[CONTRIBUTION FROM THE INSTITUTE FOR ENZYME RESEARCH, THE UNIVERSITY OF WISCONSIN, MADISON 5, WIS.]

Studies on Polynucleotides. XVI.[1] Specific Synthesis of the $C_{3'}$–$C_{5'}$ Interribonucleotidic Linkage. Examination of Routes Involving Protected Ribonucleosides and Ribonucleoside-3′ Phosphates. Syntheses of Uridylyl-(3′→5′)-adenosine, Uridylyl-(3′→5′)-cytidine, Adenylyl-(3′→5′)-adenosine and Related Compounds[2]

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N^6-Benzoylcytidine was prepared from $N^6,O^{2'},O^{3'},O^{5'}$-tetrabenzoylcytidine by sodium methoxide-catalyzed debenzoylation. Reaction of N^6-benzoylcytidine with trityl chloride and with phenyl-di-*p*-methoxyphenylmethyl-(di-*p*-methoxytrityl) chloride gave, respectively, 5′-O-trityl-N^6-benzoylcytidine and 5′-O-di-*p*-methoxytrityl-N^6-benzoylcytidine. Partial benzoylation of the latter with benzoyl chloride gave a mixture of 5′-O-di-*p*-methoxytrityl-N^6,2′-O-dibenzoylcytidine and 5′-O-di-*p*-methoxytrityl-N^6,3′-O-dibenzoylcytidine, which could not be separated. Careful treatment of $N^6,O^{2'},O^{3'},O^{5'}$-tetrabenzoylcytidine with sodium methoxide gave N^6,5′-O-dibenzoylcytidine and $N^6,O^{3'},O^{5'}$-tribenzoylcytidine. The latter compound and the isomeric $N^6,O^{2'},O^{5'}$-tribenzoylcytidine were obtained by partial benzoylation of N^6,5′-O-dibenzoylcytidine followed by chromatography on silicic acid. $N^6,O^{3'},O^{5'}$-Tribenzoylcytidine gave, after phosphorylation with a mixture of β-cyanoethyl phosphate and dicyclohexylcarbodiimide and removal of protecting groups, pure cytidine-2′ phosphate. In contrast, $N^6,O^{2'},O^{5'}$-tribenzoylcytidine gave, after similar treatments, a mixture of cytidine-3′ and cytidine-2′-phosphates. Similarly, in attempts to prepare cytidylyl-(3′→5′)-adenosine by condensation of 5′-O-di-*p*-methoxytrityl-N^6,2′-O-dibenzoylcytidine with $N^6,O^{2'},O^{3'}$-triacetyladenosine-5′ phosphate, some of the isomeric cytidylyl-(2′→5′)-adenosine was also obtained. Because of this tendency of the acyl group to migrate from the $O^{2'}$- to $O^{3'}$-positions in ribonucleosides, the use of acyl nucleosides bearing free $C_{3'}$-hydroxyl group is excluded for the specific synthesis of $C_{3'}$–$C_{5'}$ interribonucleotide linkage. $N^6,O^{2'},O^{3'}$-Tribenzoylcytidine was prepared by benzoylation of 5′-O-trityl-N^6-benzoylcytidine followed by removal of the trityl group. 2′,3′-Di-O-benzoyluridine was prepared by benzoylation of 5′-O-trityl- or 5′-O-di-*p*-methoxytrityluridine followed by acidic treatment. Acid-catalyzed reaction of uridine-3′ phosphate with dihydropyran gave 2′,5′-di-O-tetrahydropyranyluridine-3′ phosphate from which after reaction with dicyclohexylcarbodiimide in methyl alcohol, pure methyluridine-3′ phosphate was prepared. Tetrahydropyranylation of adenosine-3′ phosphate gave the 2′,5′-di-O-tetrahydropyranyl derivative and, with longer reaction time, $N,O^{2'},O^{5'}$-tritetrahydropyranyladenosine-3′ phosphate. Uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-adenosine were prepared by the condensation of 2′,5′-di-O-tetrahydropyranyluridine-3′ phosphate with, respectively, 2′,3′-di-O-benzoyluridine and $N,N,O^{2'},O^{3'}$-tetrabenzoyladenosine followed by removal of protecting groups. Uridylyl-(3′→5′)-cytidine was prepared by the condensation of 2′,5′-di-O-tetrahydropyranyluridine-3′ phosphate or 2′-O-tetrahydropyranyl-5′-O-di-*p*-methoxytrityluridine-3′ phosphate with $N^6,O^{2'},O^{3'}$-tribenzoylcytidine. Adenylyl-(3′→5′)-adenosine was prepared from N^6-acetyl-2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate and $N,N,O^{2'},O^{3'}$-tetrabenzoyladenosine. The exclusive presence of the $C_{3'}$–$C_{5'}$ interribonucleotide linkage in all the synthetic compounds was demonstrated by degradations with pancreatic ribonuclease and/or spleen phosphodiesterase.

Introduction

In previous publications from this Laboratory we have reported on the development of methods for the activation of the phosphomonoester groups in mononucleotides to form internucleotide bonds and on the application of these methods to the stepwise synthesis of deoxyribo-oligonucleotides and to the polymerization of several deoxyribomononucleotides.[4–6] As part of a comprehensive program of synthetic work, we have recently extended the above studies to the even more complex problem of the specific synthesis of the naturally occurring $C_{3'}$–$C_{5'}$ inter-ribonucleotidic linkage and have reported on the development of a route to the specific synthesis of uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-adenosine.[7] We have continued to examine other possible approaches to the problem of the synthesis of $C_{3'}$–$C_{5'}$ inter-ribonucleotide linkage and the present communication records experiments in this direction which have resulted in the synthesis of several of $C_{3'}$–$C_{5'}$ linked di-ribonucleoside phosphates, namely, uridylyl-(3′→5′)-uridine, uridylyl-(3′→5′)-adenosine, uridylyl-(3′→5′)-cytidine and adenylyl-(3′→5′)-adenosine.

In its simplest form, the problem of specific synthesis of the inter-ribonucleotidic linkage may be approached in two alternative ways. In the first, a suitably protected ribonucleoside-3′ phosphate and a suitably protected ribonucleoside bearing a free $C_{5'}$-hydroxyl group may be used as the two components in a condensation reaction. In the second approach, a suitably protected ribonucleoside-5′ phosphate and a second component, a protected ribonucleoside bearing a free $C_{3'}$-hydroxyl group, may be used. Although the relative merits of the two approaches would eventually have to be assessed in terms of the problems of the stepwise synthesis of the $C_{3'}$–$C_{5'}$ linked ribooligonucleotide chains and of the polymerization of ribomononucleotides to form $C_{3'}$–$C_{5'}$ linked ribopolynucleotides, we have carried out a systematic examination of both types of approaches. In the following, we discuss first the routes to the preparation of suitably protected ribonucleosides, then the preparation of suitably protected ribomononucleotides and finally the work on the synthesis of the inter-ribonucleotide bonds.

Protected Ribonucleoside Derivatives.—The classical approach to the synthesis of ribonucleosides bearing a free $C_{5'}$-hydroxyl group[8] has involved

(1) Paper XV, R. K. Ralph, R. A. Smith and H. G. Khorana, *Biochemistry*, **1**, 131 (1962).

(2) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, and the National Science Foundation, Washington.

(3) U. S. Public Health Service Post-doctoral Research Fellow, 1959-1961.

(4) For earlier references see H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961, Chapter 5.

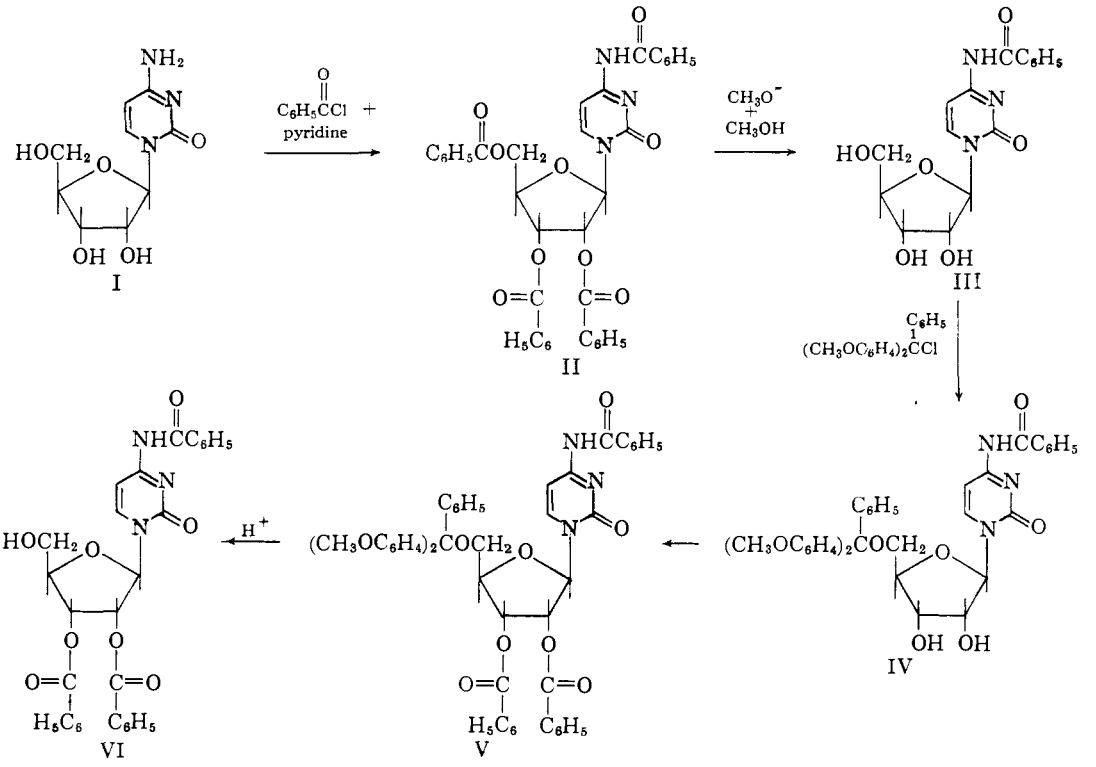
(5) H. G. Khorana, J. P. Vizsolyi and R. K. Ralph, *J. Am. Chem. Soc.*, **84**, 414 (1962).

(6) G. Weimann and H. G. Khorana, *ibid.*, **84**, 419 (1962).

(7) M. Smith, D. H. Rammler, I. H. Goldberg and H. G. Khorana, *ibid.*, **84**, 430 (1962).

