4-Nitrophenyl Phenyl Phosphorochloridate: A New Phosphorylating Agent for Oligonucleotide Synthesis

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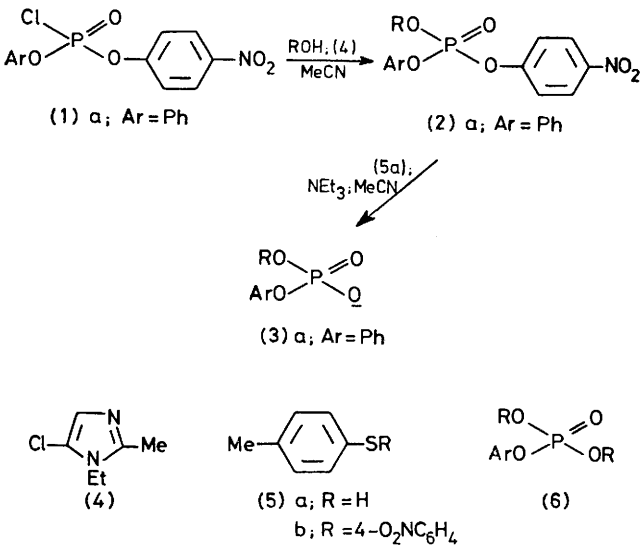
Summary 2′,5′-Protected ribonucleosides (**7**) and 5′-protected 2′-deoxyribonucleosides (**8**) readily react with 4-nitrophenyl phenyl phosphorochloridate (**1a**) in the presence of base to give good to high yields of phosphotriesters of general formula (**2a**) which, on treatment with *p*-thiocresol and triethylamine in acetonitrile are quantitatively converted into the corresponding phosphodiesters (**3a**); the latter are intermediates in oligonucleotide synthesis by the phosphotriester approach.

ALTHOUGH the phosphotriester approach[1] is beginning to emerge as the method of choice for the chemical synthesis of oligo- and poly-nucleotides, several problems remain to be solved.[2] The most important of these is the development of a really satisfactory phosphorylation procedure. We now report that 4-nitrophenyl phenyl phosphorochloridate (**1a**) is an excellent phosphorylating agent for the first step of the phosphotriester approach.

The first step of the phosphotriester approach with an aryl protecting group involves the conversion of a protected nucleoside or oligonucleotide with a free 3′-hydroxy group (ROH) into the corresponding phosphodiester intermediate (**3**; Ar = aryl). In previous studies,[1,2] like most other workers in this field, we have used a bifunctional phosphorylating agent such as an aryl phosphorodichloridate or a monoaryl phosphate in the presence of an arenesulphonyl chloride for this purpose. In the deoxy-series, this approach led to the formation of substantial quantities of symmetrical products[2] (**6**) in addition to the desired phosphodiester intermediates (**3**). A further disadvantage of the monoaryl phosphate procedure is that unacceptably low yields of products are obtained when the substrates contain guanine residues.

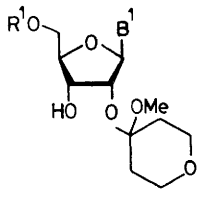


SCHEME

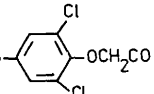
4-Nitrophenyl phenyl phosphorochloridate (**1a**), which may be obtained as a crystalline solid, in 65% isolated yield, from the products of the reaction between phenyl phosphoro-

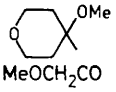
dichloridate and 4-nitrophenol† in the presence of a catalytic amount of 5-chloro-1-ethyl-2-methylimidazole[3] (**4**), is a powerful phosphorylating agent. Thus the reactions (Scheme) between the relatively hindered ribonucleoside building blocks [ROH; (**7a**),[4] (**7b**),[5] and (**7c**)[6]] and 2-3-fold excesses of (**1a**) in the presence of (**4**) [3 mol. equiv. with respect to (**1a**)] in acetonitrile solution were complete within 3 h at 20 °C and the corresponding intermediate phosphotriesters (**2a**) were isolated as colourless solids, following short chromatography[7] on silica gel, in 91, 92, and 82% yields, respectively. Under the same conditions, 5′-protected deoxyribonucleoside building blocks[1,2] [ROH; (**8a**) and (**8b**)] underwent rapid phosphorylation and the corresponding intermediate phosphotriesters (**2a**) were isolated in 86 and 75% yields, respectively. The somewhat lower yields obtained in the deoxy-series are not significant as the preparations have not yet been optimized.

