Synthesis of Bis(5-chloro-8-quinolyl) Nucleoside 5′-Phosphates in Oligoribonucleotide Synthesis by the Phosphotriester Approach[1]

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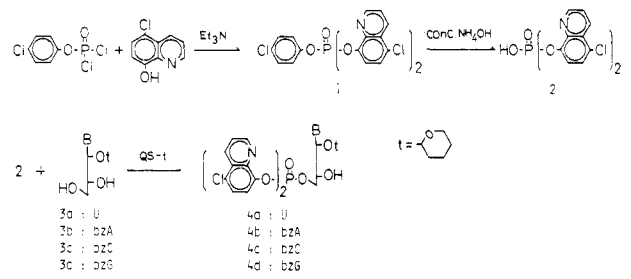
A new phosphorylating agent, bis(5-chloro-8-quinolyl) phosphate was found to be a useful agent for the phosphorylation of the 5′-hydroxyl group of unprotected nucleosides. This agent smoothly reacts with 3′,5′-unprotected nucleosides in the presence of 8-quinolinesulfonyl tetrazolide (QS-t) to give the corresponding bis(5-chloro-8-quinolyl) nucleoside 5′-phosphates in good yields without 3′-mono- and 3′,5′-diphosphorylated products. They are key intermediates for the synthesis of oligoribonucleotides bearing 5′-terminal phosphate. Zinc chloride can be used as an effective and mild agent for removal of 5-chloro-8-quinolyl group.

Recently, several methods for syntheses of oligonucleotides by the phosphotriester approach have been investigated.[2] A large number of phosphorylating agents have been developed with the use of the phosphotriester approach. However, many of these agents were designed for the phosphorylation of the 3′-hydroxyl group of nucleosides whereas only a few methods for the insertion of 5′-terminal phosphate residues have been published.[3] Such 5′-phosphorylated oligoribonucleotides may be required for native transfer ribonucleic acids, cap structure of messenger ribonucleic acids,[4] ligase reactions, and 2′,5′-oligoadenylates.[5] A few years ago, we described methods for the insertion of a 5′-terminal phosphate group at the 5′-hydroxyl group of partially protected oligoribonucleotides by use of 5-chloro-8-quinolyl phosphate in the presence of 2,2′-dipyridyl diselenide and triphenylphosphine.[6] However, these reagents were rather slow in completing the coupling reactions, and the lipophilicity of the 5′-phosphorylated ribooligonucleotides was decreased.

We have therefore undertaken the development of new phosphorylating agents for insertion of a 5′-terminal phosphate.

We examined the possibility of the selective phosphorylation of the 5′-hydroxyl groups of 2′-*O*-tetrahydropyranyl nucleosides **3** using bis(5-chloro-8-quinolyl) phosphate **2** in the presence of 8-quinolinesulfonyl tetrazolide (QS-t) (Scheme I).[7] The phosphorylating agent **2** was prepared as follows. When 4-chlorophenyl phosphorodichloridate was allowed to react with 2.2 equiv of 5-chloro-8-hydroxyquinoline in the presence of 2.2 equiv of triethylamine in dry THF for 6 h at room temperature, bis(5-chloro-8-quinolyl) 4-chlorophenyl phosphate (**1**) was obtained in 76% yield. The phosphotriester **1** thus obtained was further treated in a mixture of concentrated ammonia and pyridine (1:1 v/v) to give the desired phosphorylating agent **2** in an excellent yield of 90%. On the other hand, when *N*-methylpyridinium dichlorophosphate[8] was used for the preparation of **2**, the yield was

