200 mL of culture media). The crude metabolite mixture (840 mg) containing **16** and **17** in a ratio of 67:33 (GLC analysis) was taken up in *n*-pentane and chromatographed over 20 g of alumina.

Elution with 600 mL of *n*-pentane afforded the crude ketone **16** which was sublimed in vacuo to afford 381 mg (47.6% yield) of (−)-4-brendanone (**16**): mp 110–113 °C (in a sealed tube); $[\alpha]^{18}_D$ −26.06° (*c* 0.94, $CHCl_3$); optical purity 34.8%; CD (*c* 1.5 × 10^{-3} M, MeOH) $[\Theta]_{max}$ (λ) −2.13 × 10^3 (299 nm).

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.19; H, 8.79.

Further elution with 400 mL of *n*-pentane–ether (10:1) afforded the crude alcohol **17** which was sublimed in vacuo to afford 167 mg (20% yield) of (−)-*endo*-4-brendanol (**17**): mp 135–136 °C (in a sealed tube); $[\alpha]^{18}_D$ −31.0° (*c* 0.91, $CHCl_3$); optical purity 85.3%; NMR (CCl_4, 60 MHz) δ 4.25 (m, 1 H, CHOH).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.16; H, 10.13.

(−)-*endo*-4-Brendyl acetate (**18**): bp 110 °C (20 mm); $[\alpha]^{20}_D$ −51.2° (*c* 0.61, $CHCl_3$); optical purity 85.3%; NMR (CCl_4, 60 MHz) δ 1.93 (s, 3 H, CH_3COO), 4.98 (m, 1 H, HCOAc).

Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.89. Found: C, 73.16; H, 8.95.

(b) Oxidation of the (−)-Endo Alcohol 17. Brown's reagent[17] (0.36 mL) was added to a solution of the (−)-endo alcohol **17** (60 mg) in ether (25 mL) during 15 min at 0 °C. After an additional 15 min, the ether layer was worked up in the usual way to give the crude ketone (59.8 mg) which was taken up in *n*-pentane and chromatographed over alumina. The combined eluates were sublimed in vacuo [70 °C (20 mm)] to afford 43 mg of (+)-4-brendanone (**16**): mp 107–109 °C (in a sealed tube); $[\alpha]^{20}_D$ +63.8° (*c* 0.44, $CHCl_3$); optical purity 85.3%; CD (*c* 8.8 × 10^{-4} M, MeOH) $[\Theta]_{max}$ (λ) +4.8 × 10^3 (299 nm); UV (*c* 7.3 × 10^{-3} M, MeOH) λ_{max} 290 nm (ε 19.1).

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.41; H, 8.82.

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Registry No. (±)-**11**, 75768-02-4; (±)-**11**, 75801-41-1; (−)-**11**, 29415-46-1; (−)-**11** DNP, 75801-42-2; (+)-**11** DNP, 75801-43-3; (−)-**12**, 75801-44-4; (−)-**12** 3,5-dinitrobenzoate, 75801-45-5; (−)-**13**, 75801-46-6; (−)-**14**, 75801-47-7; (−)-**14** 3,5-dinitrobenzoate, 75801-48-8; (+)-**15**, 75801-49-9; (±)-**16**, 75768-03-5; (−)-**16**, 75801-50-2; (+)-**16**, 75801-51-3; (−)-**17**, 75768-04-6; (−)-**18**, 75768-05-7; (+)-**20**, 37167-95-6.

(8-Quinolinesulfonyl)tetrazole: A New Type of Highly Efficient Coupling Agent for the Synthesis of Ribooligonucleotides by the Phosphotriester Approach[1]

Hiroshi Takaku* and Masatoshi Yoshida

Laboratory of Organic Chemistry, Chiba Institute of Technology, Narashino-shi, Chiba 275, Japan

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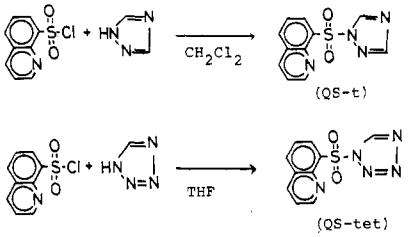
(8-Quinolinesulfonyl)Tetrazole (QS-tet) has been developed as a new type of highly efficient coupling agent in the synthesis of phosphotriester bonds. This reagent very rapidly completes the coupling reactions, and the yield is considerably higher than that obtained with conventional condensing agents.

