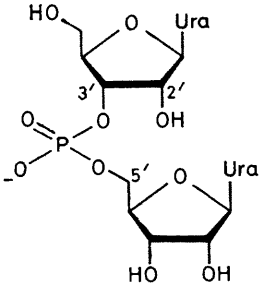
Di-uridine 3′-Phosphate

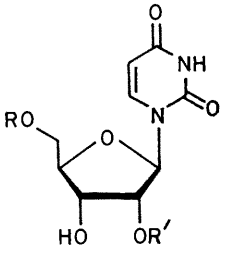
By BERNARD RAYNER, COLIN B. REESE,* and AIKO UBASAWA
(*Department of Chemistry, King's College, Strand, London* WC2R 2LS)

Summary An unambiguous synthesis of di-uridine 3′-phosphate (**7**) by the phosphotriester approach is described; (**7**) is stable in acidic solution but is very sensitive to alkaline hydrolysis.

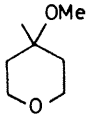
RIBONUCLEIC acids (RNA), unlike deoxyribonucleic acids (DNA) and simple dialkyl phosphates, are susceptible to alkaline hydrolysis under relatively mild conditions. It was firmly established[1] nearly thirty years ago that this characteristic property of RNA is due to the neighbouring group participation of the hydroxy-functions vicinal to the internucleotide linkages [as in uridylyl-(3′→5′)-uridine (**1**)].



(1)



(2) a; R = H, R′ = (3)
b; R = R′ = (3)

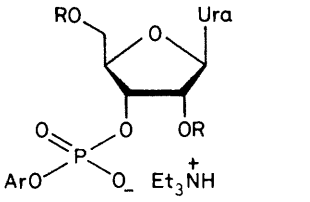


(3)

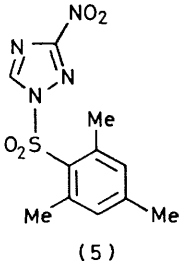
Ura = uracil-1-yl

In our approach to oligoribonucleotide synthesis,[2,3] protected mono- and di-nucleotide blocks terminating in 3′-(*o*-chlorophenyl) phosphate groups are condensed with 2′-*O*-methoxytetrahydropyranyl ribonucleosides[4] [such as (**2a**)] in which both the 3′- and 5′-hydroxy functions are unprotected. While it seems likely that phosphorylation occurs as desired on the primary (5′-) hydroxy-functions of the latter nucleoside building blocks (*e.g.* **2a**) with a very high degree of regioselectivity, the possibility of the products being contaminated with material containing 3′→3′-internucleotide linkages obviously exists. We therefore decided to investigate the chemistry of a 3′→3′-diribonucleoside phosphate. Quite apart from its relevance to oligoribonucleotide synthesis, it was clearly of considerable interest to determine the susceptibility to alkaline hydrolysis of such a dialkyl phosphate with two vicinal hydroxy functions.[5]

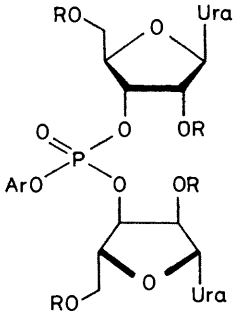
A solution of the triethylammonium salt of 2′,5′-di-*O*-methoxytetrahydropyranyluridine 3′-*o*-chlorophenyl phosphate† (**4**, 0·33 mmol), 2′,5′-di-*O*-methoxytetrahydropyranyluridine[6] (**2b**, 0·28 mmol) and 1-(mesitylen-2-yl sulphonyl)-3-nitro-1,2,4-triazole[7] (**5**, 1·53 mmol) in anhydrous pyridine (1·5 ml) was stirred at room temperature for 19 h. Examination of the products by t.l.c. then revealed two components of approximately equal intensity. Treatment, after work-up, of this crude material with 0·1M-potassium carbonate‡ in dioxan–water (4 : 1 v/v) at room temperature for 30 min and chromatography of the products on silica gel gave the fully-protected uridylyl-(3′→3′)-uridine (**6**). The latter compound (**6**) was isolated as a t.l.c. homogeneous, colourless solid in 80% yield; ^{31}P n.m.r. [$(CD_3)_2SO$]: δ −8·4(s) p.p.m.



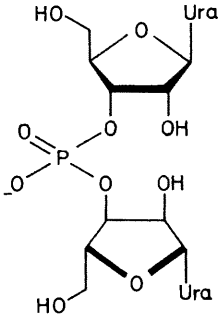
(4); R = (3), Ar = 2-ClC_6H_4



(5)



(6); R = (3), Ar = 2-ClC_6H_4



(7)

Treatment of the fully-protected dinucleoside phosphate (**6**) with a twentyfold excess of 0·8M N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-4-nitrobenzaldoximate[7] in dioxan–water (1 : 1 v/v) at room temperature for 16 h, followed by

† This material was prepared by phosphorylating (**2b**) with an excess of *o*-chlorophenyl phosphorodi-(1,2,4-triazolide) in acetonitrile–pyridine and then working-up the products with triethylamine and water (ref. 3).

