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Chemical Synthesis of Polynucleotides[**]

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Current methodology for the chemical synthesis of short chains (up to about twenty nucleotide units) of deoxyribopolynucleotides is reviewed.

1. Introduction

Methods have been developed in recent years for the chemical synthesis of short deoxyribopolynucleotide chains[1]. The availability of the synthetic deoxyribopolynucleotides with completely defined nucleotide sequences has permitted precise studies on the problems of protein biosynthesis, on the genetic code, and on DNA and RNA polymerases. Further studies of the biological functions of DNA at macromolecular level will also require the synthesis of bihelical products with defined nucleotide sequences. To this end, a general methodology has been developed which has been successfully used in the total synthesis of the gene corresponding to yeast alanine tRNA[2] (Fig. 1).

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[**] The following abbreviations have been used:
Ac = acetyl
An = anisoyl
iBu = isobutyryl
Bz = benzoyl
DCC = dicyclohexylcarbodiimide
Me = methyl
MMTr = monomethoxytriphenylmethyl (= monomethoxytrityl)
MS = mesitylenesulfonyl chloride
TEAB = triethylammonium hydrogen carbonate
TPS = 2,4,6-triisopropylbenzenesulfonyl chloride
Tr = triphenylmethyl (= trityl)

The methodology developed consists of the following three steps: 1. Chemical synthesis of deoxyribopolynucleotide segments containing eight to twelve nucleotide units. These should represent the entire two strands of the intended DNA and those belonging to the complementary strands should have an overlap of four to six nucleotides (see Fig. 1). 2. Enzymatic phosphorylation of the 5'-hydroxyl group with ATP carrying a suitable radioactive label in the γ-phosphoryl group in presence of T4 polynucleotide kinase. 3. The head-to-tail joining of the appropriate segments when they are aligned to form bihelical complexes using T4 polynucleotide ligase.

It is clear that the concept outlined in the above strategy will continue to form the basis of future syntheses of genes[2a] and manipulation of synthetic or natural DNA. Because of the relative rapidity with which steps 2 and 3 described above can be carried out, the progress-determining step in rapid and efficient syntheses of DNA will undoubtedly be the chemical synthesis of the deoxyribooligonucleotides. The aim of the present paper will principally be (1) to review the methodology which is currently available, (2) to describe some recent improvements and refinements which have been effected in our laboratory, and (3) to briefly mention the outstanding problems awaiting satisfactory solution.

2. Synthesis of the Internucleotide Bond and the Protecting Groups

The synthesis of the simplest dinucleoside phosphate, thymidylyl-thymidine (TpT), is shown in Figure 2.

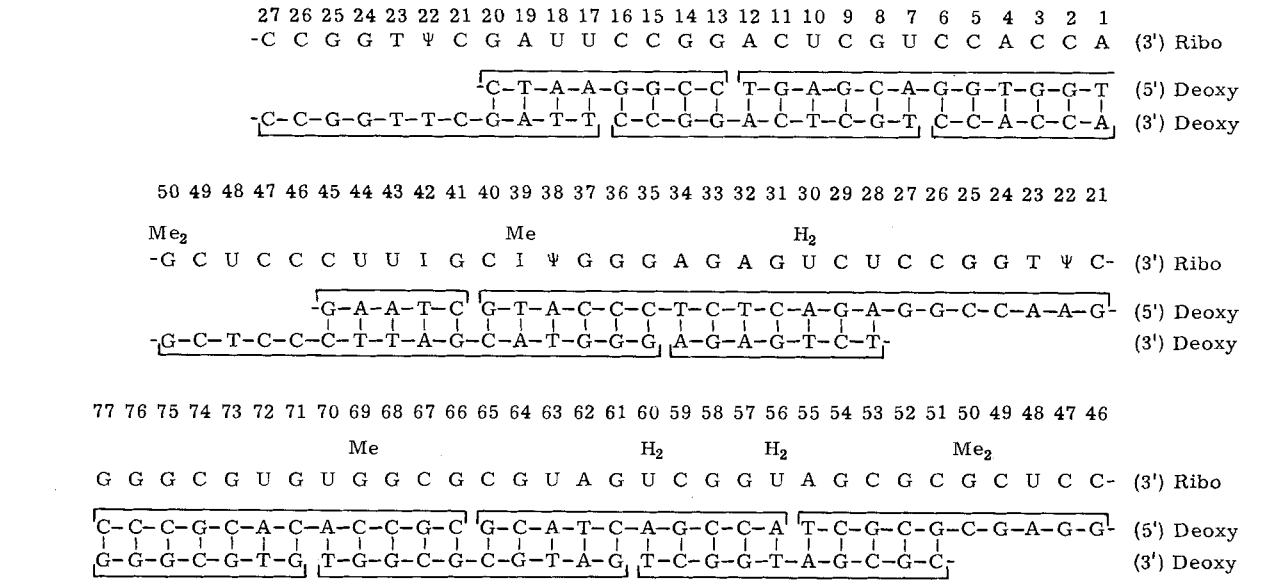


Fig. 1. Total plan for the synthesis of a $tRNA_{ala}$ gene. The chemically synthesized segments are shown in the brackets. 1st row, serial numbers; 2nd row, sequence of tRNA; 3rd and 4th row, sequence of the two strands of the $tRNA_{ala}$ gene.

In a synthesis of this kind one must note that: 1. The 5'-hydroxyl group of the nucleoside component is blocked by the classical bulky trityl group which can be removed by acid treatment when desired. 2. The 3'-hydroxyl of this component is free. 3. The second component involved in the reaction is a 5'-mononucleotide whose 3'-hydroxyl group is blocked by an acetyl group. This group is removed by very mild alkaline treatment when required. 4. The phosphate group has been used as the monoesterified component directly for the condensation step. An approach where diesterified nucleotide components were used has been suggested by *Eckstein, Letsinger*, and *Reese*, and their coworkers[3].

