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A Convenient Synthesis of the 3′-Terminal Nonaoligoribonucleotide of Rous Sarcoma Virus 35S RNA via the Modified Phosphotriester Approach[1]

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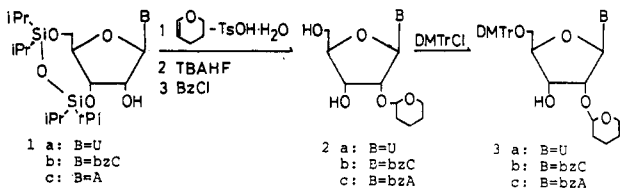
The synthesis of oligoribonucleotide UpUpCpApCpCpApCpA via the phosphotriester approach, which is located at the 3′ terminus of Rous sarcoma virus 35S RNA, is described. 5′-*O*-(Dimethoxytrityl)-2′-*O*-tetrahydropyranyl-*N*-acylnucleoside 3′-(4-chlorophenyl 5-chloro-8-quinolyl phosphates) were used as the starting materials. Treatment of the fully protected mononucleotides with 2% *p*-toluenesulfonic acid afforded the 5′-hydroxyl nucleotides, whereas treatment with *syn*-pyridine-2-carboxaldoxime to remove the 4-chlorophenyl group gave the phosphodiesters. The fully protected nonamer was prepared by utilizing these deblocking products. The fully protected nonamer was completely deprotected by treatment with zinc chloride, followed by treatment with base and acid, to give the corresponding oligoribonucleotide.

The structure of Rous sarcoma virus 35S RNA (RSV 35S RNA) has recently been determined at the 3′ and 5′ ends by two groups.[2,3] Twenty-one bases at the extreme 5′ end have been found to be identical with 21 bases adjacent to the poly(A) terminus at the 3′ end of the same molecule (Figure 1). The existence of these reiterated terminal sequences suggest mechanisms by which the growing DNA copy can jump from the 5′ end to a 3′ end of the template and become circular proviral DNA.[4] We are exploring the possibility that the oligoribonucleotide UpUpCpApCpCpApCpA corresponding to the 3′ terminus of the RSV 35S RNA may act as a competitive inhibitor of the production of RSV by interfering with the circularization step.

In this paper, we describe a convenient synthesis of UpUpCpApCpCpApCpA corresponding to the 3′ terminus of the RSV 35S RNA by the modified phosphotriester approach.

Synthesis of 5′-*O*-(Dimethoxytrityl)-2′-*O*-tetrahydropyranyl-*N*-acylnucleosides 3. We examined the synthesis of **3** as the starting material for formnation of 3′–5′ internucleotidic bonds. The first step of this synthesis is based upon the use of tetraisopropyldisiloxane-1,3-diyl as a protecting group for 3′,5′-hydroxyl groups of nucleosides.[5]

Reaction of the silylated **1** with dihydropyran and *p*-toluenesulfonic acid in anhydrous dioxane gave the corresponding 2′-*O*-tetrahydropyranyl-*N*-acylnucleoside de-



rivatives, which on immediate de-*O*-silylation using tributylammonium fluoride (TBAHF), gave the diastereoisomeric mixture of 2′-*O*-tetrahydropyranyl-*N*-acylnucleosides **2**. The diastereoisomeric mixture was separated into high R_f and low R_f by silica gel column chromatography. Treatment of **2** (low R_f) with dimethoxytrityl chloride in dry pyridine gave the corresponding 5′-*O*-(dimethoxytrityl)-2′-*O*-tetrahydropyranyl-*N*-acylnucleosides **3** in good yields.

Synthesis of the Fully Protected Mononucleotides 4 as Key Intermediates for the Synthesis of Nonanucleotide 18. Recently, we developed 4-chlorophenyl 5-chloro-8-quinolyl phosphorochloride[6] or phosphorotetrazolide[7] as new phosphorylating agents that were found

(1) This manuscript represents Part 19 in a series on oligonucleotide synthesis. For the previous paper in this series: Takaku, H.; Kamaike, K.; Kasuga, K. *J. Org. Chem.* **1982**, *47*, 4937.

(2) Haseltine, W. A.; Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 989.

(3) Schwartz, D. E.; Zamecnik, P. C.; Weith, H. L. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 994.

(4) Taylor, J. M. *Biochem. Biophy. Acta* **1977**, *473*, 57. Collett, M. S.; Faras, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1329.

