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5-CHLORO-8-QUINOLYL GROUP AS A PROTECTING GROUP ON PHOSPHOMONOESTERS IN THE SYNTHESIS OF OLIGONUCLEOTIDES

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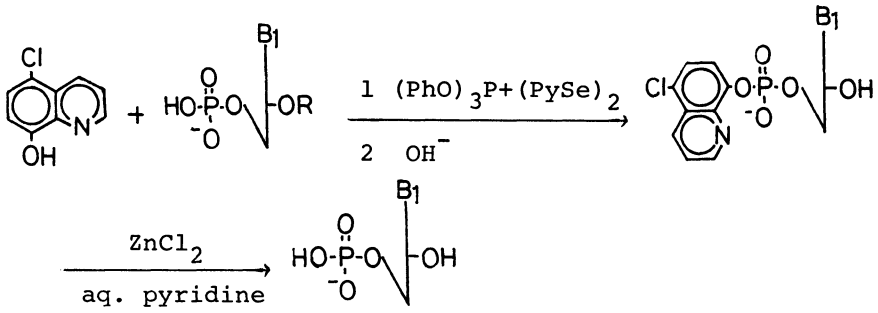
5-Chloro-8-quinolyl group (ClQ) has been used as a protecting group of phosphate in the synthesis of oligonucleotides. This group was selectively and smoothly removed by treatment with zinc chloride in aqueous pyridine at room temperature for 12 hr.

In a previous paper[1], we have reported that 8-quinolyl group could be used as a protecting group on terminal phosphate in oligonucleotide synthesis. This group was removed by treated with cupric chloride in a mixture of dimethyl sufoxide (DMSO) and water (5:1 v/v) at 50°C for 5 hr. In order to improve the removal condition of 8-quinolyl group, 5-chloro-8-quinolyl group (ClQ) was tested. It was found that 5-chloro-8-quinolyl group on phosphates was removed smoothly by use of zinc chloride in aqueous pyridine at room temperature.

5-Chloro-8-quinolyl esters of nucleotide were synthesized as follows: When the reaction of 5-chloro-8-hydroxyquinoline[2] (10 mmol) with 3'-O-acetylthymidine 5'-phosphate (d-pTOAc) (2 mmol) was carried out in the presence of triphenyl phosphite [$(PhO)_3P$] (10 mmol) and 2,2'-dipyridyl diselenide [$(PySe)_2$][3] (10 mmol) in dry pyridine (20 ml) at room temperature for 12 hr, 5-chloro-8-quinolyl thymidine 5'-phosphate (d-ClQpT) was obtained in 82% yield after removal of 3'-O-acetyl group by treatment with 1 N sodium hydroxide in usual manner.

Similarly, 5-chloro-8-quinolyl esters of N^6-benzoyldeoxyadenosine 5'-phosphate (d-ClQpA^{bz}), N^2-isobutyryldeoxyguanosine 5'-phosphate (d-ClQpG^{ibu}), and N^4-anisoyl-deoxycytidine 5'-phosphate (d-ClQpC^{an}) were obtained in 85%, 80%, and 81% yields,

respectively.



B_1=thymine, N^6-benzoyladenine, N^4-anisoylcytosine, or isobutyrylguanine.
R =acetyl.

5-Chloro-8-quinolyl group was easily removed from d-ClQpA^{bz} by zinc chloride in a mixture of pyridine and water (9:1 v/v) at room temperature for 12 hr. For example, d-ClQpA^{bz} (0.05 mmol) was treated with zinc chloride (0.15 mmol) in a mixture of pyridine and water (1.5 ml) at room temperature for 12 hr. The mixture was concentrated to dryness, and then treated with Dowex 50W-X2 (free form) for removal of zinc ion. Filtrate was concentrated in vacuo and the residue was dissolved in a small volume of water and it was applied to paper chromatography. After elution of the spot with water, d-pA^{bz} was obtained in 96% yield. Table 1 shows the effects of the metallic salts and the solvent for removal of the quinolyl group.

Table 1. Removal of the 5-chloro-8-quinolyl group from N^6-benzoyldeoxyadenosine 5'-phosphate (d-ClQpA^{bz}).