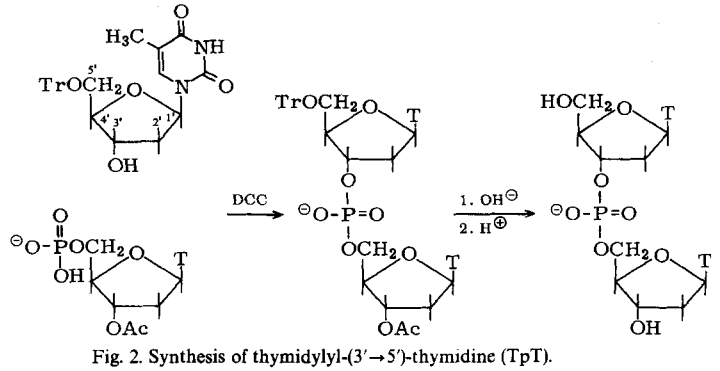


Fig. 2. Synthesis of thymidylyl-(3'→5')-thymidine (TpT).

These two components (Fig. 2) appropriately protected were condensed with dicyclohexylcarbodiimide (DCC). The condensing agents (Fig. 3), apart from DCC, currently used by us and others are aromatic sulfonyl chlorides, in particular mesitylenesulfonyl chloride (MS)[4] and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS)[5]. The reason for using the trisubstituted aromatic sulfonyl chloride is to avoid sulfonation, especially of the 3'-hydroxyl end of the nucleoside or oligonucleotide component in deoxyribo-

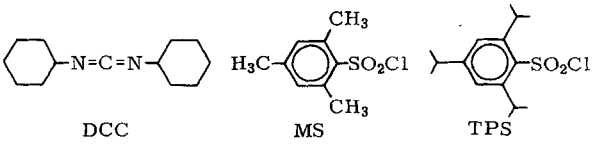


Fig. 3. Condensing agents dicyclohexylcarbodiimide (DCC), mesitylenesulfonyl chloride (MS), and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS).

oligonucleotide synthesis. The activation of phosphate by DCC, MS, or TPS and the condensation of this activated intermediate with the 3'-hydroxyl of the nucleoside component is usually carried out in one step in anhydrous pyridine.

In a condensation reaction mixture, protected dinucleoside phosphate is the main product; the other minor products are the unreacted nucleoside derivative, and unreacted 3'-acetylated nucleotide component and its symmetrical pyrophosphate. The protected dinucleoside phosphate blocked at both the 3' and 5' ends is isolated by an organic solvent extraction procedure. Mild alkaline treatment hydrolyzes the acetyl group from the 3'-hydroxyl end. Under these conditions the trityl group blocking the 5' end is completely stable.

This product can now be used for further extension of the chain simply by reaction with another nucleotide component blocked at its 3'-hydroxyl end by an acetyl group. Repetition of the condensation and alkaline treatment followed by purification of products yields a polynucleotide of desired chain length. At the end of the synthesis the trityl group is removed by acidic treatment.

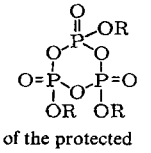


Fig. 4. Trimetaphosphate formed in the DCC reaction of the protected mononucleotide. R = amino protected nucleoside.

The mechanism of activation of phosphate by DCC, MS, or TPS is quite complex. In the case of DCC, it has been shown[6] that the initial phosphorylating species is a trimetaphosphate of the structure shown in Figure 4. The 3'-hydroxyl of the nucleoside component attacks the trimetaphosphate in a nucleophilic fashion to give an internucleotide bond. The trimetaphosphate of this type is a slow phosphorylating species. In the case of the arylsulfonyl chlorides, the activation and condensation steps are much faster, thereby suggesting that trimetaphosphates are probably not the phosphorylating species in this case[4].

We now return to the problem of the protecting groups which becomes important when nucleosides and nucleotides other than thymidine and thymidylic acid are included in the polynucleotide chains to be synthesized. Introduction of deoxyadenosine, deoxyguanosine, deoxycytidine, and their nucleotides in the synthesis of oligonucleotides requires the protection of their amino groups. If their amino groups are not protected, reaction with activated phosphate will be observed at least to some extent during the condensation reactions. The other reason for requiring the protecting groups is to increase the solubility of the nucleoside and nucleotide derivatives.

Selection of the protecting groups now requires the following considerations: 1. 3'-Hydroxyl protecting groups should be easily removed without disturbing the amino and 5'-hydroxyl protecting groups. 2. The selection of the amino protecting groups should be such that they can be removed in one step without affecting the phosphodiester bond, the 5'-hydroxyl protecting group, or the sensitive glycosyl bonds. 3. Conditions used for the removal of the 5'-hydroxyl protecting group should be such that no harm is caused to the intact oligonucleotide chain.

The protecting groups which have already been introduced in the synthesis of thymidylyl-thymidine require reconsideration in the light of these new requirements. While the use of trityl group for the protection of the 5'-hydroxyl end is satisfactory for the synthesis of oligothymidylic acid, acidic conditions required for its removal at the end also cause appreciable cleavage of glycosidic bond in purines. Therefore, a group labile to very mild acidic[7] conditions and specific for 5'-hydroxyl group is required. This is obtained by introduction of a *p*-methoxy substituent[8, 9] into the parent trityl group which has made removal possible under very mild acid or buffered conditions without causing any glycosyl bond cleavage. Such a modification of the trityl moiety did not lessen its 5'-hydroxyl group specificity. Introduction of each *p*-methoxy group into one or more phenyl rings increased the acid lability by a factor of 10. Both monomethoxy- and dimethoxy-trityl groups were used in our earlier studies for the protection of 5'-hydroxyl end; the group most favored and currently in use is the monomethoxytrityl group. The acetyl group is still satisfactory for 3'-hydroxyl protection and continues to be used in our current work. Other alternative groups such as β-benzoylpropionyl[10] and methoxyacetyl [11] have been suggested recently for protection of the 3'-hydroxyl group.