(5) Markiewicz, W. T. *J. Chem. Res., Synap.* **1979**, *24*; *J. Chem. Res. Miniprint* **1979**, 0181. Markiewicz, W. T.; Wiewiorowski, M. *Nucleic Acids Res., Spec. Publ.* **1978**, *No. 4*, 185. Gioeli, C.; Kwiatkowski, M.; Oberg, B.; Chattopadhyaya, J. B. *Tetrahedron Lett.* **1981**, 1741. Honda, S.; Tetrada, K.; Sato, Y.; Sekine, M.; Hata, T. *Chem. Lett.* **1982**, 15.

(6) Takaku, H.; Yosshida, M.; Kamaike, K.; Hata, T. *Chem. Lett.* **1981**, 197. Takaku, H.; Kamaike, K.; Tsuchiya, H. *Nucleic Acids Symp. Ser.* **1981**, *No. 10*, 171.

(7) Takaku, H.; Nomoto, T.; Kamaike, K. *Chem. Lett.*, **1981**, 543; *Nucleic Acids Symp. Ser.* **1981**, *No. 8*, s91.

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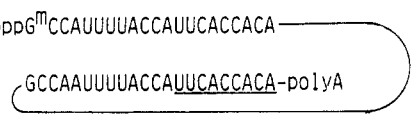


Figure 1. Terminal reiterated sequences at the 3′ and 5′ ends of Rous Sarcoma Virus 35S RNA.

Table I. Removal of Dimethoxytrityl Group with 2% *p*-Toluenesulfonic Acid Solution

	DMTrbzAtOH		
aromatic acid	condition	depyranyl,[b] %	product,[c] %
2% TsOH in CH_2Cl_2–MeOH (7:3, v/v)	0 °C, 15 min		100
	45 min		100
	1 h	5	95
2% BSA in CH_2Cl_2–MeOH (7:3, v/v)	0 °C, 5 min		100
	45 min	10	85
	1 h	27	71

[a] Yield was estimated on TLC. [b] Depyranylated product. [c] Expected detritylated product.

to give good yields in the synthesis of the fully protected mononucleotides **4**. However, we have observed side reactions leading to diminished yields of **4**.[6,7] More recently, we have found that 4-chlorophenyl 5-chloro-8-quinolyl hydrogen phosphate can be effectively used for the phosphorylating agent of 3′-hydroxyl group of nucleosides.[8]

When nucleoside **3** (1 molar equiv) was treated with 4-chlorophenyl-5-chloro-8-quinolyl hydrogen phosphate (1.5 molar equiv) in the presence of 8-quinolinesulfonyl tetrazolide (QS-t)[9] (3.0 molar equiv) in dry pyridine for 2 h, the corresponding 5′-*O*-(dimethoxytrityl)-2′-*O*-tetrahydropyranyl-*N*-acylnucleoside 3′-(4-chlorophenyl 5-chloro-8-quinolyl phosphates) **4** were obtained in 90–95% yields without detritylated[6] and O^6-phosphorylated guanosine[7] side products.

The fully protected mononucleotides **4** thus obtained were key intermediates for elongation of the chain in the 3′ and 5′ direction.

Synthesis of the Fully Protected Dinucleotides 7 and 8. The fully protected mononucleotide **4a** prepared in the above experiment was treated with 2% *p*-toluenesulfonic acid in a mixture of methylene chloride and methanol (7:3 v/v) for 15 min at 0 °C to give the 5′-hydroxyl nucleotide **5a** in 95% yield.[10] The pyranyl group was completely stable under above conditions for 1 h (see Table I).

On the other hand, treatment of **4a** with 0.06 M N^1,N^1,N^3,N^3-tetramethylguanidium salt of *syn*-pyridine-2-carboxaldoxime[11] in a mixture of dioxane and water (1:1 v/v) for 16 h at 20 °C afford phosphodiester **6a** in quantitative yield.[9] The purity of the phosphodiester **6a** was confirmed by ^{31}P NMR spectra and TLC.[7]

In a similar manner, 5′-hydroxyl nucleotides **5c** and phosphodiesters **6b** were obtained in good yields.

The phosphodiester **6** (1.5 molar equiv) thus obtained was treated with 5′-hydroxyl nucleotides **5** (1.0 molar equiv) in the presence of QS-t (3.0 molar equiv) in dry pyridine for 1 h at room temperature. The dinucleoside diphosphates **7a,b** were isolated in 93–97% yields after separation by silica gel column chromatography. The dinucleoside monophosphate **8** was obtained in 89% yield by condensing the phosphodiester **6b** (1.0 molar equiv) with N^6,2′,3′-*O*-tribenzoyladenosine (1.5 molar equiv).