Scheme I



Scheme II

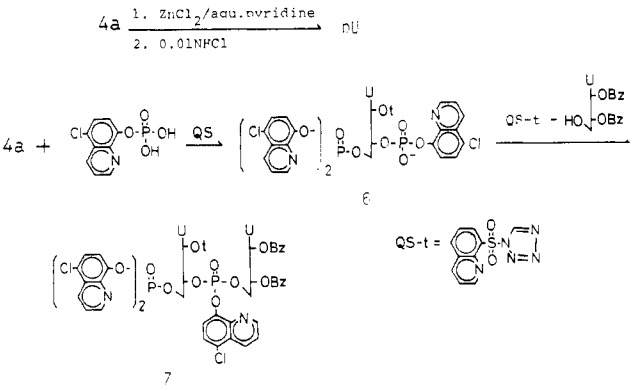


Table I. Selective Phosphorylation of the 5′-Hydroxyl Group of Nucleosides 3[a]

nucleoside	amt, mg, (mmol)	QS or QS-t (mg, mmol)	product	yield, mg (%)
3a (Ut)	328 (1.0)	QS-t (979, 3.75)	$(QCl)_2$pUt	658 (90)
3a (Ut)	328 (1.0)	QS (854, 3.75)	$(QCl)_2$pUt	629 (86)
3b (bzAt)	454 (1.0)	QS-t (979, 3.75)	$(QCl)_2$pbzAt	609 (71)
3c (bzCt)	430 (1.0)	QS-t (979, 3.75)	$(QCl)_2$pbzCt	666 (80)
3d (bzGt)	470 (1.0)	QS-t (979, 3.75)	$(QCl)_2$pbzGt	655 (75)
3d (bzGt)	470 (1.0)	QS (854, 3.75)	$(QCl)_2$pbzGt	366 (42)

[a] The above reactions were carried out in dry pyridine (5 mL) at room temperature for 2 h. The amount of bis(5-chloro-8-quinolyl) phosphate used was 439 mg (1.5 mmol) in all cases.

(1) This paper is part 18 in our series on oligonucleotide syhthesis. For the previous report in this series, see: Takaku, H.; Kono, T. *Chem. Pharm. Bull.*, in press.

(2) Amarnath, V.; Broom, A. D. *Chem. Rev.* **1977**, *77*, 183. Reese, C. B. *Tetrahedron* **1978**, *34*, 3143. Ikehara, M.; Ohtsuka, E.; Markham, A. F. *Adv. Carbohyd. Chem. Biochem.* **1978**, *36*, 135.

(3) Neilson, T.; Deugau, K. V.; England, T. E.; Wertiuk, E. S. *Can. j. Chem.* **1975**, *53*, 1093. van Boom, J. H.; Burgers, P. M.; Crea, R.; Luyten, W. C. M. M.; Vink, A. B. J.; Reese, C. B. *Tetrahedron* **1975**, *31*, 2953. Reese, C. B.%; Yau, L. *J. Chem. Soc., Chem. Commun.* **1978**, 1050. Sekine, M.; Hamaoka, K.; Hata, T. *J. Org. Chem.* **1979**, *44*, 2326. Ohtsuka, E.; Tanaka, T.; Ikehara, M. *Nucleic Acids Res.* **1979**, *7*, 1283. Imai, J.; Torrence, F. *J. Org. Chem.* **1981**, *46*, 4015.

(4) Furuichi, Y.; Miura, K. *Nature (London)* **1975**, *253*, 374. Furuichi, Y.; Morgan, M.; Muthukrishnan, S.; Shatkin, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 742. Wei, C. M.; Moss, B. *Ibid.* **1975**, *72*, 318.

(5) Brown, R.; Kerr, I. *Nature (London)* **1977**, *268*, 537, 540.

(6) Takaku, H.; Kato, M.; Yoshida, M.; Yamaguchi, R. *J. Org. Chem.* **1980**, *45*, 3347.

(7) Takaku, H.; Yoshida, M. *J. Org. Chem.* **1981**, *46*, 589. Takaku, H.; Kato, M.; Ishikawa, S. *Ibid.* **1981**, *46*, 4062. Takaku, H.; Kamaike, K.; Tsuchiya, H. *Nucleic Acids Res., Symp. Ser.* **1981**, *No.10*, 171. Takaku, H.; Kamaike, K. *Chem. Lett.* **1982**, 189.

