Synthesis and Conformational Analysis of Phosphate-Methylated RNA Dinucleotides

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Synthesis of RNA dimers having a methyl phosphotriester group as the internucleoside linkage is reported; six pairs of diastereoisomerically pure systems were prepared, i.e., r(CpU) (**15**), r(ApU) (**16**), r(CpC) (**17**), r(ApC) (**18**), r(CpG) (**19**), and r(ApG) (**20**). Compounds **15–20** are stabilized by a 2′-*O*-methyl group in the 5′-terminal residue. The present systems represent the third class of backbone-modified RNA oligomers, following the 2′-*O*-methylribonucleotide phosphorothioates and the 2′-*O*-methylribonucleotide methyl phosphonates. Our synthetic approach comprises the use of 9-fluorenylmethoxycarbonyl (Fmoc) groups for transient protection of the exocyclic NH_2 groups of the bases A, C, and G, levulinoyl (Lev) groups for the transient protection of the 2′- and 3′-OH groups of the 3′-terminal residues, methanolic K_2CO_3 for the simultaneous removal of Fmoc and Lev groups with full preservation of the methyl phosphotriester function, and finally reversed-phase HPLC separation of the S_P and R_P diastereoisomers. The availability of the six dimers in diastereoisomerically pure form enabled us to examine the molecular conformations using high-field NMR and circular dichroism (CD) spectroscopy. These studies led to the following conclusions: (i) NMR *J*-coupling analysis: the central $C_{4'}$–$C_{5'}$ (γ) and $C_{5'}$–$O_{5'}$ (β) bonds in **15–20** show less preference for the γ^+ and β^t rotamers, in comparison with their natural analogues, i.e., base stacking is diminished upon introduction of the two methyl groups on $O_{2'}$ and on the phosphate group; (ii) CD analysis: **15–20** show substantially reduced molecular ellipticities when compared to the natural counterparts, which also reveals that base stacking is reduced; (iii) UV and variable-temperature ^1H NMR measurements: (S_P)- and (R_P)-**19** show self-association, via the formation of a right-handed miniduplex with two C–G base pairs ((S_P)-**19**, T_m = 9.3 °C, concn = 36.6 μM; (R_P)-**19**, T_m = 8.7 °C, concn = 48.1 μM). The present conformational data on (R_P)- and (S_P)-**15–20** are in agreement with literature data on other phosphate-triesterified oligonucleotides, e.g., the trimer $d(T_{POEt}G_{POEt}G)$ and the tetramer $d(T_{POEt}T_{POEt}C_{POEt}A)$. While the latter systems also showed little base–base stacking, it was established that they readily form a local duplex with a complementary natural RNA sequence. Hence we anticipate that phosphate-methylated 2′-O-methyl-RNA oligomers, longer than the dimer systems described in the present work, will also hybridize easily with complementary natural RNA.

Introduction

Over the past several years there has been a surge of activity in constructing novel types of modified DNA oligomers as antisense inhibitors of gene expression.[1] In most cases, the naturally occurring phosphodiester groups were replaced with, for example, phosphorothioates, phosphorodithioates, phosphoramidates, methyl phosphonates, or alkyl phosphotriesters. The introduction of these modifications generally served a double purpose: (i) the replacement of the phosphodiester groups renders the oligomer resistant toward breakdown by enzymes (exo- and endonucleases); (ii) transport across cell membranes is usually accelerated, especially for those modifications that result in neutral internucleoside linkages. To achieve inhibition of gene expression at the level of mRNA translation, it is essential that the modified oligomer binds effectively to the RNA target sequence. Very recently, this has prompted several groups to investigate the possible utility of modified oligo*ribo*nucleotides for antisense purposes. Due to the inherent instability of natural oligoribonucleotides under physiological conditions, it is absolutely mandatory that the 2′-OH groups are protected. The logical way to accomplish this is methylation, in view of the fact that the methyl group is spatially small and also because it is known that the thermal stability of hybrids of 2′-*O*-methylribonucleotides with complementary RNA is even greater than of the corresponding RNA–RNA or DNA–RNA duplexes.[2] On the basis of this reasoning, two types of modified 2′-*O*-methylribonucleotides have been introduced recently. The first type comprises 2′-*O*-methylribonucleotide phosphorothioates.[3] These systems could be prepared from 2′-O-methylated nucleosides via the H-phosphonate method, exactly analogous to the synthesis of deoxynucleoside phosphorothioates. The second type comprises 2′-*O*-methylribonucleotide methyl phosphonates, which were prepared recently by Miller et al.[4] These systems were synthesized from the 2′-*O*-methylribonucleosides by standard methods. The pentamer UpI*p*ApU*p*C (all five nucleosides carry a 2′-*O*-methyl group, *p* represents a methyl phosphonate group, and p represents a phosphodiester group) indeed showed a greater affinity for a complementary strand GAUCA, as compared to the 2′-deoxyribonucleoside methyl phosphonate pentamer d(TpG*p*ApT*p*C) (*p* is methyl phosphonate, p is phosphodiester). As an extension of our previous work on the synthesis and structural analysis of well-defined short methyl phosphotriester DNA oligomers,[5-7] we now wish to describe studies of 2′-*O*-methylribonucleoside methyl phosphotriesters. Herein, we describe the synthesis and structural analysis of six pairs of diastereoisomerically pure phosphate-methylated dimers: r(CpU) (**15**), r(ApU)

† Eindhoven University of Technology.
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(2) Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131.
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(4) Miller, P. S.; Bhan, P.; Cushman, C.; Kean, J.; Levis, J.; Shiber, M.; Thaden, J. In *Proceedings of the 9th International Round Table on Nucleosides, Nucleotides & their Biological Implications*, July 30–August 3, Uppsala, 1990, 24.
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(7) Quaedflieg, P. J. L. M.; Broeders, N. L. H. L.; Koole, L. H.; van Genderen, M. H. P.; Buck, H. M. *J. Org. Chem.* **1990**, *55*, 122.

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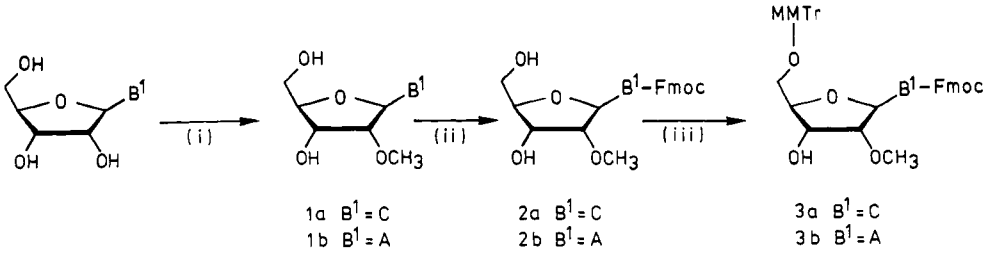
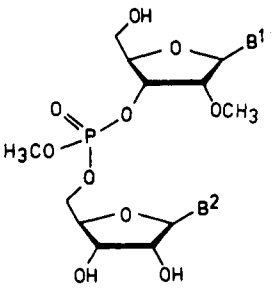
Scheme I[a]



[a] (i) NaH/CH_3I; (ii) $(CH_3)_3SiCl$; Fmoc-Cl; H_2O; (iii) MMTr-Cl.



15 B^1 = 1-cytidyl; B^2 = 1-uradyl
16 B^1 = 9-adenyl; B^2 = 1-uradyl
17 B^1 = 1-cytidyl; B^2 = 1-cytidyl
18 B^1 = 9-adenyl; B^2 = 1-cytidyl
19 B^1 = 1-cytidyl; B^2 = 9-guanyl
20 B^1 = 9-adenyl; B^2 = 9-guanyl

Figure 1. Structural formulae of the studied phosphate-methylated ribodinucleotides, which are 2′-O-methylated in their upper ribose residue.

(**16**), r(CpC) (**17**), r(ApC) (**18**), r(CpG) (**19**), and r(ApG) (**20**). The structural formulae of these systems are presented in Figure 1.

Analogous to our work on phosphate-methylated DNA dinucleotides[5] we used the 9-fluorenylmethoxycarbonyl (Fmoc) group for protection of the bases A, C, and G, and we chose the levulinoyl (Lev) group for the protection of both the 2′- and 3′-hydroxyl functions of the 3′-terminal coupling unit. Furthermore, in lieu of using a (1:1) mixture of triethylamine and pyridine (which, in some preliminary experiments, led to considerable demethylation of the methyl phosphotriester function during the Fmoc cleavage), the Fmoc groups were cleaved off by the recently published method described by Kuijpers et al.,[8] who used methanolic potassium carbonate in their solid-phase synthesis of partially phosphate-methylated DNA fragments. In this way, the methyl phosphotriester function in our ribodinucleotides was left completely unaffected and the levulinoyl esters were simultaneously saponified during the Fmoc cleavage. The S_P and R_P diastereoisomers of all six ribodinucleotides were separated with reversed-phase HPLC on a milligram scale. This enabled us to perform a detailed conformational analysis by 600-MHz ^1H NMR and circular dichroism (CD) spectroscopy.

Synthesis

The synthesis of **15–20** in diastereoisomerically pure form comprises four essential steps, which can be summarized as follows: (1) Preparation of the 5′-terminal coupling unit by methylation of the 2′-OH group of cytidine and adenosine, followed by the protection of the base amino group with Fmoc and tritylation of the 5′-OH group. (2) Preparation of the 3′-terminal coupling unit by Fmoc protection of the base amino group of cytidine and guanosine (the imino group of uridine needs no protection) and levulinoylation of the 2′-OH and 3′-OH groups via transient trityl protection of the 5′-OH group. (3) Coupling of the two protected nucleosides via an in situ generated phosphoramidite synthon, followed by oxidation to the phosphotriester. (4) Removal of Fmoc, trityl, and levulinoyl groups and reversed-phase HPLC separation of the R_P and S_P diastereoisomers. In the following, we briefly describe each of the steps.

Step 1 (Scheme I). By a slight modification of the procedure described by Yano et al.,[9] cytidine was converted into a 3:1 mixture of 2′- and 3′-*O*-methylcytidine (and some dimethylated side products) by reaction with sodium hydride and methyl iodide. Chromatographic separation and crystallization from absolute ethanol yielded 2′-*O*-methylcytidine (**1a**) as a white solid. By the same procedure 2′-*O*-methyladenosine was obtained as a white solid out of a 5:1 mixture of the 2′-*O*- and 3′-*O*-methylated compounds. Fmoc protection of the bases was carried out via transient protection of the 3′- and 5′-hydroxyl groups.[10] In this method the 3′,5′-bis(trimethylsilyl) derivatives of **1a** and **1b** were reacted with 9-fluorenylmethoxycarbonyl chloride followed by deprotection of the 3′- and 5′-OH groups. This readily furnished **2a** and **2b** as white amorphous solids. For the subsequent protection of the 5′-OH group, we used the 4-monomethoxytrityl (MMTr) group, which could be introduced under standard reaction conditions,[11] yielding **3a** and **3b** as white solids.

Step 2 (Scheme II). The base amino groups of cytidine and guanosine were protected with Fmoc via transient protection of the 2′-, 3′-, and 5′-hydroxyl functions,[10] yielding **5b** and **5c** as white amorphous solids. Tritylation of uridine, **5b**, and **5c** under standard reaction conditions yielded **6a–c** as white solids. Subsequent levulinoyl protection of the 2′- and 3′-OH groups was performed with levulinic anhydride in pyridine, yielding **7a–c**. Finally, detritylation was accomplished through treatment with aqueous 80% acetic acid (overnight, at room temperature), yielding **8a–c** as white solids.

Step 3 (Scheme III). For the 3′-5′ coupling reactions we have chosen the same synthetic approach as described earlier for the synthesis of phosphate-methylated DNA dinucleotides.[5] This approach has shown[12,13] that bifunctional phosphitylating agents are very effective for the in situ preparation of nucleoside 3′-phosphoramidites. The

(8) Kuijpers, W. H. A.; Huskens, J.; Koole, L. H.; van Boeckel, C. A. A. *Nucleic Acids Res.* **1990**, *18*, 5197.

(9) Yano, J.; Kan, L. S.; Ts'O, P. O. P. *Biochim. Biophys. Acta* **1980**, *629*, 178.

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(12) Moore, M. F.; Beaucage, S. L. *J. Org. Chem.* **1985**, *50*, 2019.

