

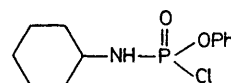
## O-Aryl S-Methyl Phosphorochloridothioates: Terminal Phosphorylating Agents in the Phosphotriester Approach to Oligonucleotide Synthesis

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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.

3'- and 5'-ends of oligonucleotide chains is therefore required. The use of aryl phosphoramidochloridates, such as phenyl phosphorocyclohexylamidochloridate (**1**), has

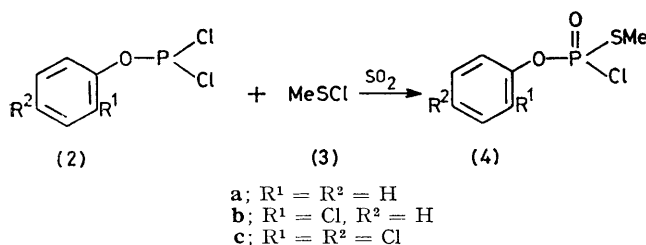


(1)

THE procedures which have been devised so far for the synthesis of oligonucleotides by the phosphotriester approach<sup>1</sup> 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

been recommended<sup>2</sup> for this purpose. However, the unblocking conditions then required<sup>2</sup> 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,<sup>3</sup> we believe that a much better solution of this problem lies in the use of *O*-aryl *S*-methyl phosphorochloridothioates (4).



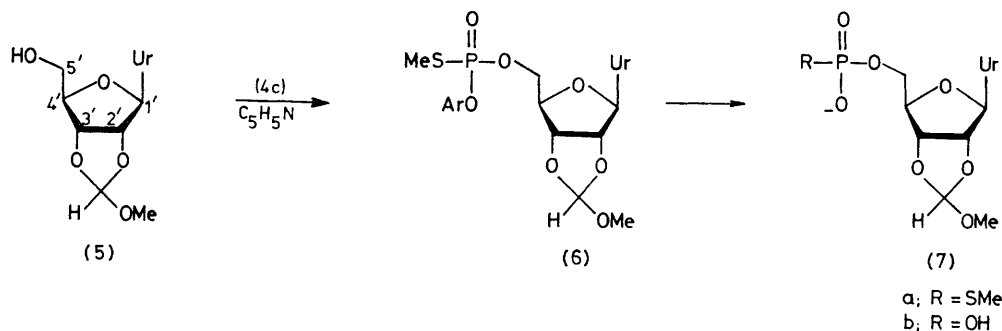
SCHEME 1

The latter reagents (4) may be readily prepared<sup>4</sup> (Scheme 1) by adding methanesulphenyl chloride (3) to a solution of the appropriate aryl phosphorodichloridite (2) in sulphur dioxide at  $-67^\circ\text{C}$ . As methanesulphenyl chloride (3) may be prepared<sup>5</sup> *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<sup>6</sup> (2c) in 64% isolated yield.<sup>†</sup> 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*-methoxymethyluridine<sup>7</sup> (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  $20^\circ\text{C}$ . The desired product (6) [<sup>31</sup>P n.m.r. ([<sup>2</sup>H<sub>6</sub>]acetone-pyridine):  $\delta$  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<sup>8</sup> and triethylamine in dioxan-water (8:5 v/v) at  $20^\circ\text{C}$  for 1 h gave (7a) [<sup>31</sup>P n.m.r. ([<sup>2</sup>H<sub>6</sub>]acetone-pyridine):  $\delta$  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<sup>‡</sup>), 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  $20^\circ\text{C}$  for 15 min gave 2',3'-*OO*-methoxymethyleneuridine 5'-phosphate (7b),  $R_F$  0.44<sup>‡</sup> as virtually the sole nucleotide product. The latter compound (7b) was converted<sup>7</sup> quantitatively into uridine 5'-phosphate ( $R_F$  0.23<sup>‡</sup>) 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.*<sup>9</sup> 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 [<sup>31</sup>P n.m.r. ([<sup>2</sup>H<sub>6</sub>]acetone-pyridine):  $\delta$  -7.24 and 26.87 p.p.m.] corresponding to (6) in 76% isolated yield.<sup>§</sup> Treatment of the latter product with 12.5 mol equiv. each of *syn*-4-nitrobenzaldoxime and *N*<sup>1</sup>*N*<sup>1</sup>*N*<sup>3</sup>*N*<sup>3</sup>-tetramethylguanidine in dioxan-water (4:3 v/v) at  $20^\circ\text{C}$  for 4 h gave (9a) [<sup>31</sup>P n.m.r. ([<sup>2</sup>H<sub>6</sub>]acetone-pyridine):  $\delta$  -1.07 and 17.69 p.p.m.;  $R_F$  0.67<sup>‡</sup>] which was isolated pure following chromatography on DEAE-Sephadex A25.<sup>¶</sup> Treatment of (9a) with a twentyfold excess of iodine in aqueous pyridine gave (9b) as virtually the sole

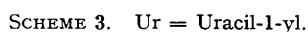
SCHEME 2. Ur = Uracil-1-yl, Ar = 2,4-Cl<sub>2</sub>C<sub>6</sub>H<sub>3</sub>.

<sup>†</sup> It is particularly convenient to prepare (3) in one arm of an H-shaped apparatus and then pour it directly into (2) and SO<sub>2</sub>, contained in the other arm. Sulphuryl chloride (50 mmol) is added dropwise to dimethyl disulphide (50 mmol) at  $-67^\circ\text{C}$  (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  $-67^\circ\text{C}$ . The orange colour is immediately discharged and a pale green solution is obtained. The products are allowed to stand at  $-67^\circ\text{C}$  for 30 min and then allowed to warm up to room temperature. Distillation gives (4c; 18.8 g, 64%), b.p.  $140\text{--}145^\circ\text{C}$  at 1.5 mmHg; <sup>31</sup>P n.m.r. (CDCl<sub>3</sub>):  $\delta$  334.71 p.p.m.; <sup>1</sup>H n.m.r. (CDCl<sub>3</sub>):  $\delta$  2.61 (3H, d, *J* 20 Hz) and 7.1–7.5 (3H, m).

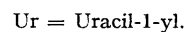
<sup>‡</sup> T.l.c. data are given for Merck DC-Alufolien Cellulose chromatograms developed (ascending) in the solvent system: EtOH-M-NH<sub>4</sub>OAc (6:4 v/v).

<sup>§</sup> 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.

<sup>¶</sup> 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.



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**)<sup>10</sup> and the product was unblocked by the reaction sequence described above to give uridine 3'-phosphate.



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