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NUCLEOSIDE 3'-PHOSPHOTRIESTERS AS KEY INTERMEDIATES FOR THE OLIGORIBONUCLEOTIDES SYNTHESIS VIA PHOSPHOTRIESTER APPROACH

Hiroshi TAKAKU, Tadaaki NOMOTO, Yukimi SAKAMOTO, and Tsujiaki HATA*

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

5'-O-Dimethoxytrityl 2'-O-tetrahydropyranyl nucleoside 3'-(2-cyanoethyl, 5-chloro-8-quinolyl) phosphates (3) were obtained in good yields by treating 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl nucleosides (1) with 5-chloro-8-quinolyl phosphate and 8-quinolinesulfonyl chloride (QS) followed by addition of 2-cyanoethanol. The compound 3 is a key intermediate for the oligoribonucleotide synthesis by the improved triester method.

In the last few years, it has been shown by several workers[1] that the fully protected mononucleotide is a key intermediate in the synthesis of oligonucleotide by the improved triester method. Narang and his coworkers[2] have carried out a synthesis of lactose operator of E. coli using the improved triester method.

Recently, it has been demonstrated in this laboratory that 5'-O-monomethoxytrityl nucleoside 3'-(2-cyanoethyl, 8-quinolyl) phosphate is a key intermediate in the synthesis of deoxyribooligonucleotide by the improved triester method.[1e] However, during work on the oligoribonucleotides synthesis using the improved triester method, we observed that the yields of 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl nucleoside 3'-(2-cyanoethyl, 5-chloro-8-quinolyl) phosphates (3) are quite low (ca. 23%). More recently, Ohtsuka et al.[3] have also reported a low yield (40%) for the synthesis of 5'-O-monomethoxytrityl 2'-O-(o-nitrobenzyl) nucleoside 3'-(2-cyanoethyl, p-chlorophenyl) phosphates as a key intermediate. We overcome this problem by using 8-quinolinesulfonyl chloride (QS)[4] as coupling reagent.

Frist, we describe an efficient method for preparation of 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl nucleoside 3'-(2-cyanoethyl, 5-chloro-8-quinolyl) phosphates (3). For example, 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl uridine (1a) (1 mmol) was phosphorylated with 5-chloro-8-quinolyl phosphate[5] (1.2 mmol) in the presence of QS (2.4 mmol) in dry pyridine (10 ml) for 6 hr at room temperature. The reaction was monitored by silica gel t.l.c..[6] The reaction was quenched with ice-water and the phosphodiester function (2a) was extracted with methylene chloride (20 ml X 3), which was back washed with water. The solvent was evaporated in vacuo. The residue was dissolved in dry pyridine (10 ml) and then 2-cyanoethanol (3 mmol) and QS (2.0 mmol) were added. After 24 hr, 8-quinolinesulfonic acid was removed by filtration. The filtrate was concentrated and the residual pyridine was removed by co-distillation with toluene. The residue was chromatographed on a silica gel column with methylene chloride-methanol (9:1 v/v) eluant to give 5'-O-dimethoxytrityl 2'-O-tetrahydropyranyl uridine 3'-(2-cyanoethyl, 5-chloro-8-quinolyl) phosphate (3a) (76%). The yields of 3 depended on the coupling reagents and the molar ratios of 2-cyanoethanol to the phosphodiester function 2, and better results were obtained by use of QS and a slight excess of 2-cyanoethanol as shown in Table. In a similar manner, the fully protected monoribonucleotides (3) were obtained as shown in Table.

