The Synthesis of Oligoribonucleotides. Part X.[1] Preparation of 2′,3′-Cyclic Phosphates of Ribonucleosides and Diribonucleoside Phosphates *via* Phosphotriester Intermediates

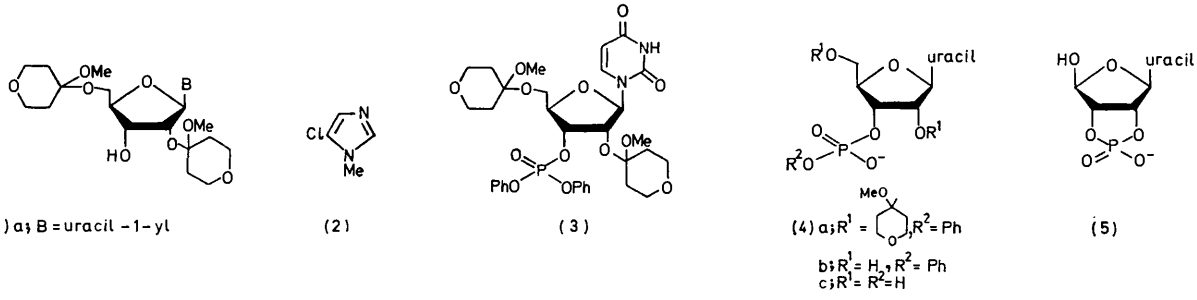
By **Jacques H. van Boom** * and **Jan F. M. de Rooy**, Gorlaeus Laboratoria der Rijksuniversiteit, Postbus 75, Leiden, The Netherlands
Colin B. Reese,*† University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW

Both 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine (1a) and 2,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridylyl-(3′ → 5′)-2′-*O*-4-methoxytetrahydropyran-4-yluridine *o*-chlorophenyl ester (7a) were converted into their diphenyl phosphate esters [(3) and (7b), respectively] by treatment with diphenyl phosphorochloridate in the presence of 5-chloro-1-methylimidazole (2). These phosphotriester derivatives, which were isolated in good yields, were converted into the corresponding mono- and di-nucleotide phenyl esters [(4a) and (8a), respectively] by alkaline hydrolysis. The latter compounds were treated with dilute acid (to remove the methoxytetrahydropyranyl groups) and the products were then treated with base, under very mild conditions, to give good yields of uridine 2′,3′-cyclic phosphate (5) and the dinucleoside phosphate cyclic phosphate (9), respectively.

We have recently come to believe that for significant progress to be made in the development of methods for the chemical synthesis of oligonucleotides in both the ribose and deoxyribose series, it is necessary to protect the internucleotide linkages and thereby work with phosphotriester intermediates. Our preliminary investigations[2] have indicated that aryl groups are particularly suitable for the protection of internucleotide linkages. We intend shortly to report the detailed results of these investigations but wish now to demonstrate the versatility of phosphotriester intermediates by describing convenient procedures for the synthesis of 2′,3′-cyclic phosphates of ribonucleosides and diribonucleoside phosphates.

As part of a systematic approach to oligoribonucleotide synthesis[3] we have developed[4] a procedure for the preparation of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yl derivatives (1) of ribonucleosides (and *N*-acylribonucleosides). Crystalline building blocks of this type (1) have been obtained[4] from each of the principal ribonucleosides. When 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine (1a) was treated with a slight excess of diphenyl phosphorochloridate in pyridine solution at 20°, none of the desired 3′-diphenyl phosphate ester (3) could be detected in the products, even after 48 h. This result was not completely unexpected in that difficulty in the phosphorylation of comparatively hindered hydroxy-functions with diphenyl phosphorochloridate had been encountered previously.[5] However, when (1a) was treated with a similar slight excess of diphenyl phosphorochloridate in acetonitrile solution, in the presence of 5-chloro-1-methylimidazole[6] (2), phosphorylation occurred readily at 20° and 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine 3′-diphenyl phosphate (3) was obtained in 60% yield. The catalytic effect of 5-chloro-1-methylimidazole (2) in phosphorylation had previously been observed[2b,3] with phenyl phosphorodichloridate in oligoribonucleotide synthesis. This imidazole derivative (2) may prove to be generally useful as a catalyst in the acylation of sterically hindered alcohols.