The chemical synthesis of deoxyribonucleotides has developed sufficiently to produce long deoxyribooligonucleotides of defined sequence,[2] whereas because of the presence of the 2′-hydroxyl group, developments for the chemical synthesis of long ribooligonucleotides of defined sequence have been much slower. In our original studies on the synthesis of ribooligonucleotides by the phosphotriester approach, we developed 8-quinolinesulfonyl chloride (QS)[3] as a new type of coupling agent and the 5-chloro-8-quinolyl group (qcl)[4] as a very effective phosphate protecting group. 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 reactions are very slow (1–2 days).[5] Cramer et al.[6] and Narang et al.[7] have also re-

Scheme I



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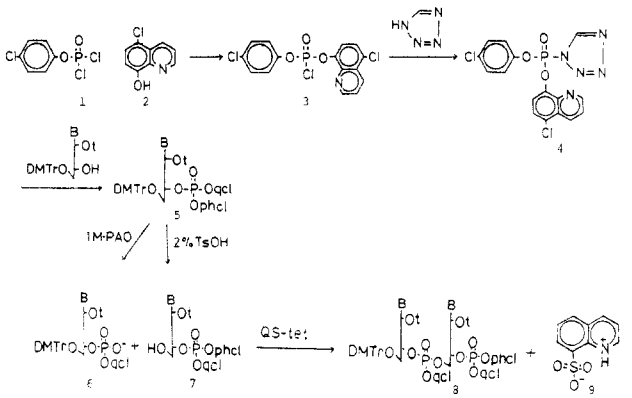
Scheme II[a]



[a] DMTr = dimethoxytrityl; t = tetrahydropyranyl; qcl = 5-chloro-8-quinolyl; phcl = 4-chlorophenyl; B = bzA, U, bzG, bzC.

ported very low yields when attempting coupling reactions with TPS of oligonucleotides containing the guanosine unit. Recently, Itakura and his co-workers[8] have developed a rapid synthetic method for trideoxyribonucleotide blocks using triisopropylbenzenesulfonyl tetrazolide as a powerful coupling agent. The rapid synthesis of trimer blocks has increased the speed in the synthesis of deoxyribooligonucleotides of defined sequence. Thus a rapid synthetic method was of considerable interest for the synthesis of ribooligonucleotides of defined sequence.

In the present paper, we report a rapid procedure for the synthesis of triribonucleotide blocks with (8-quinolinesulfonyl)tetrazole (QS-tet) as a new type of highly efficient coupling agent which is more applicable than QS in building the long chains of ribooligonucleotides.

Coupling Agent Derived from 8-Quinolinesulfonyl Chloride. (8-Quinolinesulfonyl)triazole (QS-t) and (8-quinolinesulfonyl)tetrazole (QS-tet) are prepared by condensing QS (20 mmol) with triazole or tetrazole (20 mmol) in the presence of triethylamine (20 mmol) in methylene chloride or tetrahydrofuran (40 mL) at 22 °C for 2 h (Scheme I). In the case of QS-tet, the best results were obtained by use of tetrahydrofuran as solvent. Two new types of coupling agents, QS-t and QS-tet, were obtained in 72% and 82% yields, respectively. (8-Quinolinesulfonyl)tetrazole was found to decompose on storage within 4 days. These compounds are characterized satisfactorily by their spectral and also by their elemental analyses.

Synthesis of Dinucleotides. In order to investigate coupling efficiency, we have chosen the synthesis of dinucleotide 8 containing guanosine. A mixture of the phosphodiester 6[4a] (0.24 mmol) and the partially protected phosphotriester 7[9] (0.16 mmol) in dry pyridine (0.8 mL) was treated with the coupling agents (0.48 mmol) at 22 °C for 1 to 144 h (depending upon the reactivity of coupling agents). 8-Quinolinesulfonic acid (**9**) was removed by filtration[3] (Scheme II). The filtrate was quenched with ice–water followed by extraction with methylene chloride which was washed with triethylammonium bicarbonate (0.1 M, pH 7.5). The methylene chloride was then coevaporated with toluene to a gum which was chromatographed on a short silica gel column with methylene chloride–methanol (95:5 v/v) as eluent. The dinucleotide **8** was obtained as shown in Table I. As also shown in Table I,

Table I. Comparison of the Yields for the Synthesis of Dinucleotide DMTr(bz)Atp(qcl)bzGtp(qcl, phcl) Containing the Guanosine with Various Coupling Agents[a]

coupling agents	time, h	yield, %
TPS	24	50
QS	24	56
QS-t	144	21
TPS-tet	2	71
QS-tet	1	80

[a] These reactions were carried out by use of DMTr(bz)Atp(qcl) (0.45 mmol), bzGtp(qcl, phcl) (0.3 mmol), and coupling agents (0.9 mmol) at 22 °C.

QS-tet gave better results than 2,4,6-triisopropylbenzenesulfonyl chloride (TPS)[10], QS, QS-t, and TPS-tet for the synthesis of **8** containing the guanosine unit.

