CHEMISTRY LETTERS, pp. 197-200, 1981.

4-CHLOROPHENYL 5-CHLORO-8-QUINOLYL PHOSPHOROCHLORIDATE: A PRACTICALLY USEFUL PHOSPHORYLATING AGENT FOR OLIGORIBONUCLEOTIDE SYNTHESIS VIA PHOSPHOTRIESTER APPROACH

Hiroshi TAKAKU*, Masatoshi YOSHIDA, Kazuo KAMAIKE, and Tsuyoshi HATA+

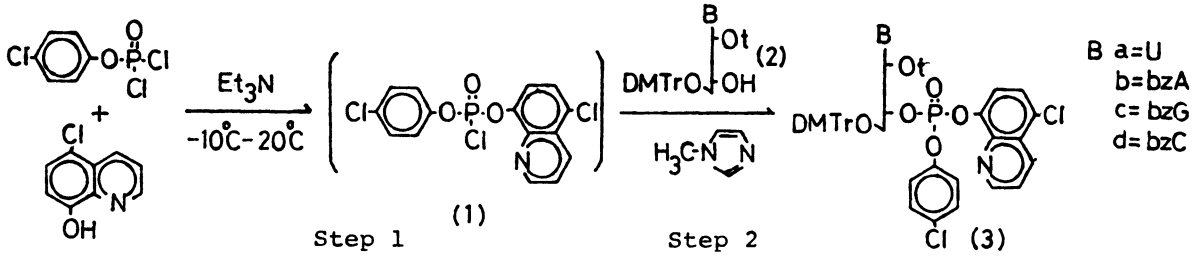
Laboratory of Organic Chemistry, Chiba Institute of Technology, Narashino, Chiba 275 and +Department of Life Chemistry, Tokyo Institute of Technology, Nagatsuta, Yokohama 227

5'-O-Dimethoxytrityl-2'-O-tetrahydropyranylnucleosides (2) smoothly reacts with 4-chlorophenyl 5-chloro-8-quinolyl phosphorochloridate (1) prepared from 4-chlorophenyl phosphodichloridate and 5-chloro-8-hydroxyquinoline to give 5'-O-dimethoxytrityl-2'-O-tetrahydropyranylnucleoside 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphates (3) in high yields. They are key intermediates for the synthesis of oligoribonucleotides via phosphotriester approach.

Recently, we have reported[1] that 5'-O-dimethoxytrityl-2'-O-tetrahydropyranylnucleoside 3'-(2-cyanoethyl, 5-chloro-8-quinolyl) phosphate is an important starting material in the synthesis of oligoribonucleotides by the phosphotriester method. The 5-chloro-8-quinolyl group is stable in acid and alkali solution, removal being achieved specifically by treatment with zinc chloride in aqueous pyridine.[1-4] However, we observed that the fully protected ribonucleoside 3'-phosphotriester intermediate could not be obtained in satisfactory yield. Phosphorylation of the 3'-hydroxyl group of 2',5'-protected nucleoside is one of the key steps in the synthesis of oligoribonucleotides by the phosphotriester method. In order to overcome this problem we tried to use 4-chlorophenyl 5-chloro-8-quinolyl phosphorochloridate (1) prepared simply from 4-chlorophenyl phosphorodichloridate and 5-chloro-8-hydroxyquinoline in one flask reaction.

We first examined phosphorylation of the 3'-hydroxyl group of nucleoside using 4-chlorophenyl 5-chloro-8-quinolyl phosphorochloridate (1): To a dry THF (5.5 ml) solution of 4-chlorophenyl phosphorodichloridate (0.52 ml, 3.18 mmol) was added a dry THF (8.0 ml) solution of 5-chloro-8-hydroxyquinoline (629 mg, 3.50 mmol) and triethylamine (0.49 ml, 3.50 mmol) at -10°C. The reaction temperature was gradually raised to room temperature, and the mixture was stirred for 45 min.[5] The reaction was monitored by tlc. After completion of the reaction, triethylammonium hydrochloride was removed by filtration. To the filtrate was added

5'-O-dimethoxytrityl-2'-O-tetrahydropyranyluridine (1.0 g, 1.59 mmol) and 1-methylimidazole (0.37 ml, 4.56 mmol). The mixture was kept for 1 h at room temperature. The reaction mixture was quenched with ice-water and extracted with methylene chloride. The methylene chloride extract was washed with water, and evaporated in vacuo. The residue was dissolved again in methylene chloride and chromatographed on a silica gel short column. The nucleoside 3'-phosphotriester 3a was isolated in 91% yield (1.43 g) by eluting the column with methylene chloride-methanol (99:1 v/v). Similarly, other ribonucleoside 3'-phosphotriesters (3) were obtained in good yields as shown in Table 1. In the above experiments, THF afforded 3 in better yields than pyridine. Furthermore, 1-methylimidazole is more suitable base than triethylamine in Step 2 (see Table 1). All of the products were homogeneous by HPLC and identified by ^{1}H-NMR and gave satisfactory elemental analysis.