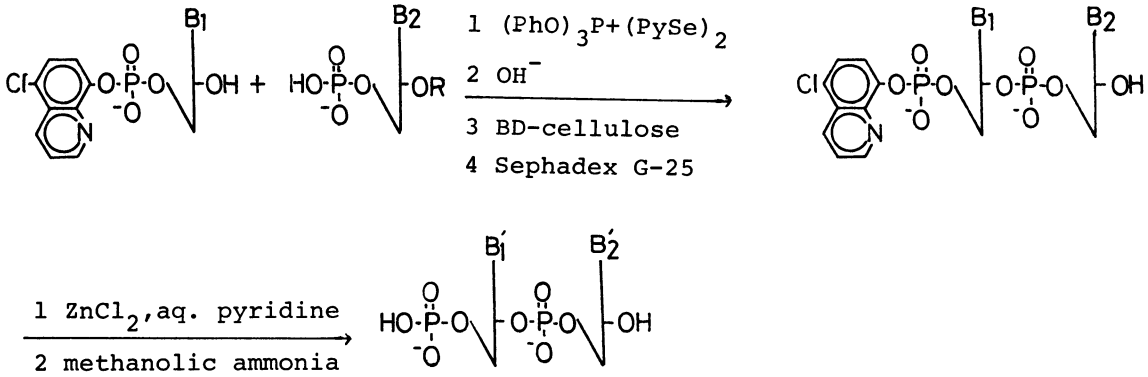
d-ClQpA^{bz} (mmol)	metallic salt (mmol)	solvent	time (hr)	temp. (C°)	yield of d-pA^{bz}* (%)
0.05	$CuCl_2$ (0.05)	DMSO-H_2O (5:1)	12	r.t.	92 (83)
0.05	$CuCl_2$ (0.15)	pyridine-H_2O(9:1)	12	r.t.	34 (30)
0.05	$ZnCl_2$ (0.15)	pyridine-H_2O(9:1)	12	r.t.	96 (28)

*Yields were determined spectrophotometrically and the values in parentheses refer to the yields by employing 8-quinolyl group under the best condition.

When d-ClQpA^{bz} synthesized in the above experiment was treated with d-pTOAc (0.24 mmol) in the presence of $(PhO)_3P$ (1.2 mmol) and $(PySe)_2$ (1.2 mmol) in dry pyridine (1.8 ml) at room temperature for 2 days, the protected dinucleotide, d-ClQpA^{bz}pT[4] was obtained in 62% yield by treatment with 1 N sodium hydroxide to remove the acetyl group.

In a similar manner, dinucleotide derivatives, d-ClQpG^{ibu}pG^{ibu} and d-ClQpC^{an}pT were obtained in 48% and 55% yields, respectively.

All of the mono- and di-nucleotides were characterized by uv spectra after elution of the spots obtained by paper chromatography[5] and paper electrophoresis.



B_1,B_2=thymine, N^6-benzoyladenine, N^4-anisoylcytosine, or N^2-isobutyryl-guanine.
B'_1,B'_2=thymine, adenine, cytosine, or guanine.
R= acetyl.

Finally, removal of 5-chloro-8-quinolyl group from the dinucleotide derivatives by using zinc chloride was examined. For example, d-ClQpA^{bz}pT (0.03 mmol) was treated with zinc chloride (0.15 mmol) in a mixture of pyridine and water (9:1 v/v) (3 ml) at room temperature for 12 hr. After treatment with Dowex 50W-X2 (free form), the resin was filtered off and the filtrate was concentrated in vacuo. The residue was treated with methanolic ammonia for removal of the benzoyl group. The corresponding dinucleotide, d-pApT, was obtained in 94% yield.

In a similar manner, d-pGpG and d-pCpT were obtained in 95% and 92% yields, respectively, based on the corresponding protected dinucleotides.

The structures of the unprotected oligonucleotides were confirmed by enzymatic degradation using snake venom phosphodiesterase after elution of the spots from

paper chromatograms.

In conclusion, there are advantageous points to remove 5-chloro-8-quinolyl group compared with 8-quinolyl group, (i) zinc chloride can be used for removal of the group, (ii) aqueous pyridine was sufficiently employed as solvent in place of a mixture of dimethyl sulfoxide and water (5:1 v/v), (iii) the reaction was carried out at room temperature.

References and notes

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4) These dinucleotide derivatives were separated and isolated by combined use of benzoylated cellulose column chromatography and sephadex G-25 gel-filtration according to the procedure of the method of Narang and his-coworkers: S.A. Narang, J.J.Michiewicz, and S.K.Dheer, J.Amer.Chem.Soc., 91, 937 (1969); S.A. Narang and S.K.Dheer, Biochemistry, 8, 3443 (1969).

5) Paper chromatography was performed by descending technique using Toyo Roshi No.51 paper. Solvent systems used were: (A) i-PrOH-NH_4OH-H_2O (7:1:2 v/v), (B) EtOH-1 M NH_4OAc (7:3 v/v), and (C) n-PrOH-NH_4OH-H_2O (55:10:35 v/v).

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