(8) The present discussion omits the easily prepared 2′,3′-O-isopropylidene- or benzylidene-ribonucleosides which bear the free $C_{5'}$-hydroxyl groups. The use of ribonucleosides thus protected would

CHART I
SYNTHESIS OF $N^6,O^{2'},O^{3'}$-TRIBENZOYLCYTIDINE



the preparation of 5′-O-tritylribonucleosides followed by acylation and removal of the 5′-O-trityl group. A modification of the above approach using 5′-O-trimethoxytrityladenosine[9] was recently used in preparation of N,N,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine.[7] The protection of the amino group in the adenine ring was desirable to avoid any formation of a phosphoroamidate linkage during the subsequent phosphorylation. In the present work, 2′,3′-di-O-benzoyluridine and N^6,$O^{2'}$,$O^{3'}$-tribenzoylcytidine have been prepared. 2′,3′-Di-O-benzoyluridine was prepared from 5′-O-trityluridine[10] as well as from 5′-O-dimethoxytrityluridine. Unambiguous synthesis of N^6,$O^{2'}$,$O^{3'}$-tribenzoylcytidine was accomplished by the method outlined in Chart I. N,$O^{2'}$,$O^{3'}$,$O^{5'}$-Tetrabenzoylcytidine (II) was prepared from cytidine (I) by the method of Brown and coworkers.[11] Careful treatment with sodium methoxide caused selective O-debenzoylation[12] and the crystalline N-benzoylcytidine (III) was obtained in good yield. Controlled reaction with di-*p*-methoxytrityl chloride[8] gave the crystalline IV which was benzoylated with benzoyl chloride in pyridine to give N^6,$O^{2'}$,$O^{3'}$-tribenzoyl-5′-O-dimethoxytritylcytidine (V). Mild acidic treatment of the latter afforded the crystalline N^6,$O^{2'}$,$O^{3'}$-tribenzoylcytidine (VI). An alternative, more direct route to this compound would be *via* 5′-O-dimethoxytritylcytidine followed by benzoylation and acidic treatment. While this route probably could be perfected readily, the lengthier route (Chart I) *via* N^6-benzoylcytidine was chosen first so as to avoid any ambiguity in the preparation of the pure 5′-O-dimethoxytritylcytidine arising from the concomitant formation of mono-N^6-dimethoxytritylcytidine.

The preparation of suitably protected ribonucleoside derivatives with free $C_{3'}$-hydroxyl groups was next investigated. Previously, Todd and coworkers have described the preparation of 3′,5′-di-O-acetyluridine[13] and the corresponding adenosine[14] derivative. These derivatives were suc-

appear to be severely limited in the synthesis of inter-nucleotide bonds because of the rather prolonged acidic treatment necessary for the cleavage of the acetal linkages. The more labile *p*-methoxybenzylideneuridine was recently tested[7] and also found not to be completely satisfactory, at least in the route used for internucleotide bond synthesis. A more promising route to the preparation of ribonucleosides containing highly acid-labile groups in the $C_{2'}$- and $C_{3'}$-hydroxyl groups would be ribonucleosides → 5′-O-acylribonucleosides → 2′,3′-di-O-tetrahydropyranyl-5′-O-acylribonucleosides → 2′,3′-di-O-tetrahydropyranylribonucleosides. The validity of this scheme is illustrated by the preparation of N^6,5′-O-dibenzoyl,2′,3′-di-O-tetrahydropyranylcytidine described in the Experimental section.

(9) Trityl is a generally accepted abbreviation for the triphenylmethyl group in the carbohydrate literature. In the present and succeeding papers we propose to extend this abbreviation to the triphenylmethyl groups bearing methoxy groups in the *p*-position of the phenyl rings. Thus *p*-$CH_3OC_6H_4C(C_6H_5)_2$- will be abbreviated to monomethoxytrityl; (*p*-$CH_3OC_6H_4)_2C(C_6H_5)$- to dimethoxytrityl and (*p*-$CH_3OC_6H_4)_3C$- to trimethoxytrityl.

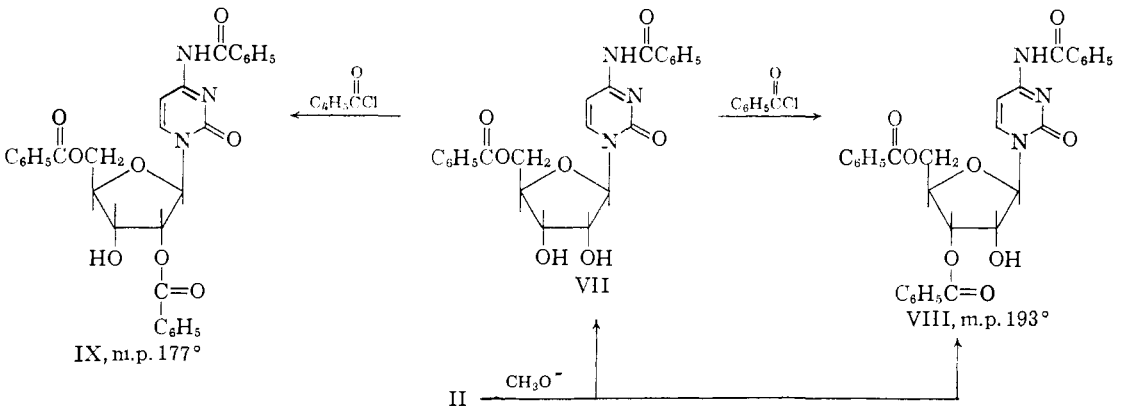
(10) *Cf.* the analogous preparation of 2′,3′-di-O-acetyluridine; G. W. Kenner, A. R. Todd, R. F. Webb and F. J. Weymouth, *J. Chem. Soc.*, 2288 (1954).

(11) D. M. Brown, A. R. Todd and S. Varadarajan, *ibid.*, 2384 (1956).

(12) For selective *O*-debenzoylations in nucleotides and deoxyribonucleosides, see H. G. Khorana, A. F. Turner and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 686 (1961); R. K. Ralph and H. G. Khorana, *ibid.*, **83**, 2926 (1961); M. Smith, G. I. Drummond and H. G. Khorana, *ibid.*, **83**, 698 (1961); H. Schaller, G. Weimann, B. Lerch and H. G. Khorana, *ibid.*, in press.

(13) D. M. Brown, A. R. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(14) (a) D. M. Brown, G. D. Fasman, D. I. Magrath and A. R. Todd, *ibid.*, 1448 (1954); (b) A. M. Michelson, L. Szabo and A. R. Todd, *ibid.*, 1546 (1956).



cessfully used in the synthesis of, respectively, uridine-[13] and adenosine-2′ phosphates[14] and, in further work, adenylyl-(2′→5′)-uridine was synthesized[14b] by using 3′,5′-di-O-acetyladenosine as one of the components. An analogous approach to the synthesis of the $C_{3'}$–$C_{5'}$ inter-ribonucleotide bonds would require the preparation of 2′,5′-di-O-substituted ribonucleosides. Although, previously by partial acetylation of ribonucleosides, only the 3′,5′-di-O-acetylribonucleosides were isolated as crystalline products, these products were obtained by fractional crystallization and it was considered possible that the isomeric 2′,5′-di-O-acyl compounds were present in the mother liquors. In the present investigation, it was further hoped that by varying the acylating agent, acylation of the $C_{2'}$-hydroxyl group might predominate, especially, in view of the findings of several groups of previous workers on the toluene-*p*-sulfonylation of ribonucleosides[15] and acylations of pyranosides.[16]

Attempts were first made to benzoylate partially N⁶-benzoyl-5′-O-dimethoxy-tritylcytidine (IV) with slightly more than 1 molar equivalent of benzoyl chloride.

Compound IV was chosen as the starting material for this purpose because after the formation of an inter-ribonucleotide bond by reaction with a protected ribonucleoside-5′ phosphate, it was hoped to remove selectively the dimethoxytrityl group from the 5′-position and to repeat condensation with a protected ribonucleoside-3′ phosphate.[7] Benzoylation of IV gave, in addition to some N⁶,O²′,O³′-tribenzoyl-5′-O-dimethoxy-tritylcytidine, two mono-O-benzoylated products which evidently were the isomeric N⁶,2′-O-dibenzoyl- and N⁶,3′-O-dibenzoyl-5′-O-dimethoxytritylcytidines. Complete separation by chromatography on a silicic acid column, which otherwise proved very useful in the present work, could not be effected and the partially resolved peaks failed to crystallize. The synthesis of cytidylyl-(2′ or 3′→5′)-adenosine using the material from one of these peaks is described below.

Partial debenzoylation under carefully controlled conditions of the fully benzoylated cytidine (II) gave the crystalline N⁶,5′-O-dibenzoylcytidine (VII) and a crystalline tribenzoyl derivative (m.p. 193°) which was identified as N⁶,O³′,O⁵′-tribenzoylcytidine (VIII). It is noteworthy that none of the isomeric IX was produced after the alkaline treatment (see below). Benzoylation of VII with slightly more than 1 molar equivalent of benzoyl chloride gave both of the expected isomers VIII and IX. These isomers could be cleanly separated by silicic acid chromatography and each crystallized to give samples with distinct and sharp melting points, which were depressed on admixture with each other.

The identification of VIII was accomplished by phosphorylation with a mixture of β-cyanoethyl phosphate and dicyclohexylcarbodiimide[17] in pyridine and subsequent removal of protecting groups under mildly alkaline conditions. Cytidine-2′ phosphate was the sole product. When the isomeric IX, which was undoubtedly a pure crystalline compound, was phosphorylated by the same procedure using an excess of the phosphorylating agent, the nucleotidic material obtained proved to be a mixture of cytidine-3′ phosphate (82%) and cytidine-2′ phosphate (18%). In repetitions of this experiment, the proportion of cytidine-2′ phosphate varied somewhat and appeared to depend upon the conditions of phosphorylation (see below).

The above results, namely, the production of the two isomeric nucleotides from phosphorylation of N⁶O²′,O⁵′-tribenzoylcytidine and the formation of cytidine-2′ phosphate alone by phosphorylation of N⁶.O³′,O⁵′-tribenzoylcytidine,[18] show that there is tendency for an acyl group to migrate from the $C_{2'}$-hydroxyl to the $C_{3'}$-hydroxyl group in the ribonucleosides. This result seems to be consistent with some previous findings.[19,20] Apparently,

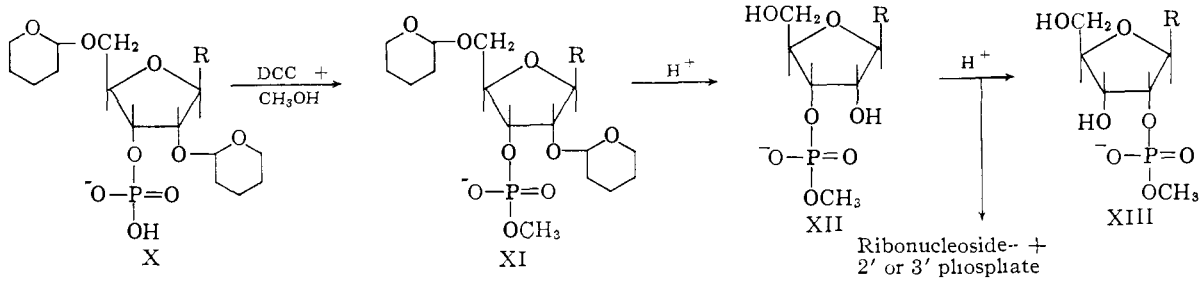
(15) For example in the reaction of 5′-O-acetyluridine with toluene-*p*-sulfonyl chloride, the main product isolated was the 2′-O-toluene-*p*-sulfonyl derivative; D. M. Brown, A. R. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956); D. M. Brown, D. B. Parihar, A. R. Todd and S. Varadarajan, *ibid.*, 3028 (1958).