In a similar manner, several fully protected dinucleotides, DMTrUtp(qcl)Utp(qcl, phcl), DMTrUtp(qcl)bzAtp(qcl, phcl), and DMTrbzCtp(qcl)bzCtp(qcl, phcl) were obtained in high yields as shown in Table II. In the above reactions, when QS-t and QS-tet as coupling agents were used, no sulfonylated and detritylated side products were observed.

Rapid Synthesis of Triribonucleotide Blocks. Next, we describe a rapid procedure for the synthesis of triribonucleotide blocks (**14**) by use of QS-tet as a highly efficient coupling agent as described in the above experiments. The phosphodiester intermediate **6** (1.7 mmol) was treated **10** (1.0 mmol) in the presence of QS-tet (3.4 mmol) in dry pyridine (10 mL) at 22 °C for 1 h (Scheme III). No 5′-hydroxyl compounds **10** could be detected on TLC of the reaction mixture. 8-Quinolinesulfonic acid (**9**) was removed by filtration. The filtrate was evaporated in vacuo, and the residual pyridine was removed by codistillation with toluene.[11] The residue was treated with 2% *p*-toluenesulfonic acid in a mixture of methylene chloride and methanol (7:3 v/v) at 0 °C for 15 min.[4] The detritylated phosphodiester **13** was removed from the reaction mixture by simple extraction with phosphate buffer (1.0 M, pH 7.5). The 5′-hydroxyl dinucleotide (**12**) was precipitated from a mixture of *n*-hexane and ether (9:1 v/v) and used for the next coupling reaction without further purification. The partially protected phosphotriester intermediate **12** thus obtained was treated with **6** (1.5 mmol) in the presence of QS-tet (3.0 mmol) in dry pyridine (10 mL) at 22 °C for 1 h. The inner salt **9** was separated by filtration. After the usual workup, the corresponding fully protected triribonucleotides DMTrUtp(qcl)Utp(qcl)U-$(OBz)_2$ (**14a**) and DMTrbzAtp(qcl)bzCtp(qcl)bzCtp(qcl, phcl) (**14b**) were isolated through short-column chromatography as pure solids in 78% and 85% yields, based on **10**, respectively. By this method, triribonucleotide blocks **14** can be prepared in 1 day.

Synthesis of Hexanucleotide. The triribonucleotide blocks **14** are useful synthetic intermediates for the synthesis of ribooligonucleotides of defined sequence. For example, the fully protected hexanucleotide DMTrbzAtp(qcl)bzCtp(qcl)bzCtp(qcl)Utp(qcl)Utp(qcl)U-$(OBz)_2$ (**17**) was synthesized by the following procedure. The dimethoxytrityl group of **14a** was removed with 2% *p*-toluenesulfonic acid solution to give the 5′-hydroxyl phosphotriester intermediate Utp(qcl)Utp(qcl)U$(OBz)_2$ (**15**). On the other hand, **14b** (0.3 mmol) was treated with

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(9) H. Takaku, M. Yoshida, and T. Hata, *Nucleic Acids Res., Spec. Publ.*, 6, s181 (1979).
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(11) When the fully protected nucleotide was treated with 2% *p*-toluenesulfonic acid solution in the presence of pyridine, the yield of detritylated product decreased remarkably.

Table II. Synthesis of the Fully Protected Dinucleotides (8)[a]

