benzoylcytosine the time was 8 hr, but for those containing N^2-acetylguanine 20 hr was given. Subsequently, after evaporation of the solvent, the residue was dissolved in aqueous pyridine and subjected to paper electrophoresis (12–14 v/cm) on Whatman No. 3 MM or 31 paper in 0.05 *M* triethylammonium bicarbonate buffer (pH 7.5) at 2°. Five major ultraviolet light absorbing bands were observed. In order of decreasing mobility, these generally corresponded to: mononucleotide (band 1), nucleoside 2′,3′-cyclic phosphate (band 2), trinucleotide (band 3), unreacted dinucleoside phosphate (band 4), and benzamide (band 5) at the origin. The desired product, trinucleotide (band 3) was not clearly resolved from band 2. After cutting out the band and eluting the nucleotidic material with water containing a little ammonium hydroxide, further purification was accomplished by rechromatography on a strip of Whatman No. 40 paper in solvent A or D. The trinucleotide band (slower travelling) was eluted and the material stored frozen. The yield, which was estimated spectrophotometrically, was based on the dinucleoside phosphate recovered unchanged.

B. Using TPS. To an anhydrous pyridine solution (0.5 ml) containing the protected dinucleoside phosphate (10 μmoles) and the fully acylated mononucleotide (3 equiv with respect to the dinucleoside phosphate) was added in the drybox TPS (1.5–2 equiv with respect to the mononucleotide) and the sealed reaction mixture kept for 4–12 hr at room temperature. An equal volume of water then was added under cooling and the mixture kept at room temperature for 12–24 hr. After evaporating to dryness, the subsequent work-up including treatment with methanolic ammonia was as described in the preceding procedure.

The details of the individual syntheses leading to the various trinucleotides are given in Table II.

Studies on Polynucleotides. LII.[1] The Use of 2,4,6-Triisopropylbenzenesulfonyl Chloride for the Synthesis of Internucleotide Bonds[2]

R. Lohrmann and H. G. Khorana

Contribution from the Institute for Enzyme Research of the University of Wisconsin, Madison, Wisconsin *Received September 2, 1965*

Abstract: Detailed studies of the $C_{3'}$–$C_{5'}$ interribonucleotide bond formation using mesitylenesulfonyl chloride and 2,4,6-triisopropylbenzenesulfonyl chloride are reported. While both reagents are efficient, the latter reagent shows, as expected, a much reduced rate of sulfonylation of the 5′-hydroxyl group of nucleosides. Syntheses of a large number of dinucleoside phosphates in high yield were accomplished using triisopropylbenzenesulfonyl chloride. The use of the reagent in the stepwise synthesis of ribotrinucleotides is given in the accompanying paper.[1]

Dicyclohexylcarbodiimide and arylsulfonyl chlorides previously have been shown to be the most satisfactory reagents for the synthesis of internucleotide bonds.[3] The characteristic features of the latter class of reagents are the great rapidity of reaction and the fact that nucleotide salts of strongly basic tertiary amines can be used.[3–5] The previous studies have, however, been all in the deoxyribonucleotide field where the synthetic work utilizes the condensation of a deoxyribonucleoside 5′-phosphate with the 3′-hydroxyl group of another nucleoside or oligonucleotide component. In the ribooligonucleotide field, where the most satisfactory approach involves the condensation of a protected ribonucleoside 3′-phosphate with the 5′-hydroxyl group of a second component,[1] the reagent used almost exclusively so far has been dicyclohexylcarbodiimide,[6,7] and no systematic studies of the utility of arylsulfonyl chlorides have been reported. In undertaking such studies which are the subject of the present paper, a further consideration was the possibility of facile sulfonylation of the primary hydroxyl group of the nucleosides in contrast with the slow and rather insignificant sulfonylation of the 3′-hydroxyl group in deoxyribonucleosides.[3] In fact, Michelson[8] had already noted that under certain conditions sulfonylation of the 5′-hydroxyl group could be a major reaction in attempted activation of nucleoside 2′,3′-cyclic phosphates for polynucleotide synthesis. It therefore was considered desirable to investigate the use of a sulfonyl chloride more hindered than mesitylenesulfonyl chloride,[8–5] which has been used successfully previously in the deoxyribopolynucleotide field. It should be added that the *ortho*-methyl groups in arylsulfonyl chlorides have been shown to offer only minor steric hindrance in sulfonylation reactions[9,10] and therefore we have investigated, in particular, the use of 2,4,6-triisopropylbenzenesulfonyl chloride[11] (TPS) for the synthesis of interribonucleotidic linkage.