Treatment of (3) with 0·1M-alkali in aqueous dioxan at 20° gave the corresponding monophenyl phosphate (4a), which was isolated as its ammonium salt in virtually quantitative yield. No by-products could be detected by t.l.c. or paper electrophoresis. Rate studies carried out in aqueous dioxan (4 : 1 v/v) solution revealed that the hydrolysis of (3) displays first-order kinetics with half-times of hydrolysis of *ca.* 18 and 115 min in 0·1M-sodium hydroxide and 5·35M-ammonia, respectively.



† *Present address:* Department of Chemistry, King's College, Strand, London, WC2R 2LS.

[1] Part IX, J. H. van Boom, G. R. Owen, J. Preston, T. Ravindranathan, and C. B. Reese, *J. Chem. Soc.* (*C*), 1971, 3230.

[2] (*a*) C. B. Reese and R. Saffhill, *Chem. Comm.*, 1968, 767; (*b*) J. H. van Boom, P. M. J. Burgers, G. R. Owen, C. B. Reese, and R. Saffhill, *ibid.*, 1971, 869; (*c*) N. J. Cusack, C. B. Reese, and J. H. van Boom, *Tetrahedron Letters*, 1973, 2209.

[3] C. B. Reese, Colloques Internationaux du C.N.R.S., Paris, 1970, vol. 182, p. 319.

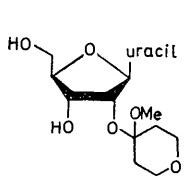
[4] D. P. L. Green, T. Ravindranathan, C. B. Reese, and R. Saffhill, *Tetrahedron*, 1970, **26**, 1031.

[5] F. Wold and C. E. Ballou, *J. Amer. Chem. Soc.*, 1959, **81**, 2368.

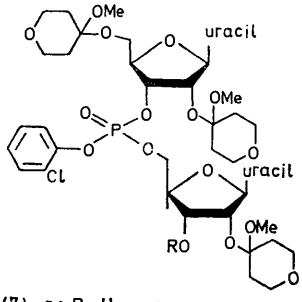
[6] F. F. Blicke and H. C. Godt, jun., *J. Amer. Chem. Soc.*, 1954, **76**, 3653.

Acidic hydrolysis (pH 2) of (4a) at 20° gave uridine 3′-phenyl phosphate (4b), contaminated with a small amount (*ca.* 2%) of uridine 2′,3′-cyclic phosphate (5). The structure of (4b) follows from the observation that it undergoes quantitative digestion in the presence of pancreatic ribonuclease to give uridine 3′-phosphate (4c) and from its ready base-catalysed cyclization to uridine 2′, 3′-cyclic phosphate (5) (see later). The foregoing procedure suggests a convenient chemical synthesis of aryl esters of 3′-ribonucleotides from the corresponding ribonucleosides. Other workers [7] have prepared α-naphthyl esters of 3′-ribonucleotides by phosphorylation of 2′,5′-bis-*O*-tetrahydropyranyl-ribonucleosides with α-naphthyl phosphorodichloridate or, more effectively, with α-naphthyl phosphate and *NN*′-dicyclohexylcarbodi-imide in pyridine solution.

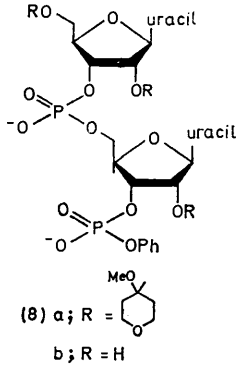
Uridine 3′-phenyl phosphate (4b) was found to be extremely labile to base; when it was dissolved in aqueous 0·155M-ammonia at 20°, it underwent a rapid and quantitative conversion into uridine 2′,3′-cyclic phosphate (5). Under these conditions, the cyclization reaction displayed first-order kinetics with $t_{\frac{1}{2}}$ 3 min. A large number of procedures have been reported for the preparation of ribonucleoside 2′,3′-cyclic phosphates.[8]



(6)

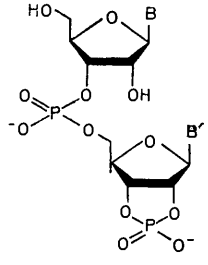


(7) a; R = H
b; R = $(PhO)_2P{=}O$



(8) a; R =

b; R = H



(9) a; B = B′ = uracil-1-yl

While it is not claimed that the present method is one of the most convenient for the preparation of such derivatives of the four common ribonucleosides, it is generally applicable and leads to products which are free from nucleotide impurities. Furthermore, it is readily adaptable to the synthesis of 2′,3′-cyclic phosphates of oligoribonucleotides with terminal 2′,3′-diol systems (see later).