(16) For example, on partial benzoylation of methyl 4,6-benzylidene-α-D-glucopyranoside with benzoyl chloride, the major product obtained was the 2-O-benzoyl derivative. Furthermore, differences were noted in the nature of the products when the above glucopyranoside was partially acylated with acetic anhydride or acetyl chloride in pyridine [R. W. Jeanloz and D. A. Jeanloz, *J. Am. Chem. Soc.*, **79**, 2579 (1957)]. Using acetyl chloride, the 2-O-acetyl derivative was the major product. Also, reaction with toluenesulfonic anhydride gave mainly the 2-O-toluenesulfonyl derivative.

(17) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

(18) *Cf.* the formation of pure ribonucleoside-2′ phosphates in the previously described phosphorylations of 3′,5′-di-O-acetylribonucleosides.[13,14]

(19) The acyl group migrations generally proceed in the direction away from the C_1 of the lactol ring; see, *e.g.*, Brown, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 1125 (1950), and E. J. Bourne, A. J. Huggard and J. C. Tatlow, *ibid.*, 735 (1953); R. K. Ness and H. G.



this migration may be acid[21] or base catalyzed. For example, during the above-described phosphorylation of N^6,$O^{2'}$,$O^{5'}$-tribenzoylcytidine, the proportion of cytidine-2′ phosphate and, therefore, the isomerized acyl-nucleoside was higher when the starting material was exposed in pyridine solution to an excess of pyridinium β-cyanoethyl phosphate than when the nucleoside IX was added to the phosphorylating agent previously prepared by adding dicyclohexylcarbodiimide to pyridinium β-cyanoethyl phosphate. The amount of the isomeric nucleoside-2′ phosphate also increased when the reaction time was increased to several days to allow the phosphorylation to go to completion. The basic catalysis of the isomerization reaction is indicated by the observation that during the partial debenzoylation of II, the only tribenzoylcytidine obtained was the N^6,$O^{3'}$,$O^{5'}$-tribenzoyl isomer. Whereas the rates of debenzoylation of the groups on the $C_{2'}$- and $C_{3'}$-hydroxyl groups would be expected to be not dissimilar, it is believed that under the basic conditions used rapid migration of the 2′-O-benzoyl group to the $C_{3'}$-hydroxyl group occurred. This interpretation is also supported by the previous experimental result of Todd and coworkers[14b] who recorded the formation in good yield of 3′,5′-di-O-acetyladenosine by fusion of an equimolar mixture of 5′-O-acetyladenosine and 2′,3′,5′-tri-O-acetyladenosine. None of 2′,5′-di-O-acetyladenosine was detected and it would appear that, presumably, the basic catalysis provided by glass resulted in the migration of the 2′-O-acetyl group to form 3′,5′-di-O-acetyladenosine.

From the standpoint of the synthesis of the $C_{3'}$–$C_{5'}$ inter-nucleotide linkage, the direct use of protected ribonucleosides bearing an acyl group on the $C_{2'}$-hydroxyl group and a free $C_{3'}$-hydroxyl group is thus excluded. The only possibility deserving of further investigation is that of using the easily accessible 3′,5′-di-O-acylribonucleosides as the starting materials and introducing on the $C_{2'}$-hydroxyl group a protecting group, such as the tetrahydropyranyl group, which would not migrate to the adjoining position. Subsequent deacylation would then give the potentially useful 2′-O-substituted ribonucleosides. Work along this line will be reported subsequently.

Protected Ribonucleoside-3′ Phosphates.—From the above discussion, the approach using a suitably protected ribonucleoside-3′ phosphate and a second component bearing a free $C_{5'}$-hydroxyl group becomes all the more important. The same approach was used in the recently described syntheses of uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-adenosine.[7] The starting material for the protected nucleotide was uridine-5′ phosphate. It was converted in high yield to uridine-3′,5′-cyclic phosphate[22] which in turn was converted to 2′-O-tetrahydropyranyluridine-3′,5′-cyclic phosphate. Subsequent base-catalyzed cleavage of the phosphate ring gave predominantly 2′-O-tetrahydropyranyluridine-3′ phosphate which was separated by further conversion to 5′-O-trityl or 5′-O-dimethoxytrityl derivative. While the same general approach is being applied to the other ribonucleotides,[23] a more direct approach to protected ribonucleoside-3′ phosphate, involving acid-catalyzed tetrahydropyranylation, has been developed in the present work.

The reaction of uridine-3′ phosphate with 2,3-dihydropyran in dimethyl sulfoxide in the presence of trifluoroacetic acid gave 2′,5′-di-O-tetrahydropyranyluridine-3′ phosphate (X) which was isolated in 96% yield by partition chromatography on a cellulose column. That no migration of the phosphoryl group to the $C_{2'}$-hydroxyl group in uridine-3′ phosphate occurred during the formation of X was shown as follows. Compound X, as the trialkylammonium salt, was treated in methyl alcohol with dicyclohexylcarbodiimide[24] (DCC). Conversion to the methyl ester XI was quantitative as determined by paper chromatography. The removal of the tetrahydropyranyl groups in XI was effected by treatment with 80% acetic acid at room temperature. It has been shown recently[7] that during prolonged treatment under these conditions measurable isomerization of the $C_{3'}$–$C_{5'}$ inter-ribonucleotide bond to the $C_{2'}$–$C_{5'}$ linkage occurs. A small degree of migration of the methylphosphoryl group in XII to form XIII was, therefore, anticipated. Consequently, the removal of the tetrahydropyranyl groups in XI was studied as a function of time. After a reaction period of 1.5 hr., when the removal of tetrahydropyranyl groups was incomplete, methyl uridine-3′ phosphate (XII) was obtained in 48% yield based on X. [In addition, the formation of a trace (about 1%) of uridine-2′ (or -3′) phosphate could

Fletcher, Jr., *J. Am. Chem. Soc.*, **78**, 4710 (1956). There are, however, exceptions; *e.g.*, R. K. Ness and H. G. Fletcher, Jr., *J. Org. Chem.*, **22**, 1470 (1957).

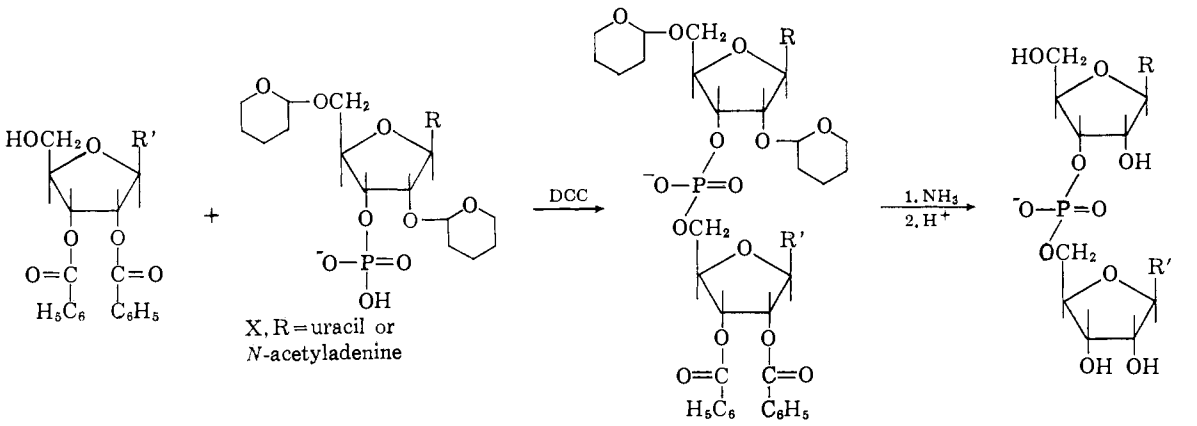
(20) See also the results on the synthesis of 2′-*O*- and 3′-*O*-phenylalanyladenosine; D. H. Rammler and H. G. Khorana, in press.

(21) A. Doerschuk, *J. Am. Chem. Soc.*, **74**, 4202 (1952).

(22) M. Smith, G. I. Drummond and H. G. Khorana, *ibid.*, **83**, 698 (1961).

(23) The approach using ribonucleoside-3′,5′ cyclic phosphates would be much less satisfactory in the case of cytidine-3′,5′ cyclic phosphate since extensive deamination occurs during the alkaline ring opening of the phosphate ring.[22]

(24) M. Smith, J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6204 (1958); H. G. Khorana, *ibid.*, **81**, 4657 (1959).



XIV, R′ = N-benzoylcytosine, uracil or N,N-dibenzoyladenine

XV, R = uracil or N-acetyladenine
R′ = uracil, N-benzoylcytosine or N,N-dibenzoyladenine

XVI, R = uracil or adenine
R′ = uracil, cytosine or adenine

be detected.] Incubation of this sample of methyl uridine-3′ phosphate with pancreatic ribonuclease under appropriate conditions caused virtually complete degradation to uridine-3′ phosphate, there being left a bare trace[25] of an ultraviolet absorbing material on paper chromatograms in the region of the starting material. When the time of hydrolysis for XI was increased to 2.5 hr., the yield of XII was 62%, but this product now contained about 5% of material, presumably XIII, which was resistant to pancreatic ribonuclease. There was also obtained at this time a substantial amount (about 20%) of nucleotidic material corresponding to uridine-2′ or -3′ phosphate.

From the above results it is concluded that the tetrahydropyranylation of the $C_{2'}$-hydroxyl group in uridine-3′ phosphate occurs before any migration of the phosphoryl group can occur.[25a] The same conclusion is reinforced below in similar experiments with adenosine-3′ phosphate. The use of these protected ribonucleoside-3′ phosphates in the synthesis of $C_{3'}$–$C_{5'}$ linked di-ribonucleoside phosphates is described below.

Acid-catalyzed reaction of adenosine-3′ phosphate with dihydropyran afforded 2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate (X, R = adenine) as the major product (70%). That no migration of the phosphoryl group had occurred in the parent nucleotide was readily shown by removal of the protecting groups in 80% acetic acid and paper chromatography of the resulting nucleotide, there being suitable solvent systems which distinguish clearly adenosine-2′ and -3′ phosphates. A second product (about 22%) formed during tetrahydropyranylation had λ_{max} at 264 mμ at *p*H 6 and it is concluded to be N.$O^{2'}$,-$O^{5'}$-tritetrahydropyranyladenosine-3′ phosphate. Thus, its paper-electrophoretic mobility at *p*H 7.5, a little less than that of 2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate, indicated it to have two acidic dissociations. On acid hydrolysis, it gave adenosine-3′ phosphate as the only product. Its amount relative to 2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate increased with increase in the reaction period given for tetrahydropyranylation.

For the synthesis of adenylyl-($C_{3'}$→$C_{5'}$)-adenosine described below, N^6-acetyl-2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate prepared by acetylation of 2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate was used. Methyl adenosine-3′ phosphate was prepared from 2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate as described for methyl uridine-3′ phosphate.