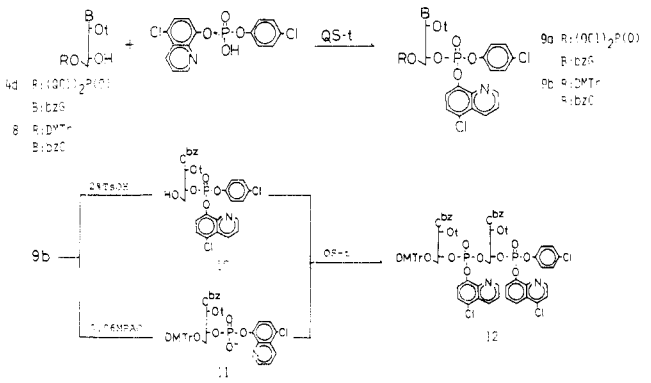
0022-3263/82/1947-4937$01.25/0

Table II. Melting Points, R_f Values, and UV Spectral Data of 4

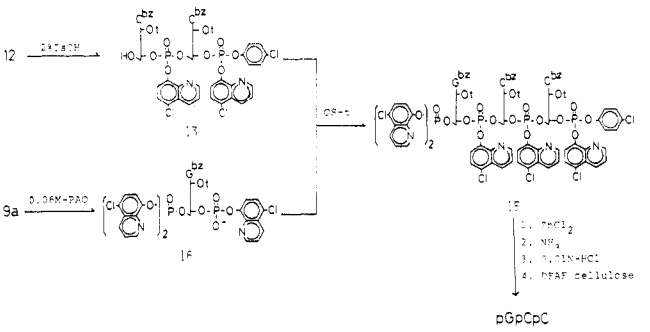
compd	mp, °C	TLC[a] R_f A	TLC[a] R_f B	UV (MeOH), nm λ_{max}	UV (MeOH), nm λ_{min}	formula[b]
4a	99-101	0.30	0.14	264, 232	246	$C_{32}H_{29}N_4O_{10}PCl_2 \cdot 2CH_3OH$
4b	120-122	0.45	0.34	278, 236	247	$C_{40}H_{34}N_7O_9PCl_2 \cdot 2H_2O$
4c	114-117	0.48	0.24	262, 233	245	$C_{39}H_{34}N_5O_{10}PCl_2 \cdot 1.5H_2O$
4d	135-137	0.57	0.21	290, 264, 233	270, 240	$C_{40}H_{34}N_7O_{10}PCl_2 \cdot 0.5H_2O$

[a] Solvent systems: A, CH_2Cl_2-MeOH (9:1 v/v); B, CH_2Cl_2-MeOH (95:5 v/v). [b] Satisfactory C, H, and N analytical data were obtained for all compounds in the table.

Scheme III



Scheme IV



considerably lower. The new phosphorylating agent **2** was found to be stable in 80% acetic acid and concentrated ammonia at room temperature for 2 days.

The phosphorylating agent **2** thus obtained was successfully applied to the selective phosphorylation of the 5′-hydroxyl group of nucleosides **3** in the presence of 8-quinolinesulfonyl tetrazolide (QS-t) to afford bis(5-chloro-8-quinolyl) nucleoside 5′-phosphates **4**. These results are summarized in Table I[9] (see Table II for physical and spectral data).

The above results indicate that 8-quinolinesulfonyl chloride (QS)[10] is unsuitable as a coupling agent for phosphorylation of guanosine. In this case, O^6-phosphorylated guanosine was found to be formed by TLC analysis.[11]

The two 5-chloro-8-quinolyl groups could be removed from **4a** by treatment with zinc chloride in aqueous pyridine at room temperature for 24 h, followed by treatment with 0.01 N HCl (pH 2) to afford the corresponding uridine 5′-phosphate **5** in quantitative yield (Scheme II).

Next, the synthesis of fully protected diuridine diphosphate **7** was examined by using **4a**. Compound **4a** was phosphorylated with 1.2 equiv of 5-chloro-8-quinolyl phosphate[12] in the presence of 2.4 equiv of QS for 2 h. The reaction was quenched with ice–water, and the mixture was extracted with CH_2Cl_2. The extracts were combined and concentrated in vacuo. The residue was dissolved in dry pyridine, and then 0.5 equiv of 2′,3′-di-*O*-benzoyluridine and 2.5 equiv of QS-t were added. After 2 h, the desired diuridine diphosphate **7** was obtained in 85% yield after separation by silica gel column chromatography.