The amino protecting groups commonly used in syntheses are anisoyl for the cytosine, benzoyl for the adenine, and isobutyryl or 2-methylbutyryl[12] for the guanine rings. These protecting groups are removed in one step by concentrated ammonium hydroxide treatment without any side reactions. The reason for choosing different protecting groups for different nucleosides and nucleotides is due to their different stabilities. It should be noted that all of the protecting groups have to survive all the chemical manipulations during the very prolonged synthesis of an oligonucleotide.

The reaction shown in Figure 5 is an example of a general method now available for preparing deoxynucleosides containing blocked amino and 5'-hydroxyl groups. Acylation of the unprotected deoxycytidine is carried out with an excess of the acylating agent. The fully protected

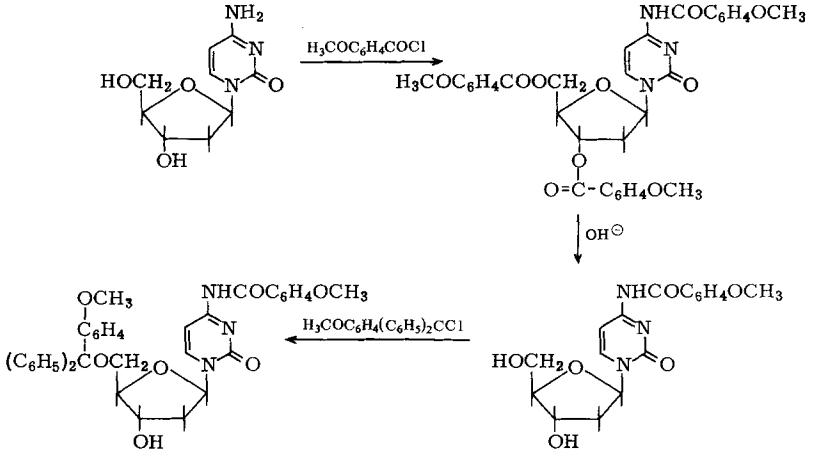


Fig. 5. Preparation of protected deoxycytidine derivatives.

derivative thus obtained is treated with alkali under carefully controlled conditions to give the desired *N*-protected deoxycytidine. Selective hydrolysis is made possible by the ionization of the amide group in the aromatic system at alkaline pH which extends the resonating system and thus stabilizes the acyl group on the amino function. The rate of hydrolysis is simply proportional to the hydroxyl ion concentration.

In this way, all of the deoxynucleosides and nucleotides can be obtained in the *N*-protected form. Protection of the 5'-hydroxyl function with monomethoxytrityl group was carried out by the standard procedure used for tritylation. Protection of the amino function of the nucleotides followed the procedure described for the nucleosides. Protection for their 3'-hydroxyl group was carried out by treatment with acetic anhydride in pyridine. The three appropriately protected nucleosides and nucleotides (apart from thymidine and thymidylic acid) are shown in Figure 6.

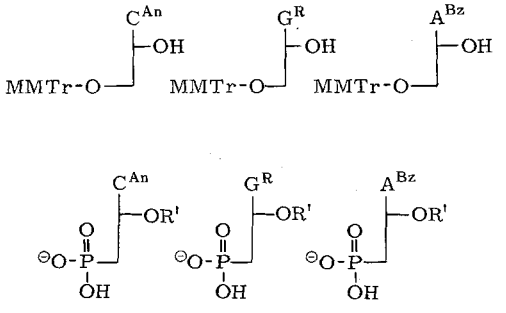


Fig. 6. Protected deoxyribonucleosides and deoxyribonucleotides (schematic). R = isobutyryl or 2-methylbutyryl, R' = H or acetyl.

3. Synthesis of Higher Deoxyribopolynucleotides of Specific Sequences

The aim of chemical synthesis is to build deoxyribopolynucleotides of defined and specific sequences. Two approaches can be imagined: one in which mononucleotides are added one by one to a growing polynucleotide chain and a second approach involving the use of preformed oligonucleotide blocks to form successively longer chains. Both of these approaches have been investigated systematically and used in synthetic work in the last few years.

3.1. Stepwise Synthesis Using Protected Mononucleotides

The approach used first with greater success was that which involves the stepwise addition of protected mononucleotide units to the 3'-hydroxyl end of a growing oligonucleotide chain[13]. An example is given in Figure 7.

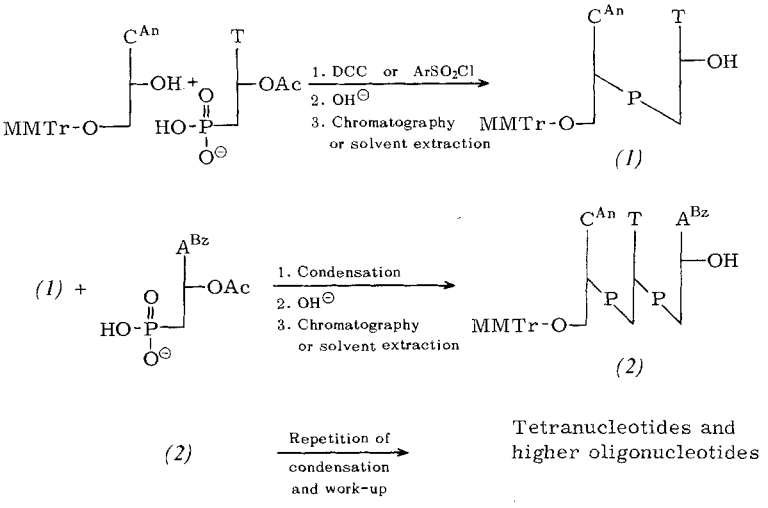


Fig. 7. Stepwise chemical synthesis of tetranucleoside triphosphate or higher oligonucleotides.