(1) Paper LI is by R. Lohrmann, D. Söll, H. Hayatsu, E. Ohtsuka, and H. G. Khorana, *J. Am. Chem. Soc.*, **88**, 819 (1966).

(2) This work has been supported by grants from the National Science Foundation (Grant No. GB-3342), the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178), and the Life Insurance Medical Research Fund (Grant No. G-62-54).

(3) T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, **86**, 1630 (1964). References to earlier relevant papers are contained in this paper.

(4) T. M. Jacob and H. G. Khorana, *ibid.*, **87** 2971 (1965).

(5) S. A. Narang and H. G. Khorana, *ibid.*, **87**, 2981 (1965).

(6) S. Chladek and J. Smrt, *Collection Czech. Chem. Commun.*, **29**, 214 (1964), and J. Smrt, *ibid.*, **29**, 2049 (1964), have reported on the use of *p*-toluenesulfonyl chloride and dicyclohexylcarbodiimide in the synthesis of dinucleoside phosphates. No detailed study of the arylsulfonyl chloride as phosphorylating agent was reported, however.

(7) The statement is restricted to the approach for the synthesis of interribonucleotide bonds which relies on the activation of phosphomonoester groups. The use of sulfonyl chlorides for the activation of ribonucleoside 2′,3′-cyclic phosphates has been described by R. Letters and A. M. Michelson, *J. Chem. Soc.*, 71 (1962).

(8) A. M. Michelson, *ibid.*, 979 (1962).

(9) W. S. Johnson, J. C. Collins, Jr., R. Pappo, M. B. Rubin, P. J. Krupp, W. F. Johns, J. E. Pike, and W. Bartmann, *ibid.*, **85**, 1409 (1963).

(10) J. F. Bunnett and J. Y. Bassett, Jr., *J. Am. Chem. Soc.*, **81**, 2104 (1959), have shown that in the nucleophilic attack on arylsulfonate sulfur, *o*-methyl groups show only minor effect.

(11) A. Newton, *ibid.*, **65**, 2439 (1943).

Table I. Relative Rates of Sulfonylation of 2′,3′-Di-O-benzoyluridine in Anhydrous Pyridine Using Different Arylsulfonyl Chlorides[a]

Time, hr	Sulfonylation, % TPS	*p*-Toluenesulfonyl chloride	Mesitylenesulfonyl chloride
5	0.5–2	36	25
12	5	55	42
24	9	74	58
52	17	93	77
192	36	95	87

[a] For details of reaction and analysis by paper chromatography see text.

Kinetic Studies Using TPS and Mesitylenesulfonyl Chloride. The first study carried out was on the rate of sulfonylation of the 5′-hydroxyl group of 2′,3′-di-O-benzoyluridine in dry pyridine and the results are reported in Table I. As seen, the rates of sulfonylation using mesitylenesulfonyl chloride and *p*-toluenesulfonyl chloride were not very different and this finding is in accord with the results of previous workers.[9,10] On the other hand, the use of TPS did, in fact, show markedly slower rate of sulfonylation.