In addition to their direct use in internucleotide bond synthesis, the 2′,5′-di-O-tetrahydropyranylribonucleoside-3′ phosphates offer an alternative route to monotetrahydropyranylribonucleoside-3′ phosphates by partial removal of one of the tetrahydropyranyl groups.[26] The monotetrahydropyranyl derivatives thus afforded would clearly be potentially useful intermediates in further elaboration of the polynucleotide synthesis and work along this line will be reported later.

Synthesis of $C_{3'}$–$C_{5'}$ Inter-ribonucleotide Bonds.—The condensation of pyridinium 2′,5′-di-O-tetrahydropyranyluridine-3′ phosphate (X, R = uracil) with the protected nucleosides, 2′,3′-di-O-benzoyluridine, N^6,$O^{2'}$,$O^{3'}$-tribenzoylcytidine and N,N,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine[7] (XIV) was effected by means of dicyclohexylcarbodiimide under the standard conditions of reaction described previously.[27] The work-up involved first an ammoniacal treatment of the initial products (general structure XV) to remove the N- and O-benzoyl groups and then treatment with 80% acetic acid at room temperature. The desired products were isolated by preparative paper chromatography. As pointed out previously, the duration of the acetic acid treatment was restricted to about 4 hr. at room

(25) This exceedingly faint band is barely detectable when the pancreatic ribonuclease digest is applied at a concentration of 1-1.5 μmoles per spot on paper chromatograms.

(25a) Since the submission of this paper, we have noted the independent work of J. Smrt and F. Sorm [*Coll. Czech. Chem. Comm.*, **27**, 73-86 (1962)] on the use of tetrahydropyranyl groups for the direct blocking of hydroxyl groups in ribonucleoside-3′ phosphates.

(26) Experiments reported separately (D. H. Rammler and H. G. Khorana, forthcoming paper) show that there are marked differences in the rates of hydrolysis of different tetrahydropyranyl groups in ribonucleotides.

(27) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212 (1958).

temperature in order to avoid any significant isomerization of the $C_{3'}$–$C_{5'}$ linkage to the $C_{2'}$–$C_{5'}$ position. Under these conditions the yields of the desired products of the type XVI ranged between 28–40%. The presence of the $C_{3'}$–$C_{5'}$ inter-ribonucleotide linkage in all the synthetic compounds was carefully checked by their susceptibility to pancreatic ribonuclease and/or spleen phosphodiesterase. It should be emphasized that the analysis by this technique was carried out by paper chromatography by using 10 or more optical density units (0.5 or more μmole of the sample) per spot. The sensitivity of this method was such that 1–2% of a product resistant to the action of the enzyme could be detected. By this technique, the synthetic samples obtained above were at least 97–98%[28] pure with respect to the $C_{3'}$–$C_{5'}$ interribonucleotidic linkage. Somewhat higher yields of the dinucleoside phosphates could be obtained by prolonging the acetic acid treatment but then the extent of isomerization of the inter-nucleotidic linkage was also higher.[28]

In several previous publications from this Laboratory,[4,6] it has been shown that the condensation using a protected nucleotide and two molar equivalents of a protected nucleoside goes virtually to completion with respect to the nucleotide in the presence of dicyclohexylcarbodiimide. Since these conditions were used throughout the present work, the formation of the condensation products of the type (XV) must have been complete. This was further evidenced by the fact that in general only small amounts of the mononucleotide components were present in the reaction products. The lowering of the yields of the desired products is attributed to the incomplete removal of the protecting groups, especially the 5′-O-tetrahydropyranyl group from the condensation products. This was clearly demonstrated in the synthesis of adenylyl-(3′→5′)-adenosine (XVI, R = R′ = adenine). Condensation of N^6-acetyl-2′,5′-di-O-tetrahydropyranyladenosine-3′ phosphate (X, R = N-acetyladenine) and N,N,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine (XIV, R′ = N,N-dibenzoyladenine) followed by ammoniacal treatment gave a product which was treated with 80% acetic acid for 4 hr. Separation by preparative paper chromatography showed, in addition to adenylyl-(3′→5′)-adenosine, a major product, presumably 5′-O-tetrahydropyranyladenylyl-(3′→5′)-adenosine. The latter on further treatment with acetic acid gave adenylyl-(3′→5′)-adenosine. The combined yield of adenylyl-(3′→5′)-adenosine after the two-step acidic treatments was 25%, the product being degraded by spleen phosphodiesterase to the nucleotide and nucleoside to at least 95%. A second synthesis of the same product (XVI, R = R′ = adenine) was accomplished by the condensation of N,$O^{2'}$,$O^{5'}$-tritetrahydropyranyladenosine-3′ phosphate and N,N,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine. The final yield (11%) was, however, much lower.

(28) The rate of isomerization (C_3′-C_5′ to C_2′-C_5′) of inter-nucleotide bonds during acidic treatment appears to vary with the nature of the components. This rate was distinctly higher in the simpler methyl uridine-3′ phosphate, where after 2.5-hr. treatment the amount of the ribonuclease-resistant material was around 5%. In the dinucleoside phosphates corresponding treatments give hardly detectable somerization.

As expected, the condensation of N^6,$O^{2'}$-$O^{3'}$-triacetyladenosine-5′ phosphate with the above described N^6,$O^{2'}$- or $O^{3'}$-dibenzoyl-5′-O-dimethoxytritylcytidine followed by removal of protecting groups gave a mixture of cytidylyl-(3′→5′)-adenosine and cytidylyl-(2′→5′)-adenosine. The mixture was degraded by pancreatic ribonuclease to the extent of 60%.

Concluding Remarks.—The present work has clearly demonstrated the superiority of that approach to the specific synthesis of $C_{3'}$–$C_{5'}$ interribonucleotide bonds which involves the condensation of a protected ribonucleoside-3′ phosphate with a second component bearing a free $C_{5'}$-hydroxyl group. The same approach was used in our previously described syntheses of uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-adenosine.[7]

The protecting groups used so far in protecting the $C_{2'}$-hydroxyl group in ribonucleoside-3′ phosphates is the tetrahydropyranyl group. Although this group is the best so far available it is not completely satisfactory because of the detectable isomerization of the internucleotidic linkage during the acidic conditions necessary for its removal. Alternative suitable protecting groups for the purpose are being sought.

Experimental[29]

General Methods.—Reagent grade pyridine[30] dried over calcium hydride for several days was used. All evaporations were carried out on a rotary evaporator at about 12 mm. pressure and a bath temperature of 30° or less. Paper chromatography was performed by the descending technique using Whatman No. 44 (double acid washed) paper.

The solvent systems used for paper chromatography were: isopropyl alcohol–concd. ammonium hydroxide–water (7:1:2; solvent A); *n*-butyl alcohol–acetic acid–water (5:2:3; solvent B); ethyl alcohol–1 *M* ammonium acetate, *p*H 7.5 (5:2; solvent C); ethyl alcohol–0.5 *M* ammonium acetate, *p*H 3.8 (solvent D); *n*-butyl alcohol–water (86:14; solvent E). Nucleosides and related compounds were detected by viewing under an ultraviolet lamp. A fluorescent screen further increased the sensitivity of the technique for the detection of small amounts of nucleoside components. The R_f's of different compounds are given in Table I. Paper electrophoresis was carried out using 12-cm. wide strips of Whatman 3 mm. or No. 31 paper in an apparatus similar to that of Crestfield and Allen.[31] The buffer used was triethylammonium bicarbonate, *p*H 7.5, 0.05 *M*. The electrophoretic mobilities of different compounds are given in Table II.

Ion exchange chromatography was carried out using the method of Cohn and Khym.[32] The standard column of Dowex-1 (formate form, 200–400 mesh, 8–10% divinylbenzene, 12 × 1.5 cm.) was eluted using 0.01 *M* formic acid at a flow rate of 1 ml. per minute. Silicic acid[33] chromatography was conducted on a standard column (28 × 2.5 cm.). A gradient elution technique was used with 1000 ml. of chloroform in the mixing vessel and 900 ml. of chloroform and 100 ml. of methyl alcohol in the reservoir unless stated otherwise. The column was under slight positive nitrogen pressure giving a flow rate of 1 ml. per minute.

Enzymic assays were carried out using pancreatic ribonuclease and spleen phosphodiesterase.[34] (a) Pancreatic ribonuclease: The incubation mixtures consisted of di-

(29) Elemental analyses were performed by A. Bernhardt, Mülheim, Germany. All melting points are uncorrected and were obtained on a Kofler block melting point apparatus.

(30) Baker and Adams, ACS reagent grade.

(31) A. M. Crestfield and F. W. Allen, *Anal. Chem.*, **27**, 422 (1955).

(32) W. W. Cohn and J. X. Khym, "Biochemical Preparations," Ed. D. Shemin, John Wiley and Sons, Inc., New York, N. Y., 1957, Vol. 5, p. 40.

(33) Mallinckrodt, analytical grade, silicic acid, 100 mesh.

(34) W. E. Razzell and H. G. Khorana, *J. Biol. Chem.*, **236**, 1144 (1961).

TABLE I

PAPER CHROMATOGRAPHY OF NUCLEOTIDES AND DERIVATIVES

	R_f Solvent A	R_f Solvent B	R_f Solvent C
Phosphate			
2',5' Di-O-tetrahydropyranyl-uridine-3'	0.42		
2'- or 5'-O-tetrahydropyranyl-uridine-3'	.26		
Uridine-3'	.07		
$N^6,O^{2'},O^{5'}$-Tritetrahydropyranyl-adenosine-3'	.69		
2',5'-Di-O-tetrahydropyranyl-adenosine-3'	.5		
2'- or 5'-O-tetrahydropyranyl-adenosine-3'	.28		
Adenosine-3'	.07		
Uridylyl-(3' → 5')-uridine	.16		
Uridylyl-(3' → 5')-adenosine	.18		
Uridylyl-(3' → 5')-cytidine	.155		
Adenylyl-(3' → 5')-adenosine	.16		
N^6-Benzoylcytidine		0.73	
N^6,5'-O-Dibenzoylcytidine		.85	
$N^6,O^{2'},O^{5'}$-Tribenzoylcytidine		.94	
$N^6,O^{3'},O^{5'}$-Tribenzoylcytidine		.91	
$N,O^{2'},O^{3'}$-Triacetyladenosine-5' phosphate			0.47
N^6-Acetyl-2',5'-di-O-tetrahydropyranyladenosine-3' phosphate			0.75

TABLE II

ELECTROPHORETIC MOBILITIES RELATIVE TO URIDINE-3' PHOSPHATE

0.05 *m* triethylammonium bicarbonate, *p*H 7.5	
2',5'-Di-O-tetrahydropyranyluridine-3' phosphate	0.84
$N^6,O^{2'},O^{5'}$-Tritetrahydropyranyladenosine-3' phosphate	.72
2',5'-Di-O-tetrahydropyranyladenosine-3' phosphate	.8

nucleoside phosphate or methyl ribonucleoside-3' phosphate (about 1 μmole), crystalline pancreatic ribonuclease (50 μg.) and tris-hydroxymethylaminomethane buffer (*p*H 7.5; 5 μmole) in a total volume of 0.1 ml. The incubation was carried out at 37° for 2–3 hr. and the products were determined by chromatography in solvent A. (b) Using spleen phosphodiesterase[34]: The phosphodiesterase preparation used had a specific activity of 123 μmoles/hr./mg. protein for hydrolysis of *p*-nitrophenyl thymidine-3' phoshate.[34] The incubation mixture contained the dinucleoside phosphate (*ca.* 1 μmole) in 0.2 ml. of 0.5 *M* ammonium acetate buffer (*p*H 6.5) and 99 μg. of the phosphodiesterase in 0.1 ml. of 0.01 *M* pyrophosphate buffer (*p*H 6.5). The incubation was carried out at 37° and the products were characterized in solvent A.