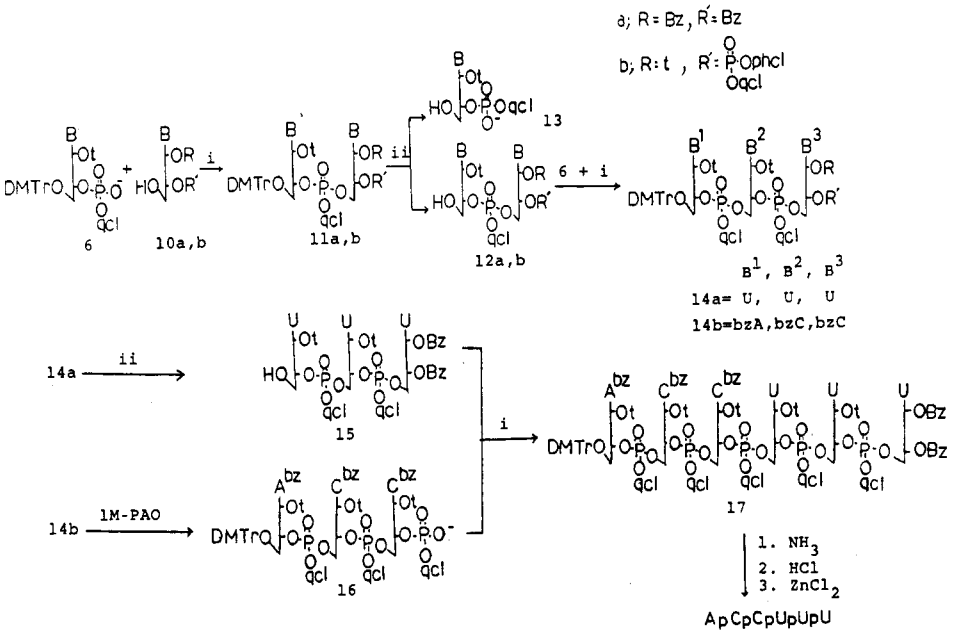
5'-protected component	amount, mmol	3'-protected component	amount, mmol	mmol of QS-tet	product	yield, % (g)
DMTrUtpqcl	0.36	Utp(qcl, phcl)	0.24	0.72	DMTrUtp(qcl)Utp(qcl, phcl)	90 (0.332)
DMTrUtpqcl	3.75	bzAtp(qcl, phcl)	2.50	7.50	DMTrUtp(qcl)bzAtp(qcl, phcl)	97 (4.055)
DMTrbzCtpqcl	2.90	bzAtp(qcl, phcl)	1.95	5.80	DMTrbzCtp(qcl)bzAtp(qcl, phcl)	97 (3.370)

[a] p, phosphate; qcl, 5-chloro-8-quinolyl; phcl, 4-chlorophenyl; t, tetrahydropyranyl; DMTr, dimethoxytrityl; bz, benzoyl.

Table III. Characterization of Ribooligonucleotides

compd	yield for complete deprotection, %	paper chromatography A	paper chromatography B	paper electrophoresis, RAp	enzymatic analyses, spleen [nuclease P1]
UpUp	91	0.12		1.01	Up [U/pU (1.0:1.1)]
ApGp	97	0.08	0.38	0.98	Ap/Gp (1.0:0.9) [A/pG (1.0:1.0)]
CpAp	90	0.21		1.00	Cp/Ap (1.0:1.1) [C/pA (1.0:0.9)]
UpAp	91	0.23			Up/Ap (1.0:1.2) [U/pA (1.0:1.1)]
UpUpU	89	0.22		0.64	2Up/U (1.9:1.0)
ApCpCp	90	0.06	0.35	1.05	Ap/2Cp (1.0:2.1) [A/2pC (1.0:1.9)]
ApCpCpUpUpU	79		0.12	0.84	Ap/2Cp/2Up/U (0.9:2.1:1.9:1.0) [A/2pC/3pU (1.0:2.1:3.1)]

Scheme III[a]



[a] i = QS-tet; ii = 2% TsOH.

the N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboxaldoxime[12] (0.38 mmol) in a mixture of dioxane and water (2:1 v/v; 6 mL) at 22 °C 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 (2 × 30 mL)[13] and extracted with methylene chloride (2 × 30 mL). The combined organic layers were dried over anhydrous sodium sulfate and evaporated. The phosphodiester DMTrbzAtp(qcl)bzCtp(qcl)bzCtp(qcl) (16) was isolated in almost quantitative yield by precipitation as the solid pyridinium salt. The phosphodiester intermediate 16 thus obtained was dissolved in dry pyridine (2 mL), and then 15 (0.2 mmol) and QS-tet (0.6 mmol) were added. After 1.5 h, the corresponding fully protected hexanucleotide (17) was isolated after silica gel column chromatography as a pure solid in 76% yield.

Complete deblocking of the fully protected di-, tri-, and hexaribonucleotides was performed as follows. Treatment of ribooligonucleotides with concentrated ammonia at 50 °C for 5 h completely removed the 4-chlorophenyl protective group, the N-protecting benzoyl group, and the hydroxyl protecting benzoyl group. The solution was concentrated to an oil which was then treated with 0.01 N hydrochloric acid (pH 2) at 22 °C for 18 h to cleave the dimethoxytrityl group and the tetrahydropyranyl group. Finally, removal of 5-chloro-8-quinolyl group was effected by treatment with zinc chloride in a mixture of pyridine and water (9:1 v/v) at room temperature for 24 h. The deblocked hexanucleotide ApCpCpUpUpU was applied to

(12) C. B. Reese, R. C. Titmas, and L. Yau, *Tetrahedron Lett.*, 2727 (1978); C. B. Reese and L. Yau, *ibid.*, 4443 (1978).
(13) 4-Chlorophenol and 2-picolinonitrile were removed from the phosphodiester by simple extraction with ether in aqueous pyridine.

