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METHODS OF STEPWISE SYNTHESIS OF THE SPECIFIC $C_{3'}$–$C_{5'}$ INTERNUCLEOTIDE BOND

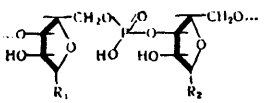
S. M. Zhenodarova

CONTENTS

I. Introduction 21
II. Methods of specific protection of functional groups in nucleosides and nucleotides 21
1. Protection of hydroxy-groups in deoxyribonucleosides and deoxyribonucleotides 21
2. Synthesis of ribonucleosides with a free hydroxy-group in position $C_{5'}$ 22
3. Synthesis of ribonucleosides with a free hydroxy-group in position $C_{3'}$ 22
4. Protection of hydroxy-groups in ribonucleotides 23
5. Protection of the amino-groups of the purine and pyrimidine bases in nucleosides and nucleotides 24
III. Methods of synthesis of the $C_{3'}$–$C_{5'}$ internucleotide bond 25
1. Synthesis of dinucleoside phosphates with a non-specific internucleotide bond 25
2. The carbodiimide method of synthesis of the $C_{3'}$–$C_{5'}$ bond 25
3. The use of other reagents for the synthesis of the specific $C_{3'}$–$C_{5'}$ internucleotide bond 27
4. Synthesis of oligonucleotides with the $C_{3'}$–$C_{5'}$ bond 28
IV. Methods of synthesis of oligonucleotides with a free phosphomonoester group 28

I. INTRODUCTION

The considerable advances in the elucidation of the role of nucleic acids in the biosynthesis of protein[1] have led to increasing interest in synthetic polynucleotides which serve as models in the investigation of the chemical, biochemical, and biological properties of nucleic acids. There is particular interest in the methods of preparation of nucleotides with a known sequence of nucleotide residues in the polynucleotide chain. One of such methods is the stepwise synthesis of the specific internucleotide bond which consists of a phosphodiester unit joined to the $C_{3'}$ atom† of one nucleotide and the $C_{5'}$ atom of another[2]:



In developing methods of synthesis of the specific $C_{3'}$–$C_{5'}$ internucleotide bond it is necessary to solve two principal problems: (1) to find ways of protecting the functional groups present in nucleoside and nucleotide molecules (the hydroxy-group of the sugar and the amino-groups of purine and pyrimidine bases) and thus obtain derivatives of the nucleosides and nucleotides with only one reactive group; (2) to achieve the condensation of these residues in such a way that only the $C_{3'}$–$C_{5'}$ phosphodiester linkage is formed. In both cases it is necessary to bear in mind the low stability of the $C_{3'}$–$C_{5'}$ linkage which is hydrolysed by acids[4] and alkalis[5] and isomerises to the $C_{2'}$–$C_{5'}$ linkage in an acid medium[6,7]. All these factors restrict the choice of protective groups, since their subsequent complete or selective removal often requires fairly severe conditions.

II. METHODS OF SPECIFIC PROTECTION OF FUNCTIONAL GROUPS IN NUCLEOSIDES AND NUCLEOTIDES

The synthesis of the $C_{3'}$–$C_{5'}$ linkage may be achieved in two ways: (1) a nucleoside-3′ phosphate is condensed with a nucleoside having a free hydroxy-group in position $C_{5'}$ (5′-OH); (2) a nucleoside-5′ phosphate is condensed with a nucleoside having a free hydroxy-group in position $C_{3'}$ (3′-OH). Thus for the synthesis of the $C_{3'}$–$C_{5'}$ internucleotide bond it is necessary to have nucleosides with free 3′-OH and 5′-OH groups (nucleoside components) and nucleotides with a phosphate residue in position $C_{3'}$ or $C_{5'}$ with the remaining functional groups completely protected (nucleotide component).

1. Protection of Hydroxy-Groups in Deoxyribonucleosides and Deoxyribonucleotides

The preparation of protected deoxyribonucleosides and deoxyribonucleotides does not present great difficulties because there is no hydroxy-group in position $C_{2'}$ and the 3′-OH and 5′-OH groups are known to differ in their reactivity. The use of triphenylmethyl chloride (trityl chloride) for the protection of the 5′-OH group is based on this difference: trityl chloride reacts much more rapidly with the primary hydroxyl[8–12]. Although the trityl group is most often used for the protection of the 5′-OH group in deoxyribonucleosides[13] and deoxyribonucleotides[14], the acetyl group[15] and the di-(*p*-anisyl)phenylmethyl group (di-*p*-APM group) are employed for this purpose too[16,17]. The acetyl group has also been used for the protection of

† The numbering of the atoms in the base and sugar residues of nucleic acids is based on that given by Kochetkov *et al.*[3].

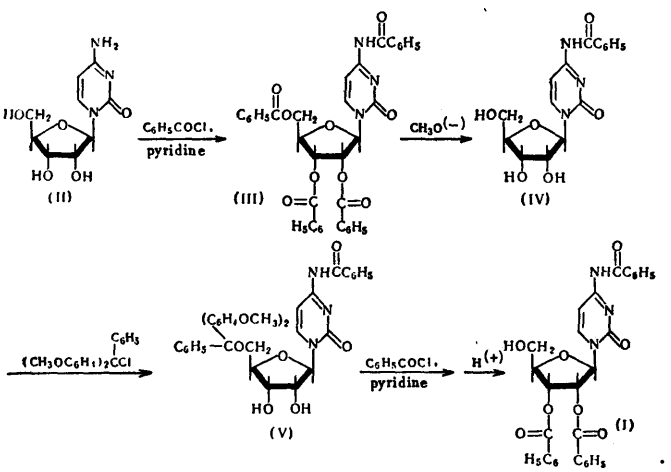
the 3'-OH group in deoxyribonucleosides[14] and deoxyribonucleotides[18], the mixture of 3'-*O*-acetyl and 5'-*O*-acetyl derivatives obtained in the first case being separated by countercurrent distribution[19].

The preparation of protected ribonucleoside and ribonucleotide derivatives is much more difficult since the hydroxy-groups in positions $C_{2'}$ and $C_{3'}$ do not differ in their properties.

2. Synthesis of Ribonucleosides with a Free Hydroxy-Group in Position $C_{5'}$

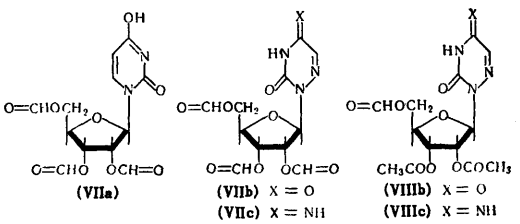
Ribonucleosides with a 5'-OH group may be obtained via the acetals of the *cis*-glycol group in the ribose residue: 2',3'-*O*-isopropylidene-[20,21], 2',3'-*O*-anisylidene-[22], 2',3'-*O*-cyclopentylidene-[22], 2',3'-*O*-cyclohexylidene-[22], or 2',3'-*O*-benzylidene-ribonucleosides[22,23]. However, the application of these ribonucleoside derivatives for the synthesis of oligonucleotides is limited, because the subsequent removal of the acetal group requires fairly severe conditions. For the same reason even the more labile *p*-methoxybenzylidene group[7] did not prove to be very suitable either. Nor was it possible to use on a preparative scale the method proposed by Todd and coworkers[24] for the synthesis of 2',3'-di-*O*-acetyluridine: by partial deacetylation of 2',3',5'-tri-*O*-acetyluridine with subsequent separation of the mixture of mono-, di-, and tri-acetyluridines by countercurrent distribution.

