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A NEW PHOSPHORYLATING AGENT, 2,6-DICHLOROPHENYL 5-CHLORO-8-QUINOLYL PHOSPHOROCHLORIDATE. ITS APPLICATION IN DEOXYRIBOOLIGONUCLEOTIDE SYNTHESIS BY THE PHOSPHOTRIESTER APPROACH

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The mononucleotide units (4) was prepared by the action of 2,6-dichlorophenyl 5-chloro-8-quinolyl phosphorochloridate on nucleosides. 2,6-Dichlorophenyl group in the mononucleotide units (4) have been employed as a protecting group for 3'-terminal phosphodiester functions; it is stable under standard operations required for the deoxyribooligonucleotide synthesis and readily removed by the action of t-$BuNH_2$ in pyridine-H_2O within 90 min at room temperature.

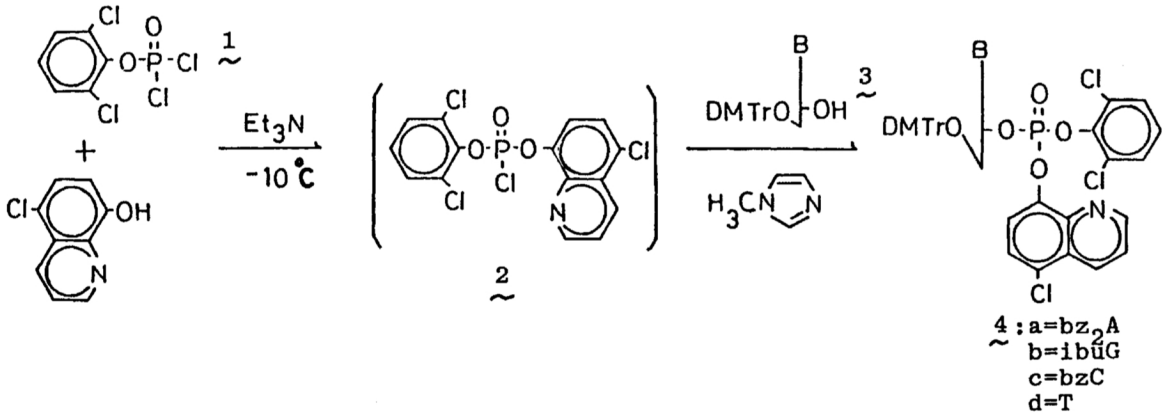
In previous paper,[1a-i] we have described the utility of nucleoside 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphates as the key intermediates for the synthesis of oligonucleotides by the phosphotriester approach. Thus, the mononucleotide units have been employed for elongation of the chain in the 3'- and 5'-directions RNA.[1b-i] Recently, we found that 5-chloro-8-quinolyl group as a protecting group for the internucleotidic bonds could be deprotected by treatment with N^1,N^1,N^3,N^3-tetramethylguanidium salt of 2-pyridinealdoximate (PAO).[2,3] However, in order to obtain the nucleoside 3'-(5-chloro-8-quinolyl) phosphates. we have used PAO for the selective removal of 4-chlorophenyl group from the mononucleotide units, whereupon the treatment of the mononucleotide units with PAO is liable to cause the formation of by-product such as nucleoside 3'-(4-chlorophenyl) phosphates. In order to prevent this side reaction, we have examined the development of a new phosphate protecting group for 3'-terminal phosphodiester functions in oligonucleotide synthesis and have found that 2,6-dichlorophenyl group is much more effective as a protecting group for 3'-terminal functions than 4-chlorophenyl and 2-cyanoethyl groups.