Although this approach requires a maximum number of synthetic steps in putting together a polynucleotide chain of desired size, it most often has the advantage of giving high yields in successive condensations. It has been used and continues to be used, either alone or in combination with the blockwise addition approach described below, in a great deal of synthetic work.

3.2. Stepwise Synthesis Using Preformed Protected Oligonucleotides Bearing 5'-Phosphate Groups

The second approach which utilizes preformed blocks[14,15] proceeds faster due to the fewer synthetic steps required for the oligonucleotide synthesis and provides a better system for chromatographic separation. However, the yields at the individual condensation steps are generally lower. On the whole, the blockwise approach is to be preferred in polynucleotide synthesis provided that the preformed protected oligonucleotides, especially the dinucleotides, can be readily prepared in quantity.

3.3. Synthesis of Protected Di- and Higher Oligonucleotides Carrying 5'-Phosphate Groups

Synthesis of the di- or trinucleotide blocks carrying 5'-phosphate end groups requires protection of the phosphomonoester group of the 5'-mononucleotide. Until recently this was done by condensation of the amino-protected nucleotides with cyanoethanol using DCC or $ArSO_2Cl$ as illustrated in Figure 8. The cyanoethylated derivative is

then brought into condensation with the second nucleotide component, appropriately blocked on the amino and the 3'-hydroxyl functions. The condensation is usually carried out with DCC or $ArSO_2Cl$ to give a fully protected dinucleotide. Cyanoethyl and acetyl groups are then removed from the dinucleotide by mild alkaline treatment. Purification of the reaction products is carried out by DEAE cellulose column chromatography, which is time consuming. By repeating the operations (cyanoethylation and condensation) higher blocks are prepared and used directly in the synthesis.

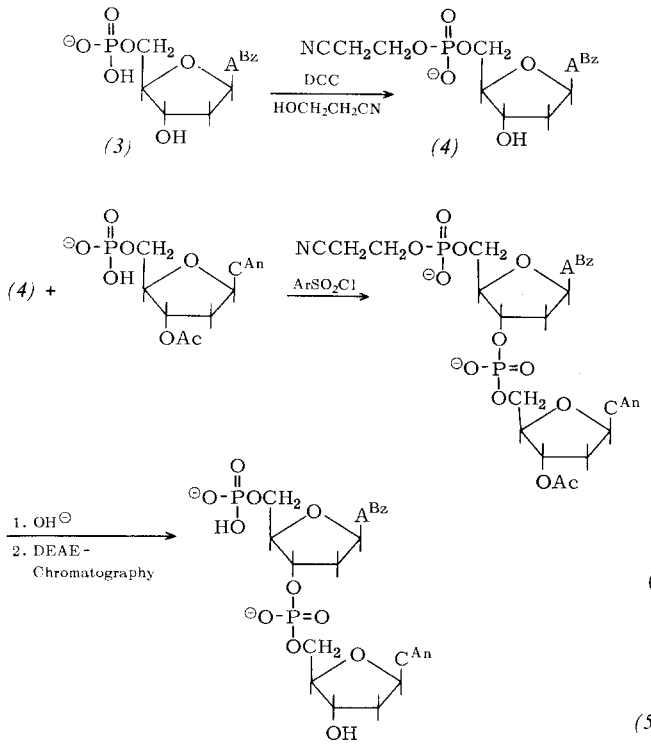


Fig. 8. Synthesis of an *N*-protected dinucleotide carrying a 5'-phosphate end group.

Alternative phosphate-protecting groups have been suggested from time to time and are shown in Figure 9. The trichloroethyl group originally introduced by *Woodward et al.*[16a] in the synthesis of cephalosporin has been suggested as a phosphate-protecting group by *Eckstein*[16b]. This group is removed by treatment with zinc in DMF. Use of substituted phosphorothioate has been suggested by *Nussbaum et al.*[17]. Conversion of phosphorothioate into phosphate was carried out by treatment with iodine in aqueous conditions. The third type, *N*-(*p*-methoxyphenyl)-carbamoylethyl, was introduced by *Narang et al.*[18] and is cleaved by alkaline treatment. The fourth type is a simple aromatic phosphoramidate. Compounds of this type were first prepared by *Moffatt* and *Khorana*[19] and used in the synthesis of nucleotide coenzymes. Recently, *Ohtsuka et al.*[20] have investigated the possible use of aromatic phosphoramidates for protection of the phosphate group and have shown that the amidate group can be cleaved by isoamyl-nitrite treatment under very mild conditions at pH 7.

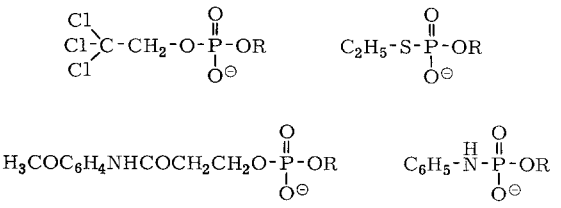


Fig. 9. Nucleosides with phosphate-protecting groups. R = thymidine or *N*-protected nucleosides.