Synthesis of Trinucleotide 10. The fully protected dinucleotide **7b** was treated with 2% *p*-toluenesulfonic acid solution to give the 5′-hydroxyl dinucleotide **9** in 88% yield.

The phosphodiester **6b** (1.5 molar equiv) was reacted together with 5′-hydroxyl dinucleotide **9** (1.0 molar equiv) and coupling agent QS-t (3.7 molar equiv) to give, after workup and chromatography of the reaction mixture, trinucleotide **10** in 70% yield.

Synthesis of Pentanucleotide 13. In order to remove the dimethoxytrityl group, we treated **8** was with 2% *p*-toluenesulfonic acid solution for 15 min at 0 °C. After workup, 5′-hydroxyl dinucleotide **11** was obtained in 85% yield by precipitation from *n*-hexane–ether and then used

(8) Takaku, H.; Kamaike, K.; Kasuga, K. *Chem. Lett.* **1982**, 197.
(9) Takaku, H.; Yoshida, M. *J. Org. Chem.* **1981**, *46*, 589.
(10) Takaku, H.; Nomoto, T.; Sakamoto, Y.; Hata, T. *Chem. Lett.*, **1979**, 1225.
(11) Reese, C. B.; Titmas, R. C.; Yau, L. *Tetrahedron Lett.* **1979**, 2727.

Table II. Reaction Conditions and Yields of Various Oligoribonucleotides

3'-phosphate component, no.	mmol	5'-hydroxyl component, no.	mmol	QS-t, mmol	reaction time, h	product no.	yield, g (%)	R_f values C	D
6a	1.20	5a	0.74	2.40	1	7a	1.05 (93)	0.48	0.32
6b	2.83	5c	1.57	5.80	1	7b	3.37 (97)	0.52	0.38
6b	1.80	$bzA(OBz)_2$	2.70	3.60	1	8	1.99 (72)	0.53	0.35
6b	1.10	9	0.73	2.75	1	10	1.50 (85)	0.48	0.33
12	0.74	11	1.11	2.03	2	13	1.94 (76)	0.46	0.32
14b	0.80	15	0.46	2.40	2.5	16	1.74 (80)	0.44	0.29
14a	0.62	17	0.31	1.90	3	18	1.13 (62)	0.41	0.25

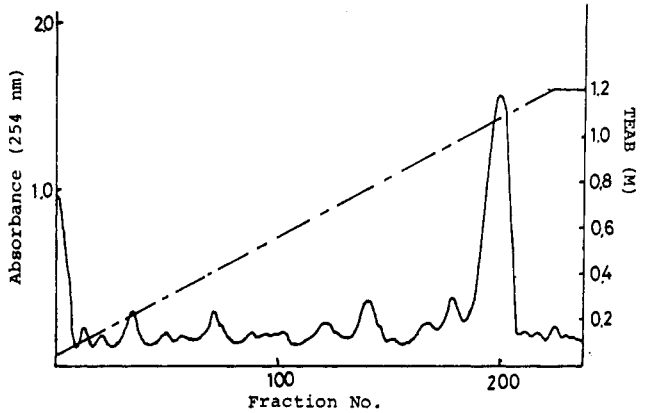
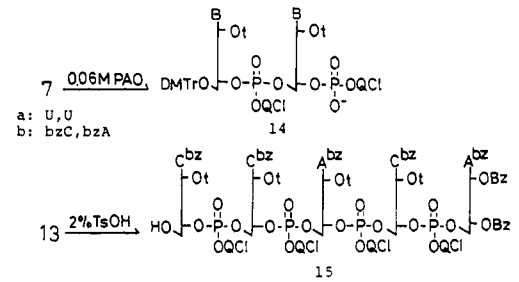