For studies on the formation of internucleotidic linkage the system used was the condensation of N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3′-phosphate[1] with 2′,3′-di-O-benzoyluridine,[1] analysis being carried out for the production of cytidylyl(3′→5′)uridine (CpU) after a brief ammoniacal treatment to remove the protecting groups. The influence of the amount of TPS, as based on the nucleotide, on the rate of synthesis and the final yield of CpU was studied first and the results are given in Table II. Thus, when stoichiometric amounts

Table II. Influence of the Amount of TPS on the Rate of Internucleotide Bond Synthesis[a]

Time, hr	Yield of CpU (%) as a function of TPS equiv[b] 1.06	1.55	2.1
1	14	26	33
2	25	43	51
5	35	65	72
8	40	73	80
19	49	78	88
28	46	77	85
48	47	74	69

[a] The condensation reaction involved pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3′-phosphate (0.1 *M*) and 2′,3′-di-O-benzoyluridine (0.11 *M*). For further details see text. [b] As based on the protected mononucleotide.

of the protected nucleotide and the nucleoside are used, as in the experiment of Table II, an excess (50–100%) of the reagent is necessary to give the maximal rate and yield of the internucleotide bond. It is noteworthy that the maximal yield using stoichiometric amounts of the nucleotide and nucleoside components is excellent, being comparable to that obtained previously in the synthesis of thymidylyl(3′→5′)thymidine.[3] In another experiment (see the Experimental Section) on the synthesis of uridylyl(3′→5′)uridine under the conditions of Table II, the maximal yield obtained was again high, being 84% when 2 equiv of TPS was used and 93% when 3 equiv of the reagent was used. The results of a further experiment in which mesitylenesulfonyl chloride

Table III. Influence of the Amount of Mesitylenesulfonyl Chloride on the Rate and Final Yield of Internucleotide Bond Synthesis[a]

Time, min	Yield of CpU (%) Mesitylenesulfonyl chloride equiv[b] 1.67	2.18
70	49	53
120	60	61
300	68	70
435	71	71
1200	70	67

[a] The condensation reaction involved N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3′-phosphate (0.1 *M*) and 2′,3′-di-O-benzoyluridine (0.1 *M*). For further details see text. [b] As based on the protected mononucleotide.

was used are given in Table III. It is thus seen that while the rate of internucleotide bond synthesis using the latter reagent was somewhat higher, the maximal yield obtained (71%) was distinctly lower. It therefore may be concluded from the results of Tables II and III that the yields of interribonucleotide bond using TPS are superior when stoichiometric amounts of the nucleotide and the nucleoside component are used.

Further experiments were carried out using an excess of the nucleotidic component over the nucleoside conditions which are used most often in polynucleotide synthesis (see, *e.g.*, ref 4 and 5). Table IV gives the results

Table IV. Influence of the Amount of TPS on he Rate of Internucleotide Bond Synthesis from Pyridinium N,O$^{2'}$,O$^{5'}$-Triacetylcytidine 3′-Phosphate (0.2 *M*) and 2′,3′-Di-O-benzoyluridine (0.1 *M*)

Time, hr	Yield of CpU (%) as a function of TPS equiv[a] 1.07	1.5	2.0
1	24	37	43
2	43	64	68
3	54	74	78
5	66	84	83
9	74	85	85
35	79	88	83

[a] As based on the protected mononucleotide.

of a study in which a 100% excess of the nucleotide was used and the amount of TPS was varied. It is noted that, in contrast with the results of Table II, the reaction proceeded to give a rather high yield (79%) even when 1 equiv of TPS, relative to the nucleotide, was used. The main effect of using a higher ratio of TPS was on the rate of internucleotide bond synthesis, although the yield was also somewhat higher.

A comparison was also made of TPS and mesitylenesulfonyl chloride using an excess of the nucleotidic component. The results, which are given in Table V, showed again that while the rate of internucleotide bond synthesis was higher with mesitylenesulfonyl chloride, the ultimate yield (77%) obtained with this reagent was lower than that (89%) obtained with TPS.