Phosphorylation of 5'-O-dimethoxytrityl-N-protected nucleosides (3) using the phosphorylating agent 2 prepared simply from 2,6-dichlorophenyl phosphorodichloridate (1) and 5-chloro-8-hydroxyquinoline in the one flask reaction was performed as follows: To a dry THF (20 ml) solution of 2,6-dichlorophneyl phosphorodichloridate (1) (1.67 g, 6.0 mmol) was added a dry THF (10 ml) solution 5-chloro-8-hydroxyquinoline (1.46 g, 6.6 mmol) at -10 °C; subsequently, a dry THF (5 ml) solution of triethylamine (1.01 ml, 6.6 mmol) was added, and the reaction mixture was gradually warmed to room temperature. After 1 h, triethylammonium hydrochloride was removed by filtration. To the filtrate was added 5'-O-dimethoxytrityl-N^6,N^6-dibenzoyldeoxyadenosine (3a)[4] (3.72 g, 4.0 mmol) and 1-methylimidazole

(9.6 ml, 12 mmol) and the mixture was kept for 45 min. The reaction mixture was quenched with ice-water (2 ml) and extracted with CH_2Cl_2 (50 ml X 3). The CH_2Cl_2 extract was washed with water (50 ml X 2), dried with Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved again in CH_2Cl_2 and chromatographyed on silica gel column. The appropriate fractions [eluted with CH_2Cl_2-MeOH (97:3, v/v)] were evaporated to give the fully protected mononucleotide (4a)[5] which was isolated as a solid (4.33 g, 94%) by precipitation from hexane.

In a similar manner, other nucleoside 3'-phosphotriester derivatives, 4b, 4c, and 4d, were obtained in 81%, 84%, and 85% yields, respectively.[5] Uhlmann and Pfleiderer have reported[6] 2,5-dichlorophenyl group is a useful protecting group for 3'-terminal phosphodiester functions in oligonucleotide synthesis. Therefore, the phosphorylation of 3d was attempted by use of 2,5-dichlorophenyl, 5-chloro-8-quinolyl phosphorochloridate in place of 2, but the corresponding 5'-O-dimethoxytritylthymidine 3'-(2,5-dichlorophenyl, 5-chloro-8-quinolyl) phosphate could not be obtained in satisfactory yield (60%) after separation by silica gel column.[7]

Next, the deprotection of 2,6-dichlorophneyl group from 4 was examined and it was found that the deprotection is successfully carried out in the presence of t-$BuNH_2$ in pyridine-H_2O for 90 min at room temperature. The stability of the 2,6-dichlorophenyl group was compared with those of previously known base-labile protecting groups[8] in pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v). The results obtained are given in Table 1. It can be seen from Table 1 that 2,6-dichlorophenyl group undergo dearylation rapidly than 4-chlorophenyl group but slowly than 2,5-dichlorophenyl and 2-cyanoethyl groups. The stability of 2,6-dichlorophenyl group is suitable situated as a protecting group for 3'-terminal phosphodiester functions. It is noteworthy that under the conditions the N-protecting groups were stable. Further, the mononucleotide units (4) could be synthesized by use of the phosphorylating agent 2 prepared in simply in one flask reaction. The results indicate clearly that 2,6-dichlorophenyl group is the most suitable 3'-terminal phosphodiester protecting group in oligonucleotide synthesis.



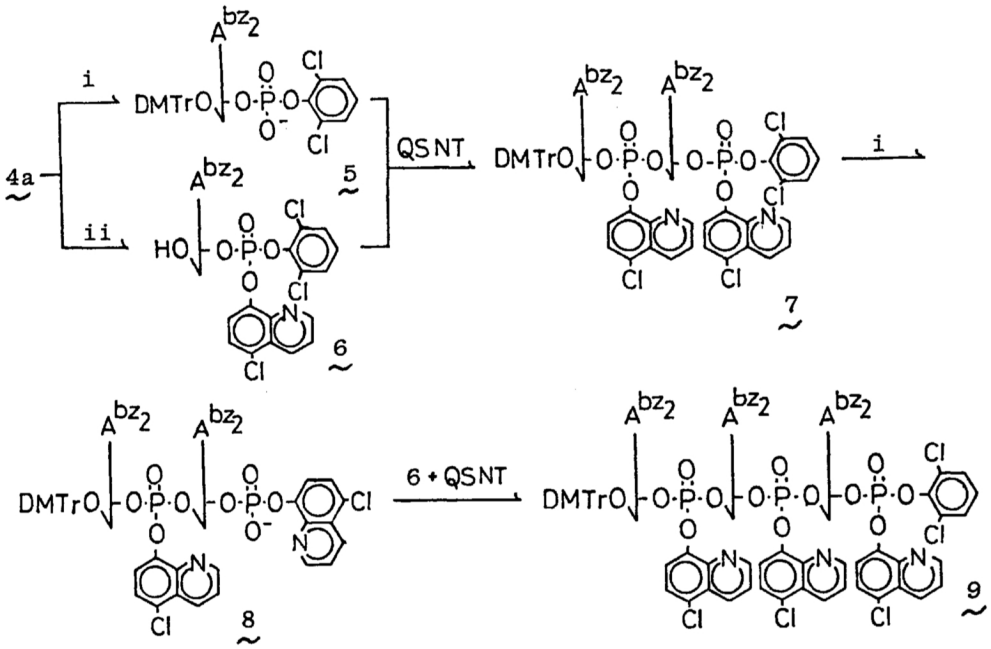
The utility of 4 can be demonstrated in the following synthesis of d-ApApAp (9). The unit 4a (1.82 g, 1.95 mmol) was treated with pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v, 20 ml) at room temperature for 90 min. Following removal of most of the sol-