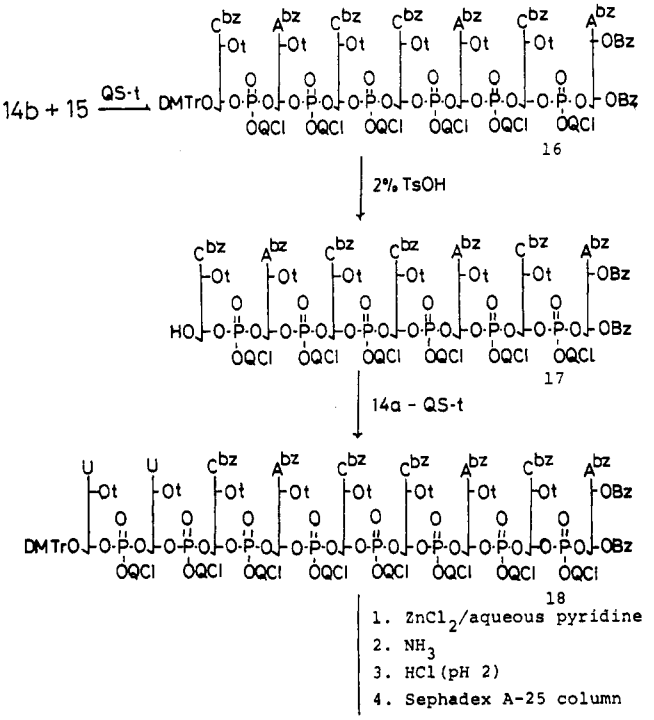
Figure 2. DEAE-Sephadex chromatography of the nonanucleotide. Elution was performed with a liner gradient of TEAB (0.05–1.2 M, total 1.2 L). Fractions of 5 mL were collected each 20 min. The main peak contained the product UpUpCpApCpCpApCpA.

for the next coupling reaction without further purification. On the other hand, a solution of the fully protected trinucleotide **10** and 0.06 M PAO in a mixture of dioxane and water (1:1 v/v) was allowed to stand for 16 h. After workup as described above, the reaction mixture afforded the corresponding phosphodiester derivative **12**. The phosphodiester **12** (1.0 molar equiv) was condensed with the 5'-hydroxyl dinucleotide **11** (1.5 molar equiv) and QS-t (3.0 molar equiv) in dry pyridine. After 1.5 h, no starting material **11** could be detected on TLC in the reaction mixture. The mixture was worked up and purified to give the fully protected pentanucleotide **13** in 76% yield.

Synthesis of Hepta- and Nonanucleotides 16 and 18. The dinucleotide diphosphates **7a,b** obtained in the above experiment were treated with 0.06 M PAO for 16 h to give the corresponding phosphodiester derivatives **14a,b**. On



the other hand, the dimethoxytrityl group of **13** was then removed by the procedure described above to give the 5'-hydroxyl pentanucleotide **15** in 88% yield. In this reaction, TLC after 15 min still showed some starting material **13**. After a total reaction time of 25 min, no starting material **13** could be detected on TLC of the reaction mixture. A solution of the latter substance **15** (1.0 molar equiv) and the phosphodiester derivative **14b** (1.7 molar equiv) in dry pyridine was then treated with QS-t (5.10 molar equiv). After 2 h, the reaction mixture was worked up and purified to give the fully protected heptanucleotide **16** in 72% yield.



The final condensation was performed starting from **16** and **7a**. The former product **16** was treated with 2% *p*-toluenesulfonic acid solution for 25 min at 0 °C to give the corresponding 5'-hydroxyl heptanucleotide **17** in 89% yield. The phosphodiester derivative **14a** (2.0 molar equiv) was treated with **17** (1.0 molar equiv) in the presence of QS-t (6.0 molar equiv) in dry pyridine for 3 h. Workup as described above afforded the corresponding nonanucleotide **18** in 62% yield. Rection conditions and yields of oligoribonucleotides are given in Tables II and III.

Removal of the Protecting Groups from the Fully Protected Nonanucleotide 18. Treatment of **18** with a large amount of zinc chloride in aqueous pyridine for 36 h at room temperature completely removed the 5-chloro-8-quinolyl groups.[12] The resulting phosphodiester compound was treated with concentrated ammonia for 5 h at 50 °C to cleave the benzoyl groups and finally 0.01 N hydrochloric acid (pH 2) for 2 days at 20 °C to remove the tetrahydropyranyl groups.[13] The completely deblocked nonanucleotide **19** was purified by DEAE Sephadex

(12) Takaku, H.; Yamaguchi, R.; Nomoto, T.; Hata, T. *Tetrahedron Lett.* **1979**, 3957. Takaku, H.; Kato, M.; Yoshida, M.; Yamaguchi, R. *J. Org. Chem.* **1980**, *45*, 3347.

(13) Smrt, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1964**, *29*, 2971. Werstiuk, E. S.; Nielson, T. *Can. J. Chem.* **1972**, *50*, 1283. Neilson, T.; Wastrodowski, E. V.; Werstiuk, E. S. *Ibid.* **1973**, *51*, 1068.