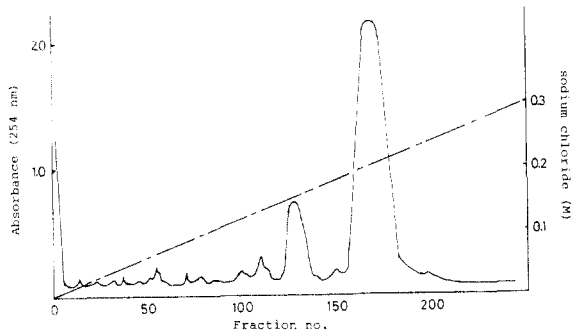


Figure 1. Chromatography of the hexanucleotide on a column (1.0 × 100 cm) of DEAE-cellulose equilibrated with 7 M urea in 20 mM Tris-HCl (pH 7.5). Elution was performed with a liner gradient of sodium chloride (0–0.3 M, total 900 mL). Fractions of 3 mL were collected every 20 min. The main peak contained the product ApCpCpUpUpU.

a column of DEAE cellulose in the presence of 7 M urea. The elution profile and conditions are shown in Figure 1. The presence of only 3′→5′ internucleotidic bonds in the deblocked products was established by complete digestion of the oligonucleotides with spleen phosphodiesterase and nuclease P1 to the expected products in the correct ratios (Table III).

(8-Quinolinesulfonyl)tetrazole (QS-tet) shows the following advantages: no detritylated and sulfonylated products were observed during the coupling reactions; 8-quinolinesulfonic acid produced in the coupling reaction forms a neutral inner salt and separated as a precipitate from the reaction mixture; the rate of the coupling reaction was much faster, and the yield was considerably higher than that obtained with other commonly used condensing agents.

Experimental Section

Thin-layer chromatography (TLC) was performed by using the ascending technique on Merck $60F_{254}$. The plates were usually developed in 5–10% methanol in methylene chloride mixtures. Paper chromatography was performed by using the descending technique on Toyo Roshi No. 51A. The solvent systems employed were the following: solvent A, 2-propanol–concentrated ammonia–water (7:1:2 v/v); solvent B, 1-propanol–concentrated ammonia–water (55:10:35 v/v); solvent C, 1-butanol–acetic acid–water (5:2:3 v/v). Paper electrophoresis was performed by using 0.05 M triethylammonium bicarbonate (pH 7.5) at 1100V/40 cm. Merck silica gel G was used for short column chromatography. DEAE cellulose (DE-52) and DEAE Sephadex A-25 were used for anion-exchange chromatography. Nucleosides and their derivatives were detected on paper chromatograms and thin-layer sheets with a UV light source (254 nm). Compounds containing the dimethoxytrityl group were detected on chromatography by spraying the samples with 10% perchloric acid solution and drying them in a stream of warm air.

Spleen phosphodiesterase and nuclease P1 were purchased from Worthington Biochemical Corp. and Yamasa Shoyu Co., respectively.

(8-Quinolinesulfonyl)triazole (QS-t). A methylene chloride (2 mL) solution of triethylamine (1.39 mL, 10 mmol) was added to a suspension of 8-quinolinesulfonyl chloride (2.28 g, 10 mmol) and 1*H*-1,2,4-triazole (0.69 g, 10 mmol) in methylene chloride (20 mL) at 0 °C. The reaction mixture was stirred at 22 °C for 2 h. The precipitate was filtered off, and the methylene chloride was washed with water (3 × 30 mL). The methylene chloride solution was dried over anhydrous sodium sulfate, and the solution was evaporated in vacuo. The residue was recrystallized from benzene to give 1.78 g (72%) of product: mp 226–229 °C; NMR ($CDCl_3$, Me_4Si) 8.90 (H_5), 8.08 (H_3) ppm. Anal. Calcd for $C_{11}H_8N_4O_2S$: C, 50.76; H, 3.10; N, 21.53. Found: C, 50.80; H, 3.12; N, 21.58.

(8-Quinolinesulfonyl)tetrazole (QS-tet). A THF (2 mL) solution of triethylamine (1.39 mL, 10 mmol) was added to a stirred suspension of 8-quinolinesulfonyl chloride (2.28 g, 10 mmol) and 1*H*-tetrazole (0.7 g, 10 mmol) in THF (20 mL) with ice cooling. After 2 h the precipitate was removed by filtration, and the filtrate was evaporated to dryness under vacuum. The crystalline residue was dissolved in methylene chloride (100 mL) which was washed with water (3 × 30 mL). The solution was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The resulting crystalline product was washed with small amount of methylene chloride, yielding 2.14 g (82%) of white powder: mp 210 ° dec; NMR (Me_2SO-d_6, Me_4Si) 9.90 (1 H, s, CH in tetrazole) ppm. Anal. Calcd for $C_{10}H_7N_5O_2S$: C, 45.79; H, 2.71; N, 26.81. Found: C, 45.20; H, 2.77; N, 26.59.