Usually ribonucleosides with a free 5'-OH group are obtained via the 5'-*O*-trityl derivatives: 2',3'-di-*O*-benzoyluridine was prepared from 5'-*O*-trityluridine[25] or from 5'-*O*-di-*p*-APM-uridine[26]. $N^6O^{2'}O^{3'}$-Tribenzoylcytidine (I) can be obtained by the reaction of cytidine (II) with benzoyl chloride in pyridine, the product being the fully benzoylated cytidine (III),[27] careful treatment of which with sodium methoxide gives crystalline N^6-benzoylcytidine (IV) in good yield. The 5'-OH group in (IV) is then protected with the di-*p*-AMP group and the 2'-OH and 3'-OH groups in the resulting N^6-benzoyl-5'-*O*-di-*p*-APM-cytidine (V) are rebenzoylated. The removal of the di-*p*-APM group in an acidic medium yields (I):



The simpler synthesis via 5'-*O*-di-*p*-APM-cytidine (VI), which is benzoylated with subsequent splitting of the di-*p*-APM group, cannot be used, since in the synthesis of (VI) some mono-N^6-di-*p*-APM-cytidine is also formed[26].

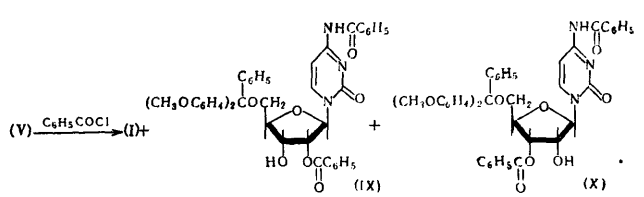
Šorm and coworkers[28] suggested the synthesis of specifically protected ribonucleosides via their *O*-formyl derivatives, which can be obtained by the usual formylation procedures[29-31]. *O*-Formyl groups are split off even on heating in methanol, i.e. under conditions in which other protecting groups, for example acetyl, are unaffected. Investigation of the methanolysis of 2',3',5'-tri-*O*-formyl derivatives of uridine (VIIa), 6-azauridine (VIIb), and 6-azacytidine (VIIc) showed that formyl groups in positions $C_{2'}$ and $C_{3'}$ are hydrolysed more rapidly than in position $C_{5'}$. The difference in the rate of methanolysis is almost independent of the structure of the heterocyclic base although the latter affects the rate of methanolysis of the formyl group in position $C_{5'}$: 5'-*O*-formyluridine hydrolyses to uridine in 1 h, while 5'-*O*-formylazauridine requires 16 h.



The 5'-*O*-formyl derivatives of (VIIIa) and (VIIIb) were prepared. The attempt at selective removal of the *O*-formyl group in the presence of an *O*-acetyl group was unsuccessful: on heating with methanol, 2',3'-*O*-diacetyl-5'-*O*-formylazauridine (VIIIb) and 2',3'-*O*-diacetyl-5'-*O*-formylazacytidine (VIIIc) were converted into (VIIb) and (VIIc).

3. Synthesis of Ribonucleosides with a Free Hydroxy-Group in Position $C_{3'}$

Numerous attempts to prepare ribonucleosides with a free 3'-OH group have been unsuccessful[26]. Partial benzoylation of (V) with a slight excess of benzoyl chloride yields a mixture of (I) and two mono-*O*-benzoylated substances, probably $N^6O^{2'}$-dibenzoyl-$O^{5'}$-di-*p*-APM-cytidine (IX) and $N^6O^{3'}$-dibenzoyl-$O^{5'}$-di-*p*-APM-cytidine (X), which could not be separated. Partial debenzoylation of (III) gives a mixture of $N^6O^{5'}$-dibenzoylcytidine (XI) and $N^6O^{3'}O^{5'}$-tribenzoylcytidine (XII) which can be separated on a silicic acid column. Treatment of (XI) with benzoyl chloride (somewhat more than one equivalent) gives both expected isomers: (XII) and $N^6O^{2'}O^{5'}$-tribenzoylcytidine (XIII).



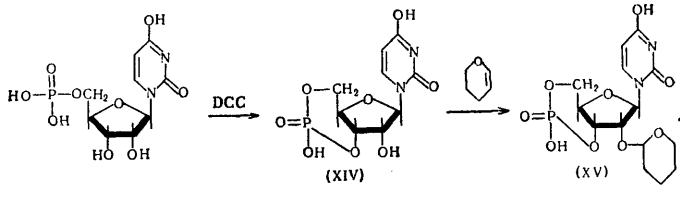
Phosphorylation of (XII) and (XIII) showed that some of the acyl groups migrate from 2'-OH to 3'-OH, in agreement with earlier data[32-34]. It is suggested that this migration may be acid-[35] or base-catalysed and that in an alkaline medium there is probably also rapid migration from 2'-OH to 3'-OH.[5] Taking all this into account, it may be assumed that direct use of ribonucleoside derivatives with a 3'-OH group and an acyl group in position C_2 is almost impossible.

4. Protection of Hydroxy-Groups in Ribonucleotides

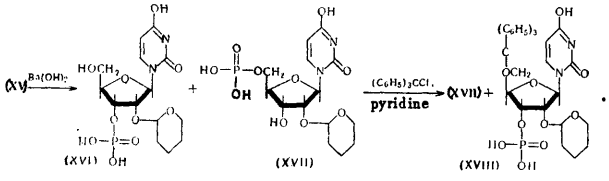
As already mentioned, the synthesis of oligonucleotides with the specific $C_{3'}-C_{5'}$ linkage requires that the nucleotides be fully converted into protected derivatives, so that the phosphate residue is the only reactive group, and that the groups protecting 5'-OH and 2'-OH or 3'-OH and 2'-OH should be removable independently of one another.

As in the case of deoxyribonucleotides, the use of trityl chloride was suggested for the protection of the 5'-OH group[36]. In order to protect the 2'-OH groups in mononucleotides, attempts were made to prepare the 2'-*O*-benzyl esters of nucleoside-3',5' cyclic phosphates using benzyl chloride and potassium t-butoxide[36,37]. However, the benzyl group migrates to the pyrimidine ring in the monobenzylated derivatives. According to the authors of these investigations, the discrepancy between their results and those of Todd and coworkers[38], who synthesised the *O*-benzyl ethers of nucleosides, is due to different reaction conditions being used.

The following method of protecting the 2'-OH group proved to be more successful[7,36]: uridine-5' phosphate in very dilute solution is converted in the presence of dicyclohexylcarbodiimide (DCC) into uridine-3',5' cyclic phosphate (XIV) which on treatment with dihydropyran in aqueous dioxan at about 20° is quantitatively converted into the 2'-*O*-tetrahydropyranyl (2'-*O*-THP) derivative (XV):



Hydrolysis of (XV) with barium hydroxide gives a mixture of 2'-*O*-THP-uridine-3' phosphate (XVI) and 2'-*O*-THP-uridine-5' phosphate (XVII) in 5 : 1 ratio, which could not be separated. Treatment of the mixture with trityl chloride in pyridine results in a quantitative yield of 2'-*O*-THP-5'-*O*-trityluridine-3' phosphate (XVIII), which can be readily separated from (XVII) (not participating in the reaction) by partition or ion-exchange chromatography:

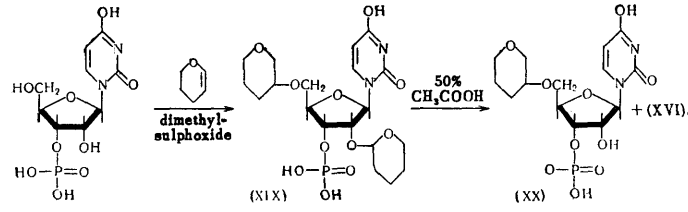


This pathway proved unsuitable for cytidine-5' phosphate, since opening of the phosphate ring with alkali is accompanied by rapid deamination of the cytosine moiety[39].

Both the trityl and the THP groups are removed on treatment of the derivative with aqueous acetic acid, the THP group being hydrolysed off at a higher rate than the trityl group[7]. Since stepwise synthesis of the $C_{3'}-C_{5'}$ linkage requires the selective removal of the 5'-*O*-trityl group, an attempt was made to use catalytic hydrogenation for this purpose, but the results were in many cases unsatisfactory[40,41]. In particular it was found that the cytosine ring may also be hydrogenated[42].