Further, we examined the synthesis of 3′- and 5′-phosphorylated trinucleotides corresponding to the 5′ end (pGpCpCp) of the Rous Sarcoma virus 35S RNA[13] by using the fully protected mononucleoside 3′-phosphotriester derivatives as the starting material.

The fully protected mononucleoside 3′-phosphotriesters **9** were prepared as follows. The compound **4d** was condensed with 1.5 molar equiv of 4-chlorophenyl 5-chloro-8-quinolyl phosphate[11] in the presence of 3.75 molar equiv of QS-t in dry pyridine at room temperature (Scheme III). After 2 h, the corresponding 3′- and 5′-phosphorylated nucleoside phosphotriester **9a** was obtained in 81% yield. In an early work, we found that 4-chlorophenyl 5-chloro-8-quinolyl phosphate can be effectively used for the phosphorylation of guanosine units without formation of side products.[11]

In a similar manner, 5′-*O*-(dimethoxytrityl)-2′-*O*-tetrahydropyranyl-N^4-benzoylcytidine 3′-(4-chlorophenyl 5-chloro-8-quinolyl phosphate) (**9b**) was obtained in 92% yield.

The dimethoxytrityl group of **9b** was selectively removed by treatment with 2% *p*-toluenesulfonic acid solution to give the 5′-hydroxyl phosphotriester intermediate **10** in 90% yield. On the other hand, the 4-chlorophenyl group could be selectively removed from **9b** by treatment with 0.06 M N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboxaldoxime in a mixture of dioxane and water (2:1 v/v) at room temperature for 12 h, giving the phosphodiester derivative **11** in almost quantitative yield.[14] The phosphodiester **11** thus obtained was dissolved in dry pyridine, and then **10** and QS-t were added. After 2 h, the corresponding fully protected dinucleotide **12** was obtained in 83% yield after separation by silica gel column chromatography. The dinucleotide **12** thus obtained was treated with 2% *p*-toluenesulfonic acid solution at 0 °C for 15 min to give the 5′-hydroxyl dinucleotide **13** in 86% yield. On the other hand, the compound **9a** was treated with the N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime to cleave the 4-chlorophenyl group, and the corresponding phosphodiester derivative **14** was

(8) Smrt, J.; Catlin, J. *Tetrahedron Lett.* **1970**, 5081. Rubinstein, M.; Patchornik, A. *Tetrahedron* **1975**, *31*, 2107.
(9) When **4** was treated with dimethoxytrityl chloride in pyridine, the tritylation of **4** did not proceed.
(10) Takaku, H.; Yoshida, M.; Kato, M.; Hata, T. *Chem. Lett.* **1979**, 811. Takaku, H.; Nomoto, T.; Sakamoto, Y.; Hata, T. *Ibid.* **1979**, 1225.
(11) Takaku, H.; Kamaike, K.; Kasuga, K. *Chem. Lett.* **1982**, 197.
(12) Takaku, H.; Yamaguchi, R.; Nomoto, T.; Hata, T. *Tetrahedron Lett.* **1979**, 3857.
(13) Haseltine, W. A.; Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 989. Schwartz, D. E.; Zamecnik, P. C.; Weith, H. L. *Ibid.* **1977**, *74*, 994. Takaku, H.; Nomoto, T.; Kamaike, K. *Nucleic Acids Res., Symp. Ser.* **1980**, *No. 8*, s91.
(14) Reese, C. B.; Titmas, R. C.; Yau, L. *Tetrahedron Lett.* **1978**, 2727. Reese, C. B.; Yau, L. *Ibid.* **1978**, 4443.

Table III. Characterization of Oligoribonucleotides[a]

compd	paper chromatography R_f[b]	electrophoresis RpA	enzymatic analyses (nuclease P1)
pUpU	0.38	1.14	pU
pGpCpCp	0.13	1.36	pG/2pC (1.00:2.09)

[a] The yield for complete deprotection was 80% in both cases. [b] The solvent system used was 1-propanol-concentrated ammonia-water (55:10:35, v/v).

