Synthesis of 2′(3′)-*O*-(Aminoacyl) Trinucleotides Incorporating All Four Common Bases[1]

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A general blocking system has been developed for four common nucleosides that allows for a specific synthesis of 2′(3′)-*O*-(aminoacyl) trinucleotides. The approach is exemplified by the synthesis of six 2′(3′)-*O*-(L-phenylalanyl) trinucleotides (**13**) with sequences containing adenosine, cytidine, guanosine, and uridine. The trinucleotides **13** were synthesized by a benzotriazolyl phosphotriester approach by using the following protecting groups: a [(9-fluorenylmethyl)oxy]carbonyl group for the adenine, cytosine, and guanine amino groups; a phenyl group for the uracil carbonyl moiety; a 2-chlorophenyl group for the internucleotide bond; a dimethoxytrityl or 4,4′,4″-tris(4,5-dichlorophthalimido)trityl group for the 5′-hydroxy functions; a 4-methoxytetrahydropyranyl group for the 2′-hydroxy functions; and a [[2-(4-biphenylyl)isopropyl]oxy]carbonyl group for the α-amino acid. The protected trinucleotides (**10**) were synthesized in a stepwise fashion and were converted in excellent yields to their 3′-*O*-[2-[[[(4-biphenylyl)isopropyl]oxy]carbonyl]-L-phenylalanyl] derivatives **11** by an aminoacylation in the presence of (mesitylenesulfonyl)tetrazole. The blocked derivatives **11** were converted in two steps to the desired 2′(3′)-*O*-(L-phenylalanyl) trinucleotides (**13**) in ca. 30% yield. Thus, this approach allows for a general synthesis of 2′(3′)-*O*-(aminoacyl) oligoribonucleotides, which are important tools for mechanistic studies in protein biosynthesis.

It has been known for more than 25 years that all tRNA's carry a common C-C-A sequence at their 3′-termini and that the 2′(3′)-*O*-hydroxy group of the 3′-terminal adenosine is the attachment site of the amino acid. It has been conclusively shown that the simple 2′(3′)-*O*-(aminoacyl) oligonucleotides (e.g. C-C-A-Phe) can mimic the role of aa-tRNA in various steps of protein biosynthesis in in vitro systems.[2] There is strong evidence that the 2′(3′)-*O*-(aminoacyl) oligonucleotides interact with the same sites on elongation factor Tu and ribosomal peptidyltransferase that the 3′-terminus of aa-tRNA would normally occupy.[2] It now appears that tRNA may base-pair with complementary sequences of ribosomal RNA during interaction of aa-tRNA with ribosomal sites.[2] Therefore, it was of interest to investigate the biochemical properties of 2′(3′)-*O*-(aminoacyl) trinucleotides with sequences other than the natural C-C-A in order to evaluate this possibility.

Chemical synthesis appears to be the only method available for the construction of 2′(3′)-*O*-(aminoacyl) oligonucleotides with nonnatural sequences. Previously, we have reported a new blocking system extremely useful for the synthesis of 2′(3′)-*O*-(aminoacyl) oligonucleotides and demonstrated its convenience with the synthesis of C-C-A-Phe (cf. ref 3).

In this paper we describe the extension of our blocking scheme to all four common nucleosides and demonstrate its generality by specific syntheses of six 2′(3′)-*O*-(L-phenylalanyl) trinucleotides incorporating the four common bases.

Results and Discussion

The major challenge to the synthesis of 2′(3′)-*O*-(aminoacyl) oligonucleotides lies in a judicious selection of protecting groups. The aim of the present work was to extend our approach to the preparation of 2′(3′)-*O*-(aminoacyl) oligonucleotides containing guanosine and uridine units. Then, presumably, the synthesis of the complete double stranded aa-tRNA aminoacyl stem would be feasible.

We have established that the exocyclic amino groups of adenosine and cytidine can be conveniently protected with a [(9-fluorenylmethyl)oxy]carbonyl group.[3] After the synthesis of the oligonucleotide chain is accomplished (via the benzotriazolyl phosphotriester method[4]) and the free 3′-OH moiety is aminoacylated[5] (with BPOC-amino acid); the Fmoc groups and the phosphorus-protecting 2-chlorophenyl groups can be removed by dry oximate treatment.[4,6] This procedure leaves the BPOC-aminoacyl residue largely intact.[3] In the next step, the remaining protecting groups (Mthp, DMT, and BPOC) are removed by a brief acidic treatment.[3]

We decided to apply this methodology to the introduction of uridine and guanosine into the 2′(3′)-*O*-(aminoacyl) oligonucleotide. In uridine and guanosine there exists the possibility of phosphorylation of the 4- and 6-carbonyl groups, respectively, with various phosphorylating agents, including that derived from 1-hydroxybenzotriazole (e.g. **7**, Scheme III, cf. ref 7a). Therefore, the carbonyl groups of both nucleosides should be protected to avoid side reactions. In the case of uridine, this

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(3) Happ, E.; Scalfi-Happ, C.; Chládek, S. *J. Org. Chem.* **1987**, *52*, 5387.