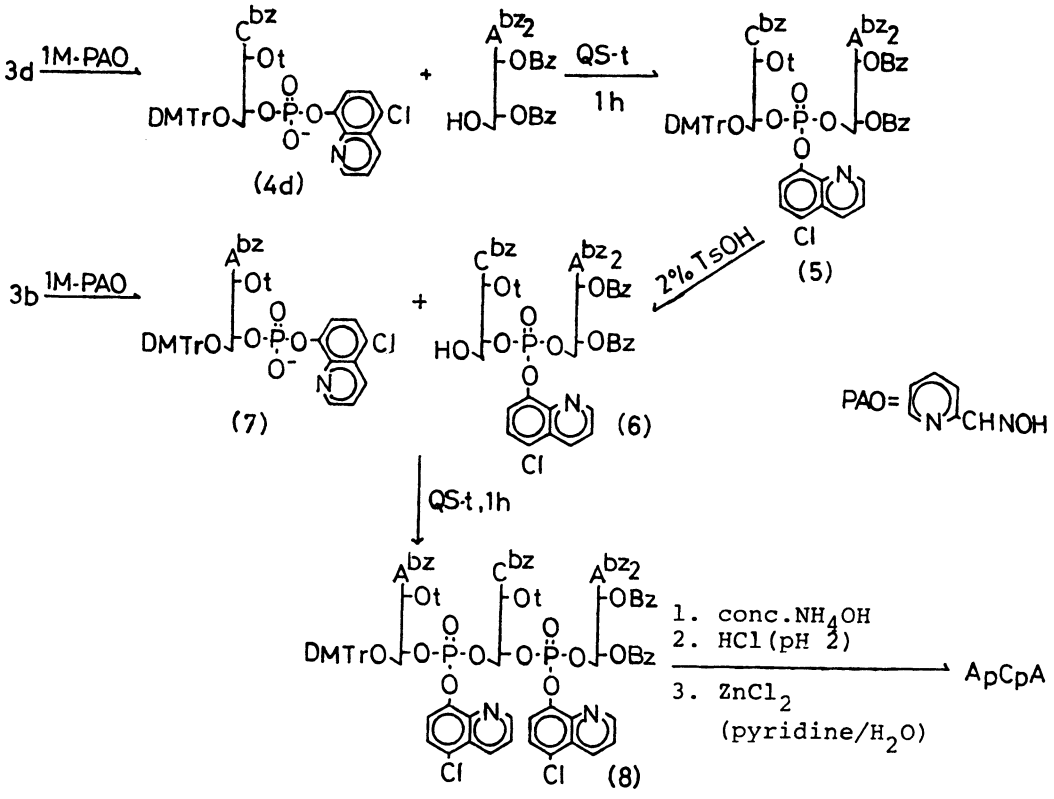
DMTr=dimethoxytriryl; t=tetrahydropyranyl

Table 1. Synthesis of ribonucleoside 3'-phosphotriesters (3)

	Step 1				Step 2			
$ClC_5H_4OP(O)Cl_2$	ClQOH	Et_3N	solvent	time	DMTrNt	1-MeIm	time	yield
mmol (ml)	mmol (mg)	mmol (ml)	ml	h	mmol (mg)	mmol (ml)	h	% (mg)
1.00 (0.16)	1.10 (198)	1.10 (0.15)	pyridine 4.5	6	U 0.50 (314)	Et_3N 1.50 (0.21)	22	40 (196)
2.00 (0.33)	2.20 (395)	3.30 (0.46)	pyridine 9.0	6	U 1.00 (629)	3.00 (0.24)	10	56 (550)
1.00 (0.16)	1.10 (198)	1.10 (0.15)	THF 4.5	0.75	U 0.50 (314)	Et_3N 1.50 (0.21)	24	30 (147)
3.18 (0.52)	3.50 (629)	3.50 (0.49)	THF 13.5	0.75	U 1.59 (1000)	4.56 (0.37)	1	91 (1425)
2.00 (0.33)	2.20 (395)	2.20 (3.00)	THF 9.0	0.75	bzA 1.00 (758)	3.00 (0.24)	1	90 (999)
2.00 (0.33)	2.20 (395)	2.20 (3.00)	THF 9.0	0.75	bzC 1.00 (734)	3.00 (0.24)	1	91 (988)
2.00 (0.33)	2.20 (395)	2.20 (3.00)	THF 9.0	0.75	bzG 1.00 (774)	3.00 (0.24)	3	72 (811)

ClQOH=5-chloro-8-hydroxyquinoline; DMTrNt=5'-O-dimethoxytrityl-2'-O-tetrahydropyranylnucleoside; 1-MeIm=1-methylimidazole.

