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CESIUM FLUORIDE PROMOTES SYNTHESIS OF RIBOOLIGONUCLEOTIDES VIA PHOSPHOTRIESTER APPROACH

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In the presence of cesium fluoride, the reactions of a fully protected 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N-acylnucleoside 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphates with 5'-hydroxyl nucleosides proceeded rapidly under mild conditions to afford the corresponding ribodinucleoside monophosphates in good yields.

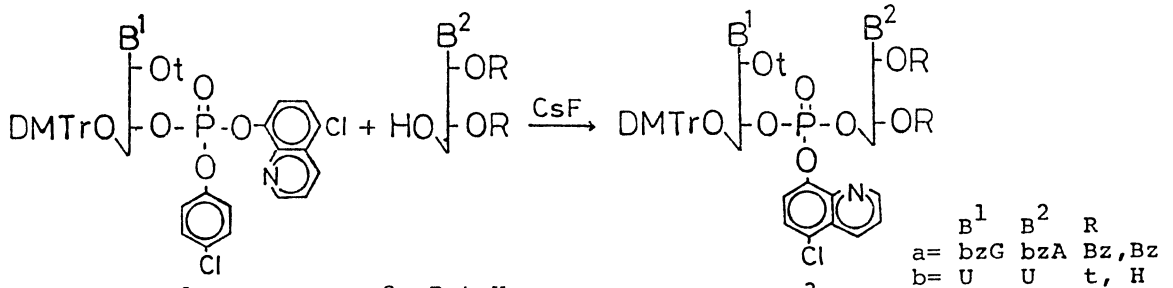
Recently, we have reported[1-4] that 8-quinolinesulfonyl chloride (QS) can be used as a new type coupling agent in the synthesis of oligonucleotides via phosphotriester approach. However, QS is unsatisfactory for the synthesis of ribooligonucleotides containing the guanosine unit owing to the liberation of hydrogen chloride during the coupling reactions and the rate of reactions is very slow (1-2 days). Consequently, we have found that 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N-acylnucleoside 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphates (1) reacted rapidly with 5'-hydroxylnucleosides (2) in the presence of cesium fluoride[5] to give dinucleosides monophosphates (3).

On the base of this fact, the present communication describes a rapid synthetic method of triribonucleotide blocks using cesium fluoride.

First, in order to investigate the effectiveness of cesium fluoride, we have performed the synthesis of dinucleotide (3) containing the guanosine unit. 5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-benzoylguanosine 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphate (1a)[6] (845 mg, 0.75 mmol) was added to N^6,2',3'-O-

tribenzoyladenosine (290 mg, 0.50 mmol) in acetonitrile (7 ml) and cesium fluoride (342 mg, 2.25 mmol). The reaction mixture was stirred for 2 hr at room temperature. The reaction was monitored by silica gel t.l.c.. The precipitate was filtered off and the acetonitrile solution was evaporated to dryness under vacumm. The residue was dissolved in methylene chloride and chromatographed on a silica gel short column. The dinucleotide 3a containing the guanosine unit was isolated in 641 mg (80%) by eluting the column with methylene chloride-methanol (95:5 v/v). Similarly, variuos dinucleoside monophosphates (3) were obtained in good yields as shown in Table 1. As shown in Table 1, cesium fluoride gave the better results than QS for the synthesis of dinucleotide 3a containing the guanosine unit. Furthermore, no detritylated and 3'- 3' linkage[7] products were observed during the synthesis of internucleotidic linkages.

The reaction seems to proceed through an intermediate, phosphofluoridate, formed from 1 and cesium fluoride. The intermediate, phosphofluoridate reacts with nucleoside to give dinucleoside monophosphate (3). This was explained by the following procedure: The phosphotriester 1a (737 mg, 0.75 mmol, Rf=0.51 in 10% acetone-methylene chloride) was treated with cesium fluoride (343 mg, 2.25 mmol) in acetonitrile (7 ml) at room temperature in the absence of 5'-hydroxyl uridine derivative. After 30 min, silica gel t.l.c. indicated complete conversion into a product of Rf=0.05 in 10% acetone-methylene chloride. Then 2',3'-O-dibenzoyluridine (226 mg, 0.5 mmol) was added, and the reaction was continued for 2 hr. The mixture was applied on a silica gel short column chromatography and the product was found to be the corresponding fully protected dinucleotide, 3c (465 mg, 70% yield, Rf=0.47 in 10% acetone-methylene chloride).



