New Phosphorylation Procedure. Activation of Phosphates with Cyclohexyl Isocyanide†

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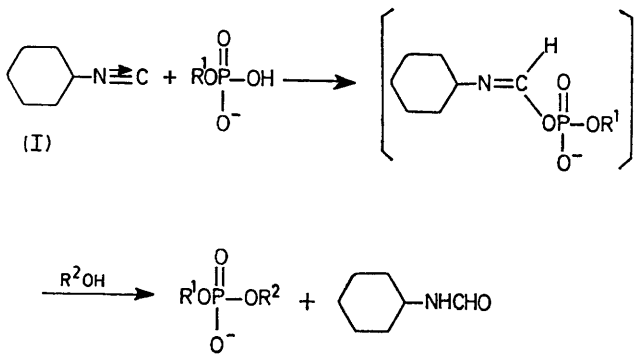
Summary Cyclohexyl isocyanide reacts with nucleoside 2′(3′)-phosphates to afford 2′,3′-cyclic phosphates or with a mixture of phosphomonoesters (*viz.*, 2′,3′-di-*O*-acetyluridine 5′-phosphate or phenyl phosphate) and alcohols to give the corresponding phosphodiesters in *ca.* 90% yields.

THE development of a procedure for the chemical synthesis of oligonucleotides, especially for the synthesis by a polymer support approach depends to a significant extent on the design of an efficient procedure for phosphate activation.[1] A characteristic reaction of isocyanides is α-addition to the isocyanide carbon,[2] and reaction of phosphoric acid should give an imidoyl phosphate.[3] Isocyanides thus might be capable of activating phosphates in a similar way to dicyclohexylcarbodi-imide (DCC).[3,4] This was found to be the case, and we find that cyclohexyl isocyanide is the most suitable reagent.

Treatment of phenyl phosphate (1·0 mmol) with absolute MeOH (10 mmol) in the presence of cyclohexyl isocyanide

† For previous paper in the nucleotides series see Y. Mizuno, S. Kitano, and A. Nomura, *Chem. Pharm. Bull.* (*Japan*), in the press.

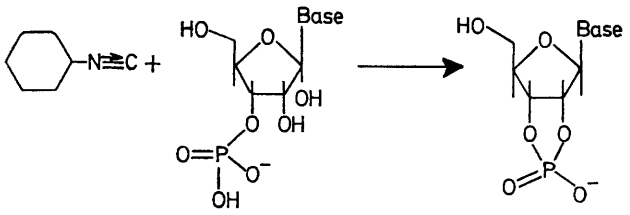
(I) (4·3 mmol) in pyridine (5 ml) at 40° for 1 day afforded an 87% (isolated) yield of methyl phenyl phosphate after work-up including purification with DEAE-cellulose (2·8 × 43 cm; linear gradient elution with 2 l of water to 2 l of 0·1M $Et_3NH^+HCO_3^-$). Analogously, a mixture of uridine-



R^1 = Ph, uridine-5′; R^2 = Me,Et

2′,3′-di-*O*-acetyluridine-5′-phosphate (pyridinium salt; 0·22 mmol)[5] and EtOH (2·2 mmol) was treated with (I) (1·2 mmol) in pyridine (2 ml) at 40° for 1 day to afford 2′,3′-di-*O*-acetyluridine-5′-ethylphosphate in 80% isolated yield (t.l.c. separation and u.v. spectral estimation[6]). The structure of the product was confirmed by u.v. spectral, electrophoretic, and enzymatic (venom phosphodiesterase) digestion data.

Uridine 2′(3′)-phosphoric acid (1·1 mmol) in pyridine (2 ml) was treated with (I) (4·8 mmol) at 40° for 1 day. Uridine-2′,3′-phosphate (II; pyridinium salt) was precipitated (90% yield) and was filtered off and washed with pyridine and then ether. Upon electrophoresis (pH 8·5), it had a mobility of 7·8 cm (monoanion) compared to 15 cm for uridine-2′(3′)-phosphate (dianion). The structural assignment was substantiated by u.v. spectral and electrophoretic comparison with an authentic sample of (II).[7] Digestion of the product with ribonuclease A afforded uridine-3′-phosphate in almost quantitative yield. Parallel



and 2′-phosphate

Base = Uracil, cytosine, adenine, and guanine

experiments starting from cytidine, adenosine, and guanosine-2′(3′)-phosphate (pyridinium salts) afforded the corresponding 2′,3′-cyclic phosphates. In these cases, however, the precipitated products were contaminated with the starting nucleotides, and they were purified by DEAE-cellulose column chromatography. Yields of cytidine-, adenosine-, and guanosine-2′,3′-cyclic phosphates ranged from 75 to 93%.

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