Next, we examined the synthesis of a dinucleotide (5) and a trinucleotide (8) by using 3: The phosphotriester (3d) (731 mg, 0.75 mmol) was treated with 1M-N^1,N^1,N^3,N^3-tetramethylguanidium salt of 2-pyridinaldoxime[6] (1.8 ml) in a mixture of dioxane and water (1:1 v/v) (27 ml) for 16 h at 20°C. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form) and the resin was removed by filtration and washed with aqueous pyridine (50%). The filtrate was washed with ether and extracted with methylene chloride. The methylene chloride extract was rendered anhydrous by repeated coevaporation with dry pyridine. The phosphodiester (4d)[7] thus obtained was dissolved in dry pyridine (2.3 ml) and then $N^6,N^6,2',3'$-O-tetrabenzoyladenosine (773 mg, 1.13 mmol) and a powerful coupling agent, 8-quinolinesulfonyltetrazolide (QS-t)[8] (396 mg, 1.5 mmol) were added. The reaction mixture was stirred for 1 h at room temperature. 8-Quinolinesulfonic acid was removed by filtration. The filtrate was then quenched with ice-water, followed by extraction with methylene chloride, and the organic layer was washed with water. The methylene chloride solution was concentrated <u>in vacuo</u>. The residue was dissolved in methylene chloride and chromatographed on a silica gel column. The fully protected dinucleotide (5) was isolated in 89% yield (1.09 g) by eluting the column with methylene chloride-methanol (95:5 v/v). The dinucleotide 5 thus obtained was treated with 2% p-toluenesulfonic acid in a mixture of dioxane and methanol (7:3 v/v) (24 ml) for 15 min at 0°C to give 6.[3] The 5'-hydroxyl dinucleotide (6) was isolated in 91% yield (740 mg) by precipitation with petroleum ether and used for next coupling reaction without further purification. On the other hand, the compound (3b) (898 mg, 0.9 mmol) was treated with N^1,N^1,N^3,N^3-tetramethylguanidium salt of 2-pyridinaldoxime to cleave the 4-chlorophenoxy group



and the corresponding phosphodiester (7) was condensed with the partially protected dinucleotide (6) (740 mg, 0.6 mmol) employing QS-t (412 mg, 1.8 mmol) for 1 h to give the fully protected trinucleotide (8) in 85% isolated yield (1.18 g).

The fully protected oligoribonucleotides were completely deblocked by treatment with concentrated ammonia for 5 h at 50°C, followed by 0.01N hydrochloric acid for 20 h at 20°C and zinc chloride in aqueous pyridine for 24 h at room temperature.[1-4] The deblocked oligoribonucleotides, CpA and ApCpA were obtained in 92% and 89% yields, respectively, after separation by ion-exchange chromatography on DEAE cellulose DE-52. The structures of the deblocked products was confirmed by complete digestion of the ribooligonucleotides with nuclease P1 to the expected products in the theoretical ratios.

The phosphorylating agent 1 is stable and can be kept in THF for 2 weeks, but the better results were obtained when 1 was prepared directly before use.

In conculsion, it was found that 4-chlorophenyl 5-chloro-8-quinolyl phosphorochloridate (1) was a useful phosphorylating agent for the synthesis of nucleoside 3'-phosphotriesters (3) and the agent can be prepared simply from 4-chlorophenyl phosphorochloridate and 5'-chloro-8-hydroxyquinoline in one flask reaction.

References and Notes

1. H.Takaku, T.Nomoto, Y.Sakamoto, and T.Hata, Chemistry Lett., 1979, 1225.
2. H.Takaku, R.Yamaguchi, and T.Hata, ibid., 1979, 5.
3. H.Takaku, R.Yamaguchi, T.Nomoto, and T.Hata, Tetrahedron Lett., 1979, 3857.
4. H.Takaku, M.Kato, M.Yoshida, and R.Yamaguchi, J.Org.Chem., 45, 3347 (1980).
5. The reaction mixture was treated with an excess of water for 12 h at room temperature and a usual work-up gave in 98% yield as a monocyclohexylammonium salt of 4-chlorophenyl 5-chloro-8-quinolyl phosphate. Mp 148-150°C; Anal. Calcd for $C_{21}H_{23}Cl_2N_4O_4P$: C, 53.75; H, 5.12; N, 5.97. Found: C, 53.69; H, 5.12; N, 5.81.
6. C.B.Reese, R.C.Titmas, and L.Yau, Tetrahedron Lett., 1978, 2727.
7. The phosphodiesters 4d and 7 were isolated in almost quantitative yields by precipitation as the pyridinium salts with petroleum ether. ^{31}P NMR spectra of 4d and 7 are shown below. 4d: +6.12 and +6.47 (pyridine, 85% H_3PO_4), Rf=0.80; 7: +5.55 and +6.08 (pyridine, 85% H_3PO_4), Rf=0.82. TLC was performed on Merck cellulose F using ethanol-1M ammonium acetate (5:3 v/v).
8. H.Takaku and M.Yoshida, J.Org.Chem., in press.

(Received November 27, 1980)