2′,5′-Bis-*O*-4-methoxytetrahydropyran-4-ylribonucleosides (1) have been designed [3,4] to function as terminal building blocks in oligoribonucleotide synthesis. Thus treatment of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine (1a) with a slight excess of *o*-chlorophenyl dihydrogen phosphate and 2·5 mol. equiv. of 2,4,6-triisopropylbenzenesulphonyl chloride [9] in pyridine solution at 20° followed, after 4 h, by the addition of a slight excess of 2′-*O*-4-methoxytetrahydropyran-4-yluridine * (6) gave the partially protected dinucleoside phosphate (7a),† isolated in 70% yield.

Reaction between (7a) and diphenyl phosphorochloridate in the presence of 5-chloro-1-methylimidazole [6] (2), under the conditions used for the preparation of (3), gave the corresponding 3′-diphenyl phosphate (7b), which was isolated as a homogeneous (t.l.c.) solid in 60% yield. Hydrolysis of this material with 0·1M-alkali in aqueous dioxan gave the protected dinucleotide (8a) as the sole nucleotide product (t.l.c. and paper electrophoresis). As in the preparation of the corresponding mononucleotide ester (4b), the methoxytetrahydropyranyl groups were then removed by acidic hydrolysis under mild conditions. The dinucleotide phenyl ester (8b), so obtained, was contaminated with 1—2% of the dinucleoside phosphate cyclic phosphate (9a). Treatment of (8b) with dilute ammonia under the same conditions as those required for the cyclization of uridine 3′-phenyl phosphate (4b) (see before) gave the desired dinucleoside phosphate cyclic phosphate (9a), contaminated with *ca.* 1% of uridine 2′,3′-cyclic phosphate (5).

Diribonucleoside phosphate 2′,3′-cyclic phosphates (9) are potentially useful intermediates in oligoribonucleotide synthesis. Thus, for example, treatment of (9; B ≠ guanin-9-yl, B′ = guanin-9-yl) with ribonuclease T_1 would promote the hydrolysis of the cyclic phosphate to give the corresponding dinucleotide while treatment of the same group of substrates with ribonuclease T_1 in the presence of an excess of a ribonucleoside can lead [10]

* In this reaction, (6) undergoes phosphorylation on its less hindered 5′-hydroxy-group with a high degree of regioselectivity. However, we do not plan to use chain-extension units with unprotected 3′-hydroxy-groups in the stepwise synthesis of large oligoribonucleotides.

† For a preliminary account of the preparation of (7a), see ref. 2*b*.

[7] R. Kole and H. Sierakowska, *Acta Biochim. Polon.*, 1971, **18**, 187; R. Kole, H. Sierakowska, and D. Shugar, *Biochim. Biophys. Acta*, 1972, **289**, 323.

[8] H. G. Khorana, 'Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest,' Wiley, New York and London, 1961, ch. 3; D. M. Brown 'Advances in Organic Chemistry: Methods and Results,' vol. 3, ed. R. A. Raphael, E. C. Taylor, and H. Wynberg, Interscience, New York and London, 1963, p. 118 *et seq.*; A. M. Michelson, 'The Chemistry of Nucleosides and Nucleotides,' Academic Press, London and New York, 1963, p. 118 *et seq.*; T. Ueda and J. J. Fox, *Adv. Carbohydrate Res.*, 1967, **22**, 307; D. Shugar in 'Methods in Enzymology,' Vol. XII, Part A, ed. L. Grossman and K. Moldave, Academic Press, New York and London, 1967, p. 131 *et seq.*

[9] R. Lohrmann and H. G. Khorana, *J. Amer. Chem. Soc.*, 1966, **88**, 829.