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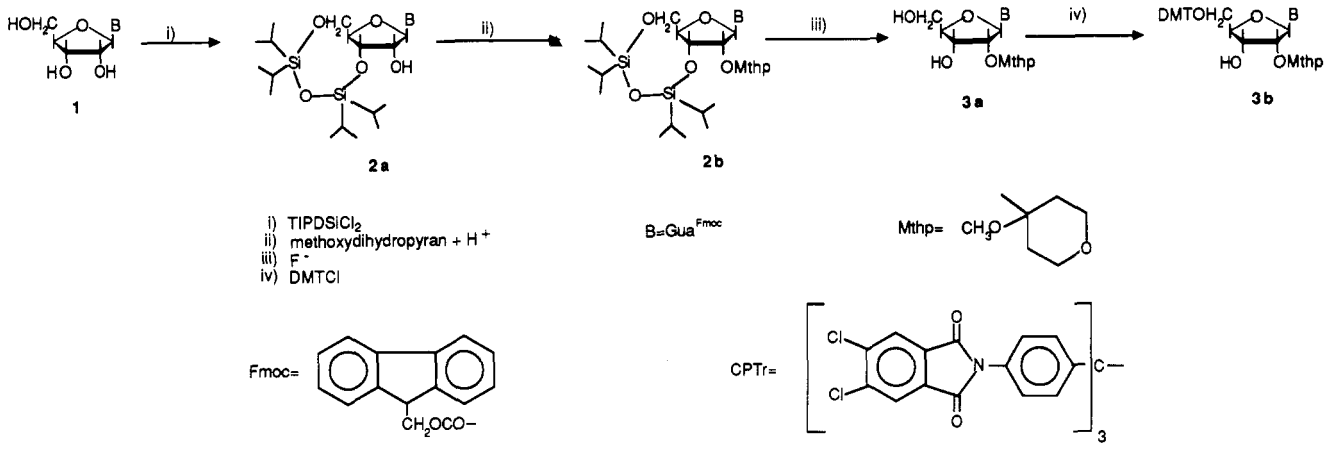
(6) Reese, C. B.; Titmes, R. C.; Yau, L. *Tetrahedron Lett.* **1978**, 2727.

(7) (a) Reese, C. B.; Richards, K. H. *Tetrahedron Lett.* **1985**, *26*, 2245. (b) De Vroom, E.; Fidder, A.; Matügg, J. E.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1986**, *14*, 5885.

0022-3263/88/1953-5040$01.50/0

Scheme I



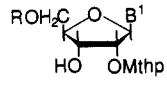
task was easily accomplished by using a 4-*O*-phenyl group. The 4-*O*-phenyl moiety can be easily removed by treatment with oximate.[8] Thus, routine dimethoxytritylation of 2′-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-phenyluridine (**4a**, cf. ref 8) afforded the desired 5′-terminal nucleoside component **4b**.

The standard combination of protecting groups for the guanosine aglycon[9] (protecting both N^2 and O^6) could not be used for our purposes due to the loss of the 2′(3′)-*O*-(aminoacyl) residue during the deblocking conditions. Since the side reactions at the O^6 carbonyl group can be minimized,[7] we decided to use the N^2-protected guanosine components for the synthesis of 2′(3′)-*O*-(aminoacyl) trinucleotides.

The starting material for the guanosine synthon **3a** was N^2-[[(9-fluorenylmethyl)oxy]carbonyl]guanosine (1; Scheme I). Although the synthesis of 1 has been previously described,[10] we have found that the acylation reaction of guanosine with FmocCl via transient protection of the ribose hydroxy groups with trimethylsilyl groups proceeds in poor yield. Moreover, compound 1 is difficult to isolate in a pure state. While part of this difficulty is undoubtedly due to the poor solubility of 1 in common solvents, formation of side products also plays an important role. One of the side products is apparently N^1,N^2-bis[[(9-fluorenylmethyl)oxy]carbonyl]guanosine (identified on the basis of NMR spectra). The Fmoc group at N^1 is quite unstable and is easily removed by brief treatment with dilute ammonia, which simultaneously cleaves off the trimethylsilyl groups but leaves the N^2-Fmoc group intact to generate 1.

Scheme I shows the synthesis of the guanosine synthons **3a** and **3b**. Compound 1 (approximately 90% pure) was first converted into derivative **2a** via reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in the presence of pyridine.[11] The 4-methoxytetrahydropyranyl group was then introduced at the 2′-position under acidic catalysis to form **2b**. Quantitative removal of the 3′,5′-tetraisopropyldisiloxanyl group was achieved with a slight excess of fluoride ion in a tetrahydrofuran–pyridine–water solution to generate **3a**. This "deactivated fluoride solution" does not affect the Fmoc group, which is rather susceptible to β-elimination and parallels the behavior of Fmoc groups of adenosine and cytidine derivatives.[3] Compound **3a** serves as an extension unit for the oligonucleotide synthesis, while compound **3b**, prepared via dimethoxytritylation of **3a**, can be utilized as the 5′-terminal unit.