Table III. Removal of Dimethoxytrilyl Group

no.	product yield, %	R_f values C	D
9	88	0.49	0.30
11	85	0.50	0.30
15	88	0.44	0.28
17	89	0.41	0.25

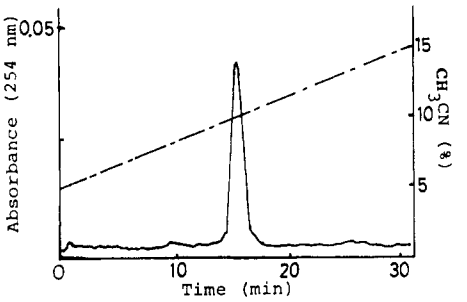


Figure 3. Reverse-phase HPLC of nonanucleotide on Finepack SIL C_{18} (4.6 × 250 mm). Elution performed with a linear gradient of acetonitrile (5–15%) in 0.1 M ammonium acetate (pH 7.0) in 32 min.

chromatography (Figure 2). The product in the main peak was analyzed by HPLC on C_{18} silica gel and found to be almost pure (Figure 3). Completely deblocked product **19** was characterized by complete digestion of the oligoribonucleotide with nuclease P1 and spleen phosphodiesterase to the expected products in the correct ratios.

Experimental Section

UV absorption spectra were measured with a Shimazu UV-200 recording spectrophotometer. ^{1}H NMR spectra were measured at 100 MHz with a JEOL JNMPS 100 spectrometer with Me_4Si as internal standard.

High-pressure liquid chromatography (HPLC) was carried out by using a JASCO TWINCLE apparatus.

Paper electrophoresis was performed on 0.05 M TEAB (pH 7.5) at 1100 V/40 cm. Paper chromatography was performed by the descending technique by using solvent systems: A, 1-propanol–concentrated ammonia–water (55:10:35 v/v); B, 2-propanol–concentrated ammonia–water (55:10:35 v/v).

Thin-layer chromatography (TLC) was performed on plates of silica gel (Merck $60F_{254}$) by using solvent systems: C, methylene chloride–methanol (9:1 v/v); D, methylene chloride–methanol (95:5 v/v). For columns, silica gel (100–200 mesh Kanto Chem. Co.) was used. RPTLC was performed on plates of silanized silica gel (Merck $60F_{254}$) by using acetone–water (7:3 v/v). For the reversed-phase column, silanized silica gel (Merck, 70–230 mesh) was eluted with 60–80% aqueous acetone.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl) nucleosides **1** were prepared according to published procedure.[5]

2′-*O*-Tetrahydropyranyluridine (2a). A solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **1a** (9.8 g, 20 mmol) in dry dioxane (50 mL) was cooled in an ice bath. *p*-Toluenesulfonic acid monohydrate (0.171 g, 0.87 mmol) was then added, followed by 2,3-dihydropyran (6.9 mL, 66 mmol). The reaction mixture was gradually warmed to room temperature and stirred for 1.5 h. The reaction mixture was cooled in an ice bath and neutralized with 1 M methanolic NaOMe solution. The sodium tosylate was filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in dry THF (80 mL), and 1 M tri-*n*-butylammonium fluoride in dry THF (80 mL) was added. After 12 h, the solution was diluted with pyridine–water–methanol (3:1:1 v/v) (340 mL) and treated with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in methylene chloride and applied to silica gel column. The column was eluted with 200 mL of methylene chloride–ether (1:1 v/v) followed by 900 mL of a stepwise gradient of methanol (0–4%) in methylene chloride. The high-R_f (0.55, solvent C) material was crystallized from AcOEt to give a diastereoisomer of **2a** (2.74 g, 43%): mp 147–149 °C (lit.[14] mp 146–148 °C); NMR (Me_2SO-d_6–D_2O) δ 7.88 (d, 1 H, H-6), 5.92 (d, 1 H, H-1′), 5.65 (d, 1 H, H-5), 4.95 (s, 1 H, Thp acetal proton), 1.65 (m, 6 H, Thp). The low-R_f (0.46, solvent C) material was crystallized from EtOH to give a diastereoisomer of **2a** (2.13 g, 33%); mp 204–205 °C (lit.[14] mp 185–187 °C); NMR (Me_2SO-d_6–D_2O) δ 7.70 (d, 1 H, H-6), 6.01 (d, 1 H, H-1′), 5.71 (d, 1 H, H-5), 4.96 (s, 1 H, Thp acetal proton), 1.69 (m, 6 H, Thp).