[10] D. Grünberger, A. Holý, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1968, **33**, 286.

to moderate yields of the appropriate trinucleoside diphosphates. Pancreatic ribonuclease would be expected [11] to catalyse similar reactions in the case of (9; B = adenin-9-yl or guanin-9-yl; B′ = uracil-1-yl or cytosin-1-yl).

Several procedures for the preparation of dinucleoside phosphate 2′,3′-cyclic phosphates (9) have been described previously [12] but we believe that the present method, based on the phosphotriester approach, is experimentally the most satisfactory: if so desired, it can be carried out on a comparatively large scale and it leads to good yields of high-quality products, free from impurities containing 2′ ⟶ 5′-internucleotide linkages. The small amounts of impurities actually obtained could, if necessary, be removed by cellulose anion-exchange chromatography. Finally, the present method is readily adaptable to the synthesis of 2′,3′-cyclic phosphates of trinucleoside diphosphates and larger oligoribonucleotides.

EXPERIMENTAL

U.v. absorption spectra were measured with a Cary C15 recording spectrophotometer. N.m.r. spectra were measured at 100 MHz with a JEOL JNM PS 100 spectrometer; tetramethylsilane was used as an internal standard.

T.l.c. plates, coated with Merck Kieselgel GF_{254} and Macherey Nagel-cellulose F_{254}, were developed in solvent systems A [$CHCl_3$–MeOH (92 : 8 v/v)] and B [aqueous M-NH_4OAc–EtOH (3 : 7 v/v)], respectively. Cellulose t.l.c. (solvent system B) was also carried out on Merck DC-Alufolien Cellulose F_{254}. Paper electrophoresis on S + S No. 2403 paper was carried out in a Camag flat-plate apparatus with the following buffer solutions: C, 0·05M-sodium phosphate (pH 8·0); D, 0·05M-sodium citrate (pH 3·5); and E, 0·1M-sodium borate (pH 8·6). Merck Kieselgel H was used for adsorption chromatography.

Acetonitrile was dried by stirring over calcium hydride for 2 days and then distilled from a small quantity of phosphorus pentoxide; it was stored in wax-sealed bottles over No. 4A molecular sieves. 5-Chloro-1-methylimidazole was wax-sealed in ampoules which were kept at −20° in the dark.

2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridine * (1a).—To a stirred solution of 3′-*O*-acetyl-2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine [4] (5·0 g, 9·7 mmol) in anhydrous methanol (45 ml) at 20° was added methanolic M-sodium methoxide (10 ml, 10 mmol). After 4 h, the solution was neutralized (to pH 7) with aqueous 0·5M-potassium dihydrogen phosphate. The products were then concentrated under reduced pressure and extracted with chloroform (3 × 50 ml). The dried ($MgSO_4$) extracts were evaporated under reduced pressure to a gum, which was triturated with anhydrous ether (80 ml) to give a finely powdered solid (4·49 g, 98%). When a solution of this material in warm chloroform–cyclohexane (1 : 2 v/v; *ca.* 45 ml) was allowed to cool, 2′,5′-*bis*-O-4-*methoxytetrahydropyran-4-yluridine* (Found: C, 52·7; H, 6·7; N, 5·65. $C_{21}H_{32}N_2O_{11}$ requires: C, 53·4; H, 6·8; N, 5·9%) was obtained as a crystalline solid (3·44 g, 75%), m.p. 97—100°; R_F 0·33 (system A); $\lambda_{max.}$ (95% EtOH) 262 (ε 9700), $\lambda_{min.}$ 230 nm (ε 2400); τ [$(CD_3)_2SO$–D_2O] 2·16 (1H, d, *J* 8 Hz), 3·98 (1H, d, *J* 7 Hz), 4·20 (1H, d, *J* 8 Hz), 5·64 (1H, dd, *J* 5 and 7 Hz), 5·96 (2H, m), 6·44 (10H, m), 6·86 (3H, s), 6·96 (3H, s), and 8·26 (8H, m).

2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridine 3′-Diphenyl Phosphate (3).—(*a*) To a stirred solution of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine (0·472 g, 1·0 mmol) in anhydrous pyridine (4 ml) at 20° was added diphenyl phosphorochloridate (0·35 g, 1·3 mmol). T.l.c. (system A) after 48 h revealed nucleoside starting material and no trace of the desired phosphorylation product.