$N^6,O^{2'},O^{3'},O^{5'}$-Tetrabenzoylcytidine was prepared in 98.5% yield essentially as described by Brown, *et al.*[11] The melting point was 208–209°, reported value 203°.

Anal. Calcd. for $C_{37}H_{29}O_9N_3$ (659.62): C, 67.4; H, 4.46; N, 6.36. Found: C, 67.64; H, 4.42; N, 6.43.

N^6-Benzoylcytidine.—To a solution of $N^6,O^{2'},O^{3'},O^{5'}$-tetrabenzoylcytidine (6.4 g., 9.7 mmoles) in either tetrahydrofuran or dioxane (300 ml.) and absolute methyl alcohol (300 ml.) was added sodium methoxide (15 ml. of 2.5 *N*). After exactly 7.5 minutes at room temperature, the reaction mixture was poured into a rapidly stirred slurry of Dowex-50 (pyridinium salt, 60 g. in 100 ml. of water). This mixture was stirred for 10 minutes, and the resin was then removed by filtration. The filtrate and washings were evaporated; N^6-benzoylcytidine crystallized during evaporation of the solution. The yield was 2.5 g. (72%), m.p. 219°. The analytical sample was prepared by crystallization from ethyl alcohol; m.p. 219–220°. Ultraviolet absorption spectrum taken in ethyl alcohol showed: λ_{max} 305 and 260 mμ, λ_{min} 285 and 229 mμ, ϵ_{max} (305 mμ) 9980, ϵ_{max} (260 mμ) 22,300.

Anal. Calcd. for $C_{16}H_{17}O_5N_3$ (347.3): C, 55.04; H, 4.94; N, 12.1. Found: C, 55.08; H, 4.91; N, 12.24.

N^6,5-O-Dibenzoylcytidine and $N^6,O^{3'},O^{5'}$-Tribenzoylcytidine.—To an ice-cold solution of $N^6,O^{2'},O^{3'},O^{5'}$-tetrabenzoylcytidine (3.4 g., 5.2 mmoles) in tetrahydrofuran (150 ml.) was added a cold solution of sodium methylate (7.5 ml. of 2.5 *N*) in methyl alcohol (150 ml.). After exactly 5 minutes, the solution was neutralized with pyridinium Dowex-50 resin as described in the preceding experiment. The total filtrate and washings were evaporated to dryness, and the residue was extracted with chloroform (4 × 25 ml.). The chloroform-insoluble portion was treated as described subsequently. The chloroform extract containing 0.64 g. of material was chromatographed on a standard silicic acid column. Two main peaks were obtained. The first at 0.5% methyl alcohol concentration contained the tetrabenzoylcytidine (0.1 g., m.p. 205°). The second peak appeared at about 1.0% methyl alcohol concentration and contained $N^6,O^{3'},O^{5'}$-tribenzoylcytidine (0.19 g.). This substance was crystallized from ethyl alcohol or a mixture of chloroform and heptane (m.p. 193–194°). Ultraviolet absorption characteristics in ethyl alcohol, λ_{max} 304, 261 mμ; λ_{min} 287 mμ.

Anal. Calcd. for $C_{30}H_{25}O_8N_3$ (555.52): C, 64.9; H, 4.54; N, 7.57. Found: C, 65.21; H, 4.59; N, 8.53.

A third minor peak eluted from the above column contained N^6,5'-O-dibenzoylcytidine.

The chloroform-insoluble material obtained above was fractionally crystallized from ethyl alcohol and furnished 0.56 g. of N^6,5'-O-dibenzoylcytidine (m.p. 184°) and 0.46 g. of N^6-benzoylcytidine with m.p. 198–213°. The melting point of the latter substance could be raised to 219°, identical with that of the pure compound, on recrystallization. N^6,5'-O-dibenzoylcytidine, after recrystallization from ethyl alcohol, melted at 185–186°, ultraviolet absorption in ethyl alcohol: λ_{max} 304 and 261 mμ, λ_{min} 287 mμ.

Anal. Calcd. for $C_{23}H_{21}O_7N_3$ (451.42): C, 61.2; H, 4.7; N, 9.3. Found: C, 60.78; H, 4.7; N, 9.73.

N^6-Benzoylcytidine and N^6,5'-O-dibenzoylcytidine were found to co-crystallize. In one experiment 0.8 g. (1.78 mmole) of N^6,5'-O-dibenzoylcytidine containing about 10% of N^6-benzoylcytidine was treated in dry pyridine (10 ml.) with di-*p*-methoxytrityl chloride (0.84 g., 2.5 mmoles) at room temperature for 18 hr. The solution was then evaporated to 0.5 ml. and the concentrate taken up in 2 ml. of chloroform. The solution was chromatographed on a standard silicic acid column. N^6-Benzoyl-5'-O-di-*p*-methoxytritylcytidine was eluted at 1.9% methyl alcohol concentration and was crystallized from a mixture of ethyl acetate–ether and light petroleum; m.p. 124–125°, undepressed on admixture with an authentic sample obtained as described below. N^6,5'-O-Dibenzoylcytidine (0.7 g.), eluted from the silicic acid column at 4% methyl alcohol concentration, melted at 183–184°.

Cytidine-2' Phosphate from $N^6,O^{3'},O^{5'}$-Tribenzoylcytidine.—Pyridinium β-cyanoethyl phosphate (0.125 mmole) was rendered anhydrous by three evaporations with dry pyridine (0.5-ml. portions) in a vacuum. To the residual gum was added dry pyridine (1.0 ml.) and DCC (0.065 g.). After 1.5 hr. at room temp., $N^6,O^{3'},O^{5'}$-tribenzoylcytidine (0.019 g., 0.034 mmole) was added. The sealed solution was kept for 5 days at room temperature and then water (1.0 ml.) was added. After 18 hours, concd. ammonium hydroxide (5 ml.) was added and the solution kept at 60° for 3 hours.[35] Methyl alcohol (10 ml.) was then added and the solution evaporated to dryness. The residue was chromatographed in solvent A. No cytidine was detected. The cytidylic acid band was eluted with water and a part (0.025 mmole) was chromatographed on a standard Dowex-1 formate column. Elution with 900 ml. of 0.01 *M* formic acid gave a single peak (0.0238 mmole) corresponding to cytidine-2' phosphate. No other nucleotidic material was obtained.

(35) In another experiment, before treatment of the reaction mixture with ammonia, DCC was removed by extraction with pentane in order to avoid the possibility of reaction of the cytidine phosphate to form cytidine-2',3' cyclic phosphate.[34]

N^6,$O^{2'}$,$O^{5'}$-Tribenzoylcytidine.—To a solution of N^6,5′-O-dibenzoylcytidine (0.63 g., 1.4 mmoles) in dry pyridine (10 ml.) was added freshly distilled benzoyl chloride (0.2 ml., 1.73 mmoles). After 2 hr. at room temperature, the solution was cooled in ice and water (0.5 ml.) was added. After a further 2 hr., chloroform (50 ml.) was added and the solution washed with water (3 × 50 ml.). After drying the chloroform solution over sodium sulfate, it was evaporated to about 10 ml. and the concentrate chromatographed on a standard silicic acid column. In addition to a small amount of starting material, two main peaks were obtained. The first (0.320 g.) was obtained at 2.7% methyl alcohol concentration. After crystallization from chloroform–light petroleum ether (30–60°) it melted at 194° and did not depress the melting point of authentic N^6,$O^{3'}$,$O^{5'}$-tribenzoylcytidine. The second peak (0.210 g.), obtained at 3% methyl alcohol concentration, corresponded to N^6,$O^{2'}$,$O^{5'}$-tribenzoylcytidine. After crystallization from chloroform–light petroleum this compound melted at 177°. On admixture with N^6,$O^{3'}$,$O^{5'}$-tribenzoylcytidine (the first peak) the melting point was 155–167°. Rechromatography of the second peak on a standard silicic acid column but with a shallower methyl alcohol gradient (5% methyl alcohol–chloroform in the reservoir) again gave a single peak at 3% methyl alcohol concentration. N^6,$O^{2'}$,$O^{5'}$-Tribenzoylcytidine was homogeneous on paper chromatography in solvents B, C and D.

Anal. Calcd. for $C_{30}H_{25}O_6N_3$ (555.52): C, 64.9; H, 4.54; N, 7.57. Found after drying in a high vacuum at 50°: C, 65.67; H, 4.66; N, 7.60.

Cytidine Phosphates from N^6,$O^{2'}$,$O^{5'}$-Tribenzoylcytidine.—To an anhydrous solution of pyridinium β-cyanoethyl phosphate (1.0 mmole) and N^6,$O^{2'}$,$O^{5'}$-tribenzoylcytidine (0.022 g., 0.0395 mmole) in dry pyridine (0.5 ml.) was added 0.100 g. of DCC and the sealed mixture kept at room temperature. After 2 days water (0.5 ml.) was added and after 12 hr. concd. ammonium hydroxide (5 ml.). The mixture was heated at 50° for 3 hr. and then kept at room temperature for 18 hr. The insoluble dicyclohexylurea was removed by filtration and the filtrate evaporated to dryness. The residue was chromatographed on a standard Dowex-1 formate column. Elution with water gave cytidine (0.013 mmole). Elution with 0.01 *M* formic acid gave two peaks. The first peak (780 ml. of eluate to beginning of the peak) corresponded to cytidine-2′ phosphate (0.0018 mmole, 13.8%). The major peak (0.0112 mmole, 86.2% of the total nucleotide) was obtained after an eluate volume of 1078 ml. and corresponded exactly in position of elution to cytidine-3′ phosphate.

In a modification of the above experiment, the phosphorylating agent was first prepared by reaction of pyridinium β-cyanoethyl phosphate (0.2 mmole) with DCC (0.1 g.) in anhydrous pyridine and the protected cytidine (0.018 mmole) was added after 45 min. The work-up was as described above except that before treatment with ammonia, unreacted DCC was extracted with pentane. The total cytidylic acid (0.0167 mmole) was found to consist of cytidine-3′ phosphate (0.014 mmole) and cytidine-2′ phosphate (0.0014 mmole).

