'Oxine' (8-Hydroxyquinoline) as a Reagent for Protection of Internucleotidic Bonds in Oligonucleotide Synthesis

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Summary The 8-quinolyl group was used as a protecting group for phosphates of internucleotidic bonds in the synthesis of oligothymidylates using the phosphotriester approach.

THERE have been several recent successful demonstrations of the synthesis of oligonucleotides using the phosphotriester approach,[1] but several problems still remain. One of these appears to lie in the selection of the protecting group of the internucleotidic bonds. An ideal phosphate-protecting group should be easily introduced and smoothly removed under specific and neutral conditions. Recently, we have found that the 8-quinolyl esters of nucleotides could be hydrolysed to the corresponding nucleotides simply by treatment with $CuCl_2$ in dimethyl sulphoxide (DMSO)–water.[2] These 8-quinolyl esters are rather hydrophobic relative to the other esters but behave as normal organic substances. Thus, the synthesis of oligothymidylates using the phosphotriester approach and the 8-quinolyl group as a protecting group of internucleotidic bonds in order to increase the yields of the coupling reactions takes advantage of the solvation effect.

The present approach to the synthesis of oligothymidylates is summarised in the Scheme.

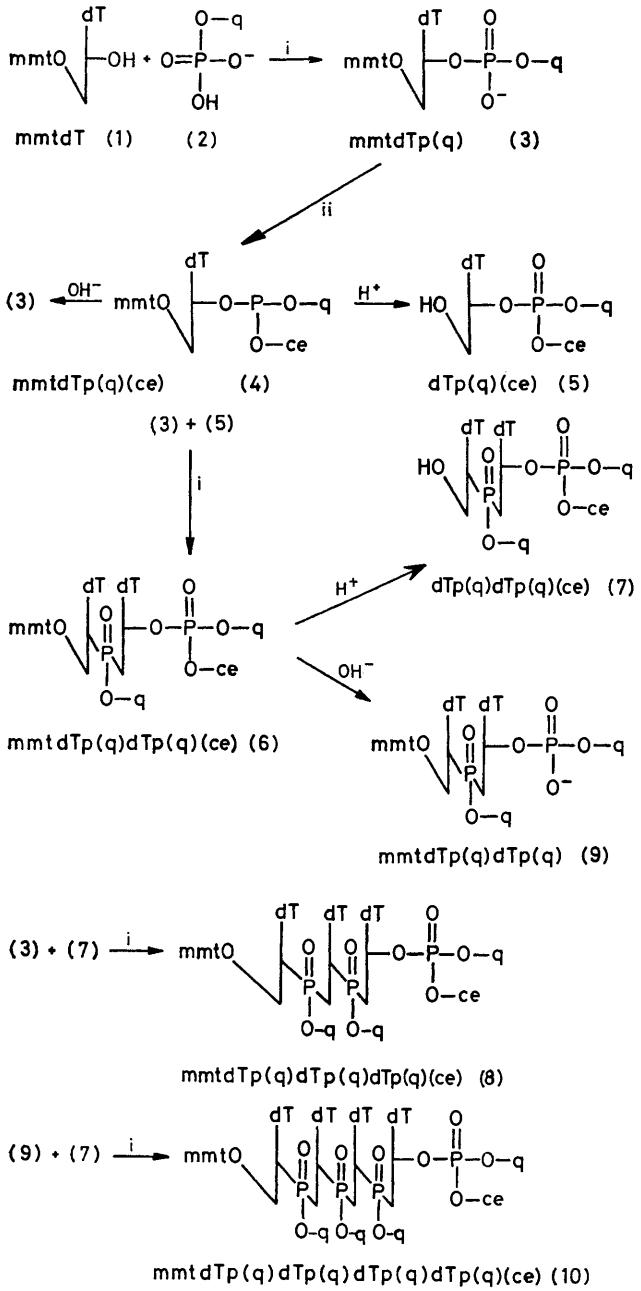
The key intermediate, 5′-*O* monomethoxytritylthymidine 3′-(2-cyanoethyl 8-quinolyl phosphate) (**4**) [mmtdTp(q)-