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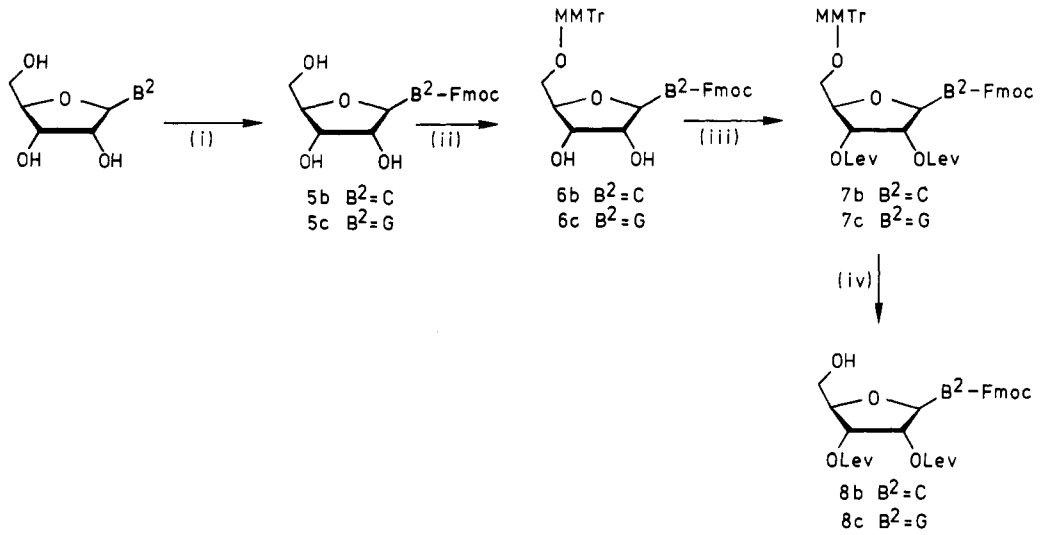
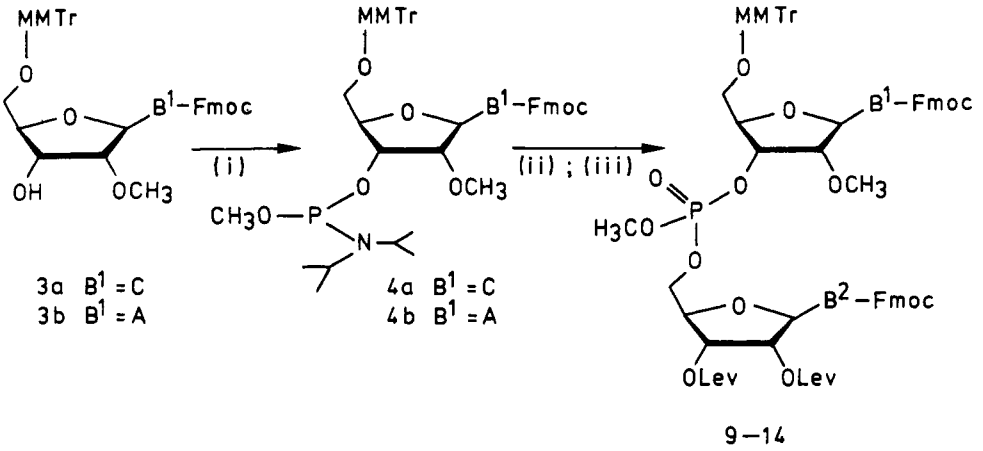
Scheme II[a]



[a] (i) $(CH_3)_3SiCl$; Fmoc-Cl; H_2O; (ii) MMTr-Cl; (iii) $(CH_3COCH_2CH_2)_2O$; (iv) CH_3COOH/H_2O (8:2). As the imino group of uridine needs no protection, steps ii, iii, and iv yielded the corresponding uridine derivatives **6a**, **7a**, and **8a**.

Scheme III[a]



[a] (i) $CH_3OP[N(iPr)_2]_2$, $^1/_2$ equiv of 1*H*-tetrazole; (ii) **8a**, **8b**, or **8c**; (iii) *t*-BuOOH.

underlying principle is that alkoxybis(dialkylamino)-phosphines are selectively activated by 1*H*-tetrazole. The 3′-phosphoramidite in situ is then further reacted with the 3′-terminal coupling unit, under activation of an excess of 1*H*-tetrazole. The advantage of this procedure is that the isolation of the reactive 3′-phosphoramidites is circumvented.

In the present synthesis we reacted the compounds **3a** and **3b** with a slight excess of methoxybis(diisopropylamino)phosphine in the presence of 1*H*-tetrazole in dry dichloromethane/acetonitrile mixtures, except in the synthesis of **9**, which was performed in dry pyridine. ^{31}P NMR revealed the quantitative formation of the desired phosphoramidites in situ, **4a** and **4b**, during 20–60 min. As a result of the diastereoisomeric nature, two distinct absorptions were seen in the ^{31}P NMR spectrum around δ 150. The 3′-terminal coupling units **8a–c** were added to both **4a** and **4b**. These reactions required addition of an extra quantity of 1*H*-tetrazole. In all six cases, ^{31}P NMR spectroscopy showed the complete conversion of **4a** and **4b** into the desired 3′-5′ phosphite triesters (two absorptions around δ 140) within 1–1 $^1/_2$ h.

Protection of both 2′- and 3′-OH functions is necessary in order to prohibit the undesired formation of 3′-3′ and 3′-2′ phosphite triesters. The choice of the levulinoyl group for this purpose was based on the fact that it is compatible with the methyl phosphotriester group (cleavage of levulinoyl and Fmoc with methanolic potassium carbonate,[8] vide infra) and even with the Fmoc group (selective cleavage of levulinoyl with hydrazine,[14] which offers the opportunity of further synthesis into the 3′-direction). In the synthesis of **9**, in which dry pyridine was used as the solvent, TLC experiments showed the formation of 9-methylenefluorene and polar side products during the coupling reaction and oxidation. Apparently, the slightly basic medium leads to partial Fmoc cleavage. All phosphite triesters were readily oxidized through reaction with *tert*-butyl hydroperoxide. This reaction proceeded smoothly without byproducts within 10 min, as was seen in the ^{31}P NMR spectra.

Step 4. Our method of deprotection was based on a recent paper by Kuijpers et al.[8] in which it is shown that the levulinoyl and Fmoc groups can be simultaneously removed by methanolic potassium carbonate, with complete preservation of the methyl phosphotriester function. The underlying principle of this method is the fact that methanolate, acting as a base, removes the Fmoc groups via a β-elimination reaction and, acting as a hard nucleophile, saponifies the levulinoyl ester. It might also cause

(14) Van Boom, J. H.; Burgers, P. M. J. *Recl. Trav. Chim. Pays-Bas* 1978, *97*, 73.

Table I. Optimal Parameters of the Preparative Isocratic Reversed-Phase HPLC Separations

compd	% acetonitrile	pH
15	8	5.0
16	12	4.7
17	3	2.5
18	8	4.5
19	4	2.5
20	4	2.5

some transesterification on the methyl phosphotriester, leading either to an exchange of the methoxy group or to chain cleavage. The latter was observed only to a very small extent (3% after 6 h).

In the present synthesis we used a 0.05 M solution of potassium carbonate in dry methanol. TLC experiments showed complete cleavage of the levulinoyl groups in 3–10 min and of the Fmoc groups in 2–3 h. Subsequent detritylation was accomplished by overnight stirring in aqueous 80% acetic acid at room temperature. This afforded **15–20** as a mixture of the S_P and R_P diastereoisomers, which were separated on a milligram scale by reversed-phase HPLC. For this the optimal separation conditions (type and concentration of organic modifier, pH) were first developed on an analytical scale. In all cases, acetonitrile was found to be the most suitable organic modifier. Table I lists the optimal parameters (% acetonitrile, pH) of the preparative isocratic reversed-phase separations. Under these conditions, the S_P was eluted prior to the R_P diastereoisomer, except in case of r(CpG). A detailed description of the optimization procedures will be published elsewhere.[15]

Structural Analysis

The second part of our investigation was dedicated to the conformational analysis of the S_P and R_P diastereoisomers of **15–20**, using high-resolution ^1H NMR at 400 MHz[16] or 600 MHz[17] as well as circular dichroism (CD) spectroscopy. Spectral assignments were made on the basis of extensive homonuclear decoupling experiments.

Configurational Assignment. Assignment of the configuration at phosphorus was performed according to the method of Summers et al.,[18] which we have formerly used in our analysis of diastereoisomerically pure phosphate-methylated DNA dinucleotides.[5] For each pair of ribodinucleotides it was found that one diastereoisomer shows a clear NOE contact between $H_{3'}$ of the 3′-phosphorylated residue and the methyl group on phosphorus, while the other diastereoisomer lacks such a contact. The structures with the NOE contact were assigned the R_P configuration. In all six cases the R_P diastereoisomer shows a ^{31}P NMR resonance at higher field than the corresponding S_P diastereoisomer, which is in accordance with our previous results on phosphate-methylated deoxyribodinucleotides[5] and with literature data on phosphate-ethylated dimers.[18]

NMR Analysis. A closer look at the molecular structure of our phosphate-methylated ribodinucleotides reveals that each of these systems has 17 essential degrees of freedom (Figure 2). These are rotation around the $C_{5'}$–$O_{5'}$ bonds β^1 and β^2, rotation around the $C_{4'}$–$C_{5'}$ bonds γ^1 and γ^2, a two-state equilibrium between a $C_{3'}$-endo (N) and a $C_{2'}$-endo (S) puckered form for the sugar rings S^1 and S^2, rotation around the $C_{2'}$–O bonds θ^1 and θ^2, rotation around the $C_{1'}$–N bonds χ^1 and χ^2 (syn ⇄ anti), rotation around the $C_{3'}$–$O_{3'}$ bonds ϵ^1 and ϵ^2, rotation around P–$O_{3'}$ (ζ^1), P–$O_{5'}$ (α^2), and P–OCH_3 (κ), and rotation around the O–CH_3 bonds λ^1 and λ^2. As is well known, only five of these (i.e., γ^1, γ^2, β^2, S^1, and S^2) can be directly and quantitatively determined with ^1H NMR.

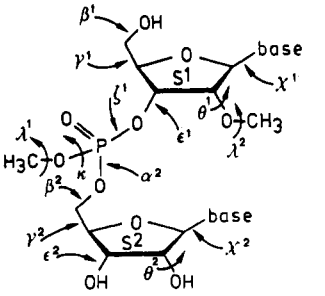


Figure 2. Representation of the 17 essential degrees of conformational freedom in a phosphate-methylated ribodinucleotide, which is 2′-O-methylated in the upper residue.

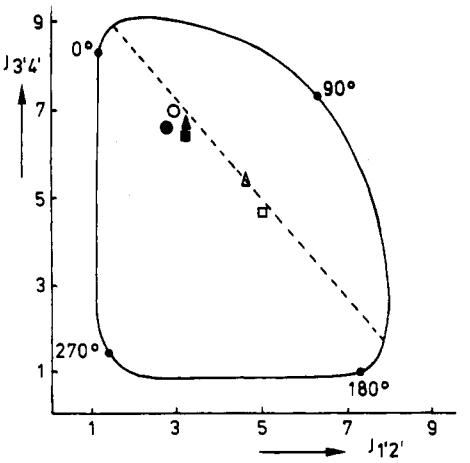


Figure 3. Calculated dependence of $J_{1'2'}$ and $J_{3'4'}$ on the phase angle of pseudorotation for ν_m = 39°. The straight line (---) connects the points for P = 18° (N pucker) and P = 162° (S pucker). For **17** (r(CpC)) the data points of both the S_P (△ for Cp-ribose, ▲ for pC-ribose) and the R_P diastereoisomer (□ for Cp-ribose, ■ for pC-ribose) as well as of their natural counterpart system (○ for Cp-ribose, ● for pC-ribose) are depicted in the graph. As can be seen the three pC residues and the Cp residue of natural r(CpC) are clearly biased toward the N puckered form ($x(C_2$-endo) = 0.25–0.30), while the Cp residue of (S_P)- and (R_P)-**17** show an approximate 1:1 blend for their ribose rings.