As pointed out above, the greater part of the effort in the preparation of the protected dinucleotide blocks goes into the purification of the synthetic products by ion exchange chromatography, which requires several days. Clearly, a procedure which would eliminate the ion exchange chromatography would certainly reduce the time and effort required for the synthesis of the dinucleotide blocks. With this aim in view, an isolation procedure involving the principle of solvent extraction has been developed[21]. A highly lipophilic amine, *p*-(triphenylmethyl)aniline (see Fig. 10) was used for protecting the phosphate group of the nucleotide as the phosphoramidate. The reason for choosing the phosphoramidate type of linkage was because it could be cleaved by isoamyl nitrite under very mild conditions without any side reactions or loss of other protecting groups. All of the four phosphoramidates of the appropriately protected nucleotides were prepared by reaction of the nucleotide and the amine with DCC. These phosphoramidates were isolated in pure form by simple organic solvent extraction procedures and were shown to exist as the salts of the substituted guanidine. Formation of this guanidine inhibits the DCC reaction which is catalyzed by phosphate. Use of alternative activating agents gave lower yields of phosphoramidates. The phosphoramidate was then condensed with protected mononucleotide in the presence of TPS to give a protected dinucleotide as shown in Figure 11.

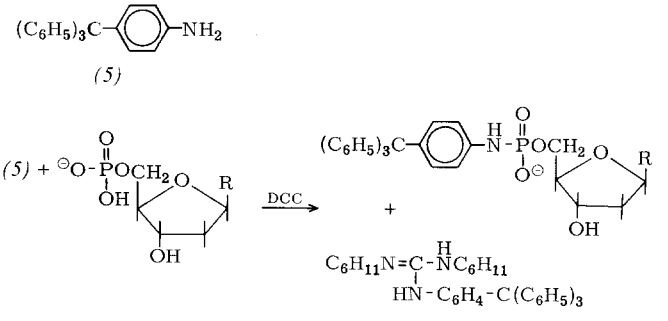


Fig. 10. Synthesis of *N*-protected phosphoramidates of nucleotides. R = thymine, *N*-anisoylcytosine, *N*-benzoyladenine, or *N*-isobutyrylguanine.

The yields of the various dinucleotides prepared in this manner were in the range of 55 to 70%. The dinucleotide phosphoramidates were isolated free from the mononucleotide and its symmetrical pyrophosphate by solvent extraction. The yield of the dinucleotides protected on the amino functions were in the range of 50 to 65% determined after isoamyl-nitrite treatment. All the sixteen dinucleotides could be prepared in relatively short time and well characterized.

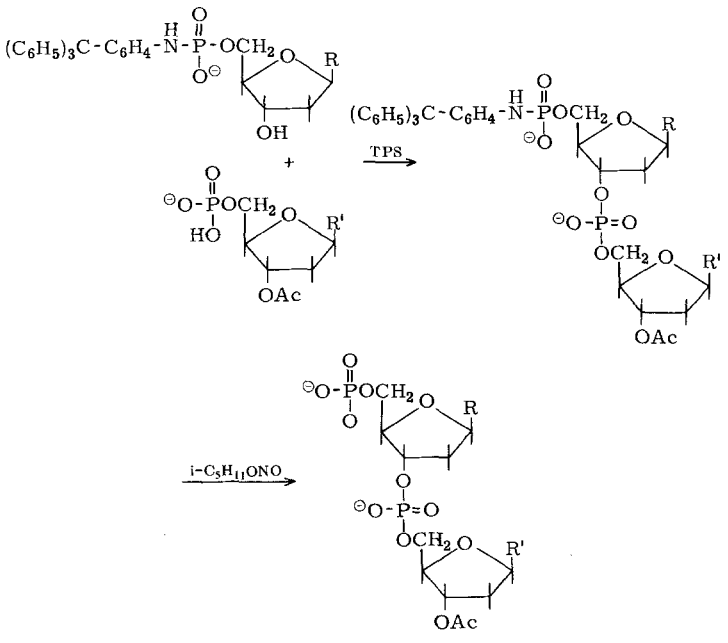


Fig. 11. Synthesis of *N*-protected dinucleotide using *p*-(triphenylmethyl)aniline as the 5′-phosphate protecting group [21]. R or R′ = thymine, *N*-benzoyladenine, *N*-anisoylcytosine, or *N*-isobutyrylguanine.

3.4. Synthesis of an Icosanucleotide

The approach using preformed oligonucleotide blocks, as mentioned above, is potentially more useful and continues to be used extensively in current work on polynucleotide synthesis. This is further illustrated by its use in the stepwise synthesis of the icosanucleotide complementary in sequence to nucleotide 21 through 40 of ala-tRNA (see Fig. 1). The synthetic steps used are shown in Figure 12. The bottom line shows the ultimate product.

The synthesis started from the 5′-end of the chain with guanosine whose 5′-hydroxyl function was protected with the monomethoxytrityl group and the amino function with isobutyryl. This was first condensed with the mononucleotide $pA^{Bz}OAc$ and the product dinucleoside phosphate was isolated by solvent extraction. Further steps involved the condensations between the 3′-hydroxyl end of the growing chain and the appropriately protected mononucleotide or di-, tri-, and tetranucleotide blocks.

