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5-CHLORO-8-QUINOLYL GROUP AS HIGH EFFICENT PHOSPHATE PROTECTING GROUP FOR THE SYNTHESIS OF OLIGORIBONUCLEOTIDES

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Summary: 5-Chloro-8-quinolyl group is found to be very suitable for the protecting group on phosphates in the internucleotidic bonds. The oligoribonucleotides were obtained in good yields by a simple procedure using this phosphate protecting group.

Phosphotriester approach to the synthesis of oligonucleotide of defined sequence has a few advantages over the diester method.[1] However, the triester method for the synthesis of oligoribonucleotide is confornted with problem to the proper choice of protecting groups for 2'-hydroxyl and phosphate functions.

Recently, we have reported that 8-quinolyl group could be use as a new protecting group on phosphates of internucleotidic bonds in the synthesis of deoxyribooligonucleotides.[2] Under conditions for removal of the above protecting group (cupric chloride in a mixture of DMSO and water, 50°C/5 hr) from the internucleotidic bonds, a number of the internucleotidic bonds were cleaved, and nucleosides and nucleotides were detected.[2] Thus loss of product during deblocking was considerable expect for oligonucleotide. Second, the oligonucleotides by phosphotriester approach are obtained only after several synthetic steps. Consequently, a simple method for the synthesis of oligonucleotides using the phosphotriester approach is required.[3]

In this communication, we wish to report a simple synthetic method for oligoribonucleotides using 5-chloro-8-quinolyl group as a new protecting group on phosphates in the internucleotidic bonds.

First, we describe a phosphorylation of the suitably protected ribonucleosides by means of monophosphates in the presence of 8-quinolinesulfonyl chloride (QS)[5] as a new coupling agent.

When 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl uridine (0.1 mmol) was treated with 5-chloro-8-quinolyl phosphate[6] (0.1 mmol) in the presence of QS (0.2 mmol) in dry pyridine (1 ml) at room temperature for 8 hr, the corresponding

5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl uridine 3'-5-chloro-8-quinolyl phosphate was obtained in quantitative yield. The best result was obtained by use of 5-chloro-8-quinolyl phosphate as monophosphates as shown in Table.

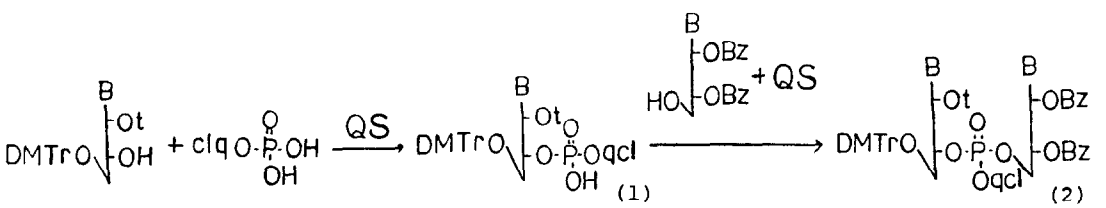
Table Preparation of phosphodiester derivatives (1)

nucleoside (0.1 mmol)	monophosphate (0.1 mmol)	time (hr)	yield* (%)
DMTrUtOH	5-chloro-8-quinolyl phosphate	8	quant.
DMTrUtOH	trichloroethyl phosphate	12	85
DMTrUtOH	4-chlorophenyl phosphate	12	90
DMTrA^{bz}tOH	5-chloro-8-quinolyl phosphate	8	quant.

8-Quinolinesulfonyl chloride (QS) (0.2 mmol) was used in the above reactions.
*Yields were determined by UV absorption.