The full set of vicinal ^1H–^1H and ^1H–^{31}P coupling constants was derived from the 600-MHz ^1H NMR spectrum (measured in D_2O at 20 °C). In some cases a routine simulation–iteration algorithm was used in order to extract the precise values for coupling constants. These data were used to determine the conformational properties of the individual ribose rings and backbone bonds γ ($C_{4'}$–$C_{5'}$) and β ($C_{5'}$–$O_{5'}$).[19]

The conformations of the ribose rings (S^1 and S^2) in **15–20** were analyzed with the help of Figure 3. Essentially, we used the pseudorotation concept of Altona and Sundaralingam[20] in which the conformations of the five ring

(15) Coenen, A. J. J. M.; Henckens, L. H. G.; Mengerink, Y.; van der Wal, S.; Quaedflieg, P. J. L. M.; Koole, L. H.; Meijer, E. M. *J. Chromatogr.* Submitted.
(16) NMR Facility at the Eindhoven University of Technology, The Netherlands.
(17) Dutch National NMR Facility at the Catholic University of Nijmegen, The Netherlands.
(18) Summers, M. F.; Powell, C.; Egan, W.; Byrd, R. A.; Wilson, W. D.; Zon, G. *Nucleic Acids Res.* **1986**, *14*, 7421.
(19) See, for instance: (a) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413. (b) Koole, L. H.; van Genderen, M. H. P.; Buck, H. M. *J. Org. Chem.* **1988**, *53*, 5266. (c) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783. (d) Lankhorst, P. P.; Haasnoot, C. A. G.; Erkelens, C.; Altona, C. *J. Biomol. Struct. Dyns.* **1984**, *1*, 1387.

torsions are mathematically related to a phase angle of pseudorotation (P) and a puckering amplitude (ν_m). The parameter P actually indicates which part of the ring is bent. P lies in the range 0–360°, thus encompassing an entire pseudorotation cycle. From the large number of X-ray crystallographic studies of nucleosides and nucleotides[21] it is known that P values occur in two distinct and relatively narrow ranges. The first range is centered around P = 18° ($C_{3'}$-endo ring conformation) and is designated as N (north).[22] The N pucker is characteristic for all RNAs and the A form of DNA. The second range is centered around P = 162° ($C_{2'}$-endo ring conformation) and is called S (south).[22] This pucker is found, for example, in B DNA. The puckering amplitude identifies the deviation from planarity of the furanose ring. Also from crystallographic studies, it is known that ν_m is confined to a narrow range[21] around ν_m = 39°. Figure 3 shows the calculated dependence of the proton–proton coupling constants $J_{1'2'}$ and $J_{3'4'}$ for a fixed value of 39° for ν_m.[23] Data points of nucleosides and nucleotides lie roughly on a straight line that connects the calculated points for P = 18° and P = 162°. This confirms that the ribose conformations can be treated as rapid N ⇄ S equilibria.[24] The population density of the $C_{2'}$-endo puckered form (S) can be extracted from a $J_{1'2'}$–$J_{3'4'}$ plot by taking the ratio of the distance of the data point to the N pucker and the total distance between the N pucker and the S pucker.

The conformation around the γ^1 and γ^2 bonds can be best described as a rapid equilibrium over the staggered rotamers γ^+, γ^t, and γ^-.[19,25] Analogously, the conformation around the β^2 bond is described in terms of a rapid equilibrium over β^+, β^t, and β^-.[19,26] Table II lists the full

Table II. ^1H–^1H and ^{31}P–^1H NMR Coupling Constants (in Hz) in D_2O at 20 °C and Calculated Time-Averaged Populations of the $C_{2'}$-endo Puckered Ring Form of the Ribose Rings, the C_4–$C_{5'}$ (γ) Rotamers, and the $C_{5'}$–$O_{5'}$ (β) Rotamers for (S_P)- and (R_P)-15–17 and Their Natural Analogues

	CpU						ApU						CpC					
	S_P		R_P		nat.		S_P		R_P		nat.		S_P		R_P		nat.	
	Cp	pU	Cp	pU	Cp	pU	Ap	pU	Ap	pU	Ap	pU	Cp	pC	Cp	pC	Cp	pC
$J_{1'2'}$	4.7	3.9	5.3	3.9	3.2	3.3	7.0	4.0	7.1	3.9	3.8	3.5	4.7	3.4	5.1	3.3	3.0	2.9
$J_{2'3'}$	5.1	5.0	4.7	5.2	5.1	5.2	4.7	5.2	4.8	5.4	5.2	5.2	4.8	5.3	5.1	5.4	5.1	5.1
$J_{3'4'}$	5.1	5.8	4.7	6.1	6.8	6.1	2.3	6.1	2.1	6.2	5.4	5.6	5.3	6.7	4.6	6.5	7.0	6.6
$J_{3'P}$	7.3		7.1		8.6		7.1		7.1		8.4		7.4		7.2		8.3	
$J_{4'5'}$	3.1	2.2	3.1	2.4	2.3	2.2	3.1	2.2	3.0	2.3	3.0	2.2	3.0	2.3	3.1	1.7	2.5	2.2
$J_{4'5''}$	3.7	4.3	3.7	4.6	3.8	2.8	3.3	4.5	3.3	4.7	2.1	2.3	3.7	4.6	3.7	4.0	3.3	2.6
$J_{5'P}$		5.9		5.9		3.6		5.9		6.3		3.5		5.9		5.6		4.0
$J_{5''P}$		6.1		6.1		3.6		6.2		6.4		3.7		5.9		5.9		3.6
$J_{5'5''}$	−12.9	−11.6	−12.8	−11.8	−13.3	−11.9	−13.0	−11.7	−13.0	−11.8	−13.1	−11.9	−12.9	−11.8	−12.9	−12.0	−13.0	−11.7
$x(C_{2'}$-endo)	0.52	0.41	0.59	0.39	0.28	0.35	0.89	0.39	0.91	0.38	0.43	0.40	0.50	0.30	0.59	0.30	0.25	0.28
$x(\gamma^+)$	0.68	0.68	0.68	0.64	0.73	0.84	0.73	0.66	0.74	0.63	0.86	0.89	0.69	0.65	0.68	0.71	0.78	0.86
$x(\gamma^t)$	0.24	0.32	0.24	0.36	0.27	0.16	0.20	0.34	0.20	0.37	0.07	0.11	0.24	0.35	0.24	0.29	0.22	0.16
$x(\gamma^-)$	0.08	0	0.08	0	0	0	0.07	0	0.06	0	0.07	0	0.07	0	0.07	0	0	0
$x(\beta^+)$		0.18		0.18		0.06		0.18		0.19		0.06		0.17		0.17		0.08
$x(\beta^t)$		0.65		0.65		0.88		0.65		0.62		0.88		0.65		0.67		0.86
$x(\beta^-)$		0.17		0.17		0.06		0.17		0.19		0.05		0.17		0.16		0.08

(20) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333.

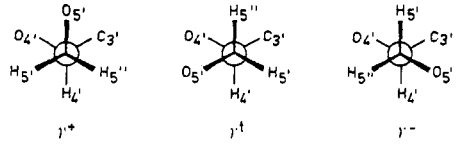
(21) (a) de Leeuw, H. P. M., Haasnoot, C. A. G.; Altona, C. *Isr. J. Chem.* **1980**, *20*, 108. (b) Harvey, S. C.; Prabhakaran, M. *J. Am. Chem. Soc.* **1986**, *108*, 6128.

(22) The $C_{2'}$-endo and $C_{3'}$-endo puckered conformations of the ribose ring can be drawn as follows:

(23) Calculations were performed by using the empirically generalized Karplus equation as developed by Altona and co-workers. See: Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.

(24) Koole, L. H.; Buck, H. M.; Nyilas, A.; Chattopadhyaya, J. *Can. J. Chem.* **1987**, *65*, 895.

(25) The Newman projections of the staggered rotamers around a C_4–$C_{5'}$ bond are defined as



For the standard right-handed A RNA conformation, the γ^+ rotamer has the highest population density. In order to obtain the population distribution over the C_4–$C_{5'}$ rotamers, we solved the set of equations:

$$J_{4'5'(5'')}(\text{exp}) = x(\gamma^+)\cdot J_{4'5'(5'')}(\gamma^+) + x(\gamma^t)\cdot J_{4'5'(5'')}(\gamma^t) + x(\gamma^-)\cdot J_{4'5'(5'')}(\gamma^-)$$

combined with $x(\gamma^+) + x(\gamma^t) + x(\gamma^-) = 1$. For the coupling constants in the individual γ rotameters, we used the values as proposed by Haasnoot et al. (ref 19c), i.e., $J_{4'5'}(\gamma^+)$ = 2.4 Hz, $J_{4'5'}(\gamma^t)$ = 2.6 Hz, $J_{4'5'}(\gamma^-)$ = 10.6 Hz, $J_{4'5''}(\gamma^+)$ = 1.3 Hz, $J_{4'5''}(\gamma^t)$ = 10.5 Hz, $J_{4'5''}(\gamma^-)$ = 3.8 Hz.

set of vicinal coupling constants and conformational data on both diastereoisomers of r(CpU), r(ApU), and r(CpC) as well as on their corresponding natural counterparts.[27,28] Table III analogously contains all data on r(ApC), r(CpG), and r(ApG). As can be seen from Tables II and III, all phosphate-methylated systems have a clear preference for the γ^+ conformation in both the 5′-terminal ($x(\gamma^+)$ = 0.68–0.79) and the 3′-terminal ($x(\gamma^+)$ = 0.55–0.68) residue. This preference, which is almost independent of the configuration at phosphorus, is lower than in the natural counterpart systems, in particular for the 3′-terminal residues ($x(\gamma^+)$ = 0.72–0.89), i.e., there is an increased flexibility around the central C_4–$C_{5'}$ bond (γ^2). The same holds true for the preference for β^t around the central β^2 bond, which is considerably lower ($x(\beta^t)$ = 0.62–0.68) than in the natural analogues ($x(\beta^t)$ = 0.84–0.89). This indicates that the C_4–$C_{5'}$ and $C_{5'}$–$O_{5'}$ bonding network in our dimers forms a stable conformational unit, which is, however, more flexible than that in the natural analogues.

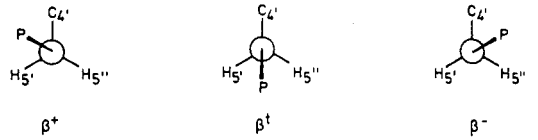
With respect to the ribose conformations, we must differentiate between the 5′-terminal and the 3′-terminal residues. In the 3′-terminal pC and pU residues there is a moderate bias toward the N form ($x(C_2$-endo) = 0.30–0.41), while in the 3′-terminal pG residues an approximate 1:1 blend over N and S is found ($x(C_2$-endo) = 0.40–0.53). In essence, these values do not differ from those of their natural analogues. In the 5′-terminal Cp residues a flexible ribose ring (i.e., no clear preference for N or S, $x(C_2$-endo) = 0.50–0.59) is found in contrast to the clear bias for the N-puckered conformation ($x(C_2$-endo) = 0.25–0.28) in their natural analogues. Even more remarkably, the 5′-terminal Ap residues possess an unusually strong bias for the S-puckered ribose conformation ($x(C_{2'}$-endo) = 0.89–0.92) in contrast to their natural counterparts, in which the Ap ribose ring is highly flexible or moderately biased toward the N-puckered form ($x(C_{2'}$-endo) = 0.36–0.52).