$$\text{DMTrTpQCl(OR)} \xrightarrow{\text{reagent}} \text{DMTrTpQCl}$$

Table 1. Comparision of the 2,6-Dichlorophenyl Group with Previously Known Base-Labile Protecting Groups[a)]

R	Reagent	$t_{1/2/min}$	$t_{complete/min}$
2,6-Cl_2Ph	pyridine-H_2O-t-$BuNH_2$(8:1:1)	15	90
2,6-Cl_2Ph	CH_3CN-H_2O-t-$BuNH_2$(8:1:1)	30	280
4-ClPh	pyridine-H_2O-t-$BuNH_2$(8:1:1)	40	300
2,5-Cl_2Ph	pyridine-H_2O-t-$BuNH_2$(8:1:1)	8	50
NCEt	pyridine-t-$BuNH_2$(9:1)	3	15

a) The reactions were estimated by TLC.



1. pyridine-H_2O-t-$BuNH_2$(8:1:1)
2. zinc acetate
3. conc. ammonia
4. 80% AcOH

→ d-ApApAp

(i) pyridine-H_2O-t-$BuNH_2$(8:1:1)
(ii) 3% Cl_3CCOOH in CH_3NO_2-MeOH(95:5)

vent in vacuo, the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 was washed with 5% $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated in vacuo. The phosphodiester 5 [^{31}P NMR ($CDCl_3$-pyridine-d_5): δ +7.31 ppm] was isolated in an almost quantitative yield. On the other hand, 4a (2.29 g, 2.0 mmol) was treated with 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5, v/v, 50 ml) at room temperature for 5 min. The mixture was quenched with pyridine and extracted with CH_2Cl_2. The extract CH_2Cl_2 was washed with water, dried over Na_2SO_4, and evaporated in vacuo. The residue was precipitated with hexane-ether (95:5, v/v) to give the corresponding 5'-hydroxyl nucleotide 6 in 92% (1.55 g) yield. The condensation reaction of 5 and 6 (1.09 g, 1.30 mmol) in the presence of 8-quinolinesulfonyl-3-nitro-1H-2,3,4-tetrazole

(QSNT)[1e] (1.78 g, 5.85 mmol) in dry pyridine (6.5 ml) for 2 h gave the fully protected dinucleotide (7) in 92% (2.21 g) yield. Treatment of 7 (1.70 g, 1.30 mmol) thus obtained with pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v) at room temperature for 90 min gave the phosphodiester derivative 8 in an almost quantitative yield. The 3'-phosphodiester component 8 and 6 (1.39 g, 1.6 mmol) were condensed by using QSNT (1.01 g, 3.30 mmol) in dry pyridine (5 ml). The condensation reaction was completed within 2 h and the usual workup gave the fully protected triadenylate (9) in 82% (2.29 g) yield.