Scheme II



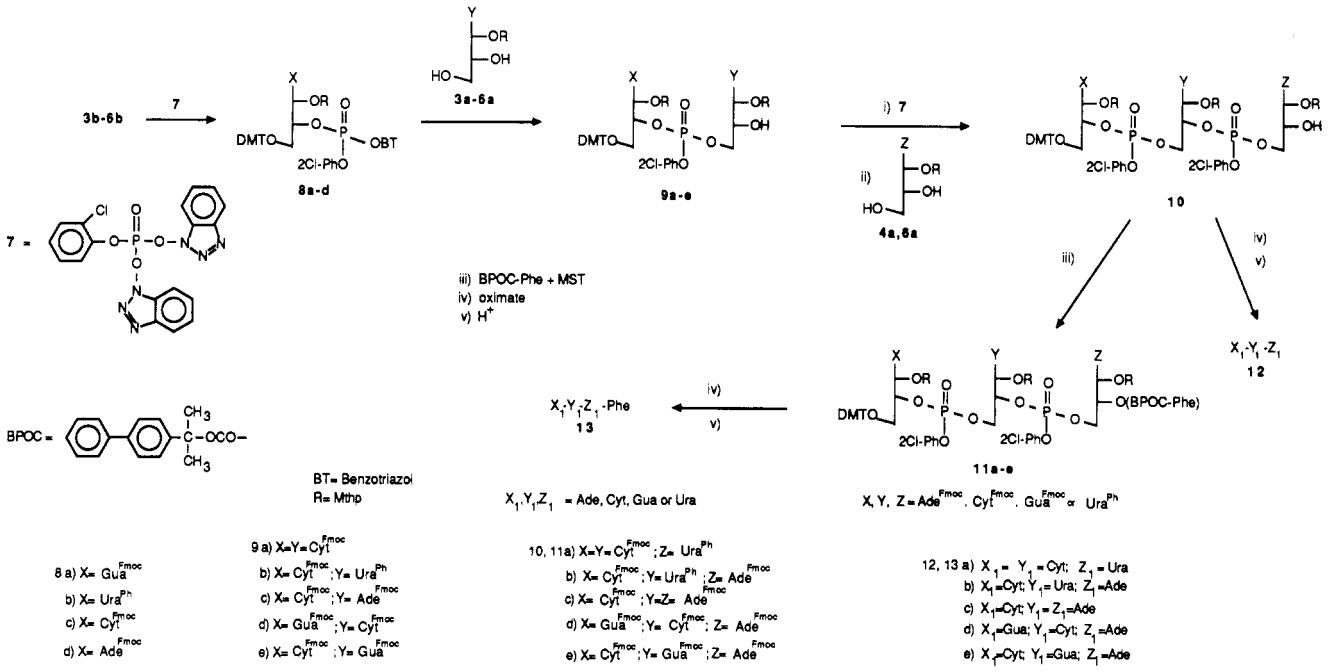
3-6

3a B^1=Gua^{Fmoc}; R=H **3b** B^1=Gua^{Fmoc}; R=DMT
4a B^1=Ura^{Ph}; R=H **4b** B^1=Ura^{Ph}; R=DMT **4c** B^1=Ura^{Ph}; R=CPTr
5a B^1=Cyt^{Fmoc}; R=H **5b** B^1=Cyt^{Fmoc}; R=DMT **5c** B^1=Cyt^{Fmoc}; R=CPTr
6a B^1=Ade^{Fmoc}; R=H **6b** B^1=Ade^{Fmoc}; R=DMT **6c** B^1=Ade^{Fmoc}; R=CPTr

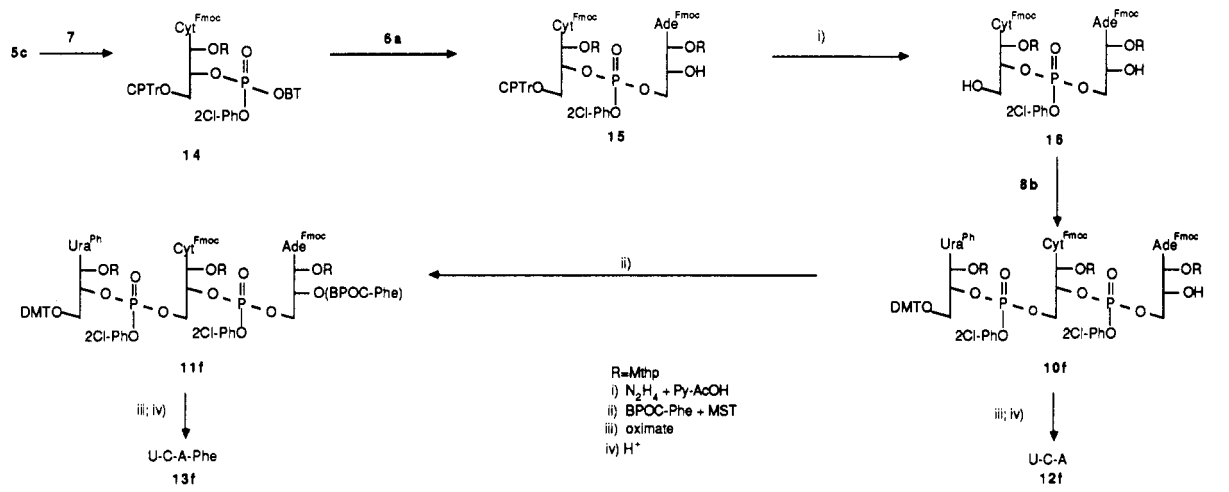
In the methodology described previously, an oligonucleotide chain is built from the 5′-end.[1a] The DMT group serves as a "permanent blocking group" and is removed at the end of the synthesis. For the synthesis of oligonucleotides by a block approach, a temporary protecting group is needed that will shield the 5′-hydroxy function and then be removed under conditions that will leave the rest of the protection system intact. In the past we have prepared oligonucleotides with free 5′-hydroxy moieties employing the 5′-levulinoyl group as the temporary protecting group, removable by a brief treatment with hydrazine.[12] While the conditions for the removal of the 5′-levulinoyl group are satisfactory, its introduction is not very selective. The recently reported 4,4′,4″-tris(4,5-dichlorophthalimido)trityl group[13] (CPTr) appeared to be more satisfactory for this purpose, since the 5′-protected nucleosides **4c**, **5c**, and **6c** (Scheme II) can be obtained in good yields. This protecting group proved successful in model experiments; for example, compound **5c** gives a high yield of **5a** upon treatment with a hydrazine and pyridine–acetic acid mixture. As our results suggest (vide infra), the CPTr group can be conveniently used for the synthesis of oligoribonucleotides with a free 5′-hydroxy group, being fully compatible with Fmoc and Mthp groups. An apparent drawback of the CPTr group is the troublesome separation of the desired product from the aromatic cleavage products (colored pararosaniline and 4,5-dichlorophthaloyl hydrazide).