In Table VI are shown the results of another series of experiments in which an excess of the protected nucleoside over the nucleotide was used. It is seen that under these conditions, practically identical yields were obtained with both TPS and mesitylenesulfonyl chloride.[12]

(12) In a similar experiment, picryl chloride (F. Cramer, R. Wittmann, K. Daneck, and G. Weimann, *Angew. Chem.*, 75, 92 (1963)) also was

Table V. Rate of Synthesis and the Yield of Cytidylyl(3′→5′)uridine from Pyridinium N,O²′,O⁵′-Triacetylcytidine 3′-Phosphate (0.2 *M*) and 2′,3′-Di-O-benzoyluridine (0.092 *M*) Using Mesitylenesulfonyl Chloride and Triisopropylbenzenesulfonyl Chloride[a]

Time, min	Yield of CpU, %: Mesitylenesulfonyl chloride	Yield of CpU, %: TPS
35	61	41
70	67	62
115	71	76
150	77	84
180	77	...
240	77	89
360	74	89
600	72	88

[a] 2 equiv as based on the mononucleotide. For details see text.

With the latter reagent, the rate again was higher, the maximal yield being obtained within 2 hr. The main result, that the yields are comparable under the conditions of Table VI, would be expected since sulfonylation, which obviously occurs slower than phosphorylation, does not effectively deplete the nucleosidic component.

Table VI. Influence of the Amount of 2′,3′-Di-O-benzoyluridine on the Rate of Synthesis of Cytidylyl(3′→5′)uridine from Pyridinium N,O²′,O⁵′-Triacetylcytidine 3′-Phosphate Using TPS and Mesitylenesulfonyl Chloride[a]

Time, hr	Yield of CpU, %: TPS, nucleoside equiv 1.5	TPS, nucleoside equiv 2.0	Mesitylenesulfonyl chloride, nucleoside equiv 1.7
0.5	26	26	...
1	37	44	74
2	58	64	82
3	69	76	...
4	76	83	...
5			84
6	84	85	
8	85	87	
12	84	85	

[a] 2.18 equiv as based on the nucleotide.

Finally, the rate of condensation of a protected dinucleoside phosphate, 2′-O-acetyluridylyl(3′→5′)-N,-O²′,O⁵′-tribenzoyluridine,[13] with the protected nucleotide N,O²′,O⁵′-triacetylcytidine 3′-phosphate was studied, the amount of the product, cytidylyl(3′→5′)-uridylyl(3′→5′)uridine, being measured after removal of the protecting groups. The results, which are reported in Table VII, showed that the rate of internucleotide bond synthesis was slower than that obtained in the above experiments in which a protected nucleoside was used. The maximum yield in 10 hr was 70%.

The Synthesis of Dinucleoside Phosphates. To establish the generality of the usefulness of TPS a large number of condensations were performed between protected ribonucleoside 3′-phosphates and protected nucleosides. The details of the conditions used and the yields of the dinucleoside phosphates obtained after removal of the protecting groups are assembled in Table VIII.[14] As is seen, the yields of the desired products were uniformly high (76–90%). That the products contained exclusively the C_3–C_5′ internucleotidic linkage was shown by appropriate enzymatic tests of selected members. Thus adenylyl(3′→5′)-uridine and guanylyl(3′→5′)uridine both were completely degraded by spleen phosphodiesterase to the respective mononucleotides and the nucleoside, and, similarly, cytidylyl(3′→5′)uridylyl(3′→5′)uridine was completely susceptible to the action of pancreatic ribonuclease.

Many additional applications of TPS in the synthesis of trinucleotides have been provided in the preceding paper.[1]

Concluding Remarks. Detailed kinetic studies on internucleotide bond synthesis using arylsulfonyl chlorides have been reported for the first time and the total information obtained is of general value in the synthesis of polynucleotides. Triisopropylbenzenesulfonyl chloride has been demonstrated to be a useful reagent for the synthesis of internucleotide bonds. The yields obtained using this reagent were superior to those obtained using mesitylenesulfonyl chloride unless an excess of the nucleosidic component was used. It seems clear that these results were due to the much reduced rate of sulfonation relative to that of phosphorylation of the hydroxylic groups in nucleosides.

Table VII. Rate of Synthesis of Cytidylyl(3′→5′)uridylyl(3′→5′)uridine from Pyridinium N,O²′,O⁶′-Triacetylcytidine 3′-Phosphate and 2′-O-Acetyluridylyl(3′→5′)-N,O²′,O³′-tribenzoyluridine in the presence of TPS[a]

Time, min	Yield of CpUpU, %[b]
30	12
100	31
210	54
270	61
330	67
615	70
1440	76

[a] For details see text. [b] As based on the protected dinucleoside phosphate.