Phosphorylation of the Partially Protected Nucleosides. An anhydrous THF (3 mL) solution of 5-chloro-8-hydroxyquinoline (287 mg, 1.6 mmol) and triethylamine (0.22 mL, 1.6 mmol) was added to 4-chlorophenyl phosphorodichloridate (0.25 mL, 1.5 mmol) in anhydrous THF (2.5 mL) at 0 °C. The reaction mixture was stirred at 22 °C for 45 min. Next, an anhydrous THF (3.5 mL) solution of 1*H*-tetrazole (122 mg, 1.7 mmol) and triethylamine (0.23 mL, 1.7 mmol) was added to the reaction mixture and allowed to stand a further 10 min. The precipitated triethylammonium hydrochloride was removed by filtration, and the filtrate was concentrated (to 5 mL) under reduced pressure. To this solution was added 5′-*O*-(dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-*N*-benzoylnucleoside (1 mmol), and the reaction mixture was stirred at 20 °C for 30 min. The mixture was quenched with ice–water (1 mL) and repeatedly extracted with methylene chloride (3 × 50 mL). Combined organic extracts were washed with water (2 × 50 mL), and the methylene chloride was evaporated in vacuo. The residue was dissolved in methylene chloride (2 mL) and chromatographed on a silica gel column (2.5 × 8 cm) by eluting with methylene chloride–methanol (98:2 v/v; 300 mL). The yields of fully protected monoribonucleotides were 82–96%.

(a) **5′-*O*-(Dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-uridine 3′-(4-Chlorophenyl 5-Chloro-8-quinolyl phosphate)**: mp 106–108 °C; UV (methanol) λ_{max} 260 nm (ϵ 11 800), 234 (sh), λ_{min} 246; R_f 0.56 (CH_2Cl_2/MeOH, 9:1 v/v). Anal. Calcd for $C_{50}H_{46}N_3O_{12}PCl_2$: C, 61.10; H, 4.73; N, 4.27. Found: C, 61.02; H, 4.71; N, 4.28.

(b) **5′-*O*-(Dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-*N*^{6}-benzoyladenosine 3′-(4-chlorophenyl 5-chloro-8-quinolyl phosphate)**: mp 97–99 °C; UV (methanol) λ_{max} 280 nm (ϵ 25 400), 233 (sh), λ_{min} 248; R_f 0.61 (CH_2Cl_2/MeOH, 9:1 v/v). Anal. Calcd for $C_{58}H_{51}N_6O_{11}PCl_2$: C, 62.76; H, 4.64; N, 7.57. Found: C, 62.80; H, 4.70; N, 7.43.

(c) **5′-*O*-(Dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-*N*^{4}-benzoylcytidine 3′-(4-chlorophenyl 5-chloro-8-quinolyl phosphate)**: mp 112–114 °C; UV (methanol) λ_{max} 305 nm (sh), 262 (ϵ 29 100), 233 (sh), λ_{min} 248; R_f 0.59 (CH_2Cl_2/MeOH, 9:1 v/v). Anal. Calcd for $C_{57}H_{51}N_4O_{11}PCl_2$: C, 63.04; H, 4.74; N, 5.16. Found: C, 63.03; H, 4.83; N, 5.11.

(d) **5′-*O*-(Dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-*N*^{2}-benzoylguanosine 3′-(4-chlorophenyl 5-Chloro-8-quinolyl phosphate)**: mp 128–129 °C; UV (methanol) λ_{max} 295 nm (sh), 283, 262 (ϵ 18 800), 232 (sh), λ_{min} 275, 255; R_f 0.58 (CH_2Cl_2/MeOH, 9:1 v/v). Anal. Calcd for $C_{58}H_{51}N_6O_{12}PCl_2$: C, 61.86; H, 4.57; N, 7.48. Found: C, 61.36; H, 4.61; N, 7.45.

Detritylation of Fully Protected Mono- and Trinucleotides. The fully protected compound was treated with 2% *p*-toluenesulfonic acid in methylene chloride–methanol (7:3 v/v) at 0 °C for 15 min.[4] The reaction mixture was neutralized with 5% sodium bicarbonate solution and transferred into methylene chloride. The organic layer was washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The residue was precipitated from *n*-hexane–ether (5:1 v/v) and used as the 5′-hydroxyl component in the subsequent condensation without purification.