(8) Jones, S. S.; Reese, C. B.; Sibanda, S.; Ubasawa, A. *Tetrahedron Lett.* **1981**, *22*, 4755.
(9) (a) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59. (b) Reese, C. B.; Skone, P. A. *J. Chem. Soc., Perkin Trans. 1* **1984** 1263. (c) Pon, R. T.; Usman, N.; Danha, M. J.; Ogilvie, K. K. *Nucleic Acids Res.* **1986**, *14*, 6453.
(10) Heikkilä, J.; Chattopadhyaya, J. *Acta Chem. Scand., Ser. B* **1983**, *B37*, 263.
(11) Markiewicz, W. T.; Wiewiorowski, M. In *Nucleic Acids Chemistry*; Townsend, L. B., Tipson, R. S., Eds.; Wiley: New York, 1986; p 229.
(12) (a) Scalfi-Happ, C.; Happ, E.; Ghag, S.; Chládek, S. *Biochemistry* **1987**, *26*, 4682. (b) The levulinoyl group had been used previously as temporary protecting group for the 5′-hydroxy moiety in the oligonucleotide synthesis with different blocking system [van Boom, J. H.; Burgers, P. M. *Recl. Trav. Chim. Pays-Bas* **1978**, *97*, 73].
(13) Sekine, M.; Hata, T. *J. Am. Chem. Soc.* **1986**, *108*, 4581.

Scheme III



Scheme IV



The trinucleotides **10a–e** (Scheme III) were synthesized in a 5′–3′ direction by using the hydroxybenzotriazolyl phosphotriester method[3,4,12a] for the phosphorylation of the 5′-terminal components **3b–6b**. Condensation of the intermediate diesters **8a–d** with synthons **3a–6a** produced dinucleotides **9**, and repetition of this reaction led to trinucleotides **10**. Although yields of these reactions were generally satisfactory, we have observed consistently lower yields for dimers and trimers containing guanosine moieties. Also, guanosine component **3b** or dinucleotides containing guanosine (entries **9d** and **9e**) consumed larger excesses of phosphorylation agent **7** than the other phosphorylations. Although analysis of deblocking products of protected compounds **9d–e**, **10d–e**, or **13d–e** did not indicate any formation of side products, it seems that some phosphorylation of the Fmoc guanine moiety with reagent **7** probably takes place under our reaction conditions (cf. ref 7a, but also cf. ref 4). Thus, syntheses of longer aa-tRNA fragments should use double-protected guanosine components.

The trinucleotide **10f** (Scheme IV) was synthesized by using the CPTr group as a temporary 5′-hydroxy shielding group. The dimer **15**, resulting from the routine condensation of **6a** and **5c**, was treated briefly with a hydrazine–pyridine–acetic acid mixture to produce **16** in good yield. Condensation of dimer **16** with the phosphorylated **8b** yielded trinucleotide **10f**.

All trinucleotides **10** were aminoacylated with BPOC–Phe in the presence of (mesitylenesulfonyl)tetrazole to obtain **11** in quantitative yields (Schemes III and IV). The deprotection of **11** proceeded in two simple steps: (i) dry oximate treatment, removing the Fmoc and Ph groups from the aglycons and 2-ClPh groups from the internucleotide linkages, and (ii) hydrolysis with formic acid, removing the remaining acid labile protecting groups (Mthp, DMT and BPOC).[3] While the lipophilic reaction products (residues of protecting groups) are easily removed by a solvent extraction, the final purification of the target compounds **13** involves a simple chromatography on Baker's 10SPE C18 column. This system separates products **13** from trace amounts of deacylated trimers **12**. The yields of the combined deprotection steps were in the 20–30% range.

Compounds **13a–e** were characterized by TLC, paper electrophoresis, UV spectroscopy, and by their hydrolysis in mild alkaline medium to parent trimers **12** and phe-

Table I. ^{1}H NMR Spectral Data of Nucleoside Derivatives (δ)[a]