Experimental Section

General Methods and Materials. Paper chromatography was carried out using a descending technique on Whatman No. 1, No. 40, or No. 3 MM paper. The solvent systems used were: solvent A, isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:2, v/v); solvent B, *n*-propyl alcohol–concentrated ammonium hydroxide–water (55:10:35, v/v); solvent C, ethanol–1 *M* ammonium acetate (pH 7.5)(7:3, v/v); solvent D, isobutyric acid–concentrated ammonia–water (pH 3.7)(60:1:33, v/v). R_f values of different compounds are listed in Table IX.

Paper electrophoresis for analytical purposes was carried out using Whatman 3 MM paper in a high-voltage apparatus in which the paper was immersed in a water-cooled, high-boiling petroleum fraction (Varsol). The buffers used were phosphate buffer (pH 7.1) (0.03*M*) and ammonium formate buffer (pH 2.7)(0.05 *M*). The electrophoretic mobilities of different compounds are included in Table IX. Preparative electrophoresis was

tried as a reagent. Using an excess of the nucleosidic component and giving a prolonged time (50 hr) for reaction, internucleotide bond synthesis proceeded to the extent of 79%.

(13) Y. Lapidot and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3852 (1963).

(14) The system of abbreviations for presentation of the protected derivatives is as has been described in the preceding paper.[1]

Table VIII. The Synthesis of Dinucleoside Phosphates from Protected Mononucleotides and Protected Nucleosides Using Triisopropylbenzenesulfonyl Chloride

Dinucleoside phosphate	Protected nucleotide[a]	Amount, mmole	Protected nucleoside[a]	Amount, mmole	Amount of TPS, mmole	Reaction time, hr	Time of ammonia treatment, hr	Yield,[b] %
ApU	BzO-A^{Bz}-OBz-p	0.1	HO-U-$(OBz)_2$	0.15	0.2	8	12	90
CpA	AcO-C^{Ac}-OAc-p	0.05	HO-A^{Bz_2}-$(OBz)_2$	0.10	0.13	5	12	81
CpC	AcO-C^{Ac}-OAc-p	0.05	HO-C^{Bz}-$(OBz)_2$	0.10	0.13	10	12	89
CpG	AcO-C^{Ac}-OAc-p	0.05	HO-G^{Ac}-$(OAc)_2$	0.10	0.13	5	20	84
CpU	AcO-C^{Ac}-OAc-p	0.05	HO-U-$(OBz)_2$	0.10	0.13	8	3	88
GpA	AcO-G^{Ac}-OAc-p	0.05	HO-A^{Bz_2}-$(OBz)_2$	0.10	0.11	8	20	76
GpC	AcO-G^{Ac}-OAc-p	0.05	HO-C^{Bz}-$(OBz)_2$	0.10	0.11	8	20	81
GpG	AcO-G^{Ac}-OAc-p	0.05	HO-G^{Ac}-$(OAc)_2$	0.10	0.15	8	20	79
GpU	AcO-G^{Ac}-OAc-p	0.05	HO-U-$(OBz)_2$	0.10	0.10	8	20	82
UpU	AcO-U-OAc-p	0.05	HO-U-$(OBz)_2$	0.06	0.11	3	1	84
	AcO-U-OAc-p	0.05	HO-U-$(OBz)_2$	0.06	0.15	15.5	1	93
	AcO-U-OAc-p	0.05	HO-U-$(OBz)_2$	0.12	0.11	3	1	97

[a] The system of abbreviations used for specification of protected mononucleotides and protected nucleosides has been described in the preceding paper.[1] [b] The yields of dinucleoside phosphates are calculated spectrophotometrically using molar extinction values obtained by summation of the established molar extinction values (at particular wavelengths) of the constituent nucleosides. Hypochromicity thus was ignored.