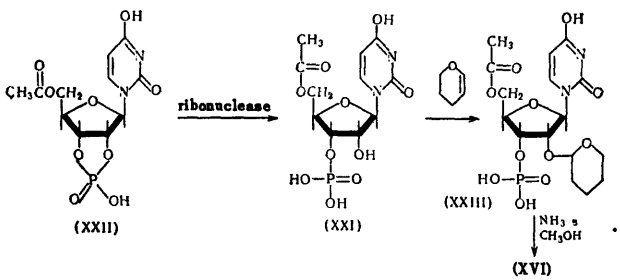
Bearing in mind that the ease of ionisation of *p*-methoxy-substituted triphenylmethanols increases greatly on introduction of each *p*-methoxy-group[43], Khorana and coworkers[7] suggested the use of a modified trityl group for the protection of the 5'-OH group. Investigation of the hydrolytic stability of 5'-*O*-*p*-anisyldiphenylmethyluridine, 5'-*O*-di-*p*-APM-uridine, and 5'-*O*-tri-*p*-anisylmethyluridine showed that the introduction of each *p*-methoxy-group increases the rate of hydrolysis in 80% acetic acid at 20° by a factor of about ten. Introduction of *p*-methoxy-groups into trityl chloride also increases the rate of its reaction with hydroxy-groups, including secondary hydroxy-groups, which may be a disadvantage in the preparation of nucleotide derivatives. It proved convenient to use di-*p*-APM chloride for the protection of the 5'-OH group, since the difference in the rate of removal of 2'-*O*-THP and 5'-*O*-*p*-anisyldiphenylmethyl groups is still insufficient for the selective elimination of the latter, while treatment of 2'-*O*-THP-5'-*O*-di-*p*-APM-uridine-3' phosphate with 80% acetic acid at 0° for 6 h gives a good yield of (XVI).

Todd[44] was the first to attempt the blocking of the 2'-OH group by treating uridine-3' phosphate with methyl iodide in the presence of silver oxide. He obtained a mixture of 2'- and 3'-phosphate derivatives. It is possible to obtain the protected ribonucleoside-3' phosphate by reacting the latter directly with dihydropyran in dimethylsulphoxide in the presence of trifluoroacetic acid[26]. In the case of uridine-3' phosphate the reaction is almost quantitative, the tetrahydropyranylation of the 2'-OH group being more rapid than the migration of the phosphate residue. Under the same conditions adenosine-3' phosphate gives 70% of 2',5'-di-*O*-THP-adenosine-3' phosphate and a small amount of *NO*$^{2'}$*O*$^{5'}$-tri-THP-adenosine-3' phosphate, which increases with the duration of tetrahydropyranylation. It was established that the rate of hydrolysis of the THP groups in positions $C_{2'}$ and $C_{5'}$ is different[45,46]: treatment of 2',5'-di-*O*-THP-uridine-3' phosphate (XIX) with 50% acetic acid at 0° for 3 h gives a mixture of (XVI) and 5'-*O*-THP-uridine-3' phosphate (XX), the latter predominating (75%). The mixture may be separated by treatment with di-*p*-APM chloride followed by chromatography on a DEAE-cellulose column:



Šorm and coworkers[47,48] suggested that the 2'-OH groups should be tetrahydropyranylated after preliminary acylation of the 5'-OH groups in ribonucleoside-3' phosphate, which makes possible subsequent selective removal of the protective groups, because the acetyl residue is more readily split off in an alkaline medium in which the THP group remains unaffected. The calcium salt of 5'-*O*-acetyluridine-3' phosphate (XXI) was obtained from 5'-*O*-acetyluridine-2',3' cyclic phosphate (XXII) by splitting the latter with pancreatic ribonuclease. Tetrahydropyranylation may be effected using the calcium salt and adding one equivalent of hydrochloric acid to form a homogeneous solution. It is also necessary to add a small excess of hydrochloric acid in order to catalyse the reaction, which

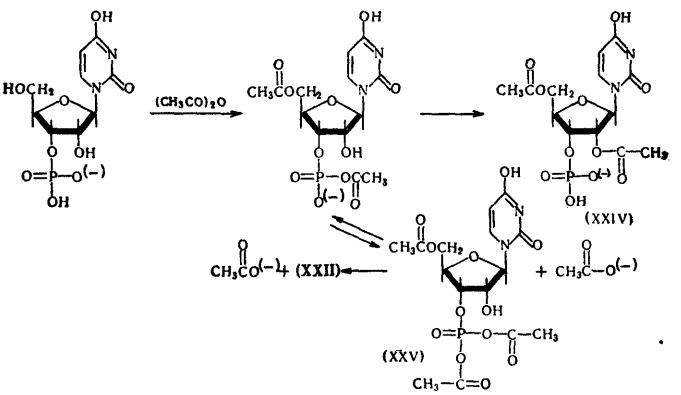
is quantitative under these conditions after 40–50 h at room temperature. There is no migration of the phosphate residue. The reacting resulting in the formation of 5′-*O*-acetyl-2′-*O*-THP-uridine-3′ phosphate (XXIII) is much more rapid (8 h) if it is carried out by adding two moles of hydrochloric acid per mole of phosphate to the ammonium or pyridinium salt of 5′-*O*-acetylnucleoside-3′ phosphate in a mixture of dimethylsulphoxide and dimethylformamide [49]. The acetyl group is split off by a mixture of methanol and ammonia at 50° (at 20° the yield of the deacetylated products is lower):



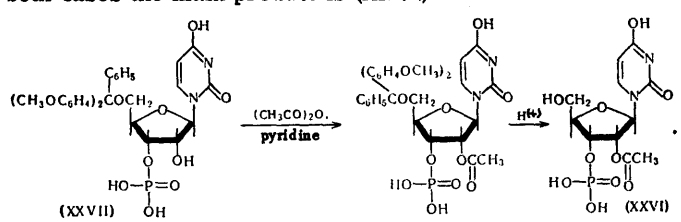
Apart from aqueous acetic acid, strongly acid ion-exchange resins, for example Dowex 50 in the pyridinium or ammonium form, can be used to remove the THP group. The rate of removal depends on the degree of cross-linking of the resin and the concentration of pyridine in solution: the THP group is readily removed if the degree of cross-linking is low, the rate of removal falling as the concentration of pyridine is increased. The most favourable conditions for the removal obtain when Dowex 50 × 8 in 30% pyridine is used.

The use of THP and di-*p*-APM nucleotide derivatives for the synthesis of dinucleoside phosphate showed that a disadvantage of these protective groups is their incomplete removal on brief treatment with acid [7] (longer treatment results in partial isomerisation of the $C_{3'}$–$C_{5'}$ linkage). Thus treatment of 2′-*O*-THP-5′-*O*-di-*p*-APM-uridylyl-(3′→5′)-uridine with 80% acetic acid for 4 h yields 50% of uridylyl-(3′→5′)-uridine. After treatment for 10 h, the yield rises to 69%, but the product contains about 1% of uridylyl-(2′→5′)-uridine. Similar results were obtained in the synthesis of uridylyl-(3′→5′)-adenosine: after a 4 h treatment with 80% acetic acid, 2′-*O*-THP-5′-*O*-di-*p*-APM-uridylyl-(3′→5′)-adenosine gave 54.5% of pure uridylyl-(3′→5′)-adenosine, while after 11 h the yield increased to 70%, but the product contained about 1.5% of uridylyl-(2′→5′)-adenosine. The low yield (28–40%) in the synthesis of uridylyl-(3′→5′)-uridine, uridylyl-(3′→5′)-cytidine, and uridylyl-(3′→5′)-adenosine from (XIX) and the corresponding derivatives of uridine, cytidine, and adenosine [26] is also due to the incomplete removal of the protecting groups, particularly the 5′-*O*-THP group.