N^4-Benzoyl-2′-*O*-tetrahydropyranylcytidine (2b). A solution of *p*-toluenesulfonic acid monohydrate (4.3 g, 22 mmol) in dry dioxane (72 mL) was cooled in an ice bath. 2,3-Dihydropyran (37 mL, 382 mmol) was then added followed by a solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-N^4-benzoylcytidine (**1b**) (17.3 g, 30 mmol) in dry dioxane (75 mL). The reaction mixture was gradually warmed to room temperature and stirred for 2 h. The mixture was cooled in an ice bath and neutralized with concentrated ammonia. The ammonium tosylate was filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in dry THF (120 mL), and 1 M tri-*n*-butylammonium fluoride in dry THF (120 mL) was added. After 12 h, the solution was diluted with pyridine–water–methanol (3:1:1 v/v) (750 mL) and treated with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in methylene chloride and applied to silica gel column. The column was eluted with 400 mL of methylene chloride–ether (1:1 v/v) followed by 1000 mL of a stepwise gradient of methanol (0–4%) in methylene chloride. The high-R_f (0.57, solvent C) material was crystallized from methylene chloride–*n*-hexane to give a diastereoisomer of **2b** (4.2 g, 34%): mp 164–165 °C (lit.[15] mp 162–164 °C); NMR (Me_2SO-d_6–D_2O) δ 8.11–7.19 (m, 6 H, Ar and H-6), 5.87 (d, 1 H, H-1′), 5.07 (s, 1 H, Thp acetal proton), 1.60 (m, 6 H, Thp). The low-R_f (0.52, solvent C) material was crystallized from methylene chloride–*n*-hexane to give a diastereoisomer of **2b** (4.65 g, 36%): mp 195–196 °C (lit.[15] mp 194–196 °C); NMR (Me_2SO-d_6–D_2O) δ 8.38 (s, 1 H, H-6), 8.11–7.21 (m, 6 H, Ar and H-5), 6.01 (d, 1 H, H-1′), 4.85 (s, 1 H, Thp acetal proton), 1.58 (m, 6 H, Thp).

N^6-Benzoyl-2′-*O*-tetrahydropyranyladenosine (2c). *p*-Toluenesulfonic acid monohydrate (2.93 g, 15 mmol) was added to 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (7.13 g, 14 mmol) in dry dioxane (50 mL). After 2 h, 2,3-dihydropyran (5 mL, 45 mmol) was added and allowed to stand for a further 20 min. The reaction mixture was cooled in an ice bath and neutralized with 1 M methanolic NaOMe solution. The sodium tosylate was filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in dry THF (56 mL) and 1 M tri-*n*-butylammonium fluoride in dry THF (40 mL) was added. After 12 h, the solution was diluted with pyridine–water–methanol (3:1:1 v/v) (500 mL) and treated with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in methylene chloride and applied to silica gel column. The column was eluted with 200 mL of methylene chloride–ether (1:1 v/v) followed by a stepwise gradient of methanol (0–5%) in methylene chloride. The high-R_f (0.43, solvent C) material was recrystallized from AcOEt to give a diastereoisomer of 2′-*O*-tetrahydropyranyladenosine (2.39 g, 43%): mp 168–170 °C (lit.[14] mp 171–172 °C); NMR (Me_2SO-d_6–D_2O) δ 8.31 (s, 2 H, H-2 and H-8), 6.12 (d, 1 H, H-1′), 4.75 (s, 1 H, Thp acetal proton), 1.61 (m, 6 H, Thp). The low-R_f (0.31, solvent C) material was recrystallized from EtOH to give a diastereoisomer of 2′-*O*-tetrahydropyranyladenosine (2.70 g, 49%): mp 197–199 °C (lit.[14] mp 199–201 °C); NMR (Me_2SO-d_6–D_2O) δ 8.26 (s, 2 H, H-2 and H-8), 6.16 (d, 1 H, H-1′), 4.76 (s, 1 H, Thp acetal proton), 1.63 (m, 6 H, Thp).