CD Analysis. In order to gather more information about the base–base stacking in the dimers **15–20**, we recorded the CD spectra in 0.01 M Tris/HCl buffer solutions (pH 7.5) at 25 °C and compared our results with literature data[29] on both the natural counterparts and the analogues that are only 2′-O-methylated in their 5′-terminal residues, measured under exactly the same conditions. The latter systems show a substantially increased molecular ellipticity compared to the natural systems due to extra stacking interactions.[2] Our phosphate-methylated dimers **15–20**

Table III. ^1H–^1H and ^{31}P–^1H NMR Coupling Constants (in Hz) in D_2O at 20 °C and Calculated Time-Averaged Populations of the $C_{2'}$-endo Puckered Ring Form of the Ribose Rings, the C_4–$C_{5'}$ (γ) Rotamers, and the $C_{5'}$–$O_{5'}$ (β) Rotamers for (S_P)- and (R_P)-18–20 and Their Natural Analogues

	ApC					CpG					ApG				
	S_P		R_P		nat.	S_P		R_P		nat.	S_P		R_P		nat.
	Ap	pC	Ap	pC	Ap	Cp	pG	Cp	pG	Cp	Ap	pG	Ap	pG	Ap	pG
$J_{1'2'}$	7.0	3.3	7.0	3.3	3.6	4.5	4.9	4.5	5.3	3.2	7.4	3.7	6.6	4.6	4.6	4.4
$J_{2'3'}$	4.7	5.3	4.7	5.2	5.3	5.2	5.0	5.3	5.5	5.1	4.7	5.7	4.6	5.3	4.9	5.2
$J_{3'4'}$	2.3	6.6	2.2	6.6	6.1	5.1	5.0	4.0	5.5	6.7	1.7	5.7	2.7	5.3	4.7	4.8
$J_{3'P}$	7.0		7.0		8.7						7.0		7.2		8.2	
$J_{4'5'}$	3.1	2.3	2.9	1.7	3.3	3.0	2.6	3.3	2.6	3.2	2.9	2.9	3.1	2.9	2.4	2.8
$J_{4'5''}$	3.3	5.0	3.1	4.4	2.2	3.7	5.4	3.6	5.4	2.8	2.9	4.0	3.3	4.0	3.4	3.6
$J_{5'P}$		5.7		5.9			5.7		5.7			5.8		5.8		4.0
$J_{5''P}$		6.0		6.3			5.4		5.4			5.8		5.8		4.0
$J_{5'5''}$	−13.0	−11.8	−12.9	−11.8	−12.9	−12.9	−11.5	−12.8		−13.0	−11.2		−13.0		−12.8	−11.8
$x(C_{2'}$-endo)	0.89	0.30	0.91	0.30	0.36	0.51	0.52	0.59	0.53	0.28	0.46	0.40	0.83	0.49	0.52	0.51
$x(\gamma^+)$	0.73	0.61	0.77	0.66	0.83	0.69	0.55	0.68	0.55	0.77	0.81	0.67	0.72	0.68	0.79	0.72
$x(\gamma^t)$	0.20	0.39	0.18	0.34	0.07	0.24	0.44	0.22	0.44	0.14	0.19	0.28	0.20	0.28	0.23	0.24
$x(\gamma^-)$	0.07	0	0.05	0	0.10	0.06	0.01	0.10	0.01	0.09	0	0.04	0.08	0.04	0	0.04
$x(\beta^t)$		0.18		0.19			0.16		0.16		0.07	0.17		0.17		0.08
$x(\beta^t)$		0.66		0.64			0.68		0.68		0.89	0.66		0.66		0.84
$x(\beta^-)$		0.16		0.17			0.16		0.16		0.04	0.17		0.17		0.08

(26) The Newman projections of the staggered rotamers around a $C_{5'}$–$O_{5'}$ bond are defined as



For the standard A RNA conformation, the β^t rotamer has the highest population density. The population distribution over the $C_{5'}$–$O_{5'}$ rotamers was solved from the equations:

$$J_{P5'(5'')}(\text{exp}) = x(\beta^+)\cdot J_{P5'(5'')}(\beta^+) + x(\beta^t)\cdot J_{P5'(5'')}(\beta^t) + x(\beta^-)\cdot J_{P5'(5'')}(\beta^-)$$

along with $x(\beta^+) + x(\beta^t) + x(\beta^-) = 1$. We used the $J_{P5'(5'')}$ values for the β rotamers, as proposed by Lankhorst et al. (ref 19d), i.e., $J_{P5'}(\beta^+) = J_{P5'}(\beta^t) = J_{P5''}(\beta^t) = J_{P5''}(\beta^-) = 2.4$ Hz, $J_{P5'}(\beta^-) = J_{P5''}(\beta^+) = 23.0$ Hz.

(27) Lee, C.-H.; Ezra, F. S.; Kondo, N. S.; Sarma, R. H.; Danyluk, S. S. *Biochemistry* **1976**, *15*, 3627.
(28) Ezra, F. S.; Lee, C.-H.; Kondo, N. S.; Danyluk, S. S.; Sarma, R. H. *Biochemistry* **1977**, *16*, 1977.
(29) Drake, A. F. In *Handbook of Biochemistry and Molecular Biology*, Vol. 1; Fasman, G. D., Ed.; CRC Press: Cleveland, 1975; pp 470–527.

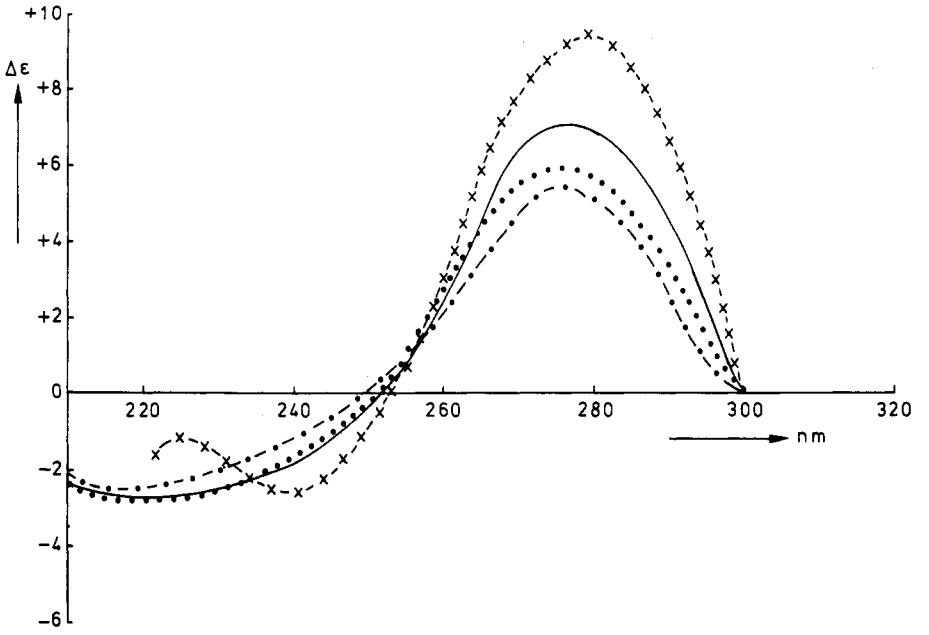


Figure 4. CD spectra of (S_P)-r(CpU) (···), (R_P)-r(CpU) (-·-), natural r(CpU) (—), and the analogue that is only 2′-O-methylated in the upper residue (x-x), recorded at 25 °C in 0.01 M Tris/HCl buffer solutions (pH 7.5) (see text).

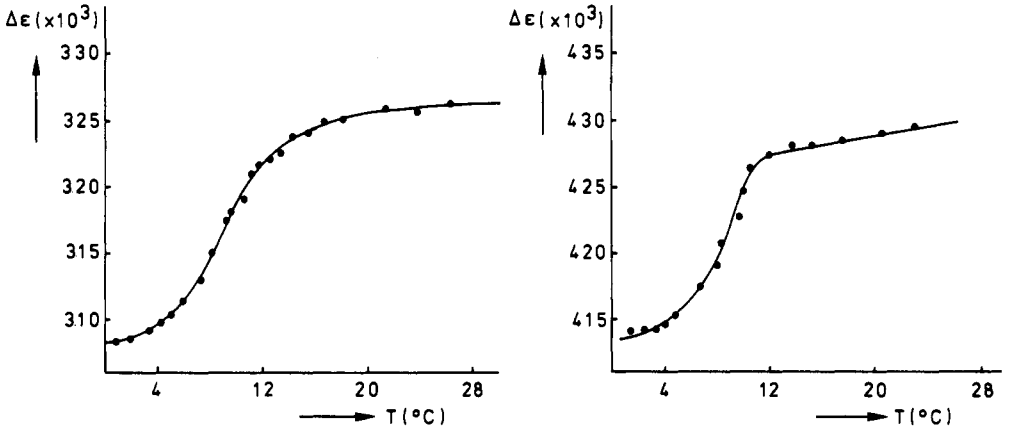


Figure 5. Left: UV extinction vs temperature profile for (S_P)-r(CpG) at a concentration of 36.6 μM (T_m value 9.3 °C). Right: UV extinction vs temperature profile for (R_P)-r(CpG) at a concentration of 48.1 μM (T_m value 8.7 °C). All experiments were performed in 0.01 M Tris/HCl buffer solutions (pH = 7.5).

showed CD spectra with a similar form but a *lower* molecular ellipticity than the natural counterparts, especially for the Ap systems. This indicates that base–base stacking occurs in the same mode but to a lesser extent. This is in perfect agreement with the conclusions previously reached on the basis of our ^{1}H NMR data (vide supra).

The CD spectra of **16–20** along with their analogues are given in the supplementary material. Figure 4 shows the spectra of (S_P)- and (R_P)-**15** (r(CpU)) and their analogues as representative examples. Interestingly, in all pairs of diastereoisomers the molecular ellipticity of S_P has a somewhat greater magnitude than that of R_P. This is in contrast to our ^{1}H NMR data in which no significant differences in ribose and backbone (γ and β) conformations were found (vide supra). Apparently, the configuration of the methyl phosphotriester group causes a difference in some of the backbone torsion angles, which cannot be monitored by means of ^{1}H NMR (e.g., the central α and ζ bonds). This is consistent with the results of Weinfeld et al.,[30] who found that in dinucleoside ethyl phosphotriesters the R_P exhibits more base unstacking than the S_P form due to a closer proximity of the ethoxy groups to the bases.

Duplex Formation. The possibility of duplex formation in both diastereoisomers of r(ApU) (**16**) and r(CpG) (**19**) was first examined with UV hyperchromicity experiments.[31] In principle, all four systems are self-complementary and thus capable of antiparallel duplex formation via two Watson and Crick type A–U or C–G base pairs.[32] The UV hyperchromicity curves showed a sigmoidal shape only in the cases of (S_P)- and (R_P)-r(CpG) (Figure 5).

The T_m values were found to be 9.3 °C in case of (S_P)-r(CpG) at a concentration of 36.6 μM and 8.7 °C in case of (R_P)-r(CpG) at a concentration of 48.1 μM. This melting transition for both r(CpG) diastereoisomers was further investigated with variable-temperature 400-MHz ^{1}H NMR experiments, in which we particularly focussed

(30) Weinfeld, M.; Drake, A. F.; Kuroda, R.; Livingston, D. C. *Anal. Biochem.* **1989**, *178*, 93.

(31) See: (a) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 141–149. (b) Porschke, D. *Biopolymers*, **1971**, *10*, 1989.

(32) For an initial study of the formation of *parallel* duplexes in **15** (r(CpU)) and **16** (r(CpC)), see: Quaedflieg, P. J. L. M.; van der Heiden, A. P.; Koole, L. H.; van Genderen, M. H. P.; Coenen, A. J. J. M.; van der Wal, S.; Buck, H. M. *Proc. K. Ned. Akad. Wet.* **1990**, *93*(1), 33. Further results will be published elsewhere.

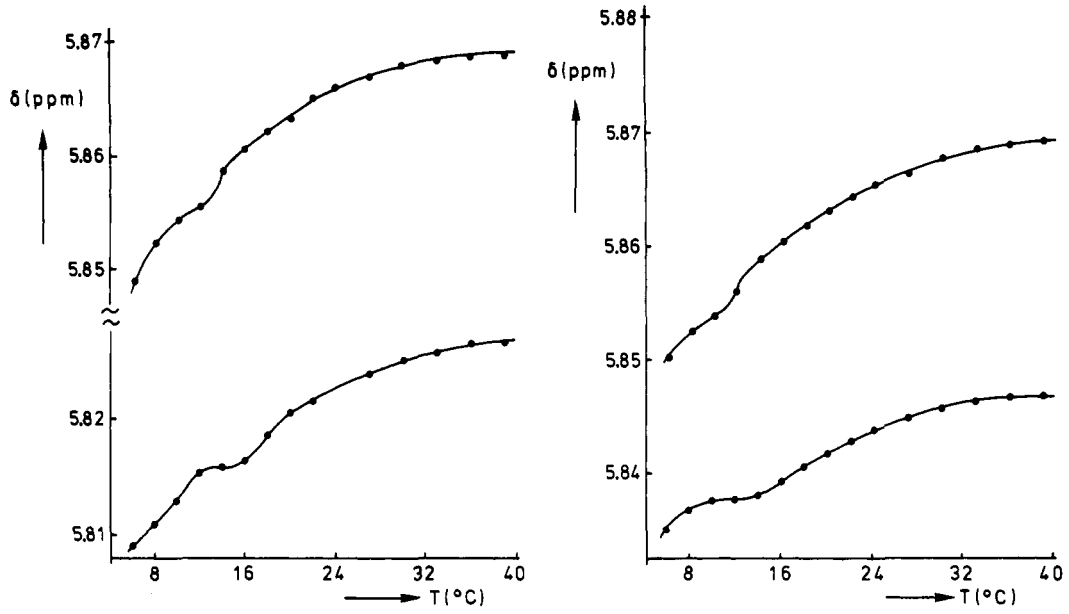


Figure 6. ^1H NMR chemical shift vs temperature profiles in D_2O of the $H_{1'}$ protons of (S_P)-r(CpG) (left) and (R_P)-r(CpG) (right). For both diastereoisomers, the upper profile corresponds with the Cp residue and the lower profile with the pG residue.

on the chemical shifts of the anomeric protons (i.e., $H_{1'}$ of Cp and pG) and the nonexchangeable base protons (i.e., H_5 and H_6 of Cp, H_2 and H_8 of pG). The chemical shifts of these protons are known to be sensitive to changes in base stacking.[33] Therefore, a melting transition gives rise to a δ vs temperature profile with a sigmoidal shape for some of these protons. In (S_P)- and (R_P)-r(CpG) this is found to be most clearly visible in the profiles of both anomeric protons (Figure 6). The T_m values extracted from these curves are 13 °C for (S_P)- and 12 °C for (R_P)-r(CpG). Furthermore, the ^1H NMR spectra in H_2O/D_2O (80:20) at 4 °C showed one single imino resonance at 14.2 ppm, demonstrating two symmetry-related G–C base pairs and thus an antiparallel duplex for both diastereoisomers. The conformational properties of (S_P)- and (R_P)-r(CpG) *in the duplex form* were determined from the 600-MHz ^1H NMR spectra recorded at 2 °C.[17] The results are shown in Table IV. These data indicate that the miniduplex adopts the standard A RNA geometry, with slightly increased population densities for the γ^+, β^t, and $C_{2'}$-endo conformers, a reflection of the enhanced intranucleotide stacking in the duplex form as compared to the single stranded conformer.