N^6,5′-O-Dibenzoyl-2′,3′-O-di-tetrahydropyranylcytidine.—To a solution of N^6,5′-O-dibenzoylcytidine (0.337 g., 0.7 mmole) in purified dioxane (5 ml.) containing dihydropyran (5 ml.) was added trifluoroacetic acid (1 ml.). After 1 hour at room temperature, the solution was evaporated to a gum. Cold ammonium hydroxide (1 *M*, 10 ml.) mixed with chloroform (20 ml.) was added and the chloroform layer was extracted several times with water. The chloroform solution was dried over sodium sulfate. It was then evaporated to a small volume and chromatographed on Whatman No. 31 paper in solvent E. The fully protected nucleoside near the front was eluted and crystallized from ethyl acetate–petroleum ether solution (0.2 g., m.p. 164–165°).

Anal. Calcd. for $C_{38}H_{37}O_9N_3$ (619.65): C, 63.9; H, 6.02; N, 6.78. Found: C, 63.47; H, 6.30; N, 6.77.

5′-O-Di-*p*-methoxytrityl-N^6-benzoylcytidine.—Di-*p*-methoxytrityl chloride (0.704 g., 2.12 mmoles) was added to a dry pyridine solution (5 ml.) of N^6-benzoylcytidine (0.65 g., 1.82 mmoles) and the clear solution kept sealed at room temperature. After 12 hr., the solution was poured into rapidly stirred ice-water (500 ml.). The precipitate which formed was collected by filtration and then dissolved in chloroform (20 ml.). The solution was dried over sodium sulfate and evaporated. The residue (1.65 g.) was dissolved in a small amount of ethyl acetate containing a drop of pyridine. Three volumes of ether were added followed by petroleum ether until opalescence appeared. 5′-O-Di-*p*-methoxytrityl-N^6-benzoylcytidine crystallized on storage at 5° for 12 hr. The yield was 0.5 g. (41%) and more could be obtained from the mother liquor. On heating it softened at 122–123° and gave a clear melt at 130°.

Anal. Calcd. for $C_{37}H_{35}O_8N_3$ (649.67): C, 68.41; H, 5.41; N, 6.47. Found: C, 67.63; H, 5.65; N, 6.22.

5′-O-Trityl-N^6-Benzoylcytidine.—To a refluxing solution of N^6-benzoylcytidine (2.8 g., 8.06 mmoles) in dry pyridine (50 ml.) was added trityl chloride (2.8 g., 8.07 mmoles). The solution was heated under reflux for 1 hr. and then kept at room temperature overnight. Some unreacted N^6-benzoylcytidine which separated from solution was removed by filtration. The pyridine solution was poured into rapidly stirred ice-water and stirred for 3 hours. After this time, chloroform (250 ml.) was added and the chloroform solution was washed with salt water (5 × 75 ml.) and dried over sodium sulfate. The dried chloroform solution was evaporated to dryness, the residue dissolved in ethyl acetate and the solution made opalescent with light petroleum ether. 5′-O-Trityl-N^6-benzoylcytidine separated as crystals (3.6 g., 75%) which melted at 202°.

Anal. Calcd. for $C_{35}H_{31}O_6N_3$ (589.62): C, 71.9; H, 5.32; N, 7.19. Found: C, 70.68; H, 5.20; N, 7.32.

5′-O-Di-*p*-methoxytrityl-N^6,$O^{2'}$ (or $O^{3'}$)-dibenzoylcytidine.—To a solution of 5′-O-di-*p*-methoxytrityl-N^6-benzoylcytidine (0.84 g., 1.29 mmoles) in dry pyridine (25 ml.) was added with the exclusion of moisture freshly distilled benzoyl chloride (0.187 ml., 1.60 mmoles). After 1.5 hours at room temperature, the clear solution was poured into ice-water (200 ml.). After 10 hr. at 5°, the mixture was extracted with chloroform (100 ml.). The chloroform solution was washed with water, dried over sodium sulfate and the dried solution was evaporated to a small volume (5 ml.). This solution was chromatographed on a standard silicic acid column.

The elution of products was followed by spotting each tube on paper and spraying the paper with 1 *N* hydrochloric acid. The rapid formation of the characteristic orange color of the di-*p*-methoxytrityl cation indicated the position of the peaks. The appropriate tubes were pooled, evaporated and weighed. The first peak (0.455 g.) appeared at 0.6% methyl alcohol concentration and corresponded to 5′-O-di-*p*-methoxytrityl-N^6,$O^{2'}$,$O^{3'}$-tribenzoylcytidine. After precipitation from ethyl acetate–petroleum ether it decomposed at 187° (shrinkage at 142°).

Anal. Calcd. for $C_{51}H_{43}O_{10}N_3$ (857.87): C, 71.5; H, 5.05; N, 4.94. Found: C, 69.61; H, 5.01; N, 4.42.

The isomeric 5′-O-di-*p*-methoxytrityl-N^6,$O^{2'}$ (or $O^{3'}$) dibenzoylcytidines appeared at 0.9% (peak II) and 1.06% (peak III) methyl alcohol concentration. There was considerable overlapping and even on rechromatography clear-cut separation was not achieved. Attempts at crystallization of either of the peaks failed.

Anal. Calcd. for $C_{44}H_{39}N_3O_9$ (753.77): C, 70.12; H, 5.22; N, 5.58. Found for peak II: C, 69.11; H, 5.08. Found for peak III: C, 66.4; H, 4.94; N, 4.29.

N^6,$O^{2'}$,$O^{3'}$-Tribenzoylcytidine.—To a solution of 5′-O-trityl-N^6-benzoylcytidine (3.0 g., 5.1 mmoles) in dry pyridine (20 ml.) was added freshly distilled benzoyl chloride (3.0 ml., 25.0 mmoles). After 12 hr. at room temperature, the solution was poured into ice-water and the mixture stirred for 3 hr. It was then extracted with water (5 × 75 ml.) and then dried over sodium sulfate. The dried solution was evaporated and the residue was dissolved in cold chloroform (20 ml.) and hydrobromic acid in acetic acid (48% by weight, 1.5 ml.) was added. After 3 min. the solution was evaporated to 5 ml. and was chromatographed on silicic acid (40 g.). The column was eluted with three bed volumes of chloroform and then with 3% methyl alcohol–chloroform solution. The solution of the first peak, which corresponded to N^6,$O^{2'}$,$O^{3'}$-tribenzoylcytidine, was extracted with water containing a drop of pyridine to remove some residual acetic acid and then evaporated. Crystallization from ethyl acetate–petroleum ether gave 1.2 g. (42%) of the desired product, m.p. 178–179°. Recrystallization afforded the analytical sample with m.p. 180–181°.

Anal. Calcd. for $C_{30}H_{25}O_8N_3$ (555.52): C, 64.9; H, 4.5; N, 7.57. Found: C, 63.64; H, 4.86; N, 7.74.

Another peak subsequently eluted appeared to contain mostly nitrogen-free material which has not been identified.

2',3'-Di-O-benzoyl-5'-O-di-*p*-methoxytrityluridine.—To a solution of 5'-O-di-*p*-methoxytrityluridine[7] (0.7 g., 1.28 mmoles) in dry pyridine (3 ml.) freshly distilled benzoyl chloride (0.5 ml., 4.2 mmoles) was added and the clear solution kept at room temperature for 3 hr. It was then poured into ice-water (500 ml.) and the precipitate (1.27 g., 99%) collected by filtration. When crystallized from ethyl alcohol, the product melted at 109–110° dec.

Anal. Calcd. for $C_{44}H_{38}O_{10}N_2$ (754.76): C, 70.1; H, 5.08; N, 3.72. Found: C, 70.34; H, 4.95; N, 3.36.

2',3'-O-Dibenzoyluridine. (a) **From 2',3'-O-Dibenzoyl-5'-O-di-*p*-methoxytrityluridine.**—A solution of 2',3'-O-dibenzoyl-5'-O-di-*p*-methoxytrityluridine (0.490 g., 0.6 mmole) in a mixture of dioxane (12 ml.) and 80% acetic acid (12 ml.) was kept at room temperature for 1 hr. The solution was then evaporated to dryness and re-evaporated with ethyl alcohol (10 ml.) and chloroform (10 ml.). The residue was dissolved in chloroform (10 ml.) and the solution applied to a silicic acid column (10 × 2 cm.). The column was first eluted with 3 bed volumes of chloroform and then with 2.5% methyl alcohol in chloroform. 2',3'-O-Dibenzoyluridine (0.22 g., 79%) thus eluted was crystallized readily from ethyl acetate–petroleum ether and from ethyl alcohol; m.p. 174–175°.

Anal. Calcd. for $C_{23}H_{20}O_8N_2C_6H_6$[36] (530.52): C, 65.6; H, 4.90; N, 5.29. Found: C, 64.47; H, 4.50; N, 5.25.

(b) *Via* **5'-O-Trityluridine.**—5'-O-Trityluridine was prepared from uridine by a modification of the procedure of Bredereck.[37] A solution of dry uridine (2.4 g., 9.8 mmoles) and trityl chloride (3.4 g., 12.2 mmoles) in dry pyridine (60 ml. was heated under reflux for 1.5 hr. After allowing it to cool to room temperature, the solution was poured into rapidly stirred ice-water (1000 ml.). The precipitate was collected by filtration, washed with heptane and dried (6.3 g.). This was dissolved into chloroform (50 ml.), the solution extracted with salt water (3 × 50 ml.) and dried over sodium sulfate. The dried chloroform solution was evaporated to dryness and the residue dissolved in boiling ethyl alcohol. The amorphous powder, which separated on cooling, was collected, dried and dissolved in anhydrous pyridine (10 ml.). Freshly distilled benzoyl chloride (3.6 ml., 30 mmoles) was added and after 12 hr. at room temperature, the dark brown solution was poured into rapidly stirred ice-water (1000 ml.). The precipitate was extracted with chloroform (100 ml.) and after the usual work-up, the dried residue was dissolved into chloroform (10 ml.) and passed onto a silicic acid column (10 × 2 cm.). Elution with chloroform gave 5'-O-trityl-2',3'-O-dibenzoyluridine (83%) which was crystallized from ethyl alcohol. The melting point was not sharp, a clear melt being obtained at 110°.

Anal. Calcd. for $C_{42}H_{34}O_8N_2$ (730.71): C, 74.0; H, 4.69; N, 3.82. Found: C, 74.08; H, 4.99; N, 3.21.

A cold chloroform solution (20 ml.) of the above product (4 g., 5.7 mmoles) was treated with hydrobromic acid in acid (0.8 ml. of 48% by weight). After 4 min. at 0°, the solution was evaporated to dryness and chloroform (10 ml.) added This solution was chromatographed on a silicic acid column and 2',3'-di-O-benzoyluridine isolated as described above under (a). The yield was 68%, m.p. 169–170°. Recrystallization afforded the pure product.