(*b*) To a stirred solution of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine (0·472 g, 1·0 mmol) and 5-chloro-1-methylimidazole [6] (0·35 ml, 3 mmol) in anhydrous acetonitrile (4 ml) at 20° was added diphenyl phosphorochloridate (0·35 g, 1·3 mmol). After 16 h, the products were concentrated under reduced pressure to an oil which was dissolved in chloroform (50 ml) and the solution washed with aqueous 10% sodium hydrogen carbonate (15 ml). The dried ($MgSO_4$) chloroform layer was filtered and concentrated to an oil which was redissolved in chloroform (3 ml). n-Pentane (150 ml) was added to this solution and the precipitate obtained was further washed with n-pentane (2 × 150 ml). A solution of this material in chloroform–methanol (97·5 : 2·5 v/v; 5 ml) was applied to a column (20 cm × 2 cm^2) of Kieselgel H (40 g) suspended in the same solvent. Elution with chloroform—methanol (97·5 : 2·5 v/v) gave a glass. Crystallization from ethanol (20 ml) gave 2′,5′-*bis*-O-4-*methoxytetrahydropyran-4-yluridine 3′-diphenyl phosphate* (Found: C, 56·3; H, 6·1; N, 3·9. $C_{33}H_{41}N_2O_{13}P$ requires C, 56·25; H, 5·8; N, 4·0%) (0·422 g, 60%), m.p. 148—149°; R_F 0·60 (system A); $\lambda_{max.}$ (95% EtOH) 260 (ε 12,300), $\lambda_{min.}$ 227 nm (ε 2750); τ ($CDCl_3$) 0·28 (1H, s), 2·18 (1H, d, *J* 8·5 Hz), 2·64 (10H, m), 3·67 (1H, d, *J* 7·5 Hz), 4·18 (1H, d, *J* 8·5 Hz), 5·00 (1H, m), 5·40 (1H, m), 6·76 (3H, s), and 6·90 (3H, s).

2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridine 3′-Phenyl Phosphate (4a).—To a stirred solution of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine 3′-diphenyl phosphate (70·4 mg, 0·10 mmol) in dioxan (2 ml) at 20° was added aqueous 0·125M-sodium hydroxide (8 ml, 1·0 mmol). After 10 h, when t.l.c. (system A) indicated that no starting material remained, the products were applied to a column of Dowex 50 cation-exchange resin (NH_4^+ form; 17 ml). The column was eluted with water; the eluate was concentrated under reduced pressure (to *ca.* 5 ml) and then lyophilized to give the ammonium salt of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine 3′-phenyl phosphate as a fluffy solid (66 mg), found to be homogeneous by t.l.c. (systems A and B) and paper electrophoresis (buffers C and D) (see Table 1).

Uridine 3′-Phenyl Phosphate (4b).—The foregoing ammonium salt (33 mg, *ca.* 0·05 mmol) was dissolved in 0·01M-hydrochloric acid (10 ml) and the pH lowered to 2·0 (pH meter) by addition of more acid. After 16 h at 20° the solution was carefully neutralized (to pH 7—8) with aqueous ammonia and then immediately lyophilized to give uridine 3′-phenyl phosphate (30 mg) as a solid. This

* This modification of the original procedure [4] is due to Dr. G. R. Owen, who first obtained this compound crystalline.

[11] L. A. Heppel, P. R. Whitfeld, and R. Markham, *Biochem. J.*, 1955, **60**, 8; M. R. Bernfield, *J. Biol. Chem.*, 1966, **241**, 2014.

[12] J. Smrt and F. Šorm, *Coll. Czech. Chem. Comm.*, 1963, **28**, 2415; A. Holý, *ibid.*, 1968, **33**, 223; A. P. Kavunenko and N. S. Thikomirova-Siderova, *Zhur. obshchei Khim.*, 1968, **38**, 2368 (*Chem. Abs.*, 1969, **70**, 68,678c); A. P. Kavunenko, E. N. Morozova, and N. S. Thikomirova-Sidorova, *ibid.*, 1971, **41**, 226 (*Chem. Abs.*, 1971, **75**, 20,877t).

material was shown by t.l.c. (system B) and paper electrophoresis (buffers C and D) to be contaminated (*ca.* 2%) with uridine 2′,3′-cyclic phosphate.