These results on the behavior of phosphate-methylated r(ApU) and r(CpG) are partly in line with the results of our previous study on the phosphate-methylated DNA congeners d(ApT), which showed no self-association,[34] and d(CpG),[6] which formed a miniduplex with T_m values of 13 °C for the S_P and 9 °C for the R_P diastereoisomer. These DNA duplexes, however, adopt the left-handed Z geometry. In general, the A → Z transition in natural RNA requires more extreme conditions than the B → Z transition in natural DNA. For instance, Hall et al.[35] showed that the RNA hybrid poly(G-C)-poly(G-C) undergoes a transition to the left-handed Z form in conditions of high ionic strength (3–6 M $NaClO_4$) and high temperatures (over 35 °C). Our studies on phosphate-methylated $d(CpG)_2$ and $r(CpG)_2$ show that factors other than phosphate shielding alone determine the transition into a left-handed Z structure.

Table IV. Some Relevant ^1H–^1H NMR Coupling Constants (in Hz), Measured for (S_P)- and (R_P)-r(CpG) (19) in D_2O at 2 °C, along with the Calculated Time-Averaged Populations of the $C_{2'}$-endo Puckered Ring Form of the Ribose Rings, the $C_{4'}$–$C_{5'}$ (γ) Rotamers, and the $C_{5'}$–$O_{5'}$ (β) Rotamers

	S_P		R_P	
	Cp	pG	Cp	pG
$J_{1'2'}$	4.1	5.0	4.1	5.2
$J_{3'4'}$	5.8	5.1	5.5	4.9
$J_{4'5'}$	2.8	2.5	3.1	2.5
$J_{4'5''}$	3.4	4.4	3.3	4.4
$J_{5'P}$		5.4		5.4
$J_{5''P}$		4.4		4.4
$x(C_{2'}\text{-endo})$	0.42	0.53	0.44	0.56
$x(\gamma^+)$	0.74	0.66	0.72	0.66
$x(\gamma^t)$	0.22	0.34	0.20	0.34
$x(\gamma^-)$	0.04	0.00	0.08	0.00
$x(\beta^+)$		0.10		0.10
$x(\beta^t)$		0.75		0.75
$x(\beta^-)$		0.15		0.15

Concluding Remarks

Phosphate-methylated RNA dimers such as **15–20** can be conveniently prepared by using 9-fluorenylmethoxycarbonyl (Fmoc) for transient protection of the exocyclic NH_2 groups of the bases A, C, and G, levulinoyl for the transient protection of the 2′-OH and 3′-OH groups in the 3′-terminal residue, and methanolic K_2CO_3 for the simultaneous removal of both the Fmoc and Lev groups in the last stage of the synthesis. We feel that this methodology could also be used for the preparation of longer oligomers of this type. Our NMR and CD conformational studies show that the contribution of base-stacked states to the conformational equilibria of **15–20** is substantially reduced as a consequence of the introduction of the two methyl groups on $O_{2'}$ and on the phosphate group. This effect must be primarily due to the methyl on the phosphate group, since it is known that the presence of methyl on merely $O_{2'}$ *enhances* base stacking. Most likely, the conformations about the bonds P–$O_{5'}$ (α) and/or P–$O_{3'}$ (ζ) are perturbed in **15–20**. Our CD data indicate that this must be especially the case for the R_P diastereoisomers, since these systems show base stacking to a lesser extent.

(33) Patel, D. In *Nucleic Acid Geometry and Dynamics*; Sarma, R. H., Ed.; Pergamon Press: New York, 1980.
(34) Koole, L. H.; Quaedflieg, P. J. L. M., unpublished results.
(35) Hall, K.; Cruz, P.; Tinoco, I., Jr.; Jovin, T. M.; van de Sande, J. H. *Nature* 1984, *311*, 584.

Independent support for our conclusion that phosphotriesterified nucleotides are inherently more flexible than their unmodified counterparts can be found in the X-ray crystal structures of two phosphotriester 5′-mononucleotides, i.e., adenosine 5′-(*O*-diethyl phosphate)[36] and cytidine 5′-(*O*-dimethyl phosphate).[37] The phosphotriester moieties were found to be disordered over two distinct conformations, and the torsion angles α and ζ do not fall in the g^-,g^- range, which is typical for a regular double-helical conformation.

The poor tendency of (R_P)- and (S_P)-**15–20** to adopt a helical stacked conformation is in line with previous work on phosphotriesterified DNA oligomers, as described by Jensen and Reed[38] and Miller and co-workers.[39] They reported that triesterification of the backbone phosphate groups does not at all inhibit the formation of Watson and Crick type double-helical structures. The "stiffer" unmodified DNA or RNA apparently forces the inherently more flexible phosphotriester to adopt a helical structure. An elegant example of this phenomenon was described by Miller et al.,[39] who showed that the tetramer d-($T_{POEt}T_{POEt}C_{POEt}A$) is able to bind specifically to the anticodon region of *Escherichia coli* and yeast $tRNA^{Phe}$, while the tetramer on its own shows virtually no base stacking. Based on this information, it might be expected that elongation of the phosphate-methylated 2′-*O*-methyl dimers, as described in the present work, can ultimately lead to a new valuable class of reagents for specific hydrogen bonding to and formation of double helices with their cellular targets.

Experimental Section

Materials and Methods. The ^1H NMR spectra were recorded on 400-MHz[16] (compounds **1–14** and (S_P)-**15**) and 600-MHz[17] (compounds (R_P)-**15** and **16–20**) NMR spectrometers. Tetramethylsilane was used as the internal standard for samples in organic solvents. For samples in aqueous solution (D_2O), the residual HDO peak was set at 4.68 ppm. For the variable-temperature measurements on (S_P)- and (R_P)-r(CpG) in D_2O, tetramethylammonium bromide (δ = 3.18 ppm) was used as the standard. ^{31}P NMR spectra were recorded at 162 MHz and referenced against 85% H_3PO_4 as external standard. For all column chromatographic separations we used Merck Silica 60, 70–230 mesh ASTM. TLC experiments were performed on Merck silica gel 60 F_{254} plates. Dimethylformamide (DMF) was distilled from CaH_2 under reduced pressure and stored on 4-Å molecular sieves. Pyridine was distilled from KOH pellets and stored on 4-Å molecular sieves. Dichloromethane (CH_2Cl_2) was distilled from potassium carbonate (K_2CO_3) and stored on 4-Å molecular sieves. Methanol (CH_3OH) was refluxed on magnesium for 2 h, distilled, and stored on 3-Å molecular sieves. 1*H*-Tetrazole, if used as a solid, was purified by sublimation prior to use. 1*H*-Tetrazole (0.50 M) in anhydrous acetonitrile (CH_3CN) was used as received (Applied Biosystems). *tert*-Butyl hydroperoxide (tBuOOH) was used as received (75% solution in di-*tert*-butyl peroxide, Merck-Schuchardt), as were 9-fluorenylmethoxycarbonyl chloride (Janssen) and chlorotrimethylsilane (Janssen). Bis(*N*,*N*-diisopropylamino)methoxyphosphine[5] and levulinic anhydride[40] were prepared as described elsewhere. Reactions were routinely run in an inert atmosphere of dry argon, and, unless otherwise stated, at ambient temperature. Prior to reactions run in pyridine or CH_2Cl_2 all nucleotide compounds were routinely dried by coevaporation with three portions of the dry solvent. Prior to deprotection reactions in methanolic K_2CO_3, all protected dinucleotides were dried by coevaporation with three portions of dry CH_2Cl_2. In the fast atom bombardment (FAB) mass spectrometrical experiments, the samples were loaded in thioglycerol solution onto a stainless steel probe and bombarded with xenon atoms having 8-keV energy. The separation of the S_P and R_P diastereoisomers of **15–20** was developed on a gradient HPLC system using a built-in diode array detector to spot the phosphate-methylated 2′-*O*-methylribodinucleotides between traces of highly absorbing Fmoc derivatives or using an absorbance detector set at 270 nm. Preparative chromatography was executed on a high-pressure liquid chromatograph consisting of a solvent delivery system equipped with a solvent select valve module for sample introduction, a RSil C18 10-μm particle size column (250 × 22 mm, Alltech), and an absorbance detector. Fractions were checked for purity on an analytical HPLC system consisting of a Nucleosil 120-3 C18 reversed-phase column (250 × 4 mm Macherey-Nagel) and an absorbance detector that monitored the eluate at 260 nm. Other reversed-phase columns used for development were a 125 × 4 mm Lichrospher C18 5-μm (Merck, Darmstadt) and a 100 × 4.6 mm Microsphere C18 3-μm column (Chrompack). Mobile phases used were acetonitrile (FSA Lab Supplies) or methanol (Lichrosolv, Merck) as organic modifiers and 0.1% (v/v) formic or acetic acid (AnalaR, BDH), 100–200 μL/L triethylamine (zur Synthese, Merck) in Milli-Q water, adjusted to the desired pH with ammonium hydroxide solution (Baker Analyzed Reagent, 25%). The purity of all title compounds was judged to be >95% by HPLC, ^1H NMR, and ^{31}P NMR determinations.

2′-*O*-Methylcytidine (1a). We used the method described by Yano et al.[9] for the synthesis of 2′-*O*-methyladenosine, with some modifications. To a cooled (0 °C) solution of cytidine (21.00 g, 86.33 mmol) in 350 mL of DMF was added sodium hydride (4.15 g, 103.75 mmol), and the reaction mixture was stirred for 45 min. Then a slight excess of methyl iodide (12.62 g, 88.92 mmol) was added, and stirring was continued for $2^1/_2$ h at 0 °C and for another 1 h at room temperature. TLC analysis (using a mixture of CH_2Cl_2 and CH_3OH (2:1 v/v) as eluent) showed the presence of dimethylated compounds (R_f = 0.64 and 0.52), monomethylated compounds (R_f = 0.37), and unreacted cytidine (R_f = 0.17). The mixture was filtered and the clear solution was evaporated to dryness. In order to remove the last traces of DMF, the yellow oil was coevaporated (three times) with CH_3OH. Then, the residue was impregnated on silica (35 g) by coevaporation with CH_3OH (three times). The impregnated powder was suspended in 100 mL of CH_2Cl_2 and the slurry was applied to a silica gel column (200 g) and eluted with a gradient of CH_3OH (5 → 33 vol %) in CH_2Cl_2. The fractions with monomethylated compounds were evaporated to afford a white solid. ^1H NMR and ^{13}C NMR experiments showed that this product consisted of an approximately 3:1 mixture of the desired 2′-*O*-methylcytidine (**1a**) and 3′-*O*-methylcytidine. Pure **1a** was obtained as a white solid by crystallization from absolute ethanol: yield 5.11 g (23%); mp 252–254 °C; ^1H NMR (D_2O) δ 3.37 (3 H, s, CH_3), 3.65 (1 H, dd, $H_{5''}$), 3.78 (1 H, dd, $H_{5'}$), 3.85 (1 H, dd, $H_{2'}$), 3.94 (1 H, m, $H_{4'}$), 4.14 (1 H, dd, $H_{3'}$), 5.82 (1 H, d, $H_{1'}$), 5.89 (1 H, d, H_5), 7.71 (1 H, d, H_6); exact mass calcd 257; FAB $(M + H)^+$ = 258. Anal. Calcd: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.51; H, 6.10; N, 16.09.