(ce)]† was prepared as follows: 5′-*O*-Monomethoxytrityl-thymidine (**1**) (1 mmol) was treated with 8-quinolyl phosphate (1 mmol) (**2**)‡ in the presence of 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPS) (1·5 mmol) in dry pyridine (10 ml) at room temperature for 12 h. The reaction was monitored by silica gel t.l.c. After completion of the reaction, the mixture was evaporated to dryness. The residue was dissolved in dry pyridine (2 ml) and then 2-cyanoethanol (5 mmol) and TPS (1 mmol) were added. The mixture was stirred at room temperature for 24 h. After the usual work-up, mmtdTp(q)(ce) (**4**) (780 mg) [R_f 0·51;§ λ_{max} (MeOH) 267 and 228; λ_{min} (MeOH) 248 and 221 nm] was obtained in 99% yield by silica gel column chromatography. Treatment of (**4**) with 80% acetic acid afforded the thymidine 5′-hydroxy-derivative, dTp(q)(ce) (**5**) [λ_{max} (MeOH) 267 and 230; λ_{min} (MeOH) 244 and 222 nm], in 95% yield, whereas mild alkaline treatment with 0·1 N sodium hydroxide–pyridine (1 : 4 v/v) to remove the 2-cyanoethyl group gave the 8-quinolyl phosphate, mmtdT(q) (**3**), in 96% yield. A solution of both compounds (**3**) (1·0 mmol) and (**5**) (0·75 mmol) in dry pyridine (1 ml) was treated with TPS (1·5 mmol) at room temperature for 24 h. The fully protected dithymidine derivative, mmtdTp(q)dTp(q)(ce) (**6**) [R_f 0·34;§ λ_{max} (MeOH) 267 and 223; λ_{min} (MeOH) 245 and 219 nm], was obtained in 86% yield after separation by silica gel column chromatography. Treatment of (**6**) with 80% acetic acid at room temperature for 6 h gave dTp(q)-dTp(q)(ce) (**7**) [λ_{max} (MeOH) 267 and 229; λ_{min} (MeOH) 245 and 221 nm] in 91% yield.

Similarly, when a mixture of (**7**) (0·4 mmol) and (**3**) (0·6 mmol) was treated with TPS (0·8 mmol), the fully protected trithymidine derivative, mmtdTp(q)dTp(q)dTp(q)(ce) (**8**) [R_f 0·37;§ λ_{max} (MeOH) 267 and 226 nm; λ_{min} (MeOH) 244 and 219 nm], was obtained in 81% yield. The fully protected tetrathymidine derivative, mmtdTp(q)dTp(q)-dTp(q)dTp(q)(ce) (**10**), was synthesised by a fragment condensation reaction. When mmtdTp(q)dTp(q) (**9**) (0·6 mmol), prepared from (**6**) in 96% yield, was treated with (**7**) (0·9 mmol) in the presence of TPS (1·2 mmol) in dry pyridine (1·2 ml) at room temperature for 24 h, compound (**10**) was obtained in 76% yield after silica gel column chromatography [R_f 0·31;§ λ_{max} (MeOH) 267 and 226; λ_{min} (MeOH) 244 and 219 nm].

Deprotection of compounds (**6**), (**8**), and (**10**) was effected in 3 stages: (i) treatment with 0·1 N sodium hydroxide–pyridine (1 : 4 v/v) at room temperature for 15 min, then neutralization with Dowex 50W-X2 (pyridinium form), and concentration of the solution; (ii) treatment with anhydrous $CuCl_2$ (equimolar amount with respect to the 8-quinolyl groups) in DMSO–water at 40 °C for 5 h[2] and evaporation to dryness; the precipitate which formed on addition of water and was removed by washing with chloroform and the aqueous layer was evaporated to dryness; (iii) treatment of the residue with 80% acetic acid at room temperature for 6 h. Finally, the oligothymidylate was separated by DEAE cellulose column chromatography. In the course of the experiment, a number of the internucleotidic bonds were cleaved, and dithymidylate, thymidine 5′-phosphate, and thymidine were detected.

The oligothymidylates, dTpdTp, dTpdTpdTp, and dTp-dTpdTpdTp were obtained in 92, 81, and 83% yields,



SCHEME.† Reagents: i, TPS; ii, $HOCH_2CH_2CN$, TPS.

† The following abbreviations have been used: mmt = monomethoxytrityl; dT = thymidine; p = 3′-phosphate; q = 8-quinolyl; ce = 2-cyanoethyl. All compounds reported gave satisfactory elemental analyses.

‡ 8-Quinolyl phosphate was prepared in 81% yield from 8-hydroxyquinoline and phosphoryl chloride in dioxan–pyridine by a modification of the procedure of Y. Murakami, J. Sunamoto, H. Sadamori, H. Kondo, and T. Takagi (*Bull. Chem. Soc. Japan*, 1970, **43**, 2518).

§ R_f values refer to t.l.c. on Merck silica gel plates 60 F_{254}, with $CHCl_3$-MeOH (9 : 1 v/v) as eluent.

respectively, based on the corresponding fully protected, oligothymidylates and no protected nucleotide derivative was detected. They were homogeneous on paper chromatography and paper electrophoresis, and characterized by degradation to thymidine 3′-phosphate with spleen phosphodiesterase.

(*Received*, 15*th November* 1976; *Com.* 1256.)

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