When the phosphotriesters (**2a**) were treated with 10 mol. equiv. of *p*-thiocresol (**5a**) and triethylamine in acetonitrile solution at 20 °C for *ca.* 1 h, they were quantitatively converted into (**5b**) and the triethylammonium salts of the corresponding phosphodiesters (**3a**). The latter intermediates (**3a**) were isolated as pure colourless solids, free from (**5a**), (**5b**), and other impurities, by precipitation with cyclohexane from dichloromethane solution. Both the acid- and base-sensitive protecting groups are quite stable under these mild reaction conditions.

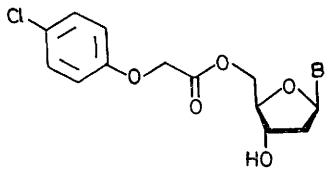


(7)

a; B^1 = uracil-1-yl, R^1 = Me- 

b; B^1 = N^2-benzoylguanin-9-yl, R^1 = 

c; B^1 = N^6-benzoyladenin-9-yl, R^1 = $MeOCH_2CO$

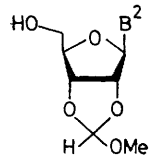


(8)

a; B = thymin-1-yl

b; B = N^4-benzoylcytosin-1-yl

The phosphodiester intermediate (**3a**) derived from the uridine building block (**7a**) was treated with *ca.* 1·6 mol. equiv. of 2,4,6-tri-isopropylbenzenesulphonyl chloride[8] (TPS) in anhydrous pyridine solution at 20 °C followed, after 1 h, by *ca.* 1·2 mol. equiv. of each of the 2′,3′-*O*-methoxymethylene derivatives[9] (**9a**—**d**). After further periods of 16 h, the corresponding fully protected dinucleoside phosphates (**10**) [*i.e.* fully protected UpU, UpC, UpG, and UpA] were isolated, following short column chromatography,[7] as pure solids in 85, 82, 70, and 81% yields, respectively. In the same way, the phosphodiester intermediate (**3a**) derived from the guanosine building block (**7b**) was converted into fully protected GpG (**10**; B^1 = B^2 = N^2-benzoylguanin-9-yl, R^1 = 4-methoxytetrahydropyran-4-yl) in 72% yield and the intermediates (**3a**) derived from the deoxynucleoside building blocks [(**8a**) and (**8b**)] were treated first with TPS and then with (**11**)[2] to give fully protected TpT and dCpT in 96 and 92% isolated yields, respectively. In a preliminary experiment, the phosphodiester intermediate (**3a**) derived from the adenosine building block (**7c**) was condensed with



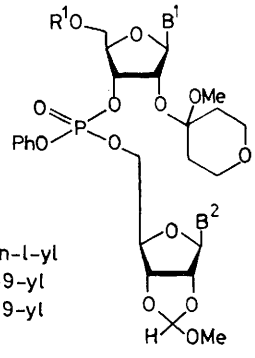
(9)

a; B^2 = uracil-1-yl

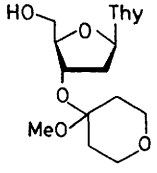
b; B^2 = N^4-*p*-anisoylcytosin-1-yl

c; B^2 = N^2-benzoylguanin-9-yl

d; B^2 = N^6-benzoyladenin-9-yl



(10)



(11)

Thy = thymin-1-yl

the partially protected dinucleoside phosphate (**10**; B^1 = uracil-1-yl, B^2 = N^4-*p*-anisoylcytosin-1-yl, R^1 = H) to give fully protected ApUpC in 60% isolated yield. After complete removal of the protecting groups, all the products underwent total digestion in the presence of appropriate enzymes (ribonuclease A, ribonuclease T_1, or spleen phosphodiesterase) and were thereby shown to contain exclusively 3′ → 5′-internucleotide linkages.

Other monofunctional phosphorylating agents have recently been suggested[10] for the first step of the phospho-

† This is more convenient than the literature preparation (I. Dilaris and G. Eliopoulos, *J. Org. Chem.*, 1965, **30**, 686) of (**1a**) from 4-nitrophenyl phosphorodichloridate and phenol.

triester approach. Nevertheless, we believe that (**1a**) or a simple derivative of it, such as (**1**; Ar = 2-ClC_6H_4), will prove to be the most useful reagent for this purpose yet proposed.

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