N,O$^{2'}$,O$^{3'}$-Triacetyladenosine-5' Phosphate.—To an anhydrous pyridine solution (20 ml.) of acetic anhydride (12 ml., 117 mmoles) was added dry adenosine-5' phosphate (0.302 g. of free acid, 0.87 mmole). The pale yellow solution was kept in the dark for 18 hours, and then cooled in ice-water. Water (5 ml.) was added to the cold solution and after 4 hours at room temperature, the solution was evaporated, at 5°, to one-half the volume. Pyridine (10 ml.) then was added and the solution was evaporated to about 3 ml. Pyridine (2 ml.) was again added and the evaporation procedure repeated. Ice-cold water (500 ml.) was added and the solution was lyophilized. The tan colored lyophilized powder was immediately dissolved into dry pyridine (10 ml.) and kept at −5° until used. On paper chromatography in solvent C only one nucleotide-containing spot (R_f 0.47) was observed. The absorption characteristics of this product in water (*p*H 5) were λ_{max} 272.5 mμ.

Another fast traveling spot (R_f, 0.88, λ_{max} 295 mμ) was observed which was non-nucleotidic. The spot is routinely encountered in acetylations and evidently arises from the reaction of acetic anhydride with pyridine.

2',5'-Di-O-tetrahydropyranyluridine-3' Phosphate.—To a solution of uridine-3' phosphate (0.624 g. of free acid, 1.9 mmoles) in dry dimethyl sulfoxide (10 ml.) was added trifluoroacetic acid (2 ml.) followed by dihydropyran (10 ml.). The homogeneous solution was kept at room temperature for 4 hr. and was then evaporated to about 12 ml. This solution was cooled and concd. ammonium hydroxide (10 ml.) was added. The solution was again evaporated to about 15 ml. and applied to a column of Whatman No. 1 cellulose powder (80 cm. × 3.5 cm.). The column was eluted with isopropyl alcohol–ammonium hydroxide–water (7:1:2) at a flow rate of 0.8 ml./min., 10-minute fractions being collected. The elution was followed by spotting each fraction on Whatman No. 1 paper and chromatographing in solvent A. The appropriate fractions were pooled and evaporated to dryness. The residue was dissolved in anhydrous pyridine and kept at 5°. Peak 1 (fractions 214–235) contained 2.5% of nucleotidic material as estimated spectrophotometrically. Peak 2 (fractions 247–315) accounted for 96% of the total nucleotidic material and contained 2',5'-di-O-tetrahydropyranyluridine-3' phosphate. Its ultraviolet absorption characteristics were identical with those of uridine-3'-phosphate; $\lambda_{max}^{pH\ 7}$ 262, λ_{min} 230; $\lambda_{max}^{pH\ 12}$ 261, λ_{min} 241, ϵ 262 mμ/*P* 12,350.

2',5'-Di-O-ditetrahydropyranyladenosine-3' Phosphate and N^6,O$^{3'}$,O$^{5'}$-Tritetrahydropyranyladenosine-3' Phosphate.—To a solution of trifluoroacetic acid (2 ml.) in dry dimethyl sulfoxide was added adenosine-3' phosphate (0.164 g. of free acid). To the clear solution was added 10 ml. of dihydropyran (freshly distilled over potassium hydroxide pellets) and the solution was kept at room temperature for 4 hours. After this time, the volume was reduced to about 10 ml. by evaporation at reduced pressure, and the solution was cooled in ice. Ammonium hydroxide (10 ml.) was added and the solution was again evaporated to about 10 ml. This was diluted with the solvent isopropyl alcohol–ammonium hydroxide–water (7:1:2); (10 ml.) and the total passed onto a cellulose column. The chromatographic procedure, method for isolation and storage of the products was the same as described above for the tetrahydropyranyl derivatives of uridine-3' phosphate. Fractions 212–220 contained N^6,O$^{2'}$,O$^{5'}$-tritetrahydropyranyladenosine-3'phosphate (22.2% as based on ϵ_{max} of 15,000 at 260 mμ). Fractions 228–252 contained 2',5'-di-O-tetrahydropyranyladenosine-3' phosphate (70%) and fractions 258–268 contained a small amount of 2'- and/or 5'-O-monotetrahydropyranyladenosine-3' phosphate. The R_f's of the various derivatives are in Tables I and II. The ultraviolet absorption spectrum of N,O$^{2'}$,O$^{5'}$-tritetrahydropyranyladenosine-3' phosphate showed $\lambda_{max}^{pH\ 6}$ at 264 mμ and $\lambda_{min}^{pH\ 6}$ at 229 mμ; $\lambda_{max\ in\ acid}$ 264 mμ.

When in the above experiment, the reaction time was increased to 24 hr., N,O$^{2'}$,O$^{5'}$-tritetrahydropyranyladenosine-3' phosphate was the major product (0.04 mmole) and the only other nucleotidic product was 2',5'-di-O-tetrahydropyranyladenosine-3' phosphate (0.0054 mmole). When the reaction time was shortened to 2 hours, the products were N^6,O$^{2'}$,O$^{5'}$-tri-O-tetrahydropyranyladenosine-3' phosphate (0.0065 mmole), 2',5'-di-O-tetrahydropyranyladenosine-3' phosphate (0.070 mmole) and 2'- and/or 5'-O-tetrahydropyranyladenosine-3' phosphate (0.032 mmole).

Methyluridine-3' Phosphate.—To an anhydrous pyridine solution (0.5 ml.) of 2',5'-di-O-tetrahydropyranyluridine-3' phosphate (0.05 mmole) and tri-*n*-butylamine (0.05 ml., 0.204 mmole) was added anhydrous methyl alcohol (1 ml.) and DCC (0.175 g.). After 12 hours at room temperature, water (1 ml.) was added and the solution concentrated to a gum. Water (1 ml.) was again added and the procedure repeated. A portion of this material was paper chromatographed in solvent A. No starting material was detected. The residue was dissolved in 80% acetic acid (10 ml.). After 1.5 hours at room temperature, the insoluble dicyclo-

(36) The mole of benzene probably originated in the ethyl alcohol sample used for crystallization.

(37) H. Bredereck, *Ber.*, **66**, 198 (1933).

hexylurea was removed by filtration and the filtrate was reduced in volume by evaporation at 5°. This solution was chromatographed on Whatman No. 31 using solvent A. In addition to methyluridine-3' phosphate (48%), a small amount of uridine-3' phosphate (0.17%) and, presumably, methyl 5'-O-tetrahydropyranyluridine-3' phosphate (52%) was detected. Under standard conditions, methyluridine-3' phosphate was completely degraded by pancreatic ribonuclease to give uridine-3' phosphate as the only ultraviolet absorbing product.

When the time for the 80% acetic acid hydrolysis was increased to 2.5 hr. and the product worked up as described above, methyl uridine-3' phosphate was obtained in 62% yield, uridine-2' or -3' phosphate in 20% yield and, presumably, methyl 5'-*O*-tetrahydropyranyluridine-3' phosphate in 17.5% yield. Treatment of the methyl uridine-3' phosphate now obtained with pancreatic ribonuclease indicated about 4% resistant diester, presumably methyl uridine-2' phosphate.

Synthesis of Uridylyl-(3'→5')-adenosine.—Ammonium 2',5'-di-*O*-tetrahydropyranyl-uridine-3' phosphate (0.057 mmole) as obtained above was converted to the sodium salt by treatment of its aqueous solution with sodium hydroxide (2 mmoles) and evaporation of the resulting alkaline solution. The residue was taken up in water and pressed through a small column of pyridinium Dowex-50 ion exchange resin. The column was washed with several bed volumes of the mixture pyridine–ethyl alcohol–water (2:7:1). The total effluent was evaporated at 5° with frequent additions of pyridine to remove water. Paper chromatography of an aliquot of the pyridine solution showed the protected nucleotide to be homogeneous. The nucleotide was rendered anhydrous by repeated evaporations of its solution in dry pyridine. To the anhydrous gum was added pyridine (0.5 ml.), then N,N,O$^{2'}$,O$^{3'}$-tetrabenzoyladenosine (0.079 g., 0.115 mmole) and finally DCC (0.175 g.). The homogeneous solution was kept sealed at room temperature for 4 days. Water (1 ml.) was then added and after a further 12 hr. dioxane (10 ml.) and concd. ammonium hydroxide (10 ml.). The solution was kept again for 18 hr. at room temperature and then evaporated. To the residue was added 80% acetic acid (10 ml.) and the mixture shaken in the presence of glass beads for 4 hr. at room temperature. The acetic acid was removed by evaporation at 5° and the residue chromatographed on a 9" wide strip of Whatman No. 31 paper in solvent A. Uridylyl-(3'→5')-adenosine was isolated by elution with water. The yield as estimated spectrophotometrically using an ϵ_{max} of 23,000 at 260 mμ was 34%.

A second broad band appearing between those of uridylyl-(3'→5')-adenosine and adenosine was eluted and treated with 80% acetic acid for 4 hr. at room temperature. Subsequent chromatography as described above gave a further amount of uridylyl-(3'→5')-adenosine (10.7% as based on the starting nucleotide), some uridine-2'(3') phosphate and adenosine also being produced. The synthetic sample of uridylyl-(3'→5')-adenosine was completely degraded by pancreatic ribonuclease to uridine-3' phosphate and adenosine. It was homogeneous as ascertained by paper chromatography (see Table for R_f's) and by paper electrophoresis.

Uridylyl-(3'→5')-uridine was prepared by the condensation of 2',5'-di-O-tetrahydropyranyluridine-3' phosphate (0.055 mmole) with 2',3'-di-O-benzoyluridine (0.111 mmole) in dry pyridine (0.5 ml.) in the presence of 0.175 g. of DCC. The work up and isolation of the product was identical to that described above. The yield was 31% using an extinction of 20,000 at 260 mμ. This product was degraded essentially completely on incubation with pancreatic ribonuclease.

Uridylyl-(3'→5')-cytidine. (a) **Using 2',5'-Di-O-tetrahydropyranyluridine-3' Phosphate.**—An anhydrous pyridine solution (0.5 ml.) of the protected nucleotide (0.055 mmole) and N^6,O$^{2'}$,O$^{3'}$-tribenzoylcytidine (0.06 g., 0.108 mmole) was treated with DCC (0.15 g.) for 4 days at room temperature. The work-up was described above for uridylyl-(3'→5')-uridine. The yield was 28% as determined spectrophotometrically using an ϵ_{max} of 19,700 at 264 mμ in water. In one experiment the product isolated by preparative paper chromatography contained over-lapping fluorescent non-nucleotidic material. In this case, the desired product was further purified by preparative paper electrophoresis at *p*H 7.5 using triethylammonium bicarbonate buffer (0.05 *M*).[7] The product was degraded by pancreatic ribonuclease to the extent of 95–97%.

(b) **Using 2'-O-Tetrahydropyranyl-5'-O-di-*p*-methoxytrityluridine-3' Phosphate.** The preparation was carried out exactly as above except that 2'-*O*-tetrahydropyranyl-5'-*O*-di-*p*-methoxytrityluridine-3' phosphate[6] (0.062 mmole) and 0.067 g. (0.122 mmole) of N^6,O$^{2'}$,O$^{3'}$-tribenzoylcytidine were used in the condensation reaction.