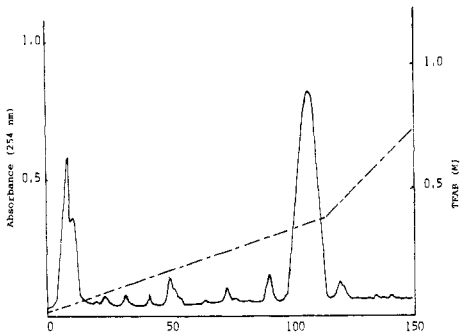


Figure 1. Chromatography of pGpCpCp on a column (1.5 × 50 cm) of DEAE cellulose DE-52 equilibrated with 0.01 M TEAB (pH 75). Elution was performed with a gradient of 0.01–0.75 M TEAB (600 mL). The main peak contained the product pGpCpCp.

condensed with the partially protected dinucleotide **13** by employing QS-t for 2 h to give the fully protected trinucleotide **15** in 71% yield after separation by silica gel column chromatography (Scheme IV).

Complete deblocking of the fully protected di- and trinucleotides was performed as follows. The oligoribonucleotides were deprotected by using first zinc chloride in aqueous pyridine at room temperature for 24 h to cleave the 5-chloro-8-quinolyl group, second concentrated ammonia at 50 °C for 5 h to remove the 4-chlorophenyl and benzoyl groups, and finally 0.01 N hydrochloric acid at 20 °C for 20 h to remove the dimethoxytrityl and tetrahydropyranyl groups. The deblocked trinucleotide pGpCpCp was applied to a column of DEAE cellullose DE-52 (Table III). The elution profile and conditions are given in Figure 1. The deblocked products were completely digested by nuclease P1 to the expected products in the correct ratios and were totally resistant to RNase A. These hydrolyses indicated that the compounds **7** and **15** had a 5'-terminal phosphate and that the starting material **4** was phosphorylated at the 5'-position.

As a phosphorylating agent for protected oligoribonucleotides, bis(5-chloro-8-quinolyl) phosphate has the following advantages: (a) it can be used for the selective phosphorylation of the 5'-hydroxyl group of nucleosides in the presence of QS-t; (b) the resultant triester products may be simply purified by silica gel column chromatography; (c) the 5-chloro-8-quinolyl group was removed by zinc chloride in aqueous pyridine under mild conditions.

The efficiency of this reagent for 5'-phosphorylation of nucleosides has been demonstrated by its application to the synthesis of the 5' end of Rous sarcoma virus 35S RNA.

Experimental Section

Thin-layer chromatography (TLC) was performed by using the ascending technique on Merck $60F_{254}$. The plates were usually developed by using a mixture of methylene chloride and methanol. For columns, silica gel (100–200 mesh, Kanto Chemical Co.) was used. Paper chromatography was performed by using the descending technique on Toyo Roshi No. 51A paper. The solvent system used was 1-propanol–concentrated ammonia–water (55:10:35 v/v). Paper electrophoresis was performed at 1100 V/40 cm by using 0.05 M triethylammonium bicarbonate (pH 7.5). DEAE cellulose DE-52 was used for anion-exchange chromatography. Enzyme analyses is described in ref 7. 8-Quinolinesulfonyl chloride and 1*H*-tetrazole were gifts from Dojin Chemical Co.

Bis(5-chloro-8-quinolyl) Phosphate (2). A dry THF (30 mL) solution of 5-chloro-8-hydroxyquinoline (5.92 g, 33 mmol) was added to a dry THF (15 mL) solution of 4-chlorophenyl phosphorodichloridate (3.18 g, 15 mmol) at 0 °C; subsequently, a dry THF (15 mL) solution of triethylamine (4.29 mL, 33 mmol) was added, and the reaction mixture was gradually warmed to room temperature. After 6 h, the mixture was quenched with ice–water, followed by extraction with methylene chloride (2 × 20 mL). The methylene chloride layer was washed with 5% sodium carbonate solution (2 × 50 mL) and then water, dried over anhydrous sodium sulfate, filtered, and evaporated to an oil. The oil was dissolved in methylene chloride and applied on a silica gel column (4 × 15 cm). The column was eluted with 500 mL of methylene chloride. The appropriate fractions were evaporated to give **1** as a yellow crystal: yield 6 g (76%); mp 118–120 °C; IR (KBr) 1595, 1499, 1360, 1220, 720 cm^{-1}. Anal. Calcd for $C_{24}H_{14}N_2O_4PCl_3$: C, 54.21; H, 2.65; N, 5.27. Found: C, 53.87; H, 2.76; N, 5.28.