At each step, the products were purified by prolonged ion exchange chromatography on DEAE cellulose. The purity of these compounds was further checked before use in the subsequent steps as follows:

1. Extensive paper chromatography of the products before and after removal of the protecting groups.

2. Determination of nucleoside and nucleotide ratios after enzymatic hydrolysis of the unprotected products.

3. DEAE cellulose chromatography in urea, a technique developed by *Tener et al.*[26] which offers very high resolution.

In syntheses of this kind, the yields tend to drop as the chain length increases and the use of large amounts of blocks becomes necessary—and even then the yields of the desired final products are only moderate in the final stages. It is, therefore, important that the choice of blocks is made after careful considerations, *e.g.*, condensations between two purines must be avoided if possible because of the low yields obtained. Pyrimidine to pyrimidine condensations are mostly favored because of the satisfactory yields obtained. The size of the blocks sometimes becomes very important because it determines the total negative charge difference between the starting chain and the product, which plays an important role in the separation of polynucleotides on ion exchange chromatography. A typical separation profile on DEAE cellulose chromatography of the reaction mixture obtained in condensation of hexadecanucleotide and tetranucleotide is shown in

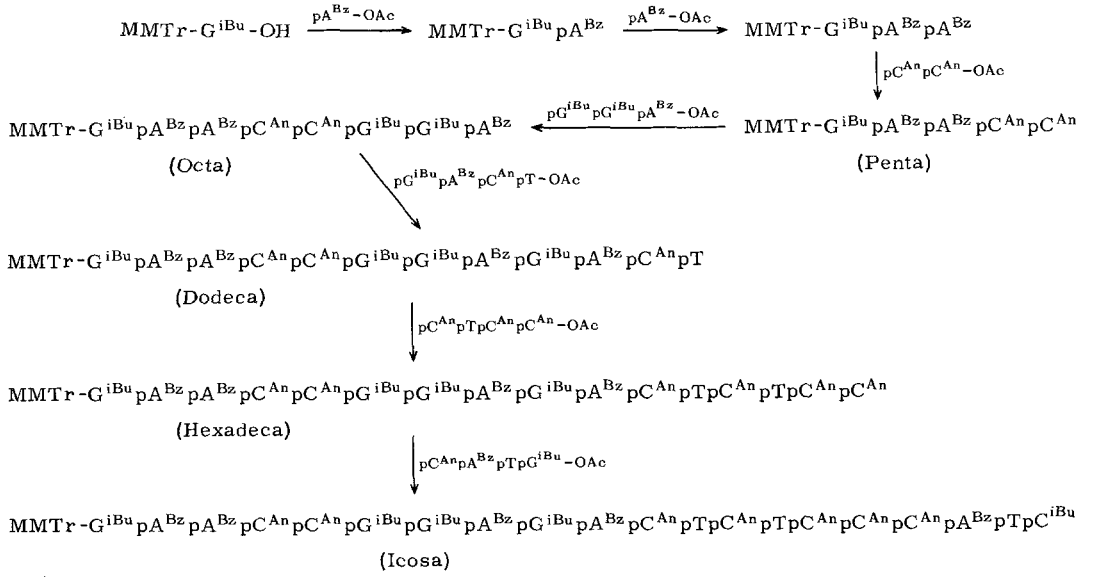


Fig. 12. Chemical synthesis of an icosanucleotide using preformed blocks.

Figure 13. The tetranucleotide block carried a tritium label so that the icosanucleotide could be distinguished from the hexadecanucleotide. Peak IV was identified as the icosanucleotide and had a constant ratio of radioactivity to ultraviolet absorbance.

formed in the synthesis of a hexanucleotide on trityl cellulose is shown in Figure 15.

The solid line shows the UV absorption and the dashed line shows the trityl estimation. Products eluted first

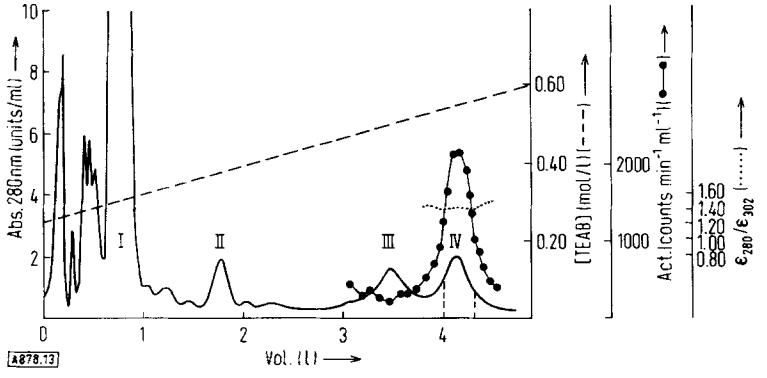


Fig. 13. Condensation of a protected hexadecanucleotide with a protected tetranucleotide (see Fig. 12). Separation of the reaction products on a DEAE cellulose (bicarbonate) column (1.8 × 60 cm) preequilibrated at 4 °C with 0.05 M triethylammonium bicarbonate (TEAB) (pH 7.5) in 40% ethanol. The bicarbonate concentration gradient used for elution was as shown by the dotted line, the solvent being 40% ethanol.

3.5. Use of Trityl Cellulose in Purification of Condensation Reaction Mixtures

The purpose of this new column adsorbent is best explained by examining the reaction products in a typical condensation reaction (Fig. 14).

1. The first type of product contains a monomethoxytrityl group at its 5'-hydroxyl function. These comprise the unreacted MMTr component and the required product.

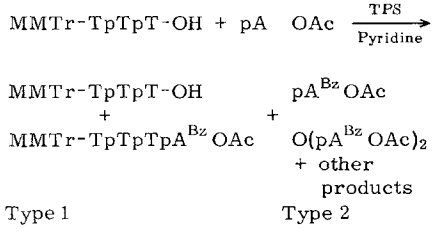


Fig. 14. Nature of products formed in a condensation reaction.