Table IX. Paper Chromatography and Electrophoresis of Different Compounds

Compound	R_f				Mobility	
	Solvent A	Solvent B	Solvent C	Solvent D	pH 7.1	pH 2.7
Uridine 3′-phosphate	0.05	0.36	0.29	0.30	1.0	1.0
Uridine	0.46	0.57	0.70	0.52	0.0	0.0
Cytidine 3′-phosphate	0.07	0.38	0.23	0.46	0.96	0.06
ApU	0.21	0.42	0.36	0.49	0.36	0.11
CpA	0.23	0.45	0.33	0.57	0.37	−0.42
CpC	0.20	0.44	0.36	0.49	0.39	−0.58
CpG	0.09	0.35	0.30	0.34	0.36	0.0
CpU	0.17	0.41	0.44	0.36	0.40	0.0
GpA	0.12	0.37	0.26	0.47	0.33	0.0
GpC	0.09	0.34	0.26	0.37	0.37	0.0
GpG	0.03	0.25	0.26	0.25	0.30	0.29
GpU	0.10	0.34	0.32	0.25	0.38	0.52
UpU	0.16	0.41	0.46	0.26	0.41	0.75
CpUpU	0.054	0.27	0.23	0.19	0.63	0.59

carried out at about 4° on Whatman 3 MM paper strips (20 cm wide) immersed in carbon tetrachloride using 0.05 *M* triethylammonium bicarbonate buffer (pH 7.5). The potential applied across the paper was about 15 v/cm.

Enzymatic degradations of the synthetic dinucleoside phosphates with pancreatic ribonuclease and spleen phosphodiesterase were performed as described in earlier papers. Anhydrous pyridine used in condensation reactions was prepared as described previously and stored over molecular sieves (Linde molecular sieves, 4 × A).

Protected Nucleosides. N,N′,$O^{2'}$,$O^{3'}$-Tetrabenzoyladenosine, N,$O^{2'}$,$O^{3'}$-tribenzoylcytidine, N,$O^{2'}$,$O^{3'}$-triacetylguanosine, and 2′,3′-di-O-benzoyluridine were prepared as described previously.

Fully Acylated Nucleoside 3′-Phosphates. Pyridinium N,$O^{2'}$,$O^{5'}$-tribenzoyladenosine 3′-phosphate, pyridinium N,$O^{2'}$,$O^{5'}$-triacetylcytidine 3′-phosphate, pyridinium N,$O^{2'}$,$O^{5'}$-triacetylguanosine 3′-phosphate, and pyridinium 2′,5′-di-O-acetyluridine 3′-phosphate were synthesized as reported in earlier papers.

2,4,6-Triisopropylbenzenesulfonyl Chloride. This was prepared by a modification of the procedure described in the literature.[11] To a mixture of triisopropylbenzene (100 ml) in chloroform (500 ml), magnetically stirred at 0°, was added chlorosulfonic acid (120 ml) within 15 min under cooling and exclusion of moisture. The stirring was continued for 45 min at room temperature and the mixture then was poured onto crushed ice. The product was extracted with chloroform (500 ml), dried over sodium sulfate, and freed from the solvent *in vacuo* at 30°. The white residue was dissolved in warm pentane, and the solution was filtered and concentrated to about 200 ml. Triisopropylbenzenesulfonyl chloride crystallized slowly on standing and was collected by filtration and washed with prechilled (−30°) pentane. After drying *in vacuo* over phosphorus pentoxide the yield was 75.1 g, mp 96–97°, that reported in literature being 97–98°.[11] Further concentration of the mother liquor gave a second crop (31.4 g), mp 94–96°. The total yield was 106.5 g (86%).

For analysis and for use as condensing agent the above product was recrystallized again from pentane and dried over phosphorus pentoxide under vacuum.

Anal. Calcd for: $C_{15}H_{23}SO_2Cl$ (302.85): C, 59.48; H, 7.65; Cl, 11.71. Found: C, 59.53; H, 7.68; Cl, 11.41.