The compound **1** (6 g, 7.9 mmol) thus obtained was treated with a mixture of concentrated ammonia and pyridine (1:1 v/v, 100 mL) at room temperature for 24 h. The precipitate was removed by filtration, and the filtrate was evaporated in vacuo. The residue was recrystallized from a mixture of acetonitrile and water (9:1 v/v) to afford pure phosphate **2**: yield 3.1 g (90%); mp 171–173 °C; IR (KBr) 3200, 1600, 1510, 1390, 1260, 720 cm^{-1}. Anal. Calcd for $C_{18}H_{11}N_2O_4PCl_2$: C, 44.64; H, 3.75; N, 5.79. Found: C, 44.35; H, 3.70; N, 5.80.

General Method for the Synthesis of Bis(5-chloro-8-quinolyl) Nucleoside 5'-Phosphates 4. Bis(5-chloro-8-quinolyl) phosphate (**2**, 1.5 molar equiv) was combined with nucleoside **3** (1 molar equiv), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-t (3.75 molar equiv) in pyridine at room temperature for 2 h. The reaction mixture was quenched with ice–water (2.5 mL), followed by extraction with methylene chloride (2 × 50 mL). The methylene chloride layer was washed with 0.5 M triethylammonium bicarbonate (pH 7.5, 50 mL) and then water (50 mL), dried over anhydrous sodium sulfate, filtered, and under reduced pressure evaporated to gum. The gum was dissolved in methylene chloride and purified by column chromatography on silica gel. The column was eluted with methylene chloride–methanol (98:2 v/v). The appropriate fractions were evaporated to give the bis(5-chloro-8-quinolyl) nucleoside 5'-phosphates **4** (see Table I).

Melting points, R_f values, and UV spectral data are summarized in Table II.

Removal of 5-Chloro-8-quinolyl Group from 4a. The compound **4a** (36 mg, 0.05 mmol) was treated with zinc chloride (27 mg, 0.2 mmol) in a mixture of pyridine and water (9:1 v/v, 3 mL) at room temperature for 24 h. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form) and filtered, and the filtrate was evaporated to a gum. The gum was dissolved in 0.01 N hydrochloric acid (pH 2)(2 mL). The resulting solution was stirred at 22 °C for 20 h and evaporated in vacuo. The residue was dissolved in water (2 mL) and chromatographed on Toyo Roshi No. 514 paper developed with 2-propanol-concentrated ammonia–water (7:1:2 v/v) to give a band of R_f 0.11. Elution of the band with water afforded pU (96%).

Synthesis of Diuridine Diphosphate 7. The compound **4a** (348 mg, 0.48 mmol) was combined with 5-chloro-8-quinolyl phosphate[12] (187 mg, 0.72 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS (329 mg, 1.44 mmol) in pyridine (3 mL) for 1.5 h. The reaction mixture was quenched with ice–water (10 mL), followed by extraction with methylene chloride (2 × 30 mL). The methylene chloride extract was washed with 0.5 M triethylammonium bicarbonate (pH 7.5, 2 × 25 mL) and then water (25 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to a gum. The gum was combined with 2',3'-di-*O*-benzoyluridine (151 mg, 0.33 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-t (329 mg, 1.25 mmol) in

dry pyridine (2 mL). After 2 h, the mixture was quenched with ice–water (10 mL), followed by extraction with methylene chloride (2 × 30 mL). The methylene chloride extract was washed with water (3 × 20 mL), dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The residue was dissolved in methylene chloride and subjected to a silica gel chromatography. The column was eluted with a stepwise gradient of methanol (0–5%) in methylene chloride. The appropriate fractions were evaporated to give **7** which was isolated (390 mg, 85%) by precipitation from *n*-hexane–ether (9:1 v/v): UV (methanol) λ_{max} 258, 230, λ_{min} 252 nm; R_f 0.56 (CH_2Cl_2/MeOH, 9:1 v/v), 0.34 (CH_2Cl_2/MeOH, 95:5 v/v).