Deprotection of 9 was performed as follows: 1) pyridine-H_2O-t-$BuNH_2$ (8:1:1, v/v) at room temperature for 2 h to remove the 2,6-dichlorophenyl group; 2) zinc acetate in pyridine-H_2O (9:1, v/v) at room temperature for 24 h to remove the 5-chloro-8-quinolyl group[3]; 3) conc. ammonia-pyridine (9:1, v/v) at 60 °C for 6 h to remove the benzoyl group; 4) 80% AcOH at room temperature for 15 min to remove the DMTr group. Thus, d-ApApAp was isolated in 79% yield after chromatographic separation using Whattman 3 MM paper (n-PrOH-conc. NH_4OH-H_2O, 6:1:3, v/v). The deblocked trimer d-ApApAp was completely degradated by spleen phosphodiesterase to give a single spot of d-Ap.

References

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5) 4a: mp 114-116 °C; Rf=0.79; UV: λmax (MeOH)272, 232 nm, λmin 254 nm; ^{1}H NMR ($CDCl_3$) δ 9.00-6.45 (m, 33H, C-8, C-2, and Ar), 6.10 (t, 1H, 1'-H), 4.67 (m, 1H, 3'-H), 3.94 (m, 1H, 4'-H), 3.75 (s, 6H, OCH_3), 3.55 (br s, 2H, 5'-H); Found: C, 62.39; H, 3.89; N, 7.47%. Calcd for $C_{60}H_{46}N_6O_{10}PCl_3$: C, 62.57; H, 4.37; N, 7.32%.
4b: mp 131-134 °C; Rf=0.60; UV: λmax (MeOH) 282, 260, 233 nm, λmin 269, 255 nm; ^{1}H NMR ($CDCl_3$) δ11,90 (br s, 1H, NH), 8.71-8.45 (m, 2H, NH and Ar), 7.82-7.05 (m, 19H, C-8 and Ar), 7.18 (d, 2H, Ar), 6.20 (m, 2H, 1'-H and 3'-H), 4.50 (m, 1H, 4'-H), 3.71 (s, 6H, OCH_3), 3.42 (br s, 2H, 5'-H); Found: C, 58.14; H, 4.29; N, 7.98%. Calcd for $C_{49}H_{44}N_6O_{10}PCl_3$: C, 58.03; H, 4.37; N, 8.23%.
4c: mp 107-111 °C; Rf=0.66; UV: λmax (MeOH) 268, 232, λmin 241 nm; ^{1}H NMR ($CDCl_3$) δ8.81-8.41 (m, 2H, Ar), 8.16 (d, 1H, C-6), 8.01-7.08 (m, 23H, NH and Ar), 6.76 (d, 2H, Ar), 6.72 (d, 1H, C-5), 6.25 (d, 1H, 1'-H), 5.90 (m, 1H, 3'-H), 4.65 (m, 1H, 4'-H), 3.75 (s, 6H, OCH_3), 3.56 (br s, 2H, 5'-H); Found: C, 61.39; H, 4.10; N, 5.59%. Calcd for $C_{52}H_{42}N_4O_{10}PCl_3$: C, 61.27; H, 4.12; N, 5.49%.
4d: mp 115-118 °C; Rf=0.63; UV: λmin (MeOH) 268, 232 nm, λmin 261 nm; ^{1}H NMR ($CDCl_3$) δ 9.85 (br s, 1H NH), 8.70-8.41 (m, 3H, C-6 and Ar), 7.70-6.65 (m, 19H, Ar), 6.50 (m, 1H, 1'-H), 6.08 (m, 1H, 3'-H), 4.05 (br s, 1H, 4'-H), 3.72 (s, 6H, OCH_3), 3.52 (m, 2H, 5'-H); Found: C, 60.85; H, 5.29; N, 4.04%. Calcd for $C_{45}H_{39}N_3O_{10}PCl_3$: C, 60.88; H, 5.39; N, 4.28%.
TLC data are given for Merck $60F_{254}$ silica gel plates developed in the solvent system: CH_2Cl_2-MeOH (9:1, v/v).
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7) 5'-O-Dimethoxytritylthymidine 3'-(2,5-dichlorophenyl, 5-chloro-8-quinolyl) phosphate was found to be formed almost quantitatively by TLC analysis, but it was not stable enough to be isolated by a silica gel column chromatography.
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