Uridine 2′,3′-Cyclic Phosphate (5).—A solution of the foregoing ammonium uridine 3′-phenyl phosphate (42 mg) in 0·155M-ammonia (3·5 ml) was kept at 20° for 4 h; t.l.c. (system B) then showed that no starting material remained. The products were lyophilized to give ammonium uridine 2′,3′-cyclic phosphate (32 mg) in quantitative (estimated spectrophotometrically) yield. This material was found to be homogeneous and identical with authentic uridine 2′,3′-cyclic phosphate by t.l.c. (system B) and paper electrophoresis (buffers C and D); $\lambda_{max.}$ (H_2O; pH 7) 260 nm (ε 9600).

Determination of the Rate of Hydrolysis of 2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridine 3′-Diphenyl Phosphate.—(a) *In 0·1M-sodium hydroxide.* A solution of the substrate (35·2 mg, 0·05 mmol) in dioxan (50 ml) at 20° was rapidly added to aqueous 0·125M-sodium hydroxide (200 ml) at 20° with thorough mixing. Some of the solution was immediately transferred to a 1 cm cuvette and the change in absorbance (A) at 292 nm with time was determined spectrophotometrically. A straight line was obtained by plotting $\log(A_\infty - A_t)$ against time (t). The first-order rate constant (k) and the half-time ($t_{\frac{1}{2}}$) for the hydrolysis were found to be $6·4 \times 10^{-4}$ s^{-1} and 18 min, respectively. The experiment was then repeated with ten times the concentration of substrate: a solution of 35·2 mg in dioxan (5 ml) at 20° was added to aqueous 0·125M-sodium hydroxide (20 ml) at 20°. First-order kinetics were again observed with k and $t_{\frac{1}{2}}$ $6·2 \times 10^{-4}$ s^{-1} and 18·5 min, respectively.

(b) *In 5·35M-ammonia.* A solution of the substrate (35·2 mg, 0·05 mmol) in dioxan (50 ml) at 20° was rapidly added to aqueous 5·35M-ammonia (10% w/w; 200 ml) at 20°. The rate of hydrolysis was again determined spectrophotometrically. First-order kinetics were observed with k $1·04 \times 10^{-4}$ s^{-1} and $t_{\frac{1}{2}}$ 115 min. The experiment was then repeated with ten times the concentration of substrate: first-order kinetics were again observed with k $0·99 \times 10^{-4}$ s^{-1} and $t_{\frac{1}{2}}$ 116 min.

Determination of the Rate of Hydrolysis of Uridine 3′-Phenyl Phosphate.—Ammonium uridine 3′-phenyl phosphate (19·5 mg, 0·05 mmol) was dissolved in aqueous 0·155M-ammonia (250 ml) at 20°. Some of the solution was rapidly transferred to a 1 cm cuvette and the change in absorbance (A) at 292 nm with time (t) was determined spectrophotometrically. A straight line was obtained by plotting $\log(A_\infty - A_t)$ against t. The first-order rate constant and $t_{\frac{1}{2}}$ for the hydrolysis were found to be $3·9 \times 10^{-3}$ s^{-1} and 3·0 min, respectively.

o-Chlorophenyl Dihydrogen Phosphate.—A flask fitted with a reflux condenser, thermometer, and stirrer, was charged with *o*-chlorophenyl phosphorodichloridate (67·0 g, 0·27 mol). Water (85 g, 4·7 mol) was added dropwise during 60 min to the stirred contents of the flask, which was maintained at 80°. The cooled products were concentrated under reduced pressure to give an oil which solidified. A solution of this material in benzene (100 ml) was evaporated under reduced pressure. After this process had been repeated, the material was dried (KOH) *in vacuo* overnight. Recrystallization twice from chloroform (150 ml) gave *o-chlorophenyl dihydrogen phosphate* (42·0 g, 75%) (Found: C, 34·4; H, 2·7; Cl, 17·0. $C_6H_6ClO_4P$ requires C, 34·5; H, 2·9; Cl, 17·0%), m.p. 97—98°.