compd	H_8 or H_2 H_6 or H_5	aromatic protons	$H_{1'}$	fluorene protons	OCH_3 (Mthp)	other signals
1[b]	8.22 (s, 1)	7.90 (d, 2),[d] 7.81 (d, 2),[d] 7.43 (t, 2),[d] 7.34 (t, 2),[d]	5.81 (d, 1) J = 5.8	4.48 (m, 3)		
2a[c]	7.93 (s, 1)	7.78 (d, 2),[d] 7.56 (d, 2),[d] 7.40 (t, 2),[d] 7.33 (t, 2),[d]	5.99 (s, 1)	4.61 (m, 3)		0.98 (m, 28) isopropyls
2b[c]	8.01 (s, 1)	7.77 (d, 2),[d] 7.58 (d, 2),[d] 7.41 (t, 2),[d] 7.32 (t, 2),[d]	5.86 (s, 1)	4.51 (m, 3)	3.35 (s, 3)	1.05 (m, 28) isopropyls
3a[c]	7.87 (s, 1)	7.76 (d, 2),[d] 7.58 (d, 2),[d] 7.40 (t, 2),[d] 7.31 (t, 2),[d]	5.87 (d, 1) J = 7.2	4.60 (d, 2) J = 6.2	2.71 (s, 3)	
3b[c]	7.81 (s, 1)	7.73 (d, 2) J = 8.0, 7.35 (m, 15), 6.79 (m, 4)	5.92 (d, 1) J = 7.3	4.47 (m, 3)	2.87 (s, 3)	3.74 (s, 6) CH_3O of DMT
4b[c]	8.38 (d, 1) J = 6.0, 5.70 (d, 1) J = 6.0	7.47–7.10 (m, 14), 6.88 (d, 4) J = 8.0	6.23 (s, 1)		3.21 (s, 3)	3.85 (s, 6) CH_3O of DMT
4c[c]	8.11 (d, 1) J = 6.0, 5.96 (d, 1) J = 6.0	8.04 (s, 6), 7.50 (dd, 12) J = 12, 7.32–7.04 (m, 5)	6.16 (s, 1)		3.20 (s, 3)	
5c[c]	8.18 (d, 1) J = 6.5, 6.87 (d, 1) J = 6.5	8.01 (s, 6), 7.55 (dd, 12) J = 12, 7.81–7.32 (m, 8)	6.19 (d, 1) J = 5.6	4.38 (m, 3)	3.22 (s, 3)	
6c[c]	8.34 (s, 1), 8.16 (s, 1)	8.04 (s, 6), 7.5 (dd, 12) J = 12, 7.70–7.30 (m, 8)	6.16 (d, 1) J = 6.0	4.50 (m, 3)	2.96 (s, 3)	

[a] δ given in ppm; J values in hertz. [b] In DMSO-d_6. [c] In $CDCl_3$. [d] J = 7.3.

nylalanine. Furthermore, compounds **13a–e** were quantitatively cleaved with the appropriate nucleases and snake venom diesterase, giving rise to the expected products in correct ratios, thus excluding the possibility of significant isomerization of the 3′–5′ phosphodiester linkages in the course of the synthesis.

Thus, this work provides for the first time a general method for the synthesis of 2′(3′)-*O*-(aminoacyl) oligoribonucleotides containing all four common nucleosides. We are currently engaged in the synthesis of double-protected guanosine synthons, which will make the construction of longer fragments of aa-tRNA feasible.

Experimental Section

General Methods. The general methods were the same as those described in previous papers of this series.[3] Elemental analyses were performed by MHW Laboratories.

Chromatography and Electrophoresis. Thin-layer chromatography (TLC) was performed on silica gel (60F-254) coated aluminum foils containing fluorescent indicator (E.M. Laboratories) and on cellulose plates (Avicel F Uniplate; Analtech). Preparative TLC was performed on silica gel GF (precoated thin-layer chromatography plates, 2000 μm; Analtech). The following chromatographic systems were used on silica gel: S_1, CH_2Cl_2–5% CH_3OH; S_2, CH_2Cl_2–10% CH_3OH; and on cellulose, S_3, 1-butanol–water–acetic acid (5:3:2). Paper electrophoresis was conducted on a Savant flat plate (at 4 °C) by using 1 M acetic acid on Whatman No. 1 paper at 50V/cm for 2 h. Column chromatography was performed on silica gel (E.M. Reagent, 70–230 mesh) with a step gradient of methanol in CH_2Cl_2. The 2′(3′)-*O*-(aminoacyl) oligonucleotides were purified on Baker's 10 SPE octadecyl column (J.T. Baker Chemical Co.) by using a step gradient of CH_3CN in ammonium acetate buffer (5 mM; pH 4.5). High-performance liquid chromatography (HPLC) was conducted on an Altex-Spectraphysics instrument with a Kratos Spectroflow 773 absorbance detector (at 254 nm).

Spectra. UV spectra were obtained by using a Perkin-Elmer Lambda 5 UV/vis spectrophotometer. Yields of oligonucleotides were determined spectrophotometrically at pH 2.0 (0.01 N HCl) by using ϵ_{260} 23.6×10^3 for C-C-U; 31.3×10^3 for C-U-A and U-C-A; 35.3×10^3 for C-A-A; 32.9×10^3 for G-C-A and C-G-A. ^{1}H NMR spectra were recorded on a QE-300 instrument (General Electric) at 300 MHz with tetramethylsilane as an internal standard. The NMR data are presented in Table I.

Enzymic Digestions. The digestions with ribonuclease A (Sigma; 25 μg) and snake venom phosphodiesterase from *Crotalus durissus* (Boehringer, Mannheim; 4 μg) were performed with ca. 0.5–2.0 A_{260} units of oligonucleotides as described previously.[3] The digestions with ribonuclease T_1 (Sigma) were carried out with ca. 0.5–2.0 A_{260} units of oligonucleotides and 1.7 μg of enzyme in 0.05 M Tris·HCl buffer (pH 7.8) by incubation at 37 °C for 3 h. The cleavage by ribonuclease T_2 (Sigma) was performed with 0.5–2.0 A_{260} units of oligonucleotides and 0.5 units of enzyme in 0.05 M ammonium acetate (pH 4.5) at 37 °C for 3 h. The digestion mixtures were analyzed by HPLC on a Synchropak RP C-18 analytical column (Altex) using system A, isocratic elution with 0.1 M KH_2PO_4 and 0.05 M NaCl (pH 4.5), and System B, linear gradient of methanol (1–5%) in 50 mM ammonium acetate (pH 4.5). The peaks of digestion products were identified by a comparison with authentic samples, and the ratio was obtained by using appropriate extinction coefficients.[14]

Starting Materials. Nucleosides, amino acids, and reagents were commercial preparations (Sigma, Aldrich, Fluka). Nucleoside blocks **5a**, **5b**, **6a**, and **6b** were prepared as described previously.[3] Reagent **7** was prepared according to ref 4.

