Oligonucleotide Synthesis *via* Phosphotriester Intermediates: the Phenyl-protecting Group

By C. B. REESE* and R. SAFFHILL

(*University Chemical Laboratory, Lensfield Road, Cambridge*)

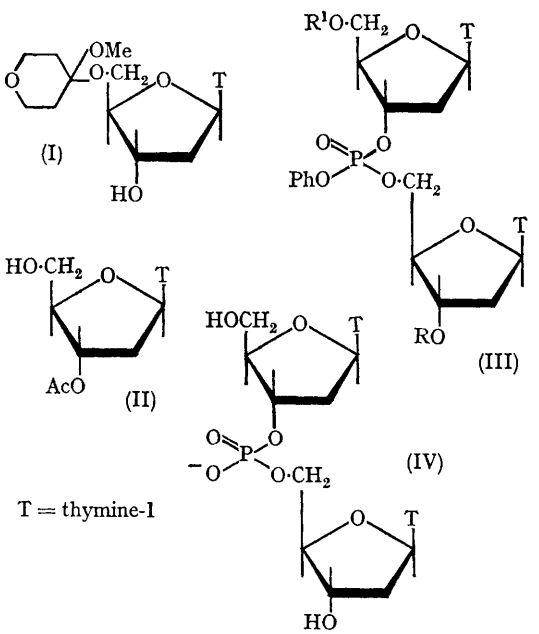
FOR oligonucleotide synthesis *via* phosphotriester intermediates a suitable protecting group for the internucleotidic linkages is required, *i.e.* a third esterifying function. This must satisfy two main criteria: firstly, it must be selectively and readily removable from the phosphotriester functions, and secondly, it must remain intact under the conditions of acidic and basic hydrolysis which are necessary for the removal of common protecting groups from the sugar hydroxyl functions. We report what we consider to be a satisfactory solution† to this problem.

The thymidine 5′-ketal[1] (I) was stirred with phenyl phosphorodichloridate[2] (1·05 mol. equiv.) and 2,6-lutidine (3 mol. equiv.) in anhydrous methyl cyanide at 20°, considerable care being taken to exclude moisture. After 15 hr., complete solution had occurred and t.l.c. [$CHCl_3$–MeOH (92 : 8, v/v)] indicated only a trace of unreacted 5′-ketal (I). After a further 21 hr., 3′-*O*-acetylthymidine (II)

† Eckstein and Rizk (*Angew. Chem. Internat. Edn.*, 1967, **6**, 695, 949) have recently advocated the use of the 2,2,2-trichloroethyl protecting group for the present purpose.

(1·0 mol. equiv.) and 2,6-lutidine (3 mol. equiv.) were added and the reactants stirred for 48 hr. The products were then concentrated and partitioned between chloroform and aqueous sodium bicarbonate. Evaporation of the dried organic layer gave the crude fully-protected dinucleoside phosphate (III; R = Ac, R^1 = $C_6H_{11}O_2$) as a discoloured glass in *ca.* 70% yield.

Approximately one-half of this material* was treated with 0·1 M-NaOH solution [in aqueous dioxane (4 : 1 v/v)] at 20° for 5 min. The products were then neutralized, worked-up and chromatographed on silicic acid to give the partially-protected dinucleoside phosphate (III; R = H, R^1 = $C_6H_{11}O_2$) in 46% overall yield, based on thymidine 5′-ketal (I). This material, which was t.l.c. homogeneous (system A), was treated with 50% aqueous formic acid at 20°. After 1 hr., the solution was evaporated and the products chromatographed on silicic acid to give the phenyl ester of thymidylyl-(3′ → 5′)-thymidine (III; R = R^1 = H) as a colourless, t.l.c. homogeneous (system A) glass in 85% yield.



When the latter compound (III; R = R^1 = H) was treated with an excess of 0·1 M-NaOH solution [in aqueous dioxane (4 : 1 v/v)] for 6 hr. at 20°, it was quantitatively converted into thymidylyl-(3′ → 5′)-thymidine (TpT) (IV). No trace of any other phosphorus-containing product could be detected. The unprotected dinucleoside phosphate, which was paper electrophoretically and chromatographically identical to authentic TpT, underwent digestion in the presence of *Crotalus adamanteus* snake venom phosphodiesterase to give thymidine and thymidine 5′-phosphate. Thus the phenyl protecting group satisfies the first criterion (see above) in that it may be selectively removed (*i.e.* displaced as phenoxide ion) from phosphotriesters such as (III; R = R^1 = H).

The base-catalyzed hydrolysis of the latter compound (III; R = R^1 = H), in an excess of 0·1 M-NaOH solution [in aqueous dioxan (4 : 1 v/v)], displayed first-order kinetics with $t_{\frac{1}{2}}$ 20 min. at 20°; under the same conditions, 3′-*O*-acetylthymidine (II) underwent hydrolysis at a much faster rate ($t_{\frac{1}{2}}$ ~ 15 sec.). Thus the phenyl protecting group remains virtually intact under the conditions of alkaline hydrolysis required to remove a 3′-*O*-acetyl function. As the ketal group in (III; R = H, R^1 = $C_6H_{11}O_2$) may also be removed without any detectable hydrolysis of the phosphotriester function, phenyl satisfies the second criterion for a phosphate-protecting group.

Phenyl phosphorodichloridate has previously been used extensively in the preparation of unsymmetrical phosphodiesters in the phosphatide field[3] and in the synthesis of a symmetrical dinucleoside phosphate.[4] Although we do not think that this phosphorylating agent will prove to be of general use in oligonucleotide synthesis, the results presented here allow us to conclude that phenyl is a suitable protecting group in the phosphotriester approach.

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* The remainder of the fully-protected dinucleoside phosphate (III; R = Ac, R^1 = $C_6H_{11}O_2$) was treated with 50% aqueous formic acid for 1 hr. at 20°, and the products chromatographed on silicic acid. The other partially-protected dinucleoside phosphate (III; R = Ac, R^1 = H) was isolated in 59% overall yield; it was contaminated (t.l.c., system A) with a trace amount (<1%) of 3′-*O*-acetylthymidine (II).

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[2] Brigl and H. Müller, *Chem. Ber.*, 1939, **72**, 2121.
[3] E. Baer, *Chem. Soc. Special Publ.* No. 8, 1957, p. 103.
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