The low-R_f 2′-*O*-tetrahydropyranyladenosine (2 g) was dissolved in dry pyridine (20 mL) and treated with benzoyl chloride (4.2 mL) for 2 h at 0 °C and allowed to stand for another 12 h at room temperature. The TLC showed the reaction to be complete (R_f 0.31 → 0.9, solvent C). The reaction mixture was quenched with ice–water (6 mL), added to ice–water (100 mL), extracted with methylene chloride (3 × 100 mL), and washed with 5% $NaHCO_3$. The organic layer was dried with Na_2SO_4 and evaporated in vacuo. The residue was dissolved in a mixture of EtOH (30 mL) and pyridine (16 mL). A 2 N NaOH (32 mL) solution and additional

(14) Griffin, B. E.; Jarman, M.; Reese, C. B. *Tetrahedron* **1968**, *24*, 639.
(15) Nielson, T.; Werstiuk, E. S. *Can. J. Chem.* **1971**, *49*, 493.

EtOH (32 mL) were added. The solution was neutralized with Dowex 50W-X2 (pyridine form), the resin was removed by filtration, and the filtrate was evaporated in vacuo. The residue was washed with ether, then dissolved in methylene chloride (100 mL), and washed with 5% $NaHCO_3$ and with water. The organic layer was dried with Na_2SO_4, and the product **2c** was precipitated with *n*-hexane–ether (95:5 v/v) (500 mL) from its solution in methylene chloride. The yield was 2.06 g (80%): UV (MeOH) λ_{max} 279 nm; NMR (Me_2SO-d_6–D_2O) δ 8.81 and 8.72 (2 s, 2 H, H-2 and H-8), 7.97–7.35 (m, 5 H, Ar), 6.26 (d, 1 H, H-1'), 4.75 (s, 1 H, Thp acetal proton), 1.60 (m, 6 H, Thp).

Tritylation of Nucleotide 2.[16] A solution of the high-R_f **2a** (329 mg, 1.0 mmol) and dimethoxytrityl chloride (375 mg, 1.1 mmol) in dry pyridine (5 mL) was kept for 12 h. The reaction was checked by TLC (solvent C), and **2a** (R_f 0.46) was found to be converted to a traveling compound **3a** (R_f 0.57). The reaction was quenched with EtOH (2 mL) and poured into water (25 mL). The mixture was extracted with methylene chloride (3 × 25 mL) and washed with water (3 × 25 mL). The organic layer was dried with Na_2SO_4 and evaporated in vacuo. The residue was dissolved in methylene chloride and applied to silica gel column. Elution with a stepwise gradient of methanol (0–2%) in methylene chloride gave the corresponding 5'-tritylated material **3a** as a solid (630 mg, 99%), which was sufficiently pure for coupling reactions. In the same way, other tritylated products **3b,c** were obtained in high yields.

General Procedure for the Preparation of the Fully Protected Mononucleotides 4. 5'-*O*-(Dimethoxytrityl)-2'-*O*-tetrahydropyranyl-*N*-acylnucleosides **3** (0.4 mmol) were treated with 4-chlorophenyl 5-chloro-8-quinolyl phosphate[8] (299 mg, 0.6 mmol) in the presence of QS-t (313 mg, 1.2 mmol) in dry pyridine (3 mL) for 2 h at room temperature. The reaction mixture was quenched with ice–water and extracted with methylene chloride (3 × 15 mL). The combined organic extracts were washed with water (3 × 15 mL), dried with Na_2SO_4, and concentrated in vacuo. The residue was dissolved in a small amount of methylene chloride and chromatographed on silica gel column. The fully protected mononucleotides **4** were isolated in 90–95% yields by eluting the column with methylene chloride–methanol (95:5 v/v). These materials were identical (NMR analysis, melting point) with that of synthetic mononucleotides **4** reported previously.[9]

Detritylation of the Fully Protected Mono- and Oligoribonucleotides. The fully protected compound was treated with 2% *p*-toluenesulfonic acid in methylene chloride–methanol (7:3 v/v) for 15–25 min at 0 °C.[10] The reaction mixture was neutralized with 5% $NaHCO_3$ solution and transferred into methylene chloride. The organic layer was washed with water, dried with Na_2SO_4, filtered, and evaporated in vacuo. The 5'-hydroxyl mono- and oligoribonucleotides were obtained as homogeneous white solids after precipitation from *n*-hexane–ether (5:1 v/v), and the 5'-hydroxyl component was used in the subsequent condensation without purification. The yields and TLC analytical data are given in Table II.