N^2-[[(9-Fluorenylmethyl)oxy]carbonyl]guanosine (1). Guanosine was dried for 18 h at 60 °C. To a suspension of the dried guanosine (2.00 g, 7.07 mmol) in anhydrous pyridine (40 mL) at 0 °C was slowly added trimethylsilyl chloride (3.20 mL, 25.1 mmol). After 1.5 h, 9-fluorenylmethyl chloroformate (3.2 g, 12.4 mmol) was added in small portions, and the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was cooled to 0 °C and quenched with 5% aqueous ammonium bicarbonate. The aqueous phase was extracted with methylene chloride, and the combined organic extracts were dried over magnesium sulfate and concentrated in vacuo.

To a solution of the crude residue in methanol (50 mL) and methylene chloride (10 mL) at 0 °C was added a saturated solution of methanolic ammonia (50 mL). The resulting mixture was stirred at 0 °C for 3 h and was then concentrated to a volume of 30 mL. The suspension was diluted with methylene chloride. Filtration of the suspension afforded 1.30 g of a solid containing 1, guanosine, and unidentified side products. This mixture proved difficult to separate. Purification was accomplished by dissolving the solid in pyridine, slurrying with silica gel (5 g), and concentrating in vacuo. The free-flowing powder was chromatographed on silica gel (20 g) with 5% methanol in methylene chloride. The semipure **1** (0.68 g, 18%) was used without any further purification. An analytical sample was obtained by prep-TLC. Anal. Calcd for $C_{25}H_{23}O_7N_5 \cdot 2H_2O$: C, 55.49; H, 5.03; N, 12.93. Found: C, 55.38; H, 5.06; N, 12.87.

N^2-[[(9-Fluorenylmethyl)oxy]carbonyl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)guanosine (2a). Compound **1** was dried by repeated evaporation of pyridine. To a suspension of **1** (0.332 g, 0.65 mmol) in anhydrous pyridine (5 mL) was slowly

(14) Van Boom, J. H.; de Rooy, J. F. M. *J. Chromatogr.* **1977**, *131*, 169.

added dichloro-1,1,3,3-tetraisopropyldisiloxane (0.25 mL, 0.79 mmol). After being stirred for 4 h, the mixture was cooled to 0 °C and quenched with 5% aqueous ammonium bicarbonate. The aqueous phase was extracted with methylene chloride, and the combined organic extracts were dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography using silica gel (10 g) and a step gradient of methanol in methylene chloride (0–2%) to afford 0.329 g (68%) of **2a**. Anal. Calcd for $C_{37}H_{49}O_8N_5Si_2$: C, 59.42; H, 6.60; N, 9.36. Found: C, 59.60; H, 6.71; N, 9.16.

N^2-[[(9-Fluorenylmethyl)oxy]carbonyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)guanosine (2b). To a solution of **2a** (0.400 g, 0.535 mmol) in anhydrous THF (2 mL) at 0 °C was added 4-methoxydihydropyran (1.20 mL, 10.7 mmol) and mesitylenesulfonic acid (0.020 g, 0.08 mmol). The solution was allowed to stand at 0 °C for 15 h. The reaction mixture was slowly added to 5% aqueous ammonium bicarbonate. The aqueous phase was extracted with methylene chloride. The combined organic extracts were dried over magnesium sulfate and concentrted in vacuo. The residue was purified by column chromatography using silica gel (20 g) and a step gradient of methanol in methylene chloride (0–2%) to afford 0.420 g (90%) of **2b**. Anal. Calcd for $C_{43}H_{59}O_{10}N_5Si_2·H_2O$: C, 58.68; H, 6.99; N, 7.95. Found: C, 59.33; H, 7.15; N, 7.12.

N^2-[[(9-Fluorenylmethyl)oxy]carbonyl]-2′-*O*-(4-methoxytetrahydropyran-4-yl)guanosine (3a). To a solution of **2b** (0.400 g, 0.46 mmol) in THF (1 mL) at 0 °C was added a solution of tetrabutylammonium fluoride (1.84 mmol) in THF–H_2O–pyridine (8 mL/1 mL/1 mL). After being stirred for 3 h at 0 °C, the reaction mixture was quenched with 5% aqueous ammonium bicarbonate and extracted with methylene chloride. The combined organic extracts were dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography using silica gel (10 g) and a step gradient of methanol in methylene chloride (0–5%) to yield 0.196 g (69%) of **3a**. Anal. Calcd for $C_{31}H_{33}O_9N_5·H_2O$: C, 58.39; H, 5.53; N, 10.98. Found: C, 59.37; H, 5.81; N, 10.79.