Khorana and coworkers [45] suggested that these groups be replaced by acetyl groups. In contrast to the data of Šorm and coworkers [48] who state that acetylation of nucleoside-3′ phosphate always results in the formation of 2′,3′ cyclic phosphates, it was found that, depending on the conditions, the reaction of acetic anhydride with ribonucleoside-3′ phosphate can take place in different ways: in an acid medium uridine-3′ phosphate yields predominantly 2′,3′ cyclic phosphate, while in an alkaline medium (pyridine) an excess of acetic anhydride gives a mixture of (XXII) and 2′,5′-*O*-diacetyluridine-3′ phosphate (XXIV), which constitutes 35% of the mixture. If acetic anhydride is replaced by acetyl bromide, a 51% yield of (XXIV) is obtained. Acetylation of other ribonucleoside-3′ phosphates takes place similarly. Bearing in mind that the presence of water in the pyridine–acetic anhydride mixture reduces the yield of cyclic phosphate and assuming that the role of water reduces to the formation of acetate ions, Khorana and coworkers [45,46,50] suggested that the acetylation be carried out in an anhydrous medium using tetraethylammonium acetate as a source of acetate ions. (XXIV) was obtained in 92% yield from the pyridinium salt of uridine-3′ phosphate in dry pyridine with an excess (30-fold) of acetic anhydride at about 20° in 1 h. It is believed that (XXII) is formed via an intermediate diacylphosphate (XXV) and that acetate ions probably inhibit its formation:



The acetyl group has also been used to protect the 2′-OH group after preliminary blocking of the 5′-OH group in the ribonucleoside-3′ phosphate with a group which is stable in an alkaline medium and is removed in an acid medium [46]. Thus 2′-*O*-acetyluridine-3′ phosphate (XXVI) was obtained from (XX) and 5′-*O*-di-*p*-APM-uridine-3′ phosphate (XXVII) by treating them with an excess of acetic anhydride in pyridine with subsequent removal of the protecting group in the $C_{5'}$ position: the THP group is removed at pH 2.8 in the presence of Dowex 50 resin after 7.5 h at room temperature and the di-*p*-APM group after 7 min at pH 2.5. In both cases the main product is (XXVI).



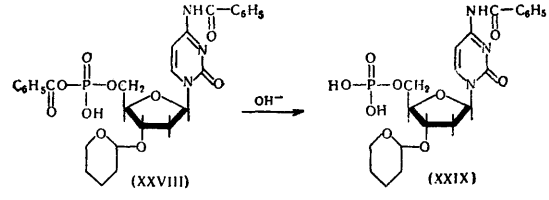
5. Protection of the Amino-Groups of the Purine and Pyrimidine Bases in Nucleosides and Nucleotides

The amino-groups in the purine and pyrimidine bases in nucleosides and nucleotides are extremely reactive [42,51–53]. Thus when 3′-*O*-acetylthymidine-5′ phosphate is condensed with 5′-*O*-trityldeoxycytidine in the presence of dicyclohexylcarbodiimide (DCC), the main reaction is phosphorylation of the amino-group of deoxycytidine by the activated nucleotide component [52]. Therefore in the synthesis of oligonucleotides with the $C_{3'}$–$C_{5'}$ linkage it is necessary to

protect the amino-groups as well as the hydroxy-groups of the nucleoside and nucleotide components. Acetyl[15,42,54], benzoyl[17,52,55], and anisyl[52,55] groups are usually employed for this purpose.

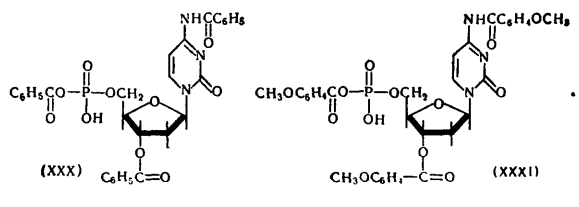
N-Acetyl derivatives of nucleosides and nucleotides are obtained from the *NO*-acetyl derivatives using the different rates of hydrolysis of the *O*-acetyl and *N*-acetyl groups[41,42]; in an alkaline medium the *O*-acetyl groups are removed much more rapidly than the *N*-acetyl groups. Nevertheless it was not possible to remove selectively the 3'-*O*-acetyl group from $N^6O^{3'}$-diacetyldeoxycytidine-5' phosphate[42]. The method of selective acetylation of the amino-group in cytidine-2',3' cyclic phosphate with a limited amount of the acetylating agent[54] proved to be unsuitable for the preparation of N^6-acetyldeoxycytidine-5' phosphate, since the acetic (or other) anhydride reacts with the mononucleotide, forming predominantly a mixed acylphosphate anhydride[56]. A disadvantage of *N*-acetyl protection is its instability in an acid medium[7,52,57]: partial removal of the *N*-acetyl group was noted after brief treatment with dilute acetic acid of N^6-acetyl-$O^{3'}$-THP-deoxycytidine-5' phosphate[52], $NO^{2'}O^{3'}$-triacetyl-$O^{5'}$-trityladenosine[57], and *N*-acetyl-$O^{5'}$--tri-*p*-anisylmethyladenosine[7]. Furthermore, *N*-acetyl derivatives of nucleosides and nucleotides are insufficiently soluble in pyridine.

N-Benzoyl derivatives[26,52,55] are more stable to acids and are more soluble. On prolonged treatment of 3'-*O*-THP--deoxycytidine-5' phosphate with benzoic anhydride in pyridine, compound (XXVIII) is probably formed, the anhydride bond in which is very stable to aqueous pyridine[58]. N^6-benzoyl-3'-*O*-THP-deoxycytidine-5' phosphate (XXIX) is obtained in high yield after brief treatment of (XXVIII) with alkali, followed by purification on DEAE-cellulose:



(XXVIII) (XXIX)

N^6-Benzoyldeoxycytidine-5' phosphate was obtained by the benzoylation of deoxycytidine-5' phosphate with an excess of benzoic anhydride or benzoyl chloride in pyridine with subsequent treatment of the product (XXX) with a mixture of pyridine and aqueous sodium hydroxide under rigorously controlled conditions[52]. *N*-Benzoyldeoxyadenosine-5' phosphate was obtained similarly[55]. The *N*-benzoyl group in the latter is more stable than in the corresponding derivative of deoxycytidine-5' phosphate. N^6-Benzoylcytidine is obtained by partial debenzoylation of $N^6O^{2'}O^{3'}O^{5'}$-tetrabenzoylcytidine with sodium methoxide[26]. Benzoylation of adenosine under mild conditions with an excess of benzoyl chloride gives a crystalline pentabenzoyl derivative[7]. At a high temperature two benzoyl groups can be added to the adenine ring[59,60]. It is suggested[7] that one of them is attached to N^6 and the other possibly to N^2.



(XXX) (XXXI)

N-Anisyl derivatives of nucleosides and nucleotides are very stable to alkali: treatment with alkali of a fully substituted derivative of deoxycytidine-5' phosphate (XXXI) gives a 90% yield of N^6-anisyldeoxycytidine-5' phosphate, no deoxycytidine-5' phosphate being detected in the product[52]. Study of the rate of hydrolysis of various *N*-acyldeoxycytidine-5' phosphates in 9 *N* ammonium and sodium hydroxides at room temperature showed that ammonium hydroxide is more effective in the hydrolysis and that the *N*-acyl residues can be arranged in the following sequence as regards their stability: *N*-anisyl > *N*-benzoyl > *N*-acetyl. After prolonged interaction of *N*-anisyl- and *N*-benzoyl--deoxycytidine-5' phosphate with alkali and with aqueous acetic acid, some deamination is observed[52]; it is even more extensive in the case of N^6-acylcytosines[27]. Ammonia does not cause deamination. When acetic acid is used, the degree of deamination varies, depending on the nature of the *N*-acyl group: the *N*-acetyl group is more readily deaminated than the *N*-benzoyl group.