‡ When 1-(mesitylen-2-yl-sulphonyl)-3-nitro-1,2,4-triazole (**5**) is used in excess in oligoribonucleotide synthesis, and when reaction times are relatively long, uracil residues may undergo modification (C. B. Reese and A. Ubasawa, *Tetrahedron Lett.*, 1980, 2265). Thus at least two products were obtained (see above) in the condensation reaction involving (**4**) and (**2b**). This base modification may readily be reversed by treatment with potassium carbonate under very mild conditions.

acidic hydrolysis (0·01M hydrochloric acid, room temperature, 6 h) gave di-uridine 3′-phosphate (**7**) § [^{1}H n.m.r. (D_2O, 250 MHz): δ 3·83 (H-5′, 2H, dd, *J* 3·8, 12·5 Hz), 3·92 (H-5′, 2H, dd, *J* 2·8, 12·5 Hz), 4·33 (H-4′, 2H, m), 4·48 (H-2′, 2H, t, *J ca.* 5·5 Hz), 4·66 (H-3′, 2H, dt, *J ca.* 7·5, 5 Hz), 5·90 (H-5, 2H, d, *J* 8·1 Hz), 5·96 (H-1′, 2H, d, *J* 5·3 Hz), and 7·89 (H-6, 2H, d, *J* 8·1 Hz)] as virtually the sole nucleotide product. The latter compound (**7**), isolated as its triethylammonium salt, had the same h.p.l.c. R_T [Partisil PXS 10/20 SAX column, eluted with 0·05M potassium phosphate buffer (pH 3·35)] as uridylyl-(3′→5′)-uridine (**1**) but had a higher R_F than (**1**) in three partition chromatographic systems.

While di-uridine 3′-phosphate (**7**) was found to be almost unchanged after it had been allowed to stand in 0·01M hydrochloric acid (pH 2) for 4 days at room temperature, it readily underwent hydrolysis to give uridine and uridine 2′,3′-cyclic phosphate under mildly alkaline conditions. For example, the half-time of hydrolysis (pseudo-first-order kinetics) of (**7**) in 0·045M sodium carbonate buffer (pH 10·9) at 20 °C was 160 min. Under the same conditions, uridylyl-(3′→5′)-uridine (**1**) underwent *ca.* 2·5% hydrolysis in 8 days. It is thus apparent that the rate of hydrolysis of (**7**) at pH 10·9 is more than three orders of magnitude faster than that of (**1**). Not unexpectedly[5] the influence of the second vicinal hydroxy function is profound. Indeed, (**7**) must be one of the most base-sensitive dialkyl phosphate esters known. From the point of view of oligoribonucleotide synthesis[2,3] (see above), it would seem that a short base treatment (say, at pH 11—12, 20 °C) would cleave any 3′→3′-internucleotide linkages present without affecting the 3′→5′-linkages to a significant extent.

The susceptibility of (**7**) to enzyme-promoted hydrolysis was then examined. Di-uridine 3′-phosphate (**7**) was found to be a substrate for ribonuclease A but a poorer substrate than uridylyl-(3′→5′)-uridine (**1**). Thus, while the latter compound (**1**) underwent *ca.* 95% ribonuclease A-promoted hydrolysis (to uridine, uridine 2′,3′-cyclic phosphate and uridine 3′-phosphate) at 37 °C in 2 h, (**7**) was only *ca.* 80% hydrolysed under the same conditions after 5 h. Finally, di-uridine 3′-phosphate (**7**) was found to be, at most, an extremely poor substrate for calf spleen phosphodiesterase.

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§ Di-uridine 3′-phosphate (**7**) has previously been obtained but not in an unambiguous way. Thus, when a partially-protected derivative of uridylyl-(3′→5′)-uridine (**1**) with a free 3′-hydroxy function was unblocked (by treatment with alkali, followed by treat-treatment with acid), (**7**) (12%) was obtained (J. H. van Boom, P. M. J. Burgers, P. H. van Deursen, J. F. M. de Rooy, and C. B. Reese, *J. Chem. Soc., Chem. Commun*, 1976, 167) in addition to (**1**) (88%). The preparation of di-(2′-*O*-benzyl)uridine 3′-phosphate has also been reported (G. Reitz and W. Pfleiderer, *Chem. Ber.*, 1975, **108**, 2878).

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