General Procedure for Dimethoxytritylation of Nucleosides 3a and 4a. The procedure of ref 3 was used. To a 0.15 M solution of nucleoside **3a** or **4a** (ref 8) in dry pyridine was added dimethoxytrityl chloride (1.3 molar equiv) and a catalytic amount of 4-(dimethylamino)pyridine. The solution was stirred at room temperature until TLC (system S_1) showed quantitative conversion to a faster moving material. The reaction mixture was quenched with 5% aqueous ammonium bicarbonate and extracted with methylene chloride. The combined organic extracts were dried over magnesium sulfate and concentrated in vacuo. Traces of pyridine were removed by co-evaporation with toluene. The residue was purified by column chromatography using silica gel and a step gradient of methanol in methylene chloride (0–5%). The product could be obtained as a white powder (75% yield) by trituration with methylene chloride and hexane. Anal. (**3b**) Calcd for $C_{52}H_{51}O_{11}N_5$: C, 67.74; H, 5.58; N, 7.60. Found: C, 67.98; H, 5.53; N, 7.39. Anal. (**4b**) Calcd for $C_{42}H_{44}O_{10}N_2$: C, 68.46; H, 6.02; N, 3.80. Found: C, 68.45; H, 6.24; N, 3.78.

2′-*O*-(4-Methoxytetrahydropyran-4-yl)-5′-*O*-[4,4′,4″-tris(4,5-dichlorophthalimido)trityl] Nucleosides (4c, 5c, and 6c). To a solution of nucleosides **4a**, **5a**, and **6a** (0.1 mmol), 2,6-lutidine (0.026 mL), and silver nitrate (0.034 g, 0.2 mmol) in DMF (1 mL) was gradually added under stirring 4,4′,4″-tris(4,5-dichlorophthalimido)trityl bromide (0.193 g, 0.2 mmol[13]). After 1 h at room temperature, TLC (system S_1) showed almost quantitative conversion to a faster moving material. Ethanol (1 mL) and methylene chloride (10 mL) were added, and the precipitate was filtered through a glass fiber filter and washed with methylene chloride (3 × 5 mL). The filtrate was extracted with an aqueous solution of ammonium acetate (5 mM, pH 6.0; 20 mL), and the aqueous solution was back-extracted with methylene chloride (3 × 20 mL). The combined organic extracts were dried over sodium sulfate and concentrated in vacuo. The residue was dried in high vacuo to remove the last traces of DMF. The product was purified by column chromatography using silica gel (10 g) and a step gradient of methanol in methylene chloride (1–2%). Most of the product was eluted with 1.5% methanol. The chromatographically uniform product was isolated in yields of 55–65%. Anal. (**4c**) Calcd for $C_{64}H_{43}O_{14}N_5Cl_6$: C, 58.28; H, 3.29; N, 5.31. Found: C, 58.66; H, 3.69; N, 5.03. (**5c**) Calcd for $C_{73}H_{50}O_{15}N_6Cl_6·H_2O$: C, 59.18; H, 3.54; N, 5.67; Cl, 14.36. Found: C, 58.92; H, 3.64; N, 5.53; Cl, 14.31. (**6c**) Calcd for $C_{74}H_{50}O_{14}N_8Cl_6$: C, 59.73; H, 3.38; N, 7.53. Found: C, 59.72; H, 3.85; N, 7.05.

Table II. Electrophoretic Mobilities of Products and Standard Specimens (Electrophoresis in 1 M Acetic Acid)

compd	electrophoretic mobility[a]	compd	electrophoretic mobility[a]
Cp	1.00	U-C-A-Phe, **13f**	2.3
A	3.7	C-C-U, **12a**	0.86
Phe	2.6	C-U-A, **12b**	0.78
C-C-U-Phe, **13a**	2.4	C-A-A, **12c**	1.59
		G-C-A, **12d**	1.51
C-U-A-Phe, **13b**	2.4	C-G-A, **12e**	1.72
		U-C-A, **12f**	0.73
C-A-A-Phe, **13c**	2.6		
G-C-A-Phe, **13d**	3.0		
C-G-A-Phe, **13e**	3.1		

[a] Mobility of Cp = 1.00.

General Procedure for Removal of the CPTr Group from Oligonucleotides. The CPTr protected oligonucleotide (e.g. **15**; 0.164 mmol) was dissolved in a 1 N hydrazine hydrate solution of pyridine–glacial acetic acid (3:1; total volume of 5.0 mL) and left at room temperature for 20 min. A pyridine–glacial acetic acid solution (1:3; 5 mL) was then added, and the total mixture was left to stand at room temperature for 5 min. The reaction mixture was quenched with demineralized water (40 mL) and extracted with methylene chloride (2 × 40 mL). The combined organic layers were extracted with aqueous ammonium acetate solution (5 mM; pH 6.0, 2 × 40 mL), and the salt solutions back-extracted with methylene chloride (80 mL). The organic extracts were dried over magnesium sulfate, concentrated in vacuo, and co-evaporated with toluene. The crude product was contaminated with purple-colored pararosaniline. TLC (system S_2) shows the double spot of the product in addition to a slower moving nonnucleotidic product. The residue was purified by column chromatography with silica gel (20 g) and a step gradient of methanol in methylene chloride (0–5%). The desired product (e.g. **16**) was isolated in yields of 70%. The NMR spectra of product shows the presence of all other protecting groups.

General Method for Preparation of Protected Oligoribonucleotides (9 or 10). The procedure was essentially the same as used in the previous reports in this series.[3,12a] All operations were performed under complete exclusion of atmospheric moisture with dry argon for bleeding the apparatus. The 5′-terminal component (0.11 mmol) was predried by repetitive coevaporation with anhydrous pyridine (a small amount of pyridine was always left after the last evaporation). The dried component was dissolved in THF (1–2 mL) and was phosphorylated with reagent **7** (ref 4; 0.12–0.15 mmol). The reaction mixture was stirred at room temperature until TLC (system S_2) showed a quantitative formation of the diester (base-line material), and then a solution of a component with a free 5′-OH (0.1 mmol, predried by coevaporation with anhydrous pyridine) and 1-methylimidazole (0.02 mL) in THF (ca. 0.5 mL) was added. After several hours, TLC (system S_2) showed formation of a new faster moving dimethoxytrityl containing material and almost quantitative disappearance of the diester. The reaction mixture was quenched with 5% aqueous ammonium bicarbonate, and the product was isolated by routine column chromatography on silica gel. Occasionally, separation of the oligonucleotides from the free 5′-OH component required additional purification by preparative TLC (system S_1). The chromatographically uniform oligonucleotides **9** and **10** were obtained as colorless solids after drying in vacuo in yields of 50–70%, except for guanosine-containing oligonucleotides, which were in the 30–50% range.