General Method for the Synthesis of Fully Protected Dinucleotides. A 5′-*O*-(dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-*N*-benzoylnucleoside 3′-(4-chlorophenyl 5-chloro-8-quinolyl phosphate) (1.5 molar equiv) was treated with N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime[12] (1.8 molar equiv) in a mixture of dioxane and water (2:1 v/v) at 22 °C 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 (25 mL/mmol of the nucleotidic component). The filtrate was washed with ether (3 × 30 mL/mmol of the nucleotidic component)[13] and extracted with methylene chloride (3 × 40 mL/mmol of the nucleotidic component). The methylene chloride solution was dried over anhydrous sodium sulfate and evaporated in vacuo. The phosphodiester 6 thus obtained was dissolved in anhydrous pyridine (3 mL/mmol of the phosphodiester component), and then 5′-hydroxyl nucleotide 7 (1 molar equiv) and the coupling agents (3 molar equiv) were added. After 1–144 h, 8-quinolinesulfonic acid was removed by filtration. The filtrate was quenched with ice–water, following by extraction with methylene chloride (3 × 40 mL/mmol of the nucleotidic material). The methylene chloride layer was washed with 0.1 M triethylammonium bicarbonate (pH 7.5) and then with water, dried over anhydrous sodium sulfate, filtered, and under reduced pressure evaporated to gum. The residue was dissolved in methylene chloride and purified by short-column chromatography on silica gel. The appropriate fractions [eluted with methylene chloride–methanol (98:2 v/v)] were evaporated to give the fully protected dinucleotide 8 which was isolated as a solid by precipitation from *n*-hexane–ether (95:5 v/v; see Tables I and II).

Rapid Synthesis of Trinucleotide Blocks. DMTrUtp(qcl) obtained from DMTrUtp(qcl, phcl) (1.48 g, 1.7 mmol) by treatment with N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime as described for 8 was combined with 2′,3′-*O*-benzoyluridine (0.452 g, 1 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-tet (0.898 g, 3.4 mmol) and pyridine (10 mL) at 22 °C for 1 h. The reaction mixture was quenched with ice–water (2 mL), and the solution was evaporated in vacuo. The residue was coevaporated with toluene three times and treated with 2% *p*-toluenesulfonic acid solution in a mixture of methylene chloride and methanol (7:3 v/v; 50 mL) at 0 °C for 15 min. The reaction mixture was washed with phosphate buffer (1.0 M, pH 7.5; 4 × 40 mL) and the water (40 mL). The detritylated phosphodiester, Utp(qcl) was removed from the reaction mixture by simple extraction. The methylene chloride layer was dried over anhydrous sodium sulfate, filtrated and evaporated in vacuo. A solution of the residual gum in methylene chloride was added dropwise to stirred *n*-hexane–ether (9:1 v/v). The resulting colorless precipitate was collected by filtration and used for the next coupling reaction without further purification. The partially protected phosphotriester intermediate Utp(qcl)U(OBz)$_2$ thus obtained was treated with DMTrUtp(qcl) (1.7 mmol) and QS-tet (0.898 g, 3.4 mmol) in pyridine (10 mL) for 1 h. The reaction mixture was then worked up as described for the synthesis of 8 and purified by short-column chromatography (2.5 × 12 cm) on silica gel. The appropriate fractions [eluted with methylene chloride–methanol (98:2 v/v)] were evaporated to give DMTrUtp(qcl)Utp(qcl)U(OBz)$_2$ (**14a**) which was isolated as a solid (1.5 g, 78%) by precipitation from *n*-hexane–ether (95:5 v/v); R_f 0.51 (CH_2Cl_2/MeOH, 9:1 v/v).

In a same way, DMTr(bz)Atp(qcl)bzCtp(qcl)bzCtp(qcl, phcl) (**14b**) was isolated as a solid: 2.01 g (85%); R_f 0.61 (CH_2Cl_2/MeOH, 9:1 v/v).

Synthesis of Hexanucleotides. DMTr(bz)Atp(qcl)bzCtp-(qcl)bzCtp(qcl) (**16**) obtained from **14b** (677 mg, 0.3 mmol) by treatment with the N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime as described for 8 was combined with Utp(qcl)Utp(qcl)U(OBz)$_2$ (**15**) (348 mg, 0.2 mmol), rendered anhydrous by coevaporation of pyridine three times, and then treated with QS-tet (158 mg, 0.6 mmol) in pyridine (2 mL) at 22 °C for 1.5 h. 8-Quinolinesulfonic acid was removed by filtration. The filtrate was quenched with ice–water (1.5 mL), following by extraction with methylene chloride (3 × 10 mL). The methylene chloride layer was washed with 0.1 M triethylammonium bicarbonate (pH 7.5) and then water, dried over anhydrous sodium sulfate, filtered, and under reduced pressure evaporated to gum. The gum was dissolved in methylene chloride and applied on a silica gel column (2.5 × 20 cm). The column was eluted with 100 mL of methylene chloride followed by 350 mL of methylene chloride–methanol (95:5 v/v). The product **17** was precipitated with *n*-hexane–ether (9:1 v/v) from its solution in methylene chloride: 603 mg (76%); R_f 0.44 (CH_2Cl_2/MeOH, 9:1 v/v).

