold excess with CEpdT to get nearly quantitative yield of dinucleotide [37]. This approach to calculation of the excess of phosphorylating compound was used in the course of the synthesis of deoxydecanucleotide complementary to 1-10 sequence of $tRNA_1^{Val}$ from yeast [42].

The protected decanucleotide $PhNHpdC^{An}pdC^{An}pdA^{Bz}pdC^{An}pdG^{iBu}pdA^{Bz}pdA^{Bz}pdA^{Bz}pdC^{An}pdC^{An}$ was prepared starting from $PhNHpdC^{An}pdC^{An}$ via consecutive condensation with pdA^{Bz}-Ac (4 eqv., 80% yield), $pdC^{An}pdG^{iBu}$-Ac (6 eqv., 60% yield), $pdA^{Bz}pdA^{Bz}$-Ac (8 eqv., 50% yield) and $pdA^{Bz}pdC^{An}pdC^{An}$-Ac (15 eqv., 42% yield).

The above considerations are in agreement with the strong influence of the side reactions of phosphodiester groups of nucleoside component on the yield of oligonucleotide increasing with the length of the oligonucleotide chain found in the previous investigations of the blocked oligonucleotide synthesis [43,44]. It was shown that with same excess of phosphorylating component the yield decreases with the chain length of nucleoside component. The reaction of tetranucleotide with 4-fold excess of dinucleotide results in 57% yield whereas the reaction of hexanucleotide with 5-fold excess of dinucleotide results in 38% of oligonucleotide. The increase of the excess of dinucleotide enhances the yield significantly - with 10-fold excess of the same dinucleotide the yield of octanucleotide is as great as 75%.

It is possible to lower the excess of the nucleotide component using greater excess of $ArSO_2Cl$ to convert poorly reactive trisubstituted pyrophosphates to reactive compounds of B type. In [37] it was demonstrated that $(CE)_2$pdTpdT-Ac may be obtained with nearly quantitative yield with equimolar amounts of pT-Ac and $(CE)_2$ pdT using 4-fold excess of TPS. Similar results were obtained for the synthesis of pdApdT and pdTpdC.

When dinucleotides are to be synthesized it is rather difficult to conclude which of these two methods (the use of the

excess either of nucleotide component or of $ArSO_2Cl$) is more advantageous. For longer oligonucleotides the use of the great excess of $ArSO_2Cl$ is disadvantageous for the formation of tetrasubstituted pyrophosphate may lead to some splitting via triester route of the oligonucleotide chain already prepared [31,45]. Besides this some losses of the nucleoside component may take place due to reaction of $ArSO_2Cl$ with hydroxy group with formation of sulphonylated products [46,47].

It is possible to exclude completely the direct contact of the nucleoside component with $ArSO_2Cl$ using preactivated nucleotide component. This may be done with cross-linked polymeric arylsulphonyl chloride ($Ps\text{-}SO_2Cl$) [19,48]. The phosphorylating derivative may be easily separated from arylsulphonic acid and the excess of arylsulphonyl chloride. It was found that the reactive derivative B obtained from pT-Ac and pdC^{An}-Ac separated from polymeric condensing reagent reacts readily with mMTr-dT forming dinucleoside phosphates in 75% yield. I.Kh.Urmanov and S.M.Yasnetskaya demonstrated the reaction of $Ps\text{-}SO_2Cl$ with dinucleotides resulting in the formation of activated dinucleotides of C or B-D type (compounds III, IV,V). The last compound may be separated from polymer to be used for oligonucleotide synthesis. Dinucleotide derivative of the C type separated from polymer reacts readily with amines and the reaction may be used for indroducing of the amide blocking group on the 5'-end of dinucleotide [49].

The most promising approach of the diester oligonucleotide synthesis seems to be based on the blocking of phosphodiester groups in the nucleoside component. For this purpose we proposed the reversible blocking of these groups with DCC. It was already mentioned that the treatment of Tr-dTpdT with DCC leads to formation of DCU-derivative (II,VII) stable in the conditions of the oligonucleotide synthesis and quantitatively hydrolyzable in aqueous medium to starting dinucleoside phosphate.

To elucidate the possibility of the use of VII as a nucleoside component its reaction with activated pdT-Ac and pdTpdT-Ac was investigated [38].

Fig.9 represents ^{31}P NMR spectrum of the reaction mixture obtained by adding of compound B derived from pdT-Ac with excess of TPS to pyridine solution of VII. It is seen, that besides the signals at -5 ppm (VII) and 5.1 ppm (B) complicated signals appear in the ranges 11-13 and 13-14 ppm typical of trisubstituted (C) and tetrasubstituted pyrophosphates. The signal at -5 ppm become more complicated (of Fig.9 with Fig.6). These data permit us to conclude that the compounds