Rates of Sulfonylation of Protected Nucleosides. A. 2′,3′-Di-O-benzoyluridine. Portions (0.5 ml each) of a standard solution (0.08 *M*) of 2′,3′-di-O-benzoyluridine in anhydrous pyridine were treated with TPS (0.083 mmole, about 2 equiv), *p*-toluenesulfonyl chloride (0.082 mmole, about 2 equiv), and mesitylenesulfonyl chloride (0.087 mmole, about 2 equiv). Aliquots were removed at intervals and treated with an equal volume of water. After standing at room temperature for a few hours, acetone was added to effect homogenization and the solutions were applied to Whatman No. 1 paper which previously had been treated twice with a mixture (1:4, v/v) of dimethyl sulfoxide and benzene. The chromatograms were developed with carbon tetrachloride and the spots corresponding to dibenzoyluridine and its sulfonylation products (in one run[15] the R_f values of the sulfonylation products relative to dibenzoyluridine were 2.1, 2.7, and 6.2 in the reaction with *p*-toluenesulfonyl chloride, mesitylenesulfonyl chloride, and triisopropylbenzenesulfonyl chloride, respectively) were eluted with ethanol and their concentrations determined spectrophotometrically. The results are given in Table I.

(15) The R_f values varied considerably in different runs due to changes in humidity in the tank.[16]

(16) R. Lohrmann and H. G. Khorana, *J. Am. Chem. Soc.*, 86, 4188 (1964).

B. N,O$^{2'}$,O$^{3'}$-Tribenzoyluridine. An experiment identical with that described above under A showed about 15% sulfonylation in 52 hr with TPS.

C. 5'-O-Tritylthymidine. An experiment identical with that described above under A showed only a trace of sulfonylation with TPS in 24 hr.

Kinetic Studies of the Synthesis of Cytidylyl(3'→5')uridine with Arylsulfonyl Chlorides as Condensing Agents. A. The Influence of the Amount of TPS. An anhydrous pyridine (0.5 ml) solution of pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3'-phosphate (0.05 mmole) and 2',3'-di-O-benzoyluridine (0.055 mmole) was prepared by repeated evaporation of added pyridine and finally adding the required volume of pyridine. A series of reaction vessels thus was set up and each was treated with varying amounts of TPS (Table II) or mesitylenesulfonyl chloride (Table III). Aliquots were removed from the sealed reaction mixtures at different time intervals and treated with an equal volume of water under cooling. After standing overnight, these were treated with 7 *M* ammonium hydroxide for 3 hr and the products then were examined by paper chromatography in solvent A. The yield of cytidylyl(3'→5')-uridine was determined spectrophotometrically after elution of the spot and measurement of the optical density at pH 6.9. The yields were calculated using the extinction values of 17,300 at 262 mμ for cytidylyluridine and the value of 7300 for cytidine 3'-phosphate or cytidine 2',3'-cyclic phosphate. The results are shown in Table II.

B. Influence of the Amount of Mesitylenesulfonyl Chloride. The experiment was carried out exactly as described above under A, the amounts of mesitylenesulfonyl chloride being used as shown in Table III. The results of the rate and the final yield of cytidylyl-uridine are given in the same table.

C. Influence of the Amount of TPS Using an Excess of Mononucleotidic Component. Three reaction mixtures were set up as described above under A except that the concentration of pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3'-phosphate was 0.2 *M* (twice that in experiment under A). The amounts (as based on the mononucleotide) of TPS used are given in Table IV which also lists the results obtained.

D. Comparison of Mesitylenesulfonyl Chloride and TPS. Two reaction mixtures were set up as described above under A using pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3'-phosphate (0.2 *M*) and 2',3'-di-O-benzoyluridine (0.092*M*). Two equivalents of the arylsulfonyl chloride as based on the mononucleotide was used. The results are given in Table V.

E. Effect of the Amount of 2',3'-Di-O-benzoyluridine. A series of reaction mixtures was set up as described above under A, the amount of dibenzoyluridine being varied as shown in Table VI. The amounts of TPS and mesitylenesulfonyl chloride used were 2.18 equiv throughout as based on pyridinium N,O$^{2'}$,O$^{5'}$-triacetyl-cytidine 3'-phosphate. The work-up was as described above and the results are given in Table VI.