Deblocking of the Fully Protected Oligoribonucleotides. The hexanucleotide **17** (19.8 mg, 0.005 mmol) was treated with concentrated ammonia (50 mL) at 50 °C for 5 h. The solution was evaporated in vacuo, and the residue was coevaporated with toluene. The residue was then treated with 0.01 N methanolic hydrochloric acid (pH 2, 10 mL) at 20 °C for 18 h to remove the dimethoxytrityl and tetrahydropyranyl groups. The solution was carefully neutralized (pH 8) with 0.5 M ammonia and concentrated. The residue was treated with zinc chloride (170 mg) in aqueous pyridine (90%, 10 mL) at room temperature for 24 h to deblock the 5-chloro-8-quinolyl group. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form) and filtered, and the filtrate was evaporated in vacuo to gum. The gum was dissolved in water and washed with ether (20 mL), and the aqueous layer was evaporated to dryness. The residue was dissolved in 7 M urea–20 nM Tris–HCl (pH 8, 20 mL) and applied to a column (1.0 × 100 cm) of DEAE cellulose (Cl^-). The elution profile and conditions are shown in Figure 1. The product was desalted by adsorption onto DEAE cellulose, washing with 0.1 M triethylammonium bicarbonate, and elution with 1 M triethylammonium bicarbonate. The yield of free hexanucleotide is summarized in Table (III).

Enzyme Assay. (a) Spleen Phosphodiesterase. An incubation solution of 1 M ammonium acetate (pH 5.7, 80 μL) containing oligonucleotide (10 OD_{260}) and enzyme (20 units/mL, 40 μL) was incubated at 37 °C for 12 h.

(b) Nuclease P1. An incubation solution of 0.05 M potassium acetate (pH 7.0, 100 μL) containing oligonucleotide (10 OD_{260}) and enzyme (100 mg/mL, 10 μL) was incubated at 37 °C for 12 h.

The results of enzymatic hydrolysis are summarized in Table III.

Registry No. **1**, 772-79-2; **2**, 130-16-5; **5** (B = U), 75933-79-8; **5** (B = bzA), 75933-80-1; **5** (B = bzC), 75933-81-2; **5** (B = bzG), 75933-82-3; **6** (B = bzA), 75933-83-4; **6** (B = U), 75933-84-5; **6** (B = bzC), 75933-85-6; **7** (B = bzG), 75933-86-7; **7** (B = U), 75933-87-8; **7** (B = bzA), 75933-88-9; **10a** (B = U), 50408-20-3; **10b** (B = bzC), 75933-89-0; **12a** (B = U), 75933-90-3; **12b** (B = bzC), 75948-73-1; **14a**, 75948-74-2; **14b**, 75933-91-4; **15**, 75933-92-5; **16**, 75933-93-6; **17**, 76036-36-7; (8-quinolinesulfonyl)triazole, 75933-94-7; 8-quinolinesulfonyl chloride, 18704-37-5; 1*H*-1,2,4-triazole, 288-88-0; (8-quinolinesulfonyl)tetrazole, 73371-06-9; 1*H*-tetrazole, 288-94-8; 5′-*O*-(dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)uridine, 51296-18-5; 5′-*O*-(dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-N^6-benzoyladenosine, 71933-61-4; 5′-*O*-(dimethoxytrityl)-2′-*O*-(tetrahydropyranyl-N^4-benzoylcytidine, 69359-38-2; 5′-*O*-(dimethoxytrityl)-2′-*O*-(tetrahydropyranyl)-N^2-benzoylguanosine, 71933-62-5; DMTr-(bz)Atp(qcl)bzGtplqcl,phcl), 75933-95-8; DMTrUtp(qcl)Utp(qcl,-phcl), 75933-96-9; DMTrUtp(qcl)bzAtp(qcl,phcl), 75933-97-0; DMTrbzCtp(qcl)bzAtp(qcl,phcl), 75933-98-1; UpUp, 3526-97-4; ApGp, 7600-31-9; CpAp, 23193-58-0; UpAp, 2617-54-1; UpUpU, 3152-53-2; ApCpCp, 6158-19-6; ApCpCpUpUpU, 75933-99-2.