Next, the reaction of phosphodiester (1) prepared in the above experiments with the suitably protected ribonucleosides in the presence of QS was tried. For example, dinucleotide, DMTrUtp(clq)U(OBz)$_2$ (2a) was prepared as follows: 5'-O-Dimethoxytrityl 2'-O-tetrahydropyranyl uridine (0.7 mmol) was phosphorylated with 5-chloro-8-quinolyl phosphate (0.7 mmol) and QS (1.4 mmol) in dry pyridine (7 ml) at room temperature for 8 hr. The reaction was monitored by silica gel t.l.c. After completion of the reaction, 2',3'-O-dibenzoyluridine (1.05 mmol) and QS (0.7 mmol) were added, and the reaction was continued for a further 20 hr. 8-Quinolinesulfonic acid was removed by filtration. The filtrate was then decomposed with ice-water, followed by exraction with methylene chloride, and the organic layer was backwashed with 0.1M triethylammonium bicarbonate pH 7.5. The methylene chloride solution was evaporated in vacuo and 2a [Rf=0.64][7] was obtained in 89% yield by silica gel column chromatograhy.

Similarly, DMTrUtp(clq)C^{bz}(OBz)$_2$ (2b) [Rf=0.63][7] and DMTrA^{bz}t(clq)U(OBz)$_2$ (2c) [Rf-0.65][7] were obtained in 91% and 87% yields, respectively.

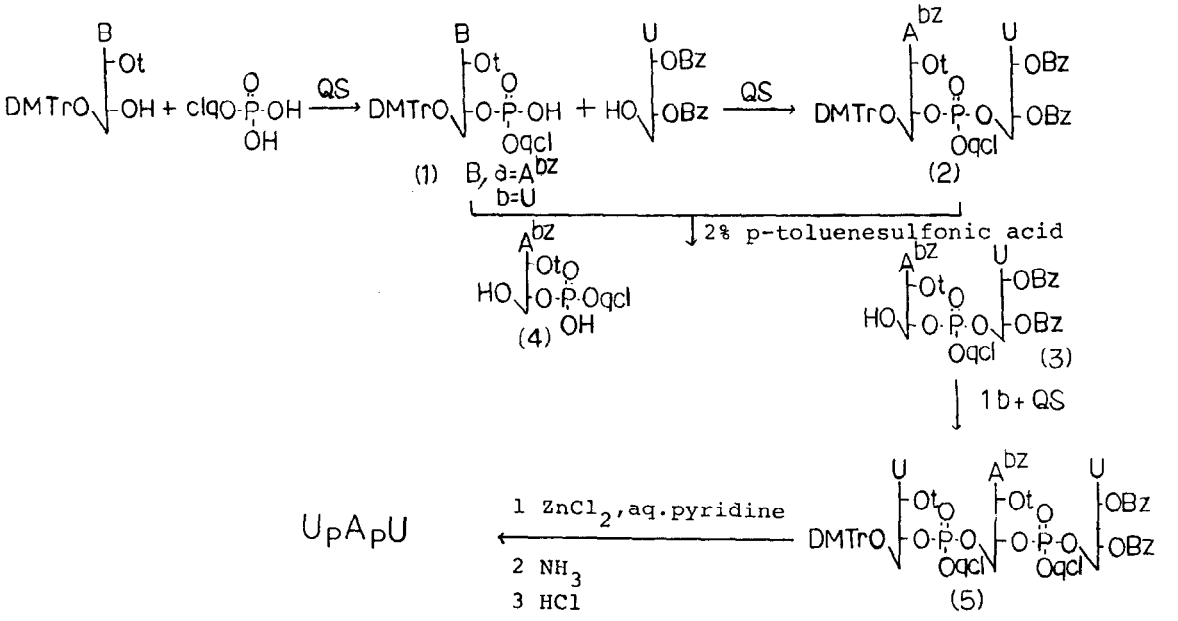


DMTr=dimethoxytrityl; clq=5-chloro-8-quinolyl; QS=8-quinolinesulfonyl chloride; t=tetrahydropyranyl; B=uracil, N^4-benzoylcytosine, N^6-benzoyladenine

Further, trinucleotide, DMTrUtp(clq)A^{bz}t(clq)U(OBz)$_2$ (5) was synthesized simply by use of Itakura's original method[3a] as illustrated in the following

Scheme.