N^6-Acetyl-2',5'-di-O-tetrahydropyranyladenosine-3' Phosphate.—An aqueous solution (5 ml.) of 2',5'-di-O-tetrahydropyranyladenosine-3' phosphate (0.25 mmole of ammonium salt) was passed through a Dowex-50 (pyridinium form) column (1 × 10 cm.). The eluent was 70% aqueous pyridine solution (cold, 50 ml.) and the effluent was collected in the cold. This solution was evaporated to dryness in the cold with repeated additions of dry pyridine to ensure complete removal of water. The residue was dissolved in dry pyridine (1 ml.). Paper chromatography of an aliquot in solvent A at this stage showed the starting material to be pure. Acetic anhydride (0.5 ml.) was then added and the solution was kept at room temperature in the dark for 2 days. After this time, the solution was cooled and methyl alcohol (1 ml.) was added. The solution then was evaporated and the residual gum dissolved in water (10 ml.). Lyophilization of this solution yielded a light tan powder which was shown to be homogeneous by paper chromatography in solvent C, the R_f (see Table I) being higher than the starting material. The product was taken up in pyridine, and used immediately in the following synthesis.

Adenylyl-(3'→5')-adenosine. (a) **Using N^6-Acetyl-2',5'-di-O-tetrahydropyranyladenosine-3' Phosphate.**—To an anhydrous pyridine solution (0.25 ml.) containing pyridinium N^6-acetyl-2',5'-O-tetrahydropyranyladenosine-3' phosphate (0.0444 mmole) was added DCC (0.150 g.). After 30 min. at room temperature, N,N,O$^{2'}$,O$^{3'}$-tetrabenzoyladenosine (0.080 g., 0.117 mmole) was added followed by dry pyridine (0.5 ml.). After 4 days at room temperature, water (1 ml.) was added. This solution was kept at room temperature for 12 hr. and concd. ammonium hydroxide (2 ml.) was then added. After 3 hr., this solution was extracted with pentane (3 × 3 ml.) to remove the excess of DCC. The aqueous solution was then evaporated to a gum and the gum was dissolved in dioxane (5 ml.) and ammonium hydroxide (5 ml.) added. Glass beads were added to break up clumps of dicyclohexylurea which were present. After 20 hr. at room temperature, the solution was carefully evaporated to a gum and this was dissolved in 80% acetic acid (5 ml.). After 4 hr. at room temperature, the acetic acid was removed by evaporation and the residue was paper chromatographed on Whatman No. 31 paper using solvent A. In addition to the bands corresponding to adenosine and adenosine-3' phosphate, two new bands were detected. The main band (R_f 0.5) was, presumably, that of 5-O-tetrahydropyranyl-adenylyl-(3'→5')-adenosine and the second band (R_f 0.2) was that of adenylyl(3'→5')-adenosine. The main band was eluted with water, lyophilized and retreated with 80% acetic acid under the conditions described. Again in addition to a small amount of adenosine and adenosine-3' phosphate, 5'-O-tetrahydropyranyladenylyl-(3'→5')-adenosine and adenylyl-(3'→5')-adenosine were obtained. The total yield of adenylyl-(3'→5')-adenosine after the two acidic treatments was 25%. This product was at least 95% hydrolyzed by spleen phosphodiesterase under standard conditions and was paper chromatographically and electrophoretically homogeneous.

(b) **From N,O$^{2'}$,O$^{5'}$-Tritetrahydropyranyladenosine-3' Phosphate.**—To an anhydrous pyridine solution (0.3 ml.) of pyridinium N,O$^{2'}$,O$^{5'}$-tritetrahydropyranyladenosine-5' phosphate (464 optical density units at 260 mμ) was added N,N,O$^{2'}$,O$^{3'}$-tetrabenzoyladenosine (0.042 g., 0.062 mmole) and DCC (0.150 g.). After 4 days the product was worked up as described above. The yield of adenylyl-(3'→5')-adenosine was 10.9%. This substance was essentially completely degraded by spleen phosphodiesterase under standard conditions.

Methyl Adenosine-3' Phosphate.—To an anhydrous pyridine (0.5 ml.) solution of 2',5'-di-O-tetrahydropyranyladenosine-3' phosphate (0.0227 mmole of ammonium salt), tri-*n*-butylamine (0.025 ml.), methyl alcohol (1 ml.) and DCC (0.175 g.) were added. After 18 hr. at room temperature, the total reaction mixture was paper chromatographed in solvent A. Methyl 2',5'-O-tetrahydropyranyladenosine-3'

phosphate was essentially the only product and was isolated in 92% yield. A portion of this material (0.01 mmole) was treated with 80% acetic acid (2 ml.) for 3.25 hr. at room temperature. On chromatography in solvent A, three bands were detected. The main band (73%) corresponded to methyl adenosine-3′ phosphate. In addition there was a small amount (8% of adenosine-3′ phosphate and a band traveling faster than methyl-adenosine-3′ phosphate corresponding, presumably, to methyl-5′-O-tetrahydropyranyl-adenosine-3′ phosphate (19%). The sample of methyl adenosine-3′ phosphate thus obtained when incubated with spleen phosphodiesterase was degraded essentially completely to adenosine-3′ phosphate.

Cytidylyl-(2′ or 3′→5′)-adenosine.—To an anhydrous pyridine solution (1 ml.) of pyridinium $N^6,O^{2'},O^{3'}$-triacetyl-adenosine-5′ phosphate (0.06 mmole) of 5′-O-dimethoxytrityl-$N^6,O^{2'}(O^{3'})$-dibenzoylcytidine, DCC (150 mg.) was added and the sealed reaction mixture was kept in the dark at room temperature for 3 days. Water (0.5 ml.) was then added and after a further 12 hr., the solution was evaporated to a gum which was taken up in 80% acetic acid (5 ml.). After 3 hr. at room temperature, the solution was evaporated to dryness and the residue shaken with 25 ml. of concd. ammonium hydroxide for 12 hr. The solution was then filtered from the insoluble material and after concentration was chromatographed in solvent A on a sheet of Whatman No. 31 paper. The band corresponding to cytidylyl-(2′ or 3′→5′)-adenosine (0.012 mmole, 26%) was eluted with water. Digestion with pancreatic ribonuclease under the standard conditions followed by chromatography in solvent A showed 40% of the synthetic product to be resistant to the action of the enzyme.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF NORTHWESTERN UNIVERSITY, EVANSTON, ILL.]

Selectivity in Solvolyses Catalyzed by Poly-(4-vinylpyridine)[1]

BY ROBERT L. LETSINGER AND THOMAS J. SAVEREIDE[2]

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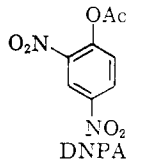
Partially protonated poly-(4-vinylpyridine) in an ethanol water solution serves as a particularly effective catalyst, relative to 4-picoline or to either non-protonated or highly protonated poly-(4-vinylpyridine), in solvolyses of nitrophenyl acetates which bear a negative electric charge. It is a poorer catalyst than 4-picoline for the solvolysis of 2,4-dinitrophenyl acetate, an electrically neutral substance. The selectivity of the partially protonated polymer with respect to charged substrates is attributed to polymer–counter ion electrostatic interaction, which increases the local concentration of an anionic substrate in the region of the polymer coil.

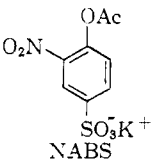
A catalyst is here termed "selective" if it distinguishes between substrates that differ only at positions far removed from the functional group undergoing transformation. Although highly selective catalysts, enzymes, are elaborated in abundance in living systems, little progress has been made in synthesizing agents with comparable properties. The action of an enzyme may be represented schematically by the sequence: E + S → E–S → E + products, where E, S and E–S represent an enzyme, the substrate or substrates, and an enzyme–substrate complex, respectively. Selectivity in these reactions may be achieved by association of the substrate with the catalyst prior to the major covalent change in the substrate.

We report in this paper a study of the catalytic properties of poly-(4-vinylpyridine) in aqueous ethanol solutions. This polymer appeared promising as a selective catalyst since in mildly acidic solution it would possess both cationic sites and basic nitrogen sites. The former should serve to bind anionic substrates[3] and the latter should act as catalytic centers for the hydrolysis of nitrophenyl esters.[4] In addition, the relative number of basic and cationic sites would be subject to control, and, as a result of the flexibility of the polymer chain, both functions would coexist within a given molecule in a great variety of spatial relationships.

2,4-Dinitrophenyl acetate (DNPA), potassium 3-nitro-4-acetoxybenzenesulfonate (NABS), 5-nitro-4-acetoxysalicylic acid (NAS) and 3-nitro-4-acetoxybenzenearsonic acid (NABA) were selected as substrates. The dinitrophenyl acetate was chosen to illustrate the solvolytic behavior of an uncharged ester; the remaining esters, to reveal the effect of charge interaction involving polymer and substrate on the course of a catalyzed solvolysis.



DNPA



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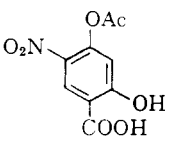

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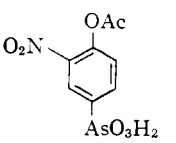

NABA

Of the previous attempts to achieve selectivity with synthetic catalysts, the most favorable results were obtained with a cross-linked sulfonated polystyrene resin, Dowex-50.[5] Whitaker and Deathe-

(1) This research was supported in part by a grant from the National Science Foundation, G7414. For a preliminary report, see R. L. Letsinger and T. J. Savereide, *J. Am. Chem. Soc.*, **84**, 114 (1962).

(2) Hercules Powder Co. Fellow, 1958; Public Health Service Research Fellow, 1960.

(3) Numerous studies of counter ion binding by polyelectrolytes have been reported. See, for example: F. T. Wall and W. B. Hill, *J. Am. Chem. Soc.*, **82**, 5599 (1960); F. T. Wall and M. J. Eitel, *ibid.*, **79**, 1550, 1556 (1957); A. M. Liquori, F. Ascoli, C. Botre, V. Cresenzi and A. Mele, *J. Polymer Sci.*, **40**, 169 (1959); M. Nagasawa and I. Kagawa, *ibid.*, **25**, 61 (1957); I. Kagawa and K. Katsuura, *ibid.*, **17**, 365 (1955); H. P. Gregor and D. H. Gold, *J. Phys. Chem.*, **61**, 1347 (1956); P. Doty and G. Ehrlich, *Ann. Rev. Chem.*, (1952); H. Morawetz, A. M. Kotliar and H. Mark, *J. Phys. Chem.*, **58**, 19 (1954). It is noteworthy that H. Ladenheim, E. M. Loebl and H. Morawetz, *J. Am. Chem. Soc.*, **81**, 20 (1959), found that poly-(4-vinylpyridine) functioned selectively in a non-catalytic reaction, quaternization with α-bromoacetamide and bromoacetate ion. See also H. Ladenheim and H. Morawetz, *ibid.*, **81**, 4860 (1959).

(4) The subject of nucleophilic catalysis in the hydrolysis of nitrophenyl esters was reviewed recently by M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

(5) J. R. Whitaker and F. E. Deatherage, *J. Am. Chem. Soc.*, **77**, 3360, 5298 (1955).