III. METHODS OF SYNTHESIS OF THE $C_{3'}$–$C_{5'}$ INTERNUCLEOTIDE BOND

1. Synthesis of Dinucleoside Phosphates with a Non--Specific Internucleotide Bond

The first attempts at the synthesis of dinucleotides led to the formation of dinucleoside phosphates in which the phosphate residue was linked to analogous carbon atoms of the respective sugar residues[61]. Thus uridylyl-(5'→5')--uridine was obtained from 2',3'-*O*-benzylideneuridine and phenyl phosphorodichloridate after the removal of the protecting groups[62]. When *p*-nitrophenyl phosphorodichloridate was used, thymidylyl-(5'→5')-thymidine was obtained from 3'-*O*-acetylthymidine[63,64]. Adenylyl-(5'→5')-uridine was prepared from the protected silver salt of adenosine-5' phosphate and 2',3'-isopropylideneuridine[65], and also by the method of mixed anhydrides[19,66]. Using the monoacyl chlorides of the activated nucleotide derivatives[19], adenylyl--(2'→5')-uridine, and also the first dinucleoside phosphate with the $C_{3'}$–$C_{5'}$ linkage, thymidylyl-(3'→5')-thymidine[19] were synthesised. Nevertheless this method did not find wide application, because the yield of the dinucleoside phosphate is small and the preparation and purification of the intermediate are difficult. Mixtures of substances with the $C_{2'}$–$C_{5'}$ and $C_{3'}$–$C_{5'}$ linkages were obtained in numerous experiments on the synthesis of dinucleoside phosphates and trinucleoside diphosphates from various derivatives of adenosine, guanosine, and cytidine using diphenyl phosphorochloridate[54]. The above methods of synthesis of dinucleoside phosphates leads to compounds with a non-specific internucleotide bond or requires starting materials which are not readily available.

2. The Carbodiimide Method of Synthesis of the $C_{3'}$–$C_{5'}$ Bond

The method providing for direct condensation of the nucleoside and nucleotide components in the presence of activating agents proved to be much more effective. The most widely used activating agent is DCC, the excess of which does not lead to side-reactions, and the dicyclohexylurea formed from it can be readily removed from the reaction mixture. Using in the reaction various nucleoside and nucleotide components (Table 1), Khorana and co-

TABLE 1. Preparation of dinucleotide phosphates by condensation in the presence of DCC.

Nucleoside component	Nucleotide component	Yield of dinucleoside phosphate, %
5′-*O*-di-*p*-APM-thymidine	3′-*O*-acetylthymidine-5′ phosphate	TpT*, 75–76
Ditto	*NO*^{6}-diacetyldeoxyguanosine-5′ phosphate	TpdG, 68
»	*NO*^{6}-diacetyldeoxyadenosine-5′ phosphate	TpdA, 70
N-Anisyl-*O*^{4}-di-*p*-APM-deoxycytidine	3′-*O*-acetylthymidine-5′ phosphate	dCpT, 75
Ditto	*N*-anisyl-*O*^{4}-acetyldeoxycytidine-5′ phosphate	dCpdC, 73
N-Benzoyl-*O*^{4}-di-*p*-APM-deoxycytidine	*NO*^{6}-diacetyldeoxyguanosine-5′ phosphate	dCpdG, 97
NO^{6}-bis(di-*p*-APM)-deoxyguanosine	3′-*O*-acetylthymidine-5′ phosphate	dGpT, 67
Ditto	*N*-anisyl-*O*^{4}-acetyldeoxycytidine-5′ phosphate	dGpdC, 64
»	*NO*^{6}-diacetyldeoxyguanosine-5′ phosphate	dGpdG, 60
N-Benzoyl-*O*^{4}-di-*p*-APM-deoxyadenosine	3′-*O*-acetylthymidine-5′ phosphate	dApT, 70

* For the nomenclature and the abbreviations used in naming the polynucleotides, see Khorana[61].

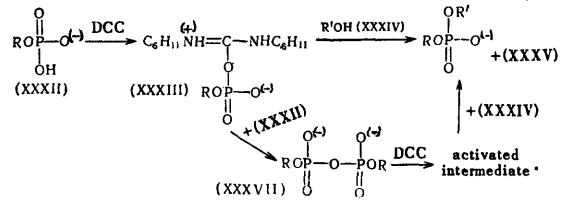
TABLE 2. Effect of the size of the protecting group in the nucleotide component on the yield of the dinucleoside phosphate.

Nucleotide component	Nucleoside component	Yield of dinucleoside phosphate, %
3′-*O*-Acetylthymidine-5′ phosphate	5′-*O*-acetylthymidine or 5′-*O*-tritylthymidine	60
5′-*O*-Tritylthymidine-3′ phosphate	3′-*O*-acetylthymidine	45
5′-*O*-Acetylthymidine-3′ phosphate	»	81
5′-*O*-Trimethylacetylthymidine-3′ phosphate	»	73
5′-*O*-Mesitylthymidine-3′ phosphate	»	60
NO^{6}-bis(di-*p*-APM)-deoxyguanosine-5′ phosphate	5′-*O*-di-*p*-APM-thymidine	12

workers[16] showed that, when equimolar amounts of both components and a fivefold excess of DCC are employed, the reaction rates are approximately the same, the yields varying from 60 to 76%. However, it should be noted that both the rate and the yield of the reaction greatly depend on the size of the protecting group in the nucleotide component[67,68] (Table 2)‡.

A 100% excess of one component raises the yield to 96–100% with respect to the second component[14]. Addition of a second mole of the nucleotide component at the end of the reaction does not increase the yield whilst addition of a second mole of the nucleoside component at the end of the reaction makes the yield almost quantitative. It is believed that the nucleoside component disappears as a result of a side-reaction[14,68]. In the reaction of deoxyribonucleotides the condensation proceeds in the same way regardless of the carbon atom in the carbohydrate residue to which the reacting groups are attached[14], but it is usual to condense nucleoside-5′ phosphates with nucleosides having a free 3′-OH group, since deoxyribonucleoside-5′ phosphates are more readily available than the 3′-phosphates and the derivatives of deoxyribonucleosides with a free 5′-OH group are in many cases unstable. On the other hand, for the specific synthesis of diribonucleoside phosphates, it is more convenient to use a procedure involving the condensation of protected ribonucleoside-3′ phosphate with a ribonucleotide having a free 5′-OH group, since ribonucleosides with a free 3′-OH group are virtually unavailable[26].

A study has been made of the mechanism of the formation of the phosphodiester linkage in the condensation of nucleotide and nucleoside derivatives in the presence of DCC.[15,42,67–71] It is believed that the first stage of the reaction is activation of the nucleotide component (XXXII) with formation of a protonated adduct (XXXIII), the alcoholysis of which with an excess of the nucleoside component (XXXIV) yields the dinucleoside phosphate (XXXV). (XXXIII), or its metaphosphate (XXXVI), forms the pyrophosphate (XXXVII) preferentially when stoichiometric amounts of (XXXII) and (XXXIV) are used. Actually in a study of the reaction of 5′-*O*-acetylthymidine (XXXVIII) with 3′-*O*-acetylthymidine-5′ phosphate (XXXIX) Khorana and coworkers[15] showed that in the first few minutes P^1P^2-di-(3′-*O*-acetylthymidine-5′) pyrophosphate (XL) is formed. Since pyrophosphates are not effective as phosphorylating agents, the next stage in the synthesis must be further activation of (XXXVII). This activation is inhibited by water or strong bases (such as tri-n-butylamine), addition of which results in pyrophosphates being the final reaction products[70], a fact which has been widely used for the synthesis of coenzymes and related compounds[72]:

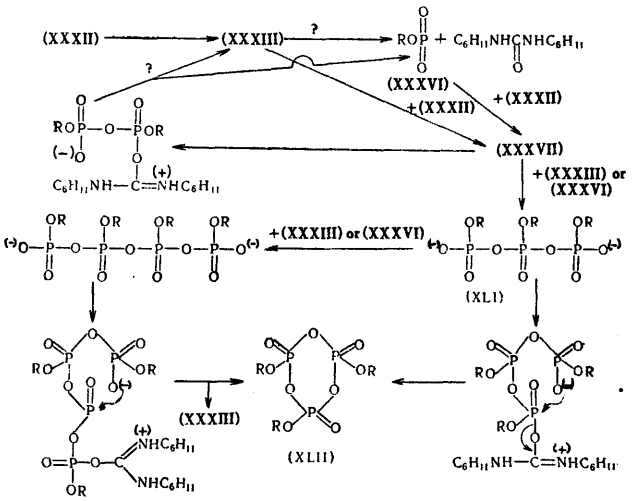


Study of substances obtained on treatment of (XXXIX) and (XL) with DCC in dry pyridine for 10–30 min established[68] that the main reaction product is $P^1P^2P^3$-tri-(3′-*O*-acetylthymidine-5′) triphosphate [(XLI), R = 3′-*O*-acetylthymidine-5′] in both cases. It is believed that the actual active phosphorylated intermediate is not (XLI) but the trimetaphosphate (XLII), which is readily converted into (XLI) in the presence of water[67,68]. Since trimetaphosphates are very labile[72,73], it was impossible to demonstrate directly the presence of (XLII) (R = 3′-*O*-acetylthymidine-5′) in the reaction mixture. However, model experiments with the more readily accessible ethyl phosphate confirmed the formation of (XLII) (R = C_2H_5), which is identical with the compound obtained in a preliminary experiment from silver trimetaphosphate and ethyl iodide[73]. The monomeric metaphosphate (XXXVI), which Todd regarded as an intermediate phosphorylating agent[74,75], can probably form as a short-lived intermediate, but according to Khorana[68] it is more likely to be attacked by the second phosphate anion (XXXII) than by the neutral hydroxy-group of (XXXIV). The possible reaction pathways of (XXXII) and (XLII) have been represented schematically by Weimann and Khorana[68] (see p. 27).

‡ More detailed information about the kinetics of the synthesis of the C_3'–C_5' linkage in the presence of DCC is given in the paper of Khorana and coworkers[69] published after this review had been written.

3. The Use of Other Reagents for the Synthesis of the Specific $C_{3'}-C_{5'}$ Internucleotide Bond

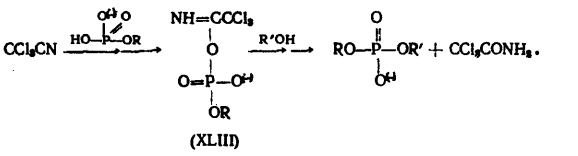
Apart from DCC, *p*-toluenesulphonyl chloride[13], mesitylenesulphonyl chloride[76], trichloroacetonitrile[77], carbonyldiimidazole[78,79], ethoxyacetylene[80-83], and *N*-alkyl-(methyl- or ethyl)-5-phenylisoxazolium salts have been proposed as activating agents for the synthesis of the $C_{3'}-C_{5'}$ linkage§[84,86].



Schematic representation of the formation of the trimetaphosphate (XLII) from the monoalkyl phosphate (XXXII).

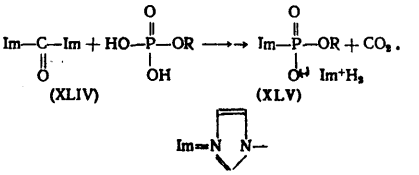
Condensation of the nucleoside and nucleotide components in the presence of *p*-toluenesulphonyl chloride also gives satisfactory yields of dinucleoside phosphates[15], but an excess of the reagent gives rise to the formation of side-products, in particular mixtures of polymers. The mechanism of the formation of the phosphodiester linkage in the presence of *p*-toluenesulphonyl chloride has not been studied in detail, but it has been suggested that the nature of the initial phosphorylating agent is the same as for the condensation in the presence of DCC¶.[68]

When a phosphomonoester is dissolved in a mixture of trichloroacetonitrile and pyridine, reactive intermediates — imidoylphosphates (XLIII)[92-95] — are formed and react with alcohols to give asymmetric phosphodiesters:



These observations were of interest for the development of methods of synthesis of the $C_{3'}-C_{5'}$ phosphodiester linkage. However, the first attempt to prepare thymidilyl-(3′→5′)-thymidine by the condensation of 5′-*O*-tritylthymidine-3′ phosphate with 3′-*O*-acetylthymidine in the presence of trichloroacetonitrile at room temperature over a period of 64 h was unsuccessful, the main product being P^1P^2-dithymidine-3′ pyrophosphate[14].

Carbonyldiimidazole (XLIV)[78,79] reacts with phosphomonoesters which are quantitatively converted into the imidazolides (XLV); these can be readily isolated as salts and possess phosphorylating properties[96]:

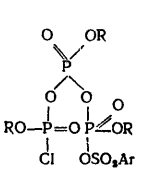


(XLV) forms phosphodiesters with alcohols but its application to the synthesis of the $C_{3'}-C_{5'}$ linkage is so far unknown†.

To compare the different activating agents, a study was made of the formation of thymidylyl-(3′→5′)-thymidine from (a) 5′-*O*-acetylthymidine-3′ phosphate and 3′-*O*-acetylthymidine or (b) from (XXXIX) and 5′-*O*-tritylthymidine, taken in stoichiometric amounts, at room temperature in pyridine in the presence of a 100% excess of *p*-toluenesulphonyl chloride or mesitylenesulphonyl chloride or a fourfold excess of DCC, ethoxyacetylene, or *N*-ethyl-5′-phenylisoxazolium fluoroborate[87,97]. It was found that the use of ethoxyacetylene in method (a) yields the symmetrical pyrophosphate after 96 h. The isoxazolium salt and DCC give approximately the same yield of the dinucleoside phosphate (68%). The sulphonyl chlorides proved to be the most effective in method (b): the yield of thymidylyl-(3′→5′)-thymidine in the presence of *p*-toluenesulphonyl chloride was 78% after 6 h, while in the presence of mesitylenesulphonyl chloride it was 71% after 1 h and 90% after 20 h. The use of activating agents such as mesitylenesulphonyl chloride permits the synthesis of the more soluble trialkylammonium salts of mononucleotides, which is impossible in the case of DCC and isoxazolium salts, since, as already mentioned, trialkylamines then lead to the formation of pyrophosphates[70].

§ The results of the use for this purpose of ethyl metaphosphate[88,89] and the product of the addition of phosgene to dimethylformamide[90,91] have also been published[87]. It was found that the first compound does not have any practical value for the synthesis of $C_{3'}-C_{5'}$ linkages, while the second gives a 30–40% yield of the dinucleoside phosphate, but side-reactions are possible.

¶ According to the latest data[87], the phosphorylating agent is in this case much more reactive than in the condensation in the presence of DCC and probably consists of the product of the addition of sulphonyl chloride to the trimetaphosphate:

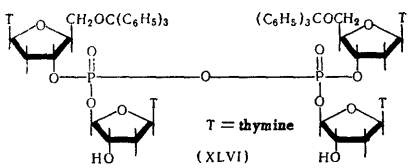


† The first attempt to obtain a dinucleoside phosphate was unsuccessful[87].

4. Synthesis of Oligonucleotides with the $C_3'-C_5'$ Bond

The methods of synthesis of oligonucleotides with a specific internucleotide bond must provide for the formation of such a bond when either the nucleoside or the nucleotide component or both simultaneously contain phosphodiester groups.

Khorana and coworkers[14,42] established that high-molecular-weight mixed oligonucleotides can be obtained by the usual methods of synthesis of dinucleoside phosphates. Three versions of the general procedure were examined. In the first version[42] the phosphodiester group was included in the nucleoside component: 5'-*O*-tritylthymidine-(3'→5')-thymidine was treated in pyridine with an excess of DCC for 1 h, to avoid the formation of an anhydride (XLVI), and then with two equivalents of 3'-*O*-acetylthymidine-5' phosphate for two days at room temperature. After treatment with alkali to remove the acetyl group, 5'-*O*-tritylthymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidine was obtained in 68% yield, which after hydrogenation gave thymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidine (83%). Thymidylyl-(3'→5')-deoxyadenylyl-(3'→5')-deoxycytidine[42] and thymidylyl-(3'→5')-deoxycytidylyl-(3'→5')-deoxycytidine[98] were obtained similarly.