Synthesis of the Nucleoside 3′-Phosphotriesters 9a,b. The compound **4d** (721 mg, 1 mmol) was combined with 4-chlorophenyl 5-chloro-8-quinolyl phosphate[11] (550 mg, 1.5 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-t (978 mg, 3.75 mmol) in pyridine (5 mL) for 2 h. The reaction mixture was quenched with ice–water (10 mL), followed by extraction with methylene chloride (2 × 25 mL). The methylene chloride extract was washed with 0.5 M triethylammonium bicarbonate (pH 7.5, 2 × 15 mL) and then water (2 × 15 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to a gum. The gum was dissolved in methylene chloride and subjected to a silica gel column chromatography. The column was eluted with a stepwise gradient of methanol (0–3%) in methylene chloride. The appropriate fractions were evaporated to give **9a** which was isolated (992 mg, 81%) by precipitation from *n*-hexane–ether (95:5 v/v): mp 121–123 °C; R_f 0.48 (CH_2Cl_2/MeOH, 9:1 v/v), 0.24 (CH_2Cl_2/MeOH, 95:5 v/v); UV (methanol) λ_{max} 290, 255, 231, λ_{min} 270, 251 nm. Anal. Calcd for $C_{55}H_{42}N_8O_{13}P_2Cl_4$: C, 52.53; H, 3.37; N, 8.91. Found: C, 52.75; H, 3.78; N, 9.01.

Nucleoside **8** (1.79 g, 2.56 mmol) was combined with 4-chlorophenyl 5-chloro-8-quinolyl phosphate[11] (1.41 g, 3.84 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-t (1.67 g, 6.40 mmol) in pyridine (13 mL) for 2 h, and the mixture was processed as described for the preparation of **9a**, giving **9b**: 3.54 g (92%); mp 111–113 °C (lit.[7] mp 112–114 °C); UV (methanol) λ_{max} 305 (sh), 264, 234, λ_{min} 250 nm; R_f 0.56 (CH_2Cl_2/MeOH, 9:1 v/v), 0.30 (CH_2Cl_2/MeOH, 95:5 v/v).

Synthesis of Dinucleotide DMTrbzCt(QCl)pbzCtp(QCl,ClPh)(12). The phosphotriester **9b** (810 mg, 0.77 mmol) was treated with a 2% solution of *p*-toluenesulfonic acid in a mixture of methylene chloride and methanol (7:3 v/v, 20 mL) at 0 °C for 15 min. The reaction mixture was neutralized with 5% sodium bicarbonate solution and transferred into methylene chloride (50 mL). The methylene chloride layer was washed wwith water (2 × 20 mL), dried over anhydrous sodium sulate, filtered, and evaporated in vacuo. The residue was dissolved in a small amount of methylene chloride and poured into *n*-hexane–ether (9:1 v/v 200 mL). A white precipitate was collected to give the 5′-hydroxyl nucleotide **10** (520 mg, 90%). On the other hand, **9b** (532 mg, 0.53 mmol) was treated with a 0.06 M solution of the N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime in a mixture of dioxane and water (2:1 v/v, 20 mL) at room temperature for 16 h. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with 50% aqueous pyridine (15 mL). The filtrate was washed with ether (3 × 10 mL) and extracted with methylene chloride (3 × 30 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The phosphodiester **11** thus obtained was combined with **10** (263 mg, 0.35 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-t (350 mg, 1.3 mmol) in pyridine (2 mL) for 2 h. The reaction mixture was then worked up as described for the preparation of **7** and purified by silica gel column (2 × 12 cm) chromatography. The appropriate fractions [eluted with a stepwise gradient of methanol (0–4%) in methylene chloride] were evaporated to ca. 3–4 mL and poured into *n*-hexane (70 mL). A white precipitate was collected to give the fully protected dinucleotide **12**: 480 mg (83%); R_f 0.53 (CH_2Cl_2/MeOH, 9:1 v/v), 0.28 (CH_2Cl_2/MeOH, 95:5 v/v); UV (methanol) λ_{max} 307 (sh), 261, 231, λ_{min} 246 nm.