2. The second type of product includes the unreacted nucleotide and its pyrophosphate. Selective adsorption of the first type of products and consequent separation from the second type have recently been investigated using naphthoyl cellulose and trityl cellulose as the column adsorbents. The adsorbed products can then be eluted from the column simply by raising the alcohol concentration to 90%. Naphthoyl cellulose was found to be rather unstable due to the slow hydrolysis of the ester bond in presence of triethylammonium bicarbonate (TEAB) pH 7.5 to 8.0. Trityl cellulose is found to be quite stable under these conditions and is now being routinely used in our synthetic work. The elution profile of a reaction mixture (Type 2) with low alcohol concentration (45%; 0.05 mol/l TEAB) were free from trityl-containing components. When all the second type of products were washed off with low

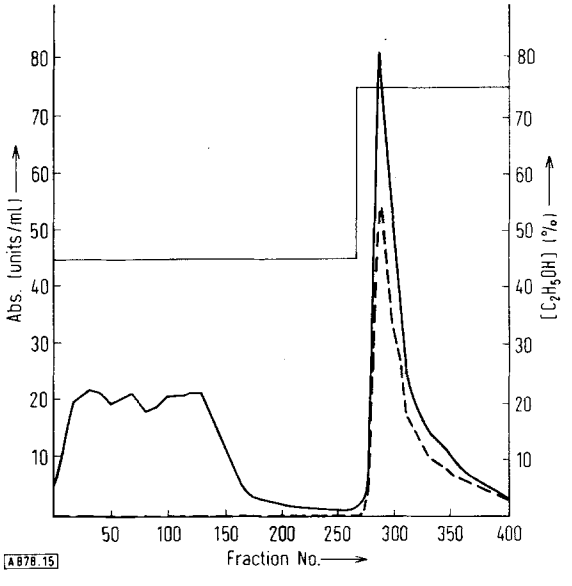


Fig. 15. Purification of condensation reaction mixture of MMTr—TpC^{An}pG^{iBu}pA^{Bz}OH and pC^{An}pC^{An}—OAc on trityl cellulose. ——, absorption at 280 nm; – – –, absorption at 472 (corresponds to content of monomethoxytrityl groups); ——, ethanol concentration.

alcohol concentration, the alcohol and TEAB concentrations were increased to 75% and 0.5 mol/l TEAB to elute the trityl-containing products. Examination of these products (Type 1) by extensive paper chromatography showed the absence of Type 2 products. Type 1 products were further purified by DEAE cellulose chromatography. A separation pattern is shown in Figure 16.

Only trityl-containing components were eluted from the column and the pattern thus obtained is relatively simple. This example clearly indicates that the introduction of trityl cellulose as a purification step prior to DEAE cellulose chromatography, not only increases the efficiency of the purification, but also reduces the overall time required.

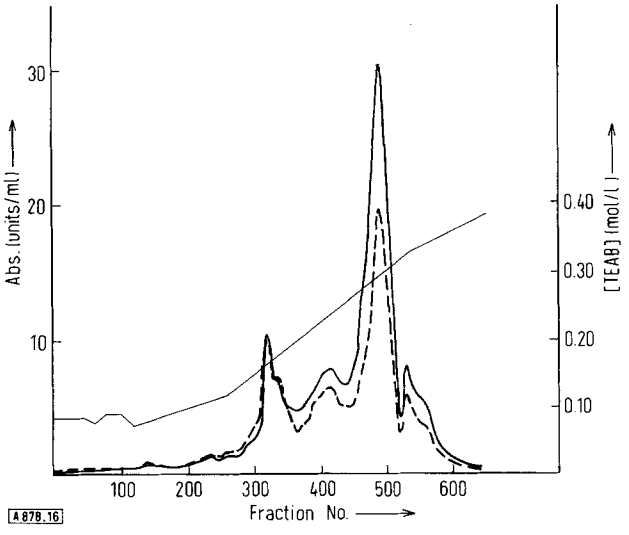


Fig. 16. Separation of monomethoxytrityl containing hexa- and tetranucleotide on DEAE cellulose. ——, absorption at 280 nm; – – –, absorption at 472 nm (corresponds to content of monomethoxytrityl groups); ——, TEAB concentration.

3.6. Polynucleotide Synthesis on Polymer Supports

There has been a great deal of interest in carrying out synthesis of biopolymers on polymer supports. This concept has been used with striking success by *Merrifield* in the synthesis of peptides and enzymes[22], and by *Katchalsky*[23] in the synthesis of insoluble enzymes. Naturally the question arises whether similar concepts can be developed in the polynucleotide field. This would accelerate the synthesis of polynucleotides and would avoid separation of products at every condensation step. A number of groups have been investigating various approaches. We, ourselves, have investigated both insoluble and organic-solvent-soluble polymers[27] for this purpose, as suggested by *Shemyakin et al.*[24]. This concept is very attractive because the condensation reactions could be carried out in homogeneous medium. In our approach, commercially available polystyrene was converted into a polymer containing a few percent of methoxytrityl chloride groups by the reaction sequence shown in Figure 17.

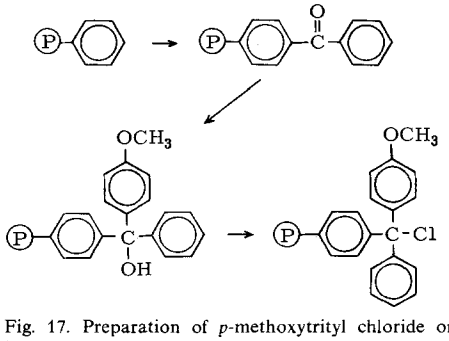


Fig. 17. Preparation of *p*-methoxytrityl chloride on a polystyrene backbone. Ⓟ = polystyrene backbone.