2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridylyl-(3′ → 5′)-2′-O-4-methoxytetrahydropyran-4-yluridine o-Chlorophenyl Ester (7a).—Anhydrous pyridine (10 ml) was added to a dry mixture of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine (0·944 g, 2·0 mmol), *o*-chlorophenyl dihydrogen phosphate (0·438 g, 2·1 mmol), and 2,4,6-tri-isopropylbenzenesulphonyl chloride [8] (1·510 g, 5·0 mmol). The solution was stirred at 20° with precautions taken to exclude moisture. After 4 h, 2′-*O*-4-methoxytetrahydropyran-4-yluridine [13] (0·788 g, 2·2 mmol) was added and, after a further 15 h, the products were concentrated under reduced pressure. A solution of the residual oil in chloroform (75 ml) was washed with aqueous 10% sodium hydrogen carbonate (40 ml) and water (40 ml), dried ($MgSO_4$), filtered, and concentrated to a glass. This material, dissolved in chloroform–methanol (95 : 5 v/v; 6 ml), was applied to a column (20 cm × 4 cm^2) of Kieselgel H (100 g) suspended in the same solvent. Elution of the column with the same solvent (1 l) gave a glass (1·50 g). A A solution of the latter in chloroform (7 ml) was added dropwise with stirring to anhydrous ether (200 ml). The precipitate of (7a) was filtered off and dried (KOH) *in vacuo*; yield 1·40 g (70%); t.l.c. (system A) revealed the presence of only one component (R_F 0·28).

Reaction between 2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridylyl-(3′ → 5′)-2′-O-4-methoxytetrahydropyran-4-yluridine o-Chlorophenyl Ester (7a) *and Diphenyl Phosphorochloridate.*—Diphenyl phosphorochloridate (0·40 g, 1·5 mmol) was added to a stirred solution of (7a) (1·0 g, 1·0 mmol) and 5-chloro-1-methylimidazole [6] (0·35 ml, 3 mmol) in anhydrous acetonitrile (4 ml) at 20°. After 24 h the products were concentrated under reduced pressure to give an oil, which was dissolved in chloroform (60 ml). The solution was washed with aqueous 10% sodium hydrogen carbonate (20 ml) and then water (20 ml), dried ($MgSO_4$), and concentrated to an oil which was redissolved in chloroform (5 ml). n-Pentane (170 ml) was added and the yellow precipitate was further washed with n-pentane (2 × 170 ml). A solution of this material in chloroform–methanol (97·5 : 2·5 v/v; 5 ml) was applied to a column (20 cm × 4 cm^2) of Kieselgel H (100 g) suspended in the same solvent. Elution of the column with the same solvent gave a glass (0·80 g). Anhydrous ether (150 ml) was added to a solution of this material in chloroform (4 ml) and, after vigorous shaking, a colourless solid (0·73 g, 60%) which was assumed to be the fully-protected dinucleotide (7b) was filtered off. This material was shown by t.l.c. (system A) to contain two components (R_F 0·49 and 0·55) in the approximate proportions of 3 : 1.

2′,5′-Bis-O-4-methoxytetrahydropyran-4-yluridylyl-(3′ → 5′)-2′-O-4-methoxytetrahydropyran-4-yluridine 3′-Phenyl Phosphate (8a).—To a stirred solution of the fully-protected dinucleotide (7b) (0·12 g, 0·1 mmol) in dioxan (1 ml) was added aqueous 0·125M-sodium hydroxide (4 ml). After 10 h at 20° the products were worked up according to the procedure described for the preparation of the ammonium salt of 2′,5′-bis-*O*-4-methoxytetrahydropyran-4-yluridine 3′-phenyl phosphate, to give the ammonium salt of the protected dinucleotide (8a) as a fluffy solid (102 mg), homogeneous by t.l.c. (system B) and by paper electrophoresis (buffers C and E) (see Table 2).

Uridylyl-(3′ → 5′)-uridine 3′-Phenyl Phosphate (8b).—Compound (8a) (50 mg, *ca.* 0·05 mmol) was dissolved in 0·01M-hydrochloric acid (10 ml) and the pH lowered to 2·0

[13] C. B. Reese, R. Saffhill, and J. E. Sulston, *J. Amer. Chem. Soc.*, 1967, **89**, 3366; *Tetrahedron*, 1970, **26**, 1023.