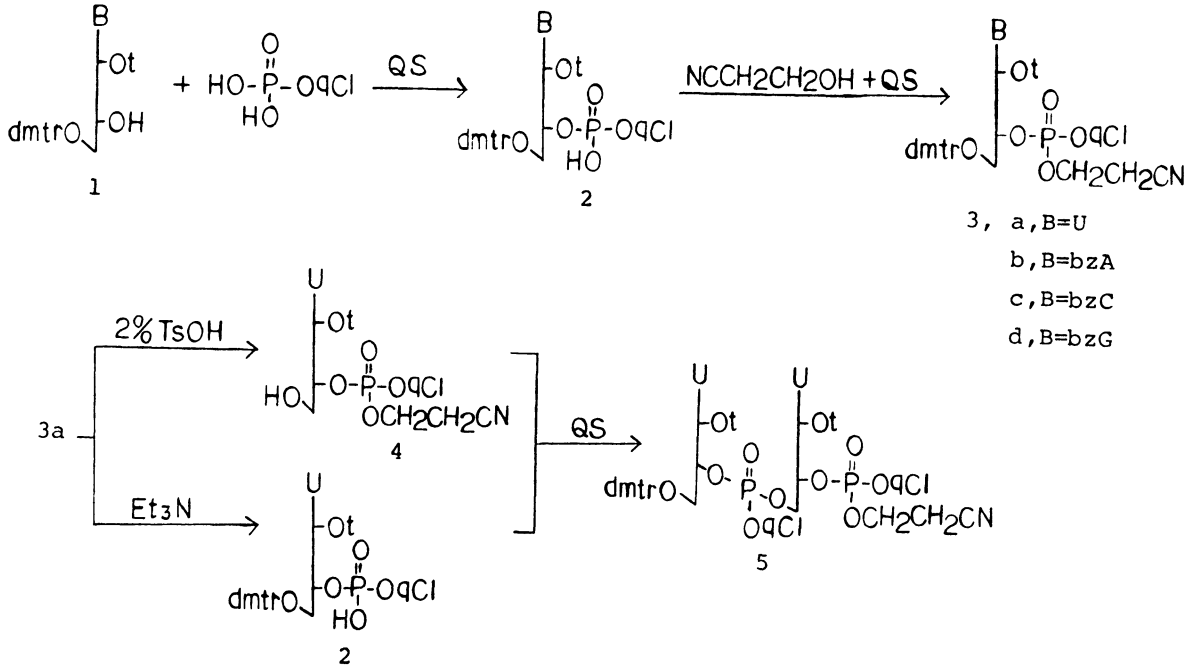
Next, the synthesis of dinucleotide, dmtUtp(qcl)Utp(qcl, ce) (5) was tried by use of 3a prepared in the above experiment as starting material. The compound 3a (0.66 mmol) was treated with 2% p-toluenesulfonic acid in a mixture of methylene chloride and methanol (7:3 v/v) (7 ml) for 15 min at 0°C. The reaction mixture was washed with 5% sodium hydrogencarbonate solution and then water. The methylene chloride was evaporated in vacuo. The residue was dissolved in methylene chloride (2 ml), applied to a silica gel column and eluted with methylene chloride-methanol (9:1 v/v). The 5'-hydroxyl mononucleotide (4) was obtained in 90% yield.[5] Under the above conditions, the other protecting groups were untouched. On the other hand, when 3a (0.6 mmol) was treated with triethylamine (12 mmol) in dry pyridine (3.6 ml) for 8 hr at room temperature, the phosphodiester 2a was obtained in quantitative yield.[7] After evaporation of excess triethylamine, pyridine, and acrylonitrile in vacuo, the residue was dissolved in dry pyridine (3 ml) and then 4 (0.45 mmol) and QS (1.2 mmol) were added. The mixture was stirred for 24 hr at room temperature. After the usual work-up, the fully protected dinucleotide 5

was isolated through short column chromatography, as pure solid in 94% yield.

Table. Syntheses of the fully protected mononucleotides (3)

Step I nucleoside component (1 mmol)	coupling reagent (2.4 mmol)	time (hr)	Step II 2-cyanoethanol (mmol)	coupling reagent (2 mmol)	time (hr)	yield of 3 (%)
mmtU[a]	TPS	12	6	TPS	48	80
mmtT[a]	TPS	12	6	TPS	48	93
mmtbzA[a]	TPS	12	6	TPS	48	96
dmtUt	TPS	12	6	TPS	48	23
dmtUt	QS	6	6	QS	24	35
dmtUt	TPS	12	6	MST[b]	24	31
dmtUt	TPS	12	3	TPS	48	40
dmtUt	TPS	12	3	MST[b]	24	48
dmtUt	QS	6	3	QS	24	76
mmtT[a]	QS	6	3	QS	20	95
mmtbzA[a]	QS	6	3	QS	18	93
dmtbzAt	QS	6	3	QS	24	79
dmtbzCt	QS	6	3	QS	24	81
dmtbzGt	QS	12	3	QS	24	65

a) 5'-O-Monomethoxytrityldeoxyribonucleoside. b) Mesitylenesulfonyl tetrazolide. c) In the above reactions, 5-chloro-8-quinolyl phosphate (1.2 mmol) was used in Step I.



dmtr=dimethoxytrityl; t=tetrahydropyranyl; qcl=5-chloro-8-quinolyl; QS=8-quinolinesulfonyl chloride.

The fully protected dinucleotide 5 was completely deblocked by treatment with triethylamine in pyridine for 18 hr at room temperature, followed by 0.01N hydrochloric acid (pH=2) for 20 hr at 20°C[8] and zinc chloride in aqueous pyridine for 24 hr at room temperature[5]. The dinucleotide, UpUp was obtained in 95% yield after separation by DEAE cellulose DE-52 column chromatography. The removal of the phosphate end group with bacterial alkaline phosphatase and subsequent treatment with pancreatic RNase afforded Up and U in correct ratio 1.08:1.00.

In conclision, it may be noted that the present procedure provides an efficient method for the synthesis of fully protected monoribonucleotides (3). The compound 3 is a suitable intermediate for elongation of the chain in the 3'- and 5'-directions.

Reference and Notes

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6) No starting material 1 can be detected on t.l.c. plate of the reaction mixture.

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