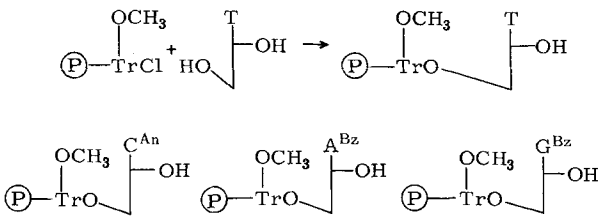


Fig. 18. Preparation of 5′-methoxytrityl-*N*-protected deoxyribonucleosides linked to a polystyrene backbone (= Ⓟ).

Deoxynucleosides appropriately protected on their amino functions were then allowed to react with this polystyrene derivative to give all four 5′-*O*-protected deoxynucleosides linked to a polystyrene backbone (Fig. 18). The subsequent chain elongation by stepwise addition of appropriately protected mononucleotides gave di- and trinucleoside phosphates as shown in Figure 19.

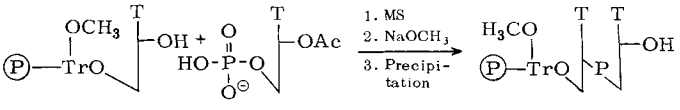


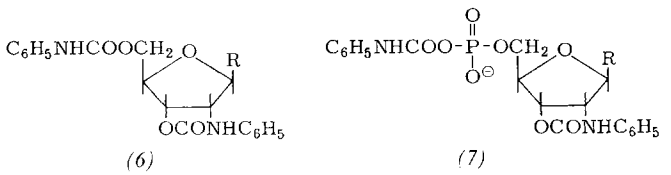
Fig. 19. Synthesis of a dinucleoside phosphate on a polystyrene backbone (= Ⓟ).

At the end of the synthesis the products were removed from the polymer by acid treatment. Although the yields were quite high in the condensation steps, they were not close to quantitative. A similar technique was investigated independently by *Cramer et al.*[25]. This approach seems quite promising but there are still some problems to be solved.

3.7. Selective Blocking of the 3′-Hydroxyl End Groups in Protected Deoxyribooligonucleotides

One main problem in the synthesis of deoxyribopolynucleotides is that the yields of the individual condensation steps are not quantitative. Synthesis cannot therefore be carried out on polymer supports because sequential isomers would result. Synthesis in solution requires time-consuming separation of starting material and elongated chain. An important advantage would accrue if the 3′-hydroxyl end groups in the unreacted components could be *specifically* and *quantitatively* blocked. A method of doing so has been found[28].

Aromatic isocyanates have been found to react with the appropriately *N*-protected nucleosides to give dicarbamoyl derivatives *(6)* (Fig. 20) in quantitative yields. Fully protected nucleosides (3′,5′-hydroxyl and amino functions appropriately blocked) did not react with the aromatic isocyanate. On the other hand, mononucleotides protected on the amino function reacted with aromatic isocyanate to give dicarbamoyl derivatives *(7)* (Fig. 20), the reaction occurring with the 3′-hydroxyl and the phosphomonoester groups. Phosphodiesters (internucleotide bonds) were resistant to aromatic isocyanate reaction, *e.g.*, the dinucleoside phosphate MMTr-TpG^{iBu}-OH reacted only on the 3′-hydroxyl group to give MMTr-TpG^{iBu}OCONHAr. Higher oligodeoxynucleotides of the size of a pentanu-



(6) (7)

Fig. 20. Dicarbamoyl derivatives of *N*-protected nucleosides (left) and mononucleotides (right). R = thymine, *N*-benzoyladenine, *N*-anisoylcytosine, or *N*-isobutyrylguanine.

cleotide appropriately protected on the 5'-hydroxyl end and the amino functions were also found to undergo quantitative reaction with the aromatic isocyanate on the 3'-hydroxyl group.

A practical application of the principle of blocking the 3'-hydroxyl end after every condensation step has been made in the synthesis of a pentanucleotide. The synthetic steps

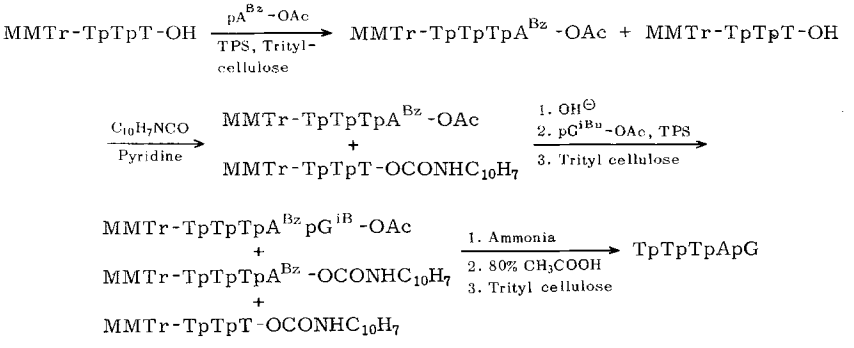


Fig. 21. Synthesis of a pentanucleotide.

are shown in Figure 21. After the condensation, the reaction mixture was first partially purified on trityl cellulose and then treated with naphthyl isocyanate to block the unreacted 3'-hydroxyl groups. Starting from a trinucleotide, the cycle: addition of appropriate mononucleotide, purification on trityl cellulose, and reaction with naphthyl isocyanate is repeated twice to give a pentanucleotide. At the end of the synthesis, the protecting groups were removed by standard procedures followed by filtration through trityl cellulose. The oligonucleotides bearing the 3'-naphthylcarbamoyl groups were selectively retained on the trityl cellulose whereas the product was present in the eluate. The product was about 90% pure. The application of this approach is now being studied in detail for the synthesis of oligonucleotides in both solution phase and solid phase synthesis.

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