(pH meter) by addition of 0·1M-hydrochloric acid. After 16 h at 20° the solution was carefully neutralized (to pH 7—8) and lyophilized as in the preparation of uridine 3′-phenyl phosphate, to give the ammonium salt of uridylyl-(3′ → 5′)-uridine 3′-phenyl phosphate (35 mg). This material was found [t.l.c. (system B) and paper electrophoresis (buffers C and D)] to be contaminated (*ca.* 1—2%) with uridylyl-(3′ → 5′)-uridine 2′,3′-cyclic phosphate (see below).

Uridylyl-(3′ → 5′)-uridine 2′,3′-Cyclic Phosphate (9a).—Uridylyl-(3′ → 5′)-uridine 3′-phenyl phosphate (31 mg) was dissolved in aqueous 0·155M-ammonia (4 ml) and set aside at 20°. After 4 h, the products were lyophilized to give the ammonium salt of uridylyl-(3′ → 5′)-uridine 2′,3′-cyclic phosphate (31 mg) in virtually quantitative yield (estimated spectrophotometrically *). This material was found [t.l.c. (system B) and paper electrophoresis (buffers C—E)] to be contaminated (*ca.* 1%) with uridine 2′,3′-cyclic phosphate.

Enzymic Hydrolysis with Pancreatic Ribonuclease.—The action of pancreatic ribonuclease on (*a*) uridine 3′-phenyl phosphate (4b), (*b*) uridine 2′,3′-cyclic phosphate (5), (*c*) uridylyl-(3′ → 5′)-uridine 3′-phenyl phosphate (8b), and (d) uridylyl-(3′ → 5′) uridine 2′,3′-cyclic phosphate (9a) was examined by the following procedure. A solution of each substrate (1·0 mg) and pancreatic ribonuclease (*ca.* 0·1 mg) in pH 7·5 Tris hydrochloride buffer (0·05M; 0·1 ml) was incubated at 37° for 2 h. T.l.c. (system B) and paper electrophoresis (buffers C—E) showed that quantitative digestion to uridine 3′-phosphate had occurred in all four experiments. Control experiments revealed that the substrates were all stable under the reaction conditions.

Acidic Hydrolysis of Uridylyl-(3′ → 5′)-uridine 2′,3′-Cyclic Phosphate (9).—A solution of (9) (10 A_{260} units) in M-hydrochloric acid (0·1 ml) was kept at 20° for 1 h. After careful neutralization (to pH 7) with aqueous ammonia, t.l.c. (system B) showed no starting material, a major component (R_F 0·05), and a minor component (R_F 0·15) corresponding to uridine 2′(3′)-phosphates. Paper electrophoresis (buffer C) revealed that the mobility of the major component was 1·02 times that of uridine 3′-phosphate. Under the above conditions, uridine 2′,3′-cyclic phosphate was completely converted into uridine 2′(3′)-phosphates.

TABLE 1

T.l.c. data

Compound	R_F System A [a]	R_F System B [b]
(1a)	0·33	
(3)	0·60	
(4a)		0·80
(4b)		0·73
(4c)		0·15
(5)		0·44
(6)	0·77	
(7a)	0·28	
(7b)	0·49, 0·55 [c]	
(8a)		0·77
(8b)		0·49
(9a)		0·20

[a] $CHCl_3$–MeOH (92 : 8 v/v) on Merck Kieselgel GF_{254}. [b] Aqueous M-NH_4OAc–EtOH (3 : 7 v/v) on Macherey Nagel-Cellulose F_{254}. [c] Compound (7b) runs as two spots in system A.

TABLE 2

Paper electrophoretic mobilities relative to uridine 3′-phosphate (4c)

Compound	Relative mobility Buffer C [a]	Buffer D [a]	Buffer E [a]
(4a)	0·35		
(4b)	0·46	0·88	
(4c)	1·00	1·00	1·00
(5)	0·61	1·19	
(8a)	0·50		0·73
(8b)	0·58	1·26	0·77
(9a)	0·71	1·47	0·83

[a] See beginning of Experimental section for compositions of buffers.

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* Based on the assumption that (9a) has ε 20,000 at 260 nm.