O-Aryl *S*-Methyl Phosphorochloridothioates: Terminal Phosphorylating Agents in the Phosphotriester Approach to Oligonucleotide Synthesis

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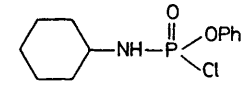
Summary *O*-Aryl *S*-methyl phosphorothioates (*e.g.*, **6**), prepared by the action of *O*-aryl *S*-methyl phosphorochloridothioates (*e.g.*, **4c**) on alcohols may be converted into the corresponding phosphate esters (*e.g.*, **7b**) by treatment first with 4-nitrobenzaldoximate ion and then with iodine in aqueous pyridine.

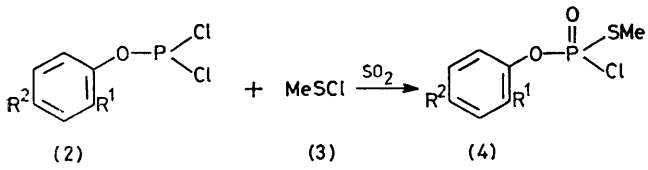
THE procedures which have been devised so far for the synthesis of oligonucleotides by the phosphotriester approach[1] lead usually to products containing one more base-residue than phosphate ester group. A method which is suitable for the introduction of phosphate groups at the 3′- and 5′-ends of oligonucleotide chains is therefore required. The use of aryl phosphoramidochloridates, such as phenyl phosphorocyclohexylamidochloridate (**1**), has



(1)

been recommended[2] for this purpose. However, the unblocking conditions then required[2] include an acidic hydrolysis step which makes the method unsuitable for

use in oligodeoxyribonucleotide synthesis. Although the acidic hydrolysis could possibly be replaced by a nitrosation step,[3] we believe that a much better solution of this problem lies in the use of *O*-aryl *S*-methyl phosphorochloridothioates (**4**).



(2) (3) (4)

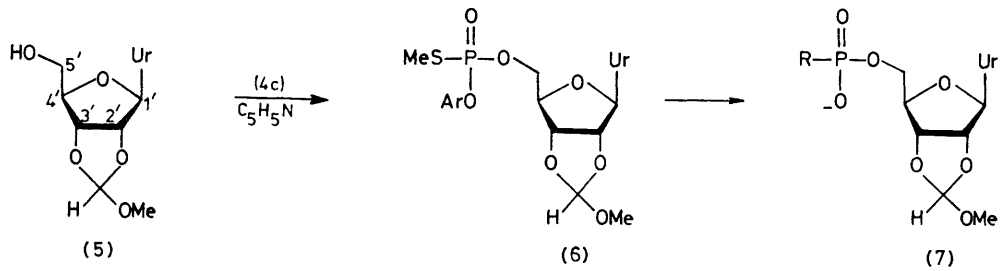
a; $R^1 = R^2 = H$
b; $R^1 = Cl$, $R^2 = H$
c; $R^1 = R^2 = Cl$

SCHEME 1

The latter reagents (**4**) may be readily prepared[4] (Scheme 1) by adding methanesulphenyl chloride (**3**) to a solution of the appropriate aryl phosphorodichloridite (**2**) in sulphur dioxide at −67 °C. As methanesulphenyl chloride (**3**) may be prepared[5] *in situ* by the action of sulphuryl chloride on commercially available dimethyl disulphide, *O*-aryl *S*-methyl phosphorochloridothioates (**4**) are easily accessible. Thus the 2,4-dichlorophenyl derivative (**4c**), which we consider to be a particularly suitable reagent for the present purpose, may be prepared from 2,4-dichlorophenyl phosphorodichloridite[6] (**2c**) in 64% isolated yield.† The corresponding phenyl and 2-chlorophenyl derivatives (**4a** and **4b**) have been prepared in 32 and 33% yields, respectively, by the same procedure.

The utility of (**4c**) as a terminal phosphorylating agent was first demonstrated with 2′, 3′-*OO*-methoxymethyleneuridine[7] (**5**) as the substrate. The reaction between (**5**) and 1·5 mol equiv. of (**4c**) in pyridine solution (Scheme 2) was complete within 30 min at 20 °C. The desired product (**6**) [^{31}P n.m.r. ([$^2H_6$]acetone–pyridine): δ 25·93 and 26·07 p.p.m.] was isolated pure in 74% yield. Treatment of (**6**) with 5 mol equiv. each of *syn*-4-nitrobenzaldoxime[8] and triethylamine in dioxan–water (8:5 v/v) at 20 °C for 1 h gave (**7a**) [^{31}P n.m.r. ([$^2H_6$]acetone–pyridine): δ 16·75 p.p.m.] as the sole phosphorus-containing product. The purity of this material was confirmed by t.l.c. (R_F 0·77‡), chromatography on DEAE-Sephadex A25, and paper electrophoresis (0·05 M phosphate buffer, pH 7·6). Treatment of (**7a**) with *ca.* 15 mol equiv. of iodine in pyridine–water (1:1 v/v) at 20 °C for 15 min gave 2′,3′-*OO*-methoxymethyleneuridine 5′-phosphate (**7b**), R_F 0·44‡ as virtually the sole nucleotide product. The latter compound (**7b**) was converted[7] quantitatively into uridine 5′-phosphate (R_F 0·23‡) by treatment first with dilute hydrochloric acid and then with dilute aqueous ammonia. The conversion of (**7a**) into (**7b**) follows the procedure developed by Cook *et al.*[9] for the similar transformation of nucleoside *S*-ethyl phosphorothioates.