Studies of the Synthesis of Uridylyl(3'→5')uridine Using TPS. A. An anhydrous pyridine solution (1 ml) of pyridinium 2',5'-di-O-acetyluridine 3'-phosphate (0.05 mmole) and 2',3'-di-O-benzoyluridine (0.06 mmole) was treated with TPS (0.11 mmole) for 3 hr at room temperature. An equal volume of water then was added and, after standing further for several hours, the mixture was evaporated *in vacuo* with added pyridine. The anhydrous residue was kept in 15 *M* methanolic ammonia for 1 hr at room temperature and the products were examined by high-voltage paper electrophoresis. The yield of uridylyluridine was determined spectrophotometrically to be 84%.

B. The experiment was exactly as described above under A except that twice the amount of dibenzoyluridine (0.12 mmole) was used. The yield of dinucleoside phosphate as based on the mononucleotide was 97%.

C. The experiment was as described above under A except that the amount of TPS used was increased to 3 equiv. After 15.5 hr of reaction time the yield of uridylyluridine was 93% (Table VIII).

Rate of Synthesis of Cytidylyl(3'→5')uridylyl(3'→5')uridine. An anhydrous pyridine solution (0.5 ml) of pyridinium N,O$^{2'}$,-O$^{5'}$-triacetylcytidine 3'-phosphate (0.031 mmole) and 2'-O-acetyl-uridylyl(3'→5')-N,O$^{2'}$,O$^{3'}$-tribenzoyluridine[1] (0.01 mmole) was treated with TPS (0.046 mmole) and the sealed reaction mixture was kept at room temperature. Aliquots were removed at intervals with exclusion of moisture and worked up as described above for cytidylyl(3'→5')uridine. The slowest band (R_f 0.05) on paper chromatography in solvent A corresponded to CpUpU. Other ultraviolet light absorbing bands detected were: cytidine 3'-phosphate, uridylyl(3'→5')uridine, cytidine 2',3'-cyclic phosphate, benzamide, and triisopropylbenzenesulfonic acid.

The yield of CpUpU (using an extinction value of 27,300 at 262 mμ, pH 6.9) as based on unreacted UpU in different aliquots is given in Table VII.

The Synthesis of Dinucleoside Phosphates. General Procedure. A mixture of the protected nucleotide (0.05 mmole) and the protected nucleoside (0.10 mmole) was rendered anhydrous by repeated evaporation of added dry pyridine leaving about 0.5 ml of pyridine in the reaction flask. Then, TPS (0.10–0.15 mmole) was added under exclusion of moisture and the reaction mixture was kept sealed for 8 hr. Water (0.5 ml) then was added under cooling and the mixture was kept for 12–24 hr at room temperature. After addition of 50% aqueous pyridine (5 ml) the mixture was extracted twice with carbon tetrachloride to remove unreacted nucleoside. (This extraction was omitted in the case of protected guanosine.) The carbon tetrachloride layer was back extracted with aqueous pyridine, the combined aqueous pyridine phase was evaporated to dryness, and the residual gum was rendered anhydrous by evaporation of added pyridine. Cold 15 *M* methanolic ammonia (7 ml) was added to the residue and the sealed reaction mixture was shaken for an appropriate time at room temperature. (For duration of this treatment in different cases see Table VIII.) The solution then was evaporated and the products were separated by preparative paper electrophoresis using triethylammonium bicarbonate buffer (0.05 *M*) in the cold. The band corresponding to the dinucleoside phosphate was eluted with aqueous ammonia (0.5%) and the eluate lyophilized.

The yields of the dinucleoside phosphates were determined spectrophotometrically after separation of the products either by paper chromatography of aliquots in solvent A or after separation by paper electrophoresis.

The dinucleoside phosphates prepared using different protected nucleotides and nucleosides, the conditions used in individual syntheses, and the yields obtained are assembled in Table VIII.