(XLVI)

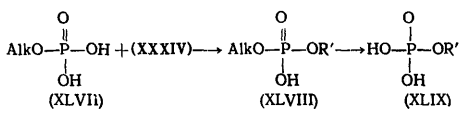
In the second version the phosphodiester group was included in the nucleotide component: 5'-*O*-tritylthymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidine was obtained by the carbodiimide method in 49% yield from 5'-*O*-tritylthymidylyl-(3'→5')-thymidine-3' phosphate and 3'-*O*-acetylthymidine. Finally, the condensation was carried out with the phosphodiester group in both components: 5'-*O*-tritylthymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidine was obtained from 5'-*O*-tritylthymidylyl-(3'→5')-thymidine-3' phosphate and thymidylyl-(3'→5')-3'-*O*-acetylthymidine in 23% yield[14]‡. The structure of the oligonucleotide was in all cases confirmed by enzymic hydrolysis. Thus it is possible to increase the chain length by stepwise synthesis of polynucleotides using components already containing internucleotide bonds. The optimum conditions for the stepwise synthesis of oligonucleotides with a specific internucleotide bond cannot be regarded as finally established. In particular, it should be borne in mind that the solubility of intermediate compounds diminishes with increase in chain length and this must complicate the requirements which have to be met by protecting groups.

‡ Recent communications have described the preparation of two triribonucleotides[99,100]: uridylyl-(3'→5')-uridylyl-(3'→5')-uridine (yield 80%) and adenylyl-(3'→5')-uridylyl-(3'→5')-uridine (yield 75%), two tetranucleotides: thymidylyl-(3'→5')-deoxycytidylyl-(3'→5')-deoxycytidylyl-(3'→5')-deoxycytidine[101] (yield 59%) and uridylyl-(3'→5')-adenylyl-(3'→5')-uridylyl-(3'→5')-uridine[100] (yield 75%), and two pentanucleotides: thymidylyl-(3'→5')-deoxyadenylyl-(3'→5')-thymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidine[102] (yield 12%) and deoxycytidylyl-(3'→5')-deoxyguanylyl-(3'→5')-deoxycytidylyl-(3'→5')-deoxycytidylyl-(3'→5')-deoxycytidine[101] (yield 15–17%).

IV. METHODS OF SYNTHESIS OF OLIGONUCLEOTIDES WITH A FREE PHOSPHOMONOESTER GROUP

True oligonucleotides, i.e. oligonucleotides with a free phosphate residue at the end of the polynucleotide chain, are obtained in two ways: either by introducing the phosphate residue into the oligonucleotide itself or by synthesising the phosphodiester linkage from the nucleoside component in which the phosphate group is protected.

The phosphate residue is nowadays introduced into the oligonucleotide by a method based on the condensation of the alcohol (XXXIV) and the monoalkyl phosphate (XLVII) in the presence of DCC:



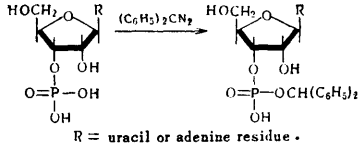
(XLVII) is chosen in such a way that the alkyl group is selectively removed after the formation of the phosphodiester (XLVIII), the final result of the reaction being the conversion of (XXXIV) into its phosphate (XLIX).[103] The phosphorylating agents investigated were monobenzyl phosphate[68,70], β-cyanoethyl phosphate[104], and tetra-*p*-nitrophenyl pyrophosphate[105]. β-Cyanoethyl phosphate, which in contrast to other phosphorylating agents is stable and becomes active only in the presence of DCC, proved to be the most convenient for the phosphorylation of oligonucleotides[106]. Since DCC is always used in a large excess, the reaction does not require absolutely anhydrous conditions. Usually a 1.5–2-fold excess of β-cyanoethyl phosphate is employed for phosphorylation. The excess of the reagent is completely decomposed after 5 min in 1 *N* sodium hydroxide at 100° and after 30 min in 0.1 *N* sodium hydroxide at 100°. The β-cyanoethyl group is removed from the nucleotide after 2 min at 100° in 1 *N* sodium hydroxide while at 50° in 0.1 *N* sodium hydroxide the rate of removal is much lower than in the reagent itself. 2 *N* sodium hydroxide removes the β-cyanoethyl group from the dinucleotide derivative in about 1 min at 0°,[14] i.e. the group can probably be selectively eliminated in the presence of certain other protecting groups, such as *N*-benzyl groups, in purine and pyrimidine bases. Free amino-groups are phosphorylated by β-cyanoethyl phosphate but the resulting phosphoramidate bonds can be readily broken: in the case of guanosine and adenosine in a weakly acid or even neutral medium and in the case of cytidine in a weakly alkaline or neutral medium on heating to 100°. Glycoside bonds are not broken under these conditions. This method was used to phosphorylate 5'-*O*-tritylthymidylyl-(3'→5')-thymidine[14], which was treated with an excess of a mixture of β-cyanoethyl phosphate and DCC, the mixture containing three times more DCC than β-cyanoethyl phosphate. When the above reagents are present in an equimolar ratio, the yield of the phosphorylated product does not exceed 23% even if the mixture were taken in a fivefold excess with respect to the dinucleoside phosphate. After treatment of the phosphorylation product with alkali, the pure dinucleotide was isolated in 68% yield. 5'-*O*-Phosphorylthymidylyl-(3'→5')-thymidine-3' phosphate and the tetranucleotide thymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidylyl-(3'→5')-thymidine-3' phosphate[14] were obtained similarly from thymidylyl-(3'→5')-thymidine[52]. Thymidylyl-(3'→5')-thymidine-3' phosphate was also obtained using tetra-*p*-nitrophenyl pyrophosphate[15].

Another phosphorylating agent which has been used is dibenzyl phosphorochloridate[15]: thymidylyl-(5′→3′)-thymidine-5′ phosphate was obtained as the sodium salt in 50% yield. Phosphorylation of 5′-*O*-tritylthymidylyl-(3′→5′)--thymidine gives thymidylyl-(3′→5′)-thymidine-3′ phosphate in low yield, probably because of the relatively low activity of the secondary 3′-OH group.

The second method of preparing oligonucleotides with a terminal phosphomonoester group, based on a nucleoside with a protected phosphate group as one of the starting materials, has been used in several versions, differing in the nature of the protecting groups in the phosphate residue being "introduced" and in the condensation conditions. Thymidylyl-(5′→3′)-thymidine-5′ phosphate was obtained from 3′-*O*-acetylthymidine-5′ phosphate and thymidine-5′ dibenzyl phosphate using DCC.[15,18] The benzyl groups were removed by hydrogenation in 10% acetic acid in the presence of palladium oxide on barium sulphate[107] and the acetyl group by mild alkaline treatment. The crystalline trisodium salt of the dinucleotide was isolated in a yield not exceeding 40%. The relatively low yield is probably due to complications which arise as a result of the monodebenzylation of the dibenzyl phosphate group. Lowering of the temperature to 0° hardly increases the yield: condensation of *NO*$^{3'}$-diacetyldeoxyadenosine-5′ phosphate with thymidine-5′ dibenzyl phosphate followed by removal of the protecting groups gave 40% of deoxyadenylyl-(5′→3′)--thymidine-5′ phosphate[15].

The use of β-cyanoethyl derivatives of nucleoside phosphates as the nucleoside components was more successful[14]: 5′-*O*-tritylthymidine-3′ phosphate and thymidine-3′ β-cyanoethyl phosphate were condensed in the presence of DCC, using 1 : 1, 2 : 1, and 1 : 2 ratios of the initial components. The yield of the dinucleotide was approximately the same in all the experiments (55–60%). It is interesting that with the 1 : 1 ratio the condensation proceeds better if the nucleoside component contains the phosphate group: when 3′-*O*-acetylthymidine was used instead of thymidine-3′ β-cyanoethyl phosphate, the yield of the dinucleoside phosphate was 45% instead of 55–60% in the case of the dinucleotide. Condensation of 5′-*O*-tritylthymidylyl-(3′→5′)-thymidine-3′ phosphate with 1.5 moles of thymidine-3′ β-cyanoethyl phosphate gave thymidylyl-(3′→5′)-thymidylyl-(3′→5′)--thymidine-3′ phosphate in 44% yield.