2′-*O*-Methyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidine (2a). To a cooled (0 °C) suspension of compound **1a** (1.06 g, 4.12 mmol) in 60 mL of pyridine was added chlorotrimethylsilane (2.16 g, 19.88 mmol), and the reaction mixture was stirred for $^1/_2$ h. After the addition of 9-fluorenylmethoxycarbonyl chloride (1.21 g, 4.68 mmol) the mixture was stirred for another 2 h. Hydrolysis of the trimethylsilyl groups and excess chlorides was effected by addition of water (20 mL) at 0 °C. After stirring for 18 h, the mixture was evaporated to a yellow oil and coevaporated with toluene. Upon addition of water (50 mL) a white precipitate appeared. The mixture was shaken vigorously until no more yellow oil was visible. After addition of ethyl acetate (25 mL) and vigorous shaking, the precipitate that was formed on the separation layer was isolated by filtration and washed with ethyl acetate. After drying in vacuo, **2a** was obtained as a white solid; yield 1.70 g (86%). This product decomposed upon heating (approximately 70 °C): ^1H NMR (pyridine-d_5) δ 3.77 (3 H, s,

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2′-OCH_3), 4.21 (1 H, dd, $H_{2'}$), 4.28 (1 H, dd, $H_{5''}$), 4.36 (1 H, t, CH Fmoc), 4.39 (1 H, dd, $H_{5'}$), 4.55 (1 H, m, $H_{4'}$), 4.79 (2 H, dd, CH_2 Fmoc), 4.91 (1 H, dd, $H_{3'}$), 6.54 (1 H, d, $H_{1'}$), 7.28 (2 H, m, arom Fmoc), 7.39 (2 H, m, arom Fmoc), 7.43 (1 H, d, H_5), 7.68 (2 H, m, arom Fmoc), 7.85 (2 H, d, arom Fmoc), 9.11 (1 H, d, H_6); exact mass calcd 479; FAB $(M + H)^+ = 480$.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidine (3a). To a suspension of compound **2a** (0.80 g, 1.79 mmol) in 24 mL of pyridine was added 4-monomethoxytrityl chloride (0.64 g, 2.07 mmol), and the solution was stirred for 15 h in darkness. The mixture was then poured into saturated aqueous $NaHCO_3$ (80 mL) and extracted with three 50-mL portions of CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated. Removal of all pyridine was accomplished by coevaporation with toluene (twice) and $CHCl_3$ (three times). The resulting yellow oil was purified by column chromatography using a gradient of CH_3OH (1 → 3 vol %) in CH_2Cl_2 as eluent, R_f 0.20 (CH_2Cl_2/CH_3OH 97:3 v/v). Compound **3a** was obtained as a white solid: yield 1.01 g (75%); the product decomposed upon heating (approximately 115 °C); ^1H NMR ($CDCl_3$) δ 3.54 (1 H, dd, $H_{5''}$), 3.62 (1 H, dd, $H_{5'}$), 3.74 (3 H, s, CH_3), 3.78 (1 H, d, $H_{2'}$), 3.80 (3 H, s, CH_3), 4.02 (1 H, m, $H_{4'}$), 4.28 (1 H, t, CH Fmoc), 4.42 (1 H, m, $H_{3'}$), 4.49 (2 H, dd, CH_2 Fmoc), 6.00 (1 H, s, $H_{1'}$), 6.88 (2 H, d, arom MMTr), 6.89 (1 H, d, H_5), 7.25–7.46 (16 H, m, arom MMTr/arom Fmoc), 7.58 (2 H, d, arom Fmoc), 7.79 (2 H, d, arom Fmoc), 7.88 (1 H, bs, NH), 8.52 (1 H, d, H_6); exact mass calcd 751; FAB $(M + H)^+ = 752$.

2′-*O*-Methyladenosine (1b). A solution of adenosine (26.70 g, 100.00 mmol) in 450 mL of DMF was reacted with sodium hydride (4.80 g, 120.00 mmol) and methyl iodide (15.60 g, 110.00 mmol) for 4 h at 0 °C. After a similar workup procedure as described in the preparation of **1a**, the product was impregnated on silica (40 g) and eluted with a gradient of CH_3OH (5 → 15 vol %) in CH_2Cl_2. Evaporation of the fractions with monomethylated compounds (R_f = 0.29 in $CHCl_3/CH_3OH$ 85:15 v/v) yielded a white solid. ^1H NMR analysis revealed that this product consisted of a 5:1 mixture of the desired 2′-*O*-methyladenosine (**1b**) and 3′-*O*-methyladenosine. Pure **1b** was obtained as a white solid by crystallization from absolute ethanol: yield 7.46 g (27%); mp 202–203 °C; ^1H NMR (D_2O) δ 3.30 (3 H, s, CH_3), 3.68 (1 H, dd, $H_{5''}$), 3.78 (1 H, dd, $H_{5'}$), 4.14 (1 H, m, $H_{4'}$), 4.33 (1 H, dd, $H_{2'}$), 4.46 (1 H, dd, $H_{3'}$), 5.93 (1 H, d, $H_{1'}$), 7.98 (1 H, s, H_2), 8.12 (1 H, s, H_8); exact mass calcd 281; FAB $(M + H)^+ = 282$. Anal. Calcd: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.82; H, 5.40; N, 24.46.

2′-*O*-Methyl-6-*N*-(9-fluorenylmethoxycarbonyl)adenosine (2b). To a cooled (0 °C) solution of compound **1b** (6.19 g, 22.00 mmol) in 110 mL of pyridine was added chlorotrimethylsilane (12.00 g, 110.00 mmol), and the reaction mixture was stirred for 15 min. Then, 9-fluorenylmethoxycarbonyl chloride (6.83 g, 26.40 mmol) was transferred into the reaction flask and stirring was continued for $2^1/_2$ h. The reaction mixture was cooled to 0 °C and 70 mL of water was added in order to hydrolyze the trimethylsilyl groups and excess of chlorides. After stirring for 18 h the mixture was evaporated to near dryness and coevaporated with toluene (three times) and CH_2Cl_2 (twice) to remove the last traces of pyridine from the yellow oil. Subsequently, 250 mL of water and 100 mL of ethyl acetate were added. The mixture was shaken vigorously, and the ethyl acetate layer was separated. The water layer was washed again with two 50-mL portions of ethyl acetate. The collected organic layers were dried ($MgSO_4$) and concentrated to yield a crude product (11.80 g). ^1H NMR analysis indicated the presence of two products, namely, the desired 2′-*O*-methyl-6-*N*-(9-fluorenylmethoxycarbonyl)adenosine and 2′-*O*-methyl-6-bis-*N*-(9-fluorenylmethoxycarbonyl)adenosine. Removal of the latter (more apolar) compound was accomplished by suspending the crude product in a mixture of ethyl acetate and CH_3OH (60 mL, 95:5 v/v), followed by 5 min of vigorous shaking. The solid was separated from the yellow solution by filtration and washed with two small portions of cold ethyl acetate. After drying in vacuo, pure **2b** was obtained as a white solid: yield 6.10 g (55%); this product decomposed upon heating (approximately 174 °C); ^1H NMR ($CDCl_3$) δ 2.82 (1 H, bs, 3′ OH), 3.32 (3 H, s, OCH_3), 3.77 (1 H, m, $H_{5''}$), 3.98 (1 H, m, $H_{5'}$), 4.32 (1 H, t, CH Fmoc), 4.39 (1 H, m, $H_{4'}$), 4.60 (1 H, m, $H_{3'}$), 4.66 (2 H, d, CH_2 Fmoc), 4.69 (1 H, dd, $H_{2'}$), 5.90 (1 H, d, $H_{1'}$), 6.03 (1 H, dd, 5′ OH), 7.38 (4 H, m, arom Fmoc), 7.66 (2 H, d, arom Fmoc), 7.79 (2 H, d, arom Fmoc), 8.03 (1 H, s, H_2), 8.67 (1 H, s, NH), 8.78 (1 H, s, H_8); exact mass calcd 503; FAB $(M + H)^+ = 504$.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-6-*N*-(9-fluorenylmethoxycarbonyl)adenosine (3b). A solution of compound **2b** (4.03 g, 8.00 mmol) and 4-monomethoxytrityl chloride (2.96 g, 9.60 mmol) in 50 mL of pyridine was stirred for 18 h in darkness. The mixture was then processed as described for the preparation of **3a**. Column separation was performed with a gradient of CH_3OH (0 → 2 vol %) in CH_2Cl_2 as eluent, R_f 0.53 ($CHCl_3/CH_3OH$ 95:5 v/v), yielding pure **3b** (5.78 g, 93%) as a yellowish solid, which decomposed upon heating (approximately 105 °C): ^1H NMR ($CDCl_3$) δ 2.72 (1 H, bs, 3′ OH), 3.43 (1 H, dd, $H_{5''}$), 3.54 (1 H, dd, $H_{5'}$), 3.58 (3 H, s, 2′-OCH_3), 3.79 (3 H, s, CH_3O MMTr), 4.22 (1 H, m, $H_{4'}$), 4.32 (1 H, t, CH Fmoc), 4.43 (1 H, dd, $H_{2'}$), 4.50 (1 H, dd, $H_{3'}$), 4.61 (2 H, d, CH_2 Fmoc), 6.18 (1 H, d, $H_{1'}$), 6.81 (2 H, d, arom MMTr), 7.20–7.50 (16 H, m, arom Fmoc/arom MMTr), 7.63 (2 H, d, arom Fmoc), 7.78 (2 H, d, arom Fmoc), 8.20 (1 H, s, H_2), 8.34 (1 H, bs, NH), 8.71 (1 H, s, H_8).

5′-*O*-(4-Monomethoxytrityl)uridine (6a). To a solution of uridine (**5a**, 4.88 g, 20.00 mmol) in 100 mL of pyridine was added 4-monomethoxytrityl chloride (9.26 g, 30.00 mmol), and the reaction mixture was stirred for 20 h in darkness. After evaporation the oil was dissolved in 150 mL of ethyl acetate and washed with three 100-mL portions of water. The ethyl acetate layer was dried ($MgSO_4$) and concentrated. The residue was coevaporated with toluene (three times) in order to remove the last traces of pyridine. The resulting yellow foam was purified by column chromatography, using a gradient of CH_3OH (5 → 10 vol %) in CH_2Cl_2 as eluent, R_f 0.36 ($CHCl_3/CH_3OH$ 9:1 v/v). Compound **6a** was obtained as a white solid: yield 7.33 g (71%); this product decomposed upon heating (approximately 105 °C); ^1H NMR ($CDCl_3$) 3.35 (1 H, bs, OH), 3.50 (1 H, dd, $H_{5''}$), 3.53 (1 H, dd, $H_{5'}$), 3.78 (3 H, s, OCH_3 MMTr), 4.18 (1 H, m, $H_{4'}$), 4.35 (1 H, dd, $H_{2'}$), 4.45 (1 H, m, $H_{3'}$), 5.33 (1 H, d, H_5), 5.50 (1 H, bs, OH), 5.90 (1 H, d, $H_{1'}$), 6.84 (2 H, d, arom MMTr), 7.22–7.38 (12 H, arom MMTr), 8.02 (1 H, d, H_6); exact mass calcd 516; FAB $(M + H)^+ = 517$.