In the same way (Scheme 3), the partially-protected dinucleoside phosphate (**8**) was converted into the dinucleotide, pUpU (**10**). Phosphorylation of (**8**) with (**4c**) gave the fully-protected dinucleotide [^{31}P n.m.r. ([$^2H_6$]acetone–pyridine): δ −7·24 and 26·87 p.p.m.] corresponding to (**6**) in 76% isolated yield.§ Treatment of the latter product with 12·5 mol equiv. each of *syn*-4-nitrobenzaldoxime and $N^1N^1N^3N^3$-tetramethylguanidine in dioxan–water (4:3 v/v) at 20 °C for 4 h gave (**9a**) [^{31}P n.m.r. ([$^2H_6$]acetone–pyridine): δ −1·07 and 17·69 p.p.m.; R_F 0·67‡] which was isolated pure following chromatography on DEAE-Sephadex A25.¶ Treatment of (**9a**) with a twentyfold excess of iodine in aqueous pyridine gave (**9b**) as virtually the sole



(5) (6) (7)

a; R = SMe
b; R = OH

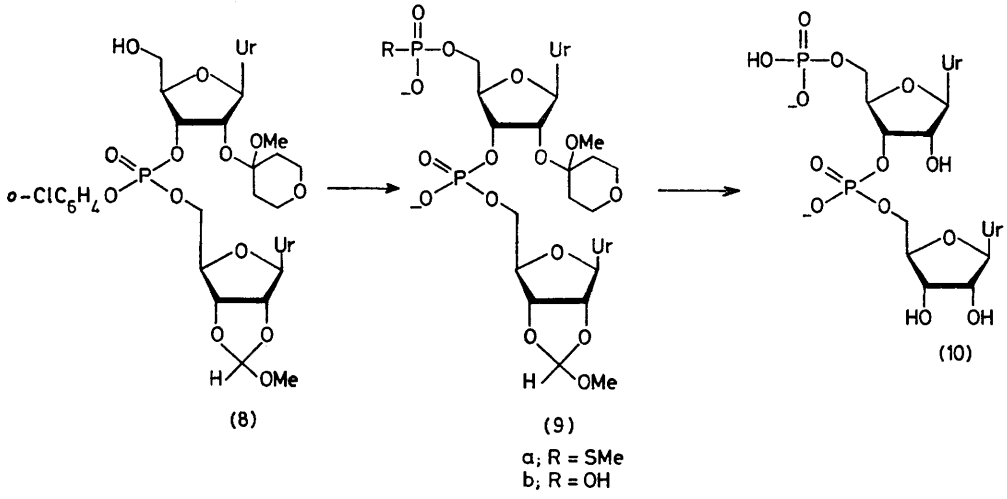
SCHEME 2. Ur = Uracil-1-yl, Ar = 2,4,-$Cl_2C_6H_3$.

† It is particularly convenient to prepare (**3**) in one arm of an H-shaped apparatus and then pour it directly into (**2**) and SO_2, contained in the other arm. Sulphuryl chloride (50 mmol) is added dropwise to dimethyl disulphide (50 mmol) at −67 °C (acetone–dry ice bath). The resulting crude orange (**3**) is added to a solution of (**2c**; 100 mmol) in sulphur dioxide (*ca.* 20 ml) also at −67 °C. The orange colour is immediately discharged and a pale green solution is obtained. The products are allowed to stand at −67 °C for 30 min and then allowed to warm up to room temperature. Distillation gives (**4c**; 18·8 g, 64%), b.p. 140–145 °C at 1·5 mmHg; ^{31}P n.m.r. ($CDCl_3$): δ34·71 p.p.m.; ^{1}H n.m.r. ($CDCl_3$): δ 2·61 (3H, d, *J* 20 Hz) and 7·1–7·5 (3H, m).

‡ T.l.c. data are given for Merck DC-Alufolien Cellulose chromatograms developed (ascending) in the solvent system: EtOH–M-NH_4OAc (6:4 v/v).

§ Yields of (**6**) and the phosphorylation product of (**8**) have not yet been optimized. If the phosphorylation reactions were carried out in acetonitrile solution in the presence of a 1-alkylimidazole catalyst, yields would very probably be much higher.

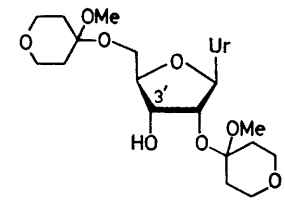
¶ Compound (**9a**) accounted for only 77% of the total nucleotide products eluted from the column. The DEAE-Sephadex chromatogram obtained suggested that a longer reaction time (say, 8–10 h) with 4-nitrobenzaldoximate ion is required for the complete unblocking of the 2-chlorophenyl-protected internucleotide linkage.



SCHEME 3. Ur = Uracil-1-yl.

nucleotide product. The desired dinucleotide, pUpU (**10**) (R_F 0·16‡) was obtained in almost quantitative yield by treating (**9b**) first with dilute hydrochloric acid (pH 2, 20 °C, 6 h) and then with dilute aqueous ammonia; this material was completely digested to uridine 5′-phosphate (R_F 0·25‡) in the presence of *Crotalus adamanteus* snake venom phosphodiesterase.

Preliminary experiments suggest that *O*-aryl *S*-methyl phosphorochloridothioates (**4**) are equally suitable for the phosphorylation of relatively hindered terminal 3′-hydroxy groups in oligoribonucleotide synthesis. Thus (**4b**) reacted readily with (**11**)[10] and the product was unblocked by the reaction sequence described above to give uridine 3′-phosphate.



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Ur = Uracil-1-yl.

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