1. a; B^1=bzG
   b; B^1=U
   c; B^1=bzC

2. R=t,H
   R=Bz,Bz
   B^2=bzA,U

3

	B^1	B^2	R
a=	bzG	bzA	Bz,Bz
b=	U	U	t, H
c=	U	U	Bz,Bz
d=	U	bzA	t,H
e=	bzC	bzA	Bz,Bz

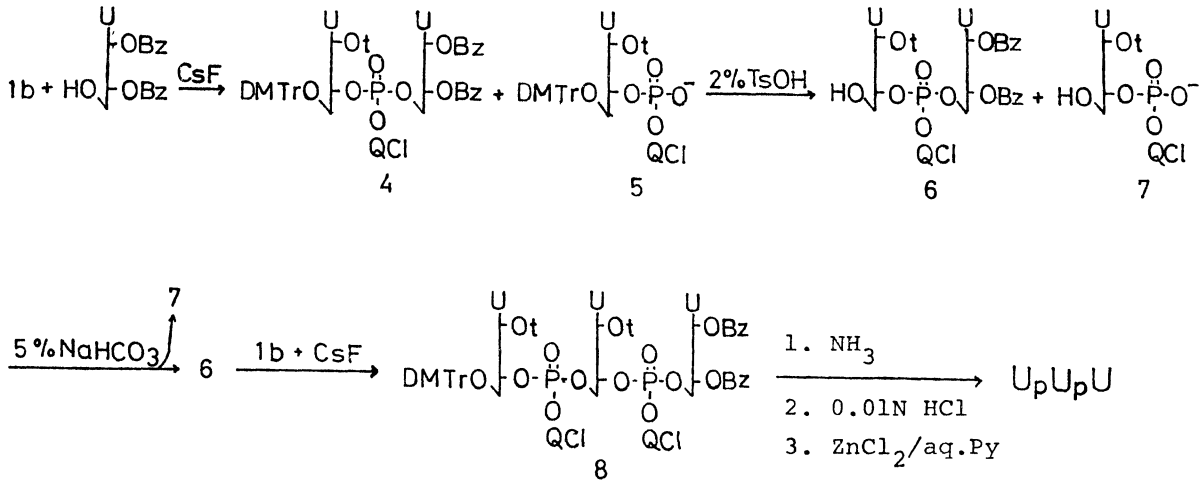
DMTr=dimethoxytrityl; t=tetrahydropyranyl; Bz=benzoyl.

Table 1. Synthesis of dinucleoside monophosphates (3).

fully protected mononucleotide	nucleoside	product	yield(%)
DMTrbzGtpQCl(PhCl)	bzA(OBz)$_2$	DMTrbzGtp(QCl)bzA(OBz)$_2$	80
DMTrbzGtpQCl	bzA(OBz)$_2$	DMTrbzGtp(QCl)bzA(OBz)$_2$	58[a]
DMTrUtpQCl(PhCl)	Ut	DMTrUtp(QCl)Ut	81
DMTrUtpQCl(PhCl)	U(OBz)$_2$	DMTrUtp(QCl)U(OBz)$_2$	81
DMTrUtpQCl(PhCl)	bzAt	DMTrUtp(QCl)bzAt	74
DMTrbzCtpQCl(PhCl)	bzA(OBz)$_2$	DMTrbzCtp(QCl)bzA(OBz)$_2$	82

a) The reaction was carried out using QS in dry pyridine for 24 hr.

Next, we have successfully applied cesium fluoride to a rapid synthetic method of triribonucleotide block (8) as illustrated in the following Scheme.



5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyluridine 3'-(4-chlorophenyl, 5-chloro-8-quinolyl) phosphate (1b) (786 mg, 0.8 mmol) was added to 2',3'-O-dibenzoyluridine (181 mg, 0.4 mmol) in acetonitrile (8 ml) and cesium fluoride (486 mg, 3.2 mmol) and the reaction mixture was stirred for 2 hr at room temperature. No 2',3'-O-dibenzoyluridine can be detected on silica gel t.l.c. in the above reaction. The reaction mixture was quenched with ice-water, followed by extraction with methylene chloride (20 ml X 3), and the organic layer was dried over anhydrous sodium sulfate. The sodium sulfate was filtered off and the methylene chloride was evaporated in vacuo. The residue was treated with 2% p-toluenesulfonic acid solution in methylene chloride-methanol (7:3 v/v) (32 ml) for 15 min at 0°C.[2-4] The detritylated product (7) was removed from the reaction mixture by extraction with 5% sodium hydrogen bicarbonate in methylene chloride. The 5'-hydroxyl dinucleotide (6) was precipitated from n-hexane-ether (9:1 v/v) and used for the next coupling reaction without further

purification. The partially protected dinucleotide (6) thus obtained was treated with 1b (786 mg, 0.8 mmol) and cesium fluoride (486 mg, 3.2 mmol) in acetonitrile (8 ml). The reaction mixture was stirred for 2 hr. The mixture was then work up as described for the synthesis of 3 and the triribonucleotide block (8) was isolated in 702 mg (92%) based on 2',3'-O-dibenzoyluridine after separation by silica gel short column chromatography.

After complete removal of the protecting groups[2-4], the deblocked ribo-oligonucleotides, UpU, UpA, GpA, and UpUpU were isolated 89, 91, 88, 90, and 89% yields, respectively, by ion-exchange chromatography on DEAE cellulose DE-52. The presence of only 3'→5' internucleotidic linkages in thus obtained completely deblocked products was established by complete digestion of the ribooligonucleotides with Nuclease P1 to the expected products in the correct ratios.

In conclusion, the present reaction using cesium fluoride is one flask reaction for the synthesis of internucleotidic linkages starting from the fully protected nucleotides.

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