5′-*O*-(4-Monomethoxytrityl)-2′,3′-di-*O*-levulinoyluridine (7a). Levulinic anhydride (6.86 g, 32.00 mmol) was added to a solution of compound **6a** (4.13 g, 8.00 mmol) in 30 mL of pyridine. After 2 h of stirring, the red solution was poured into saturated aqueous $NaHCO_3$ (200 mL), extracted with three 100-mL portions of CH_2Cl_2, dried ($MgSO_4$), and concentrated. The last traces of pyridine were removed by coevaporation with toluene (twice) and $CHCl_3$ (twice). The resulting brown foam was purified by column chromatography, using a gradient (0 → 5 vol %) of CH_3OH in CH_2Cl_2 as eluent, R_f 0.65 ($CHCl_3/CH_3OH$ 9:1 v/v). This yielded 5.60 g (98%) of pure **7a** as a yellowish solid; ^1H NMR ($CDCl_3$) δ 2.18 (3 H, s, CH_3 Lev), 2.19 (3 H, s, CH_3 Lev), 2.62–2.79 (8 H, m, CH_2CH_2 Lev), 3.48 (1 H, dd, $H_{5''}$), 3.52 (1 H, dd, $H_{5'}$), 3.80 (3 H, s, OCH_3 MMTr), 4.22 (1 H, m, $H_{4'}$), 5.29 (1 H, dd, H_5), 5.58 (2 H, m, $H_{2'}$ and $H_{3'}$), 6.20 (1 H, d, $H_{1'}$), 6.86 (2 H, d, arom MMTr), 7.2–7.4 (12 H, m, arom MMTr), 7.66 (1 H, d, H_6); exact mass calcd 712; FAB $(M + H)^+ = 713$.

2′,3′-Di-*O*-levulinoyluridine (8a). Compound **7a** (4.28 g, 6.00 mmol) was stirred for 14 h in 20 mL of a mixture of acetic acid and water (8:2 v/v). After evaporation the residue was dissolved in 100 mL of CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (200 mL). The aqueous layer was extracted with two 50-mL portions of CH_2Cl_2 and the collected organic phase was dried ($MgSO_4$) and concentrated. Purification of the resulting brownish oil was accomplished by column chromatography, using a gradient of CH_3OH (5 → 10 vol %) in CH_2Cl_2 as eluent, R_f 0.36 ($CHCl_3/CH_3OH$ 9:1 v/v). Pure **8a** was obtained as a white foam: yield 1.87 g (70%); ^1H NMR ($CDCl_3$) 2.17 (3 H, s, CH_3 Lev), 2.22 (3 H, s, CH_3 Lev), 2.60–2.83 (8 H, m, CH_2CH_2 Lev), 3.85 (1 H, dd, $H_{5''}$), 3.93 (1 H, dd, $H_{5'}$), 4.22 (1 H, m, $H_{4'}$), 5.45 (2 H, m, $H_{2'}$ and $H_{3'}$), 5.80 (1 H, d, H_5), 6.07 (1 H, d, $H_{1'}$), 7.84 (1 H, d, H_6), 8.85 (1 H, bs, NH).

4-*N*-(9-Fluorenylmethoxycarbonyl)cytidine (5b). During 5 min, chlorotrimethylsilane (32.4 mL, 253.98 mmol) was added dropwise to a suspension of cytidine (12.00 g, 49.32 mmol) in 240 mL of pyridine, and the reaction mixture was stirred for 1 h. Then, 9-fluorenylmethoxycarbonyl chloride (15.30 g, 59.28 mmol) was added, and the reaction mixture was stirred for 2 h; a white precipitate of pyridinium hydrochloride appeared in the yellow

solution. After the addition of water (240 mL) and 45 min of stirring, the yellow solution was evaporated to near dryness. Upon addition of water (750 mL) a white precipitate appeared. The mixture was shaken vigorously until no more yellow oil was visible. After addition of ethyl acetate (360 mL) and shaking, the precipitate formed on the separation layer was isolated by filtration and washed with cold ethyl acetate. After drying in vacuo, the product was recrystallized from ethanol, yielding 20.70 g (90%) of pure **5b**, which decomposed upon heating (approximately 112 °C): ^{1}H NMR (DMSO-d_6) δ 3.60 (1 H, dd, $H_{5''}$), 3.74 (1 H, dd, $H_{5'}$), 3.90 (1 H, m, $H_{4'}$), 3.98 (2 H, m, $H_{2'}/H_{3'}$), 4.29 (1 H, t, CH Fmoc), 4.39 (2 H, d, CH_2 Fmoc), 5.78 (1 H, d, $H_{1'}$), 6.95 (1 H, d, H_5), 7.2–7.5 (4 H, m, arom Fmoc), 7.7–8.0 (4 H, m, arom Fmoc), 8.39 (1 H, d, H_6); exact mass calcd 465; FAB $(M + H)^+$ = 466, $(M + Na)^+$ = 488.

5′-*O*-(4-Monomethoxytrityl)-4-*N*-(9-fluorenylmethoxycarbonyl)cytidine (6b). A mixture of compound **5b** (12.35 g, 26.53 mmol) and 4-monomethoxytrityl chloride (10.10 g, 32.71 mmol) in 260 mL of pyridine was stirred for 34 h in darkness. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (500 mL), extracted with three 200-mL portions of CH_2Cl_2, dried ($MgSO_4$), and concentrated. Removal of all pyridine from the residue was accomplished by coevaporation with toluene (three times) and CH_2Cl_2 (three times). The resulting yellow foam was purified by column chromatography, using a gradient of CH_3OH (4 → 6 vol %) in CH_2Cl_2 as eluent, R_f 0.33 (CH_2Cl_2/CH_3OH 94:6 v/v). This afforded 15.60 g (80%) of compound **6b**: ^{1}H NMR ($CDCl_3$) δ 3.42 (1 H, dd, $H_{5''}$), 3.49 (1 H, dd, $H_{5'}$), 3.77 (3 H, s, CH_3O), 4.27 (1 H, t, CH Fmoc), 4.36 (3 H, m, $H_{2'}/H_{3'}/H_{4'}$), 4.50 (2 H, d, CH_2 Fmoc), 5.86 (1 H, d, $H_{1'}$), 6.83 (2 H, d, arom MMTr), 7.05 (1 H, d, H_5), 7.20–7.46 (16 H, m, arom Fmoc/arom MMTr), 7.68 (4 H, m, arom Fmoc), 8.29 (1 H, d, H_6); exact mass calcd 737; FAB $(M + H)^+$ = 738, $(M + Na)^+$ = 760.

2′,3′-Di-*O*-levulinoyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidine (8b). A mixture of compound **6b** (4.88 g, 6.61 mmol), levulinic anhydride (5.70 g, 26.64 mmol), and 50 mL of pyridine was stirred for $5^1/_2$ h. The red reaction mixture was then processed as described in the preparation of **7a**. Column chromatography of the resulting yellow oil, using a gradient of CH_3OH (0 → 3 vol %) in CH_2Cl_2 as eluent, yielded compound **7b** as a brownish foam (5.99 g), R_f 0.39 ($CHCl_3/CH_3OH$ 97:3 v/v). This product was stirred for 14 h in 20 mL of a mixture of acetic acid and water (8:2 v/v) and processed as described in the synthesis of **8a**. Purification of the resulting brown oil was effected by column chromatography, using a gradient of CH_3OH (0 → 5 vol %) in CH_2Cl_2 as eluent, R_f 0.36 ($CHCl_3/CH_3OH$ 95:5 v/v). This afforded **8b** as a white foam: yield 3.47 g (79% from **6b**); product decomposed upon heating (approximately 100 °C); ^{1}H NMR ($CDCl_3$) δ 2.14 (3 H, s, CH_3 Lev), 2.17 (3 H, s, CH_3 Lev), 2.5–2.9 (8 H, m, CH_2CH_2 Lev), 3.60 (1 H, bs, 5′ OH), 3.84 (1 H, m, $H_{5''}$), 4.00 (1 H, m, $H_{5'}$), 4.23 (2 H, m, $H_{4'}$/CH Fmoc), 4.41 (2 H, d, CH_2 Fmoc), 5.50 (1 H, t, $H_{3'}$), 5.57 (1 H, t, $H_{2'}$), 6.04 (1 H, d, $H_{1'}$), 7.23 (1 H, d, H_5), 7.28 (2 H, t, arom Fmoc), 7.39 (2 H, t, arom Fmoc), 7.53 (2 H, d, arom Fmoc), 7.74 (2 H, d, arom Fmoc), 8.10 (1 H, bs, NH), 8.20 (1 H, d, H_6); exact mass calcd 661; FAB $(M + H)^+$ = 662, $(M + Na)^+$ = 684.

2-*N*-(9-Fluorenylmethoxycarbonyl)guanosine (5c). Chlorotrimethylsilane (28.5 mL, 224.56 mmol) was added dropwise to a cooled (0 °C) suspension of guanosine (8.00 g, 28.27 mmol) in 150 mL of pyridine, and the reaction mixture was stirred for $1^1/_2$ h. During this time the guanosine dissolved completely, and a precipitate of pyridinium hydrochloride appeared. 9-Fluorenylmethoxycarbonyl chloride (8.54 g, 33.00 mmol) was transferred into the reaction flask, and stirring was continued for $2^1/_2$ h. After the addition of water (15 mL) at 0 °C and 30 min of stirring, the clear solution was poured into saturated aqueous $NaHCO_3$ (300 mL) and extracted with three 150-mL portions of ethyl acetate. The combined organic layers were washed with two 100-mL portions of water, dried ($MgSO_4$), and concentrated. The last traces of pyridine were removed by coevaporation with two 100-mL portions of toluene. CH_2Cl_2 (100 mL) was added to the residue, and the white precipitate was filtered off, washed with CH_2Cl_2 three times, and dried in vacuo. ^{1}H NMR analysis showed that the solid consisted of **5c** (R_f = 0.30 in $CHCl_3/CH_3OH$ 8:2 v/v) and an unidentified side product (5%, R_f = 0.40 in $CHCl_3/CH_3OH$ 8:2 v/v), which were difficult to separate. The semipure **5c** (10.14 g, 71%) was used without further purification: ^{1}H NMR (DMSO-d_6) δ 3.55 (1 H, m, $H_{5''}$), 3.65 (1 H, m, $H_{5'}$), 3.91 (1 H, m, $H_{4'}$), 4.14 (1 H, m, $H_{3'}$), 4.35 (1 H, t, CH Fmoc), 4.47 (1 H, m, $H_{2'}$), 4.49 (2 H, d, CH_2 Fmoc), 5.04 (1 H, t, 5′ OH), 5.20 (1 H, d, OH), 5.50 (1 H, d, OH), 5.82 (1 H, d, $H_{1'}$), 7.34–7.47 (4 H, m, arom Fmoc), 7.87 (4 H, m, arom Fmoc), 8.25 (1 H, s, H_8); exact mass calcd 505; FAB $(M + H)^+$ = 506, $(M + Na)^+$ = 528.

5′-*O*-(4-Monomethoxytrityl)-2-*N*-(9-fluorenylmethoxycarbonyl)guanosine (6c). A solution of compound **5c** (6.00 g, 11.87 mmol) and 4-monomethoxytrityl chloride (4.40 g, 14.25 mmol) in pyridine (70 mL) was stirred for 36 h in darkness and processed as described in the preparation of **6b**. Column chromatography of the resulting brown foam, using a mixture of CH_2Cl_2 and CH_3OH (93:7 v/v) as eluent (R_f = 0.32), yielded compound **6c** as a yellowish solid (5.64 g, 61%), which decomposed upon heating (approximately 110 °C): ^{1}H NMR (DMSO-d_6) δ 3.18 (1 H, m, $H_{5''}$), 3.26 (1 H, m, $H_{5'}$), 3.72 (3 H, s, OCH_3 MMTr), 4.04 (1 H, m, $H_{4'}$), 4.22 (1 H, m, $H_{3'}$), 4.34 (1 H, t, CH Fmoc), 4.49 (2 H, d, CH_2 Fmoc), 4.54 (1 H, m, $H_{2'}$), 5.20 (1 H, bs, OH), 5.62 (1 H, bs, OH), 5.87 (1 H, d, $H_{1'}$), 6.84 (2 H, d, arom MMTr), 7.2–7.5 (16 H, m, arom MMTr/arom Fmoc), 7.81 (2 H, d, arom Fmoc), 7.92 (2 H, d, arom Fmoc), 8.10 (1 H, bs, NH); exact mass calcd 777; FAB $(M + H)^+$ = 778.