It has also been suggested that the hydroxy-group in the phosphate residue could be protected with a t-butyl group[86] which is introduced by the trichloroacetonitrile method[108] and is removed with 20% acetic acid. In addition, the reaction with diphenyldiazomethane may be used for this purpose[86]:



R = uracil or adenine residue.

Because of the large size of the diphenylmethyl residue, the 2′-OH group becomes "inaccessible" and does not participate in condensation. This method has been used to prepare the first synthetic diribonucleotide, uridylyl--(3′→5′)-uridine-3′ phosphate[108]: the pyridinium salt of 5′-*O*-acetyl-2′-*O*-THP-uridine-3′ phosphate[48] was reacted with the ammonium salt of uridine-3′ diphenylmethyl phosphate in the presence of a sevenfold excess of DCC in pyridine. After four days the protecting groups were removed and uridylyl-(3′→5′)-uridine-3′ phosphate was isolated in 30% yield. Uridylyl-(3′→5′)-adenosine-3′ phosphate (yield 39%) was synthesised similarly.

In one of the latest papers on stepwise synthesis of oligonucleotides with the $C_{3'}$–$C_{5'}$ linkage[98] it is suggested that the introduction of the phosphomonoester group be carried out by transphosphorylation, using phenyl phosphate and the appropriate enzyme[109].

All the oligonucleotides synthesised, starting with dinucleoside phosphates, are listed in Table 3.

TABLE 3. Synthetic oligonucleotides with the $C_{3'}$–$C_{5'}$ linkage.

Substance	References	Substance	References
Thymidylyl-(3′→5′)-thymidine	13–16, 69	Thymidylyl-(3′→5′)-thymidine-3′ phosphate	15
Thymidylyl-(3′→5′)-deoxyguanosine	16,69	Uridylyl-(3′→5′)-uridine-3′ phosphate	108
Thymidylyl-(3′→5′)-deoxyadenosine	15,16,42, 69	Uridylyl-(3′→5′)-adenosine-3′ phosphate	108
Thymidylyl-(3′→5′)-deoxycytidine	15	Thymidylyl-(3′→5′)-thymidylyl--(3′→5′)-thymidine	14,42
Deoxycytidylyl-(3′→5′)-thymidine	15,16,69	Thymidylyl-(3′→5′)-deoxyadenylyl--(3′→5′)-deoxycytidine	42
Deoxycytidylyl-(3′→5′)-deoxycytidine	16,69	Uridylyl-(3′→5′)-uridylyl-(3′→5′)--uridine	99,100
Deoxycytidylyl-(3′→5′)-deoxyguanosine	16,69	Adenylyl-(3′→5′)-uridylyl-(3′→5′)--uridine	99,100
Deoxyguanylyl-(3′→5′)-deoxycytidine	16,69	(5′-Phosphoryl)thymidylyl--(3′→5′)-deoxycytidylyl-(3′→5′)--deoxycytidine	98
Deoxyguanylyl-(3′→5′)-thymidine	16,69	Thymidylyl-(3′→5′)-thymidylyl--(3′→5′)-thymidylyl-(3′→5′)--thymidine	14
Deoxyguanylyl-(3′→5′)-deoxyguanosine	15,16,69	Thymidylyl-(3′→5′)-deoxycytidyl-yl-(3′→5′)-deoxycytidylyl--(3′→5′)-deoxycytidine	101
Deoxyadenylyl-(3′→5′)-thymidine	15,16,69	Uridylyl-(3′→5′)-adenylyl-(3′→5′)--uridylyl-(3′→5′)-uridine	100
Uridylyl-(3′→5′)-uridine	7,36	Thymidylyl-(3′→5′)-deoxyadenylyl--(3′→5′)-thymidylyl-(3′→5′)-thymidylyl-(3′→5′)-thymidine	102
Adenylyl-(3′→5′)-adenosine	26	Deoxycytidylyl-(3′→5′)-deoxyguanylyl-(3′→5′)-deoxycytidylyl--(3′→5′)-deoxycytidylyl-(3′→5′)--deoxycytidine	101
Uridylyl-(3′→5′)-cytidine	26		
Uridylyl-(3′→5′)-adenosine	7,26		
Cytidylyl-(3′→5′)-uridine	49		
Uridylyl-(3′→5′)-azauridine	48		
Cytidylyl-(3′→5′)-azauridine	49		
(5′-Phosphoryl)thymidylyl--(3′→5′)-thymidine	15,18		
(5′-Phosphoryl)thymidylyl--(3′→5′)-deoxyadenosine	15,18		

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RECENT DEVELOPMENTS IN METAL CHEMISTRY

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CONTENTS

1. Historical 31
2. Definition of the science of metal chemistry 31
3. Principal factors which determine the interaction between metals 32
Atomic radii 32
Position in the electronegativity series of the elements 32
Valency and ionisation potential 34
4. Formation of solid solutions of metals 34
5. Formation of intermetallic compounds 38
6. Crystal chemical reactions in metallic systems 42
7. Interaction between intermetallic compounds 44

1. HISTORICAL

Metals and alloys have played an important role in the development of human civilisation since the earliest times. However, they have only been the object of scientific investigation since the 17th–18th centuries. The development of the chemical approach to the formation of alloys is discussed in a number of papers [1-6].

Mendeleev's Periodic Law[7] of the chemical elements was by far the most important single contribution to the understanding of the regularities of chemical interactions between metals. The application of this law to the study of interactions between metals is due to Kurnakov[8]. It was he who proposed the term "metallic compounds or metallides", now widely used in the chemistry and physics of metals and in metal science, for the compounds formed by the combination of metals. The term will be used in the present article [but is hereafter translated "intermetallic compounds" (Ed. of Translation)].

The thermodynamic works of Gibbs[9] were of the utmost relevance to the development of equilibrium studies in chemical systems, including metallic systems. His papers on heterogeneous equilibria, as interpreted by Kurnakov, led to the creation of an international language based on the geometrical representation of the equilibrium diagrams of systems. Since the beginning of the 20th century, the work of Tammann[10], Roberts-Austen[11], Le Châtelier[12], and Howe[13] has contributed to progress in this direction. Systematic studies of metal interactions and of equilibrium and phase transformation diagrams received further impetus from the general advances in physicochemical analysis[14,15], metallography[16-19], and physical metallurgy[20-23].

2. DEFINITION OF THE SCIENCE OF METAL CHEMISTRY

The chemical approach to the science of alloys, which began with Kurnakov's application of the Periodic Law and with his equilibrium studies, has been called the chemistry of alloys[24,25], but is now unified under the general heading of metal chemistry[26-28]. This science straddles the boundary between inorganic and physical chemistry, and is also related to crystal chemistry, solid state physics, and metallography.

The general problems with which metal chemistry is concerned, identified during its development, were formulated as follows in a paper[28] presented to the Plenary Session of the Division of Chemical Sciences of the Academy of Sciences of the USSR in 1956: "the study of chemical interactions involving the metallic elements of the Periodic Table, with each other or with metalloids, with formation of solutions and compounds showing metallic and covalent bond character, and the establishment of the general principles underlying the formation, structure, and properties of this broad range of solutions and compounds of metals as a function of their composition".

Metal chemistry is a subdivision of inorganic chemistry, dealing with the formation (as a result of the interaction between atoms of metals and metalloids) of solid solutions or of intermetallic compounds of variable or fixed composition. These compounds do not, as a rule, obey the theory of valency thus: the chemistry of metallic solutions and intermetallic compounds is richer and more comprehensive than the chemistry of compounds of non-metals.