5'-O-Dimethoxytrityl 2'-O-tetrahydropyranyl N^6-benzoyladenosine (1.0 mmol) was phosphorylated with 5-chloro-8-quinolyl phosphate (1.0 mmol) and QS (2.0 mmol) in dry pyridine at room temperature for 8 hr. After completion of the reaction, 2',3'-O-dibenzoyluridine (0.5 mmol) and QS (0.25 mmol) were added and stirred for for 24 hr[8]. The reaction mixture was treated with 2% p-toluenesulfonic acid solution at 0°C for 15 min. The compound (4) was removed from the reaction mixture by extraction with aqueous sodium hydrogen carbonate in methylene chloride. The 5'-hydroxyl dinucleotide (3) was directly isolated from the solvent by precipitation in ether. By use of 2% p-toluenesulfonic acid solution, no depyranylated product was detected during the detritylation reaction. After 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl uridine (1.0 mmol) was phosphorylated with 5-chloro-8-quinolyl phosphate (1.0 mmol) and QS (2.0 mmol), the reaction mixture was treated with 3 and QS (0.25 mmol) for 36 hr. The trinucleotide 5 [Rf=0.45][7] was obtained in 73% yield based on 2',3'-O-dibenzoyluridine after separation by silica gel column chromatography.



Complete deblocking to give free oligoribonucleotide was effected by three-step deblocking procedure: (a) treatment with zinc chloride[9] in aqueous pyridine at room temperature for 24 hr, then treatment with Dowex 50W-X2 (pyridinium form), and concentration of the solution; (b) treatment with concentrated ammonia[10] at 50°C for 5 hr and evaporation to dryness; (c) treatment of the residue with 0.01N hydrochloric acid at 20°C for 20 hr. Finally, the oligoribonucleotides, UpU, UpC, ApU, and UpApU were obtained in 88%, 86%, 89%, and 85% yields after

separation by DEAE Sephadex A-25 column chromatography. The presence of only 3'-5' internucleotidic bonds in the thus obtained completely deblocked products was established by complete digestion of the oligoribonucleotides with snake venom phosphodiesterase and spleen phosphodiesterase to the expected products in the correct ratios.

In conclusion, it is noted that di- and tri-ribonucleotides can be prepared simply from the suitably protected ribonucleosides using 5-chloro-8-quinolyl group as phosphate protecting group of internucleotidic bonds. The adevantages of this method are being applied to the synthesis of polynucleotides. 5-Chloro-8-quinolyl group was selectively and smoothly removed from the internucleotidic bonds by treatment with zinc chloride in aqueous pyridine.

References and Notes

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5. H.Takaku, M.Yoshida, M.Kato, and T.Hata, ibid., 811(.979).
6. 5-Chloro-8-quinolyl phosphate (m.p. 127-129°C) was prepared in 92% yield from 5-chloro-8-hydroxyquinoline and phosphoryl chloride in dry pyridine by modification of the procedure for 8-quinolyl phosphate synthesis [H.Takaku, Chem.Pharm.Bull., 25,2121(1977)]. This phosphate was stable to acid and alkali solutions (hydrochloric acid, pH 2 or aqueous ammonia 6M) over a period of 48 hr.
7. Rf values refer to t.l.c. on Merk silica gel plates $60F_{254}$, with methylene chloride and methanol (9:1 v/v).
8. No 2',3'-O-dibenzoyluridine can be detected on t.l.c. of the reaction mixture.
9. The phosphotriester derivative [DMTrUt(clq)A^{bz}t(clq)U(OBz)$_2$ was transformed to the corresponding phosphodiester derivative [DMTrUtpA^{bz}tpU(OBz)$_2$] in 95% yield by treatment with zinc chloride in aqueous pyridine at room temperature for 24 hr.
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