2′,3′-Di-*O*-levulinoyl-2-*N*-(9-fluorenylmethoxycarbonyl)guanosine (8c). Levulinic anhydride (2.29 g, 10.70 mmol) was added to a solution of compound **6c** (2.08 g, 2.67 mmol) in 20 mL of pyridine. After 4 h of stirring the red solution was processed as described in the preparation of **7a**. This afforded a yellow oil (1.85 g), which mainly consisted of **7c**. Part of this product (1.80 g) was stirred for 15 h in 20 mL of a mixture of acetic acid and water (8:2 v/v). After evaporation of all acetic acid (coevaporation with water), the yellow viscous substance was purified by column chromatography, using a mixture of CH_2Cl_2 and CH_3OH (95:5 v/v) as eluent (R_f = 0.22). Compound **8c** was obtained as a yellowish solid, yield 0.96 g (53% from **6c**), which decomposed upon heating (approximately 120 °C): ^{1}H NMR (acetone-d_6) δ 2.08 (3 H, s, CH_3 Lev), 2.17 (3 H, s, CH_3 Lev), 2.51–2.88 (8 H, m, CH_2CH_2 Lev), 3.84 (2 H, m, $H_{5'}/H_{5''}$), 4.27 (1 H, m, $H_{4'}$), 4.38 (1 H, t, CH Fmoc), 4.65 (2 H, d, CH_2 Fmoc), 5.56 (1 H, dd, $H_{3'}$), 5.83 (1 H, dd, $H_{2'}$), 6.12 (1 H, d, $H_{1'}$), 7.34–7.47 (4 H, m, arom Fmoc), 7.81 (2 H, d, arom Fmoc), 7.90 (2 H, d, arom Fmoc), 8.11 (1 H, s, H_8); exact mass calcd 701; FAB $(M + H)^+$ = 702, $(M + Na)^+$ = 724.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidyl-(3′→5′)-2′,3′-di-*O*-levulinoyluridine *O*-(Methyl phosphate) (9). To a solution of compound **3a** (1.14 g, 1.51 mmol) in 9 mL of pyridine were added 1*H*-tetrazole (0.054 g, 0.76 mmol) and bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.435 mg, 1.66 mmol). After 20 min of stirring, ^{31}P NMR analysis showed complete conversion into the corresponding phosphoramidite coupling synthon **4a** as a mixture of two diastereoisomers ($CDCl_3$: δ 151.0 and 150.8). Then, a solution of compound **8a** (0.70 g, 1.59 mmol) and of 1*H*-tetrazole (0.321 g, 4.53 mmol) in 9 mL of pyridine was added to the reaction mixture, and stirring was continued for 1 h. ^{31}P NMR spectroscopy showed that all phosphoramidite had been converted into the phosphite triester (two diastereoisomers ($CDCl_3$): δ 141.3 and 140.7). Oxidation to the corresponding phosphate triester **9** (^{31}P NMR ($CDCl_3$): δ −0.3 and −0.6) was accomplished by the addition of tBuOOH (1.2 mL), followed by 10 min of stirring. The mixture was concentrated to near dryness and coevaporated with toluene (three times) and $CHCl_3$ (twice). TLC and ^{31}P NMR analyses showed the formation of polar side products and 9-methylenefluorene during the coupling reaction and oxidation. Apparently, part of the Fmoc groups had been cleaved off due to the slightly basic medium. The product was purified by column chromatography, using a gradient of CH_3OH (2 → 5 vol %) in CH_2Cl_2 as eluent, R_f = 0.30 (CH_2Cl_2/CH_3OH 95:5 v/v). Pure **9** was obtained as a white solid, which decomposed upon heating (approximately 104 °C): yield 0.47 g (24%); ^{31}P NMR ($CDCl_3$) −0.08 and −0.32; ^{1}H NMR ($CDCl_3$) δ 2.16 (6 H, 4 × s, 2 × CH_3 Lev), 2.5–2.8 (8 H, m, 2 × CH_2CH_2 Lev), 3.50 (2 H, m, $H_{5'}$(Cp)/$H_{5''}$(Cp)), 3.68 (3 H, 2 × s, 2′-OCH_3), 3.79 (3 H, s, CH_3O MMTr), 3.59 and 3.81 (3 H, 2 × d, $POCH_3$, J = 11.4 Hz), 4.1–4.5 (8 H, m, $H_{2'}$(Cp)/$H_{4'}$(Cp)/$H_{4'}$(pU)/$H_{5'}$(pU)/$H_{5''}$(pU)/CH_2 Fmoc/CH Fmoc), 5.08 (1 H, m, $H_{3'}$(Cp)), 5.27 (1 H, 2 × dd,

$H_{2'}$(pU)), 5.38 (1 H, 2 × dd, $H_{3'}$(pU)), 5.69 (1 H, 2 × d, H_5(pU)), 5.89 and 6.00 (1 H, 2 × d, $H_{1'}$(pU)), 6.03 (1 H, s, $H_{1'}$(Cp)), 6.85 (2 H, d, arom MMTr), 6.86 (1 H, d, H_5(Cp)), 7.2–7.5 (16 H, m, arom Fmoc/arom MMTr), 7.37 (2 H, d, arom Fmoc), 7.49 (1 H, d, H_6(pU)), 7.78 (2 H, d, arom Fmoc), 8.50 (1 H, d, H_6(Cp)); exact mass calcd 1268; FAB $(M + H)^+$ = 1269.

2′-*O*-Methylcytidyl-(3′→5′)-uridine *O*-(Methyl phosphate) (15). Compound **9** (150 mg, 0.118 mmol) was dissolved in 5.0 mL of a 0.05 M solution of K_2CO_3 in CH_3OH (0.25 mmol). The reaction mixture was stirred and the course of the deprotection steps was followed with TLC. After the cleavage of both the Lev groups (10 min) and the Fmoc group (2 h), the solution was neutralized (to pH ≈ 6) by addition of Dowex-H^+ resin. After filtration over a glass filter the solution was evaporated to yield a white solid, R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.16; ^{31}P NMR (CD_3OD) δ 0.13 (peaks overlap). The product was dissolved in 5 mL of 80% aqueous acetic acid and stirred for 20 h. The reaction mixture was evaporated to dryness, coevaporated (four times) with water, and partitioned between water and CH_2Cl_2. The aqueous layer and the precipitate on the separation layer were collected and evaporated to dryness, yielding **15** as a colorless film (44 mg, 65%), R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.05. **(S_P)-15**: ^{31}P NMR (D_2O) δ −0.21; ^1H NMR (D_2O) δ 3.40 (3 H, s, 2′-OCH_3), 3.68 (1 H, dd, $H_{5''}$(Cp)), 3.75 (3 H, d, $POCH_3$, J = 11.4 Hz), 3.78 (1 H, dd, $H_{5'}$(Cp)), 4.10 (1 H, ddd, $H_{2'}$(Cp)), 4.15 (1 H, dd, $H_{3'}$(pU)), 4.18 (1 H, m, $H_{4'}$(pU)), 4.21 (1 H, dd, $H_{2'}$(pU)), 4.21 (1 H, ddd, $H_{4'}$(Cp)), 4.28 (1 H, ddd, $H_{5''}$(pU)), 4.37 (1 H, ddd, $H_{5'}$(pU)), 4.82 (1 H, ddd, $H_{3'}$(Cp)), 5.73 (1 H, d, H_5(pU)), 5.74 (1 H, d, $H_{1'}$(pU)), 5.87 (1 H, d, $H_{1'}$(Cp)), 5.90 (1 H, d, H_5(Cp)), 7.58 (1 H, d, H_6(pU)), 7.73 (1 H, d, H_6(Cp)); Cp residue $J_{1'2'}$ = 4.7 Hz, $J_{2'3'}$ = 5.1 Hz, $J_{3'4'}$ = 5.1 Hz, $J_{3'P}$ = 7.3 Hz, $J_{4'5'}$ = 3.1 Hz, $J_{4'5''}$ = 3.7 Hz, $J_{5'5''}$ = −12.9 Hz; pU residue $J_{1'2'}$ = 3.9 Hz, $J_{2'3'}$ = 5.0 Hz, $J_{3'4'}$ = 5.8 Hz, $J_{4'5'}$ = 2.2 Hz, $J_{4'5''}$ = 4.3 Hz, $J_{5'P}$ = 5.9 Hz, $J_{5''P}$ = 6.1 Hz, $J_{5'5''}$ = −11.6 Hz. **(R_P)-15**: ^{31}P NMR (D_2O) δ −0.46; ^1H NMR (D_2O) δ 3.46 (3 H, s, 2′-OCH_3), 3.73 (1 H, dd, $H_{5''}$(Cp)), 3.81 (1 H, dd, $H_{5'}$(Cp)), 3.83 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.17 (1 H, ddd, $H_{2'}$(Cp)), 4.20 (1 H, ddd, $H_{4'}$(pU)), 4.23 (1 H, dd, $H_{3'}$(pU)), 4.26 (1 H, ddd, $H_{4'}$(Cp)), 4.30 (1 H, dd, $H_{2'}$(pU)), 4.32 (1 H, ddd, $H_{5''}$(pU)), 4.41 (1 H, ddd, $H_{5'}$(pU)), 4.92 (1 H, ddd, $H_{3'}$(Cp)), 5.79 (1 H, d, $H_{1'}$(pU)), 5.79 (1 H, d, H_5(pU)), 5.93 (1 H, d, $H_{1'}$(Cp)), 6.01 (1 H, d, H_5(Cp)), 7.62 (1 H, d, H_6(pU)), 7.80 (1 H, d, H_6(Cp)); Cp residue $J_{1'2'}$ = 5.3 Hz, $J_{2'3'}$ = 4.7 Hz, $J_{3'4'}$ = 4.7 Hz, $J_{3'P}$ = 7.1 Hz, $J_{4'5'}$ = 3.1 Hz, $J_{4'5''}$ = 3.7 Hz, $J_{5'5''}$ = −12.8 Hz; pU residue $J_{1'2'}$ = 3.9 Hz, $J_{2'3'}$ = 5.2 Hz, $J_{3'4'}$ = 6.1 Hz, $J_{4'5'}$ = 2.4 Hz, $J_{4'5''}$ = 4.6 Hz, $J_{5'P}$ = 5.9 Hz, $J_{5''P}$ = 6.1 Hz, $J_{5'5''}$ = −11.8 Hz.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-6-*N*-(9-fluorenylmethoxycarbonyl)adenylyl-(3′→5′)-2′,3′-di-*O*-levulinoyluridine *O*-(Methyl phosphate) (10). To a solution of compound **3b** (0.50 g, 0.65 mmol) in 2 mL of CH_2Cl_2 were added 1*H*-tetrazole (0.65 mL of a 0.50 M solution in CH_3CN, 0.33 mmol) and a solution of bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.19 g, 0.72 mmol) in 0.5 mL of CH_2Cl_2, and the reaction mixture was stirred for 40 min. Formation of the phosphoramidite coupling synthon in situ **4b** was evident from the ^{31}P NMR data (two diastereoisomers ($CDCl_3$): δ 152.0 and 151.1). Then a solution of compound **8a** (0.30 g, 0.68 mmol) in 2 mL of CH_2Cl_2 and 1*H*-tetrazole (2.60 mL of a 0.50 M solution in CH_3CN, 1.30 mmol) were transferred into the reaction vessel, and the mixture was stirred for 90 min. ^{31}P NMR analysis showed the complete conversion of **4b** into the corresponding phosphite triester (two diastereoisomers ($CDCl_3$): δ 142.0 and 141.4), which were readily oxidized through the addition of tBuOOH (0.5 mL) and 5 min of stirring. The mixture was evaporated to dryness and the residue was coevaporated with toluene (twice) and $CHCl_3$ (three times). The product was purified by column chromatography, using a mixture of CH_2Cl_2 and CH_3OH (95:5 v/v) as eluent (R_f = 0.36). This afforded 0.44 g (53%) of pure **10** as a white solid, which decomposed upon heating (approximately 90 °C): ^{31}P NMR ($CDCl_3$) δ 0.26 and −0.10; ^1H NMR ($CDCl_3$) δ 2.18 (6 H, 4 × s, 2 × CH_3 Lev), 2.5–2.8 (8 H, m, 2 × CH_2CH_2 Lev), 3.45 (3 H, 2 × s, 2′-OCH_3), 3.78 (3 H, s, CH_3O MMTr), 3.76 and 3.86 (3 H, 2 × d, $POCH_3$, J = 11.4 Hz), 4.2–4.5 (4 H, m, $H_{4'}$(Ap)/$H_{4'}$(pU)/$H_{5'}$(pU)/$H_{5''}$(pU)), 4.34 (1 H, t, CH Fmoc), 4.61 (2 H, d, CH_2 Fmoc), 4.92 and 5.20 (1 H, 2 × m, $H_{2'}$(Ap)), 5.20 (1 H, m, $H_{3'}$(Ap)), 5.30 (1 H, 2 × dd, $H_{2'}$(pU)), 5.40 and 5.47 (1 H, 2 × dd, $H_{3'}$(pU)), 5.75 (1 H, 2 × d, H_5(pU)