Synthesis of Trinucleotide (QCl)$_2$pbzGtp(QCl)bzCtp(QCl)bzCtp(QCl,ClPh) (15). The phosphotriester **12** (417 mg, 0.25 mmol) was treated wtih 2% solution of *p*-toluenesulfonic acid in a mixture of methylene chloride and methanol (7:3 v/v, 45 mL) at 0 °C for 15 min. The reaction mixture was neutralized with 5% sodium bicarbonate solution and transferred into methylene chloride (100 mL). The methylene chloride layer was washed with water (2 × 50 mL), dried over anhydrous sodium sulfate, filtered, concentrated to ca. 5–6 mL, and poured into *n*-hexane–ether (9:1 v/v, 150 mL). The precipitate was collected to give the 5′-hydroxyl dinucleotide **13** (295 mg, 89%). On the other hand, the compound **9a** (385 mg, 0.32 mmol) was treated with 0.06 M solution of the N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboxime in a mixture of dioxane and water (2:1 v/v, 14 mL) at room temperature for 16 h. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with 50% aqueous pyridine (10 mL). The filtrate was washed with ether (3 × 15 mL) and extracted with methylene chloride (3 × 30 mL). The methylene chloride extract was dried over anhydrous sodium sulfate, filtered, and concentrated to a gum. The phosphodiester **14** thus obtained was combined with **13** (295 mg, 0.21 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-t (311 mg, 1.2 mmol) in pyridine (1.5 mL) for 2 h. The reaction mixture was then worked up as described for the preparation of **7** and purified by silica gel column (2 × 10 cm) chromatography. The appropriate fractions [eluted with a stepwise gradient of methenol (0–3%) in methylene chloride] were evaporated to ca. 3–4 mL and poured into *n*-hexane. A white precipitate was collected to give the trinucleotide **15**: 581 mg (71%); R_f 0.54 (CH_2Cl_2/MeOH 9:1 v/v), 0.27 (CH_2Cl_2/MeOH, 95:5 v/v); UV (methanol) λ_{max} 295 (sh), 256, 235, λ_{min} 246 nm.

Deblocking of the Fully Protected Oligoribonucleotides. The trinucleotide **15** (0.02 mmol) was dissolved in pyridine (1 mL). Concentrated ammonia (28%, 25 mL) was added to the solution, and the mixture was kept at 50 °C for 5 h. Ammonia was removed by evaporation, and the residue was treated with zinc chloride (27 mg) in aqueous pyridine (10%, 10 mL) at room temperature for 24 h. The mixture was treated with Dowex 50W-X2 (pyridinum form), the resin was removed by filtration and then washed with aqueous pyridine (50%), and the combined solution was concentrated to dryness. The residue was treated with 0.01 N methanolic hydrochloric acid (pH 2, 25 mL) at 20 °C for 18 h. The solution was carefully neutralized (pH 8) with 0.5 M ammonia and concentrated to dryness. The residue was dissolved in water, washed with ether, and then concentrated to an oil. The oil was dissolved in 0.01 M triethylammonium bicarbonate (pH 7.5, 1 mL) and applied to a column of DEAE cellulose DE-52 (bicarbonate form). The elution profile and conditions are shown in Figure 1. The yields of free oligoribonucleotides are summarized in Table III.

Registry No. **1**, 83416-83-5; **2**, 83416-84-6; **3a**, 50826-90-9; **3b**, 31505-87-0; **3c**, 31505-92-7; **3d**, 60324-96-1; **4a**, 83416-85-7; **4b**, 83435-76-1; **4c**, 83416-86-8; **4d**, 83416-87-9; **6**, 83416-88-0; **7**, 83416-89-1; **8**, 69359-38-2; **9a**, 83416-90-4; **9b**, 75933-81-2; **10**, 75933-89-0; **11**, 71933-72-7; **12**, 77181-82-9; **13**, 75948-73-1; **14**, 83416-91-5; **15**, 83416-92-6; pU, 58-97-9; pUpU, 17489-65-5; pGpCpCp, 30805-69-7; 5-chloro-8-hydroxyquinoline, 130-16-5; 4-chlorophenyl phosphorodichloridate, 772-79-2; 2′,3′-di-*O*-benzoyluridine, 50408-20-3.