Aminoacylation of Protected Trinucleoside Diphosphates (11). The original methods of ref 3 and 5 were used. The protected trinucleotide (**10**; 0.015 mmol) and dicyclohexylammonium salt of BPOC–Phe (0.027 g, 0.045 mmol) were dried by co-evap-

Table III. UV Data of 2′(3′)-*O*-(L-Phenylalanyl) Trinucleotides

compd	UV spectrum (0.01 N HCl)				
	λ_{max}, nm	λ_{min}, nm	250/260	280/260	290/260
C-C-U-Phe, **13a**	274	243	0.73	1.17	0.84
C-U-A-Phe, **13b**	264	220	0.70	0.62	0.28
C-A-A-Phe, **13c**	260	241	0.83	0.63	0.42
G-C-A-Phe, **13d**	258	245	0.87	0.73	0.46
C-G-A-Phe, **13e**	259	242	0.84	0.72	0.47
U-C-A-Phe, **13f**	263		0.75	0.65	0.36

Table IV. Enzymatic Characterization of 2′(3′)-*O*-(L-Phenylalanyl) Trinucleotides

compd	nuclease digestion[a]		phosphodiesterase digestion[b]	
	% 2′–5′ isomer	cleavage products ratio	% 3′–3′ or 3′–2′ isomer	products
C-C-U-Phe, **13a**	0	Cp:U = 1.87:1.00[c]	0	C + pC + pU[c]
C-U-A-Phe, **13b**	0	Cp:Up:A = 1.02:1.03:1.00[c]	0	C + pU + pA[d]
C-A-A-Phe, **13c**	0	Cp:Ap:A = 1.02:0.88:1.00[c]	0	C + pA[c]
G-C-A-Phe, **13d**	0	Gp:Cp:A = 0.96:0.87:1.00[c]	0	G + pC + pA[c]
C-G-A-Phe, **13e**	0	Cp:Gp:A = 1.03:0.87:1.00[c]	0	C + pG + pA[c]
U-C-A-Phe, **13f**	0	(Cp + Up):A = 1.09:1.00[d]	0	U + pC + pA[c]

[a] Determined by RNAse A digestion (compounds **13a,b,f**); RNAse T_2 and RNAse A digestion (compound **13c**); RNAse T_1 and RNAse A digestion (compounds **13d,e**). [b] Determined by *Crotalus durissus* phosphodiesterase digestion. [c] Determined by HPLC in system A. [d] Determined by HPLC in system B; presence of Up and Cp or C and pU verified by paper electrophoresis, since these compounds did not separate on the HPLC column in system B.

oration with anhydrous pyridine and dissolved in methylene chloride (1.0 mL). To this solution was added (mesitylenesulfonyl)tetrazole (0.017 g, 0.06 mmol) and 1-methylimidazole (0.02 mL). After 1 h at room temperature, the reaction was shown to be quantitative by TLC (system S_1). The reaction mixture was diluted with methylene chloride, and directly purified by preparative TLC (system S_2). The product **11** was isolated in a quantitative yield as a chromatographically uniform amorphous solid.

2′(3′)-*O*-(L-Phenylalanyl) Trinucleotides (13). The two-step deprotection procedure was carried out essentially as described previously.[3] The protected derivative **11** (0.01 mmol) was treated with a freshly prepared solution of N^1,N^1,N^3,N^3-tetramethylguanidine (0.33 M) and *o*-nitrobenzaldoxime (0.38 M) in dry acetonitrile (1 mL). After 3 h at room temperature, the reaction was shown to be complete (quantitative formation of dimethoxytrityl positive baseline material) by TLC (system S_2). The reaction product was precipitated with an excess of dry ether, centrifuged, washed twice with dry ether, and dried in vacuo over P_2O_5. The residue was treated with cold 80% formic acid (2.0 mL) at 0 °C for 30 min. The product was precipitated with an excess of dry ether, isolated by centrifugation, dissolved in cold 80% acetic acid (0.2 mL), and reprecipitated with excess ether. TLC (system S_3) showed only one ninhydrin positive spot and a small amount of the deacylated oligonucleotide **12**. The solid product was dissolved in ammonium acetate buffer (5 mM, pH 4.5; 1 mL) and purified on a C_{18} SPE Baker column (3 mL) with use of a step gradient of acetonitrile (0, 5, 10, 20, 50, and 100%) in 5 mM ammonium acetate (pH 4.5). The product **13** was eluted with 20% acetonitrile; the eluate was evaporated in vacuo and co-evaporated with 80% acetic acid at low temperature to remove the last traces of salt. The yields of chromatographically and electrophoretically (Table II) uniform products **13** were determined spectrophotometrically and were in the 20–30% range. The products were further characterized by UV spectroscopy, mild alkaline hydrolysis to phenylalanine and parent oligonucleotides, and enzymatic digestion with appropriate nucleases. The data are presented in Tables III and IV.