General Procedure for the Preparation of the Fully Protected Oligoribonucleotides. The 5'-*O*-(dimethoxytrityl)-2'-*O*-tetrahydropyranyl-*N*-acyl(oligo)ribonucleoside 3'-(4-chlorophenyl 5-chloro-8-quinolyl phosphates) (1.5–2.0 molar equiv) were treated with 0.06 M N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime in dioxane–water (2:1 v/v) for 16 h at 22 °C. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with 50% aqueous pyridine. The filtrate was washed with ether and extracted with methylene chloride. The methylene chloride solution was dried with Na_2SO_4, filtered, and evaporated in vacuo. The phosphodiesters thus obtained and 5'-hydroxyl mono- and oligoribonucleotides(sides) (1.0 molar equiv) were dried by repeated coevaporation with dry pyridine (3 × 10 mL) and then treated with QS-t (3.7–6.0 molar equiv) in dry pyridine (10 mL/mmol of the phosphodiester component). After 1–3 h, 8-quinolinesulfonic acid was removed by filtration. The filtrate was quenched with ice–water, followed by extraction with methylene chloride. The methylene chloride layer was washed with 0.1 M TEAB (pH 7.5) and then with water, dried with Na_2SO_4, filtered, and under reduced pressure evaporated to oil. The oil was dissolved in a small amount of methylene chloride and applied to silica gel column chromatography. The appropriate fractions (eluted with a stepwise gradient of methanol (0–6%) in methylene chloride) were evaporated to give the fully protected oligoribonucleotides, which were isolated as white solids by precipitation from *n*-hexane–ether (9:1 v/v). Reaction conditions and yields of these compounds are given in Table II.

Deblocking of the Fully Protected Nonanucleotide 18. To a solution of **18** (52 mg, 81 μmol) in 10% aqueous pyridine (6 mL) was added zinc chloride (1.71 g, 12.9 mmol, 20 molar equiv per phosphotriester moiety) at room temperature. After 36 h, the reaction mixture was quenched with water (10 mL) and treated with Dowex 50W-X2 (pyridinium form). The resin was filtered off and washed with pyridine (3 × 5 mL). The solution was concentrated to an oil. The oil was dissolved in concentrated ammonia (10 mL) and kept at 50 °C. After 5 h, the solution was concentrated to an oil, which was dissolved in 0.01 N hydrochloric acid (pH 2) (25 mL). After 2 days at 20 °C, the solution was neutralized with with 1 M ammonia and evaporated in vacuo. The residue was dissolved in 0.05 M TEAB (pH 7.5) (5 mL) and washed with ether (3 × 3 mL). The solution was applied to Sephadex A-25 (HCO_3 form). The column chromatography was performed with a linear gradient of 0.05–1.2 M TEAB, and the main part of the peak was concentrated in vacuo. The yield was 385 A_{260}, 5.1 μmol, 51%. The oligoribonucleotide **19** was found by paper chromatography, paper electrophoresis, and HPLC (Figure 3) to be pure: R_f 0.23 (solvent A), 0.40 (solvent B); R_m(Cp) = 0.88. The coumpound **19** was characterized by enzymatic digestion with nuclease P1 and spleen phosphodiesterase[17] to yield 1.00:1.03:3.10:3.98 U:pU:pA:pC (=1:1:3:4) and 1.00:1.97:2.11:4.03 A:Ap:Up:Cp (=1:2:2:4).

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Registry No. **1a**, 69304-38-7; **1b**, 69304-43-4; **1c**, 69304-45-6; **2a**, 50826-90-9; α-**2a**, 85280-92-8; **2b**, 31505-92-7; α-**2b**, 85236-83-5; **2c**, 31505-87-0; **3a**, 51296-18-5; **3b**, 69359-38-2; **3c**, 71933-61-4; **4a**, 75933-79-8; **4b**, 75933-81-2; **4c**, 75933-80-1; **5a**, 75933-87-8; **5c**, 75933-88-9; **6a**, 75933-84-5; **6b**, 75933-85-6; **7a**, 75933-96-9; **7b**, 75933-98-1; **8**, 76963-61-6; **9**, 78529-97-2; **10**, 77195-90-5; **11**, 77181-84-1; **12**, 85250-04-0; **13**, 77195-91-6; **14a**, 85236-85-7; **14b**, 85236-84-6; **15**, 77181-86-3; **16**, 85304-51-4; **17**, 85304-50-3; **18**, 85304-52-5; **19**, 85250-05-1; 2'-*O*-tetrahydropyranyladenosine, 3256-14-2; α-2'-*O*-tetrahydropyranyladenosine, 85280-93-9.

(16) Smrt, J.; Malckievicz, A. *Collect. Czech. Chem. Commun.* **1973**, *38*, 2962.

(17) Takaku, H.; Kato, M.; Ishikawa, S. *J. Org. Chem.* **1981**, *46*, 4062.