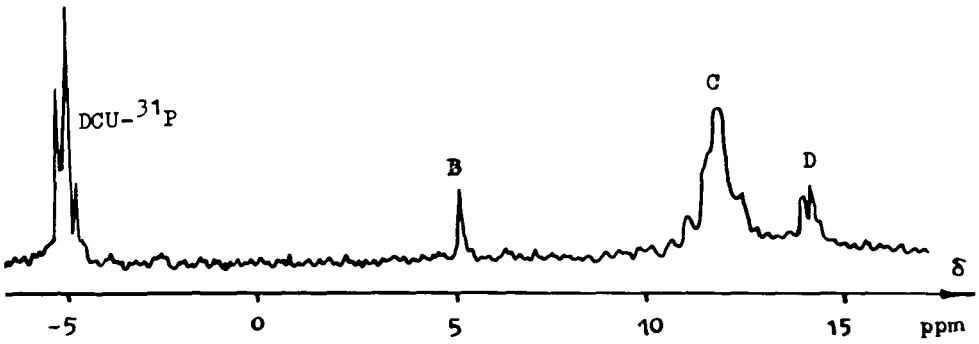
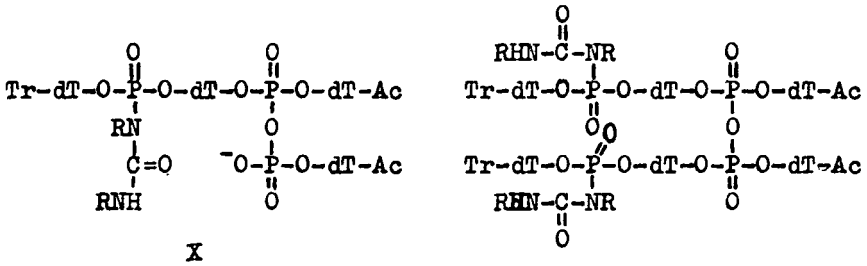


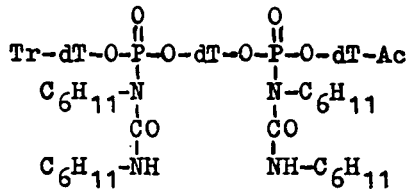
Figure 9
^{31}P NMR spectra of the reaction mixture of the compound B (0.08M) and the compound VII (0.07M), 2 hours.

are formed containing besides DCU-phosphoryl residue tri- and tetrasubstituted pyrophosphates. The structures X and XI may be ascribed to these compounds



$R = C_6H_{11}$

After long incubation the intensity of the signals at -5 ppm increases. That means that new blocked phosphodiester groups appear, probably XII

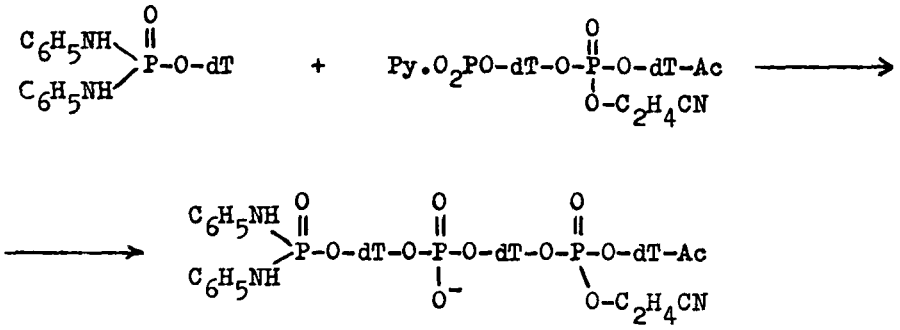


XII

The treatment of the final reaction mixture with water results in formation of Tr-dTpdTpdT-Ac and pdT-Ac. The conversion of the nucleoside component reaches 92-95%.

The results obtained permit to expect that this type of the reversible blocking of internucleotide phosphates may be successfully used in the oligonucleotide synthesis to lower the excess of nucleotide component necessary for the synthesis. The blocking DCU-group is easily hydrolyzed with water and therefore ion-exchange chromatography may be used to purify oligonucleotide prepared after each step of condensation.

Phosphodiester groups of the nucleotide component may not be blocked in a similar way for they react much more readily with activated phosphomonoester group. In the same time it was demonstrated by several authors that the reaction rate of oligonucleotide synthesis with di-, tri- and tetranucleotides is significantly lower than that of mononucleotide. A number of by-products is accumulated in the reaction mixture [44-46,50] . As it was already discussed these complications are in the first line connected with the reactions of phosphodiester groups. To overcome these difficulties it seems to be promising to use oligonucleotides containing one phosphomonoester and phosphotriester groups. This approach was proposed by Smrt [51-53]. As it was already mentioned using pdTp(CE)dT-Ac it was demonstrated that these compounds react with TPS forming highly reactive derivative of the B type without any damage to other completely protected phosphate residue [36]. The reaction was performed with nearly quantitative yield. High yields of tetranucleoside triphosphate (70%) were also obtained using mMTrTpT and pTpT-Ac with phosphodiester groups protected with p-chlorophenyl residues [54].



Therefore the most promising scheme of the oligonucleotide synthesis based on the diester approach of Khorana and on the use of ion-exchange chromatography to purify intermediate oligonucleotides may be formulated as a combination of the methods discussed above:

1) highly reactive mononucleotide derivatives or similar derivatives of oligonucleotides with protected phosphodiester groups are used as nucleotide components thus permitting the reaction to proceed with high rate and high yield;

2) the contact of arylsulphonyl chloride with the nucleoside component may be excluded using polymeric cross-linked arylsulphonyl chloride for preactivation of the nucleotide component;

3) twofold excess of the reactive derivative of nucleotide component may suffice using reversible blocking of internucleotide phosphates of nucleoside component with DCC;

4) deblocking of phosphate groups in mild conditions after each step of condensation permits to use ion-exchange chromatography for purification of the intermediate oligonucleotide as a most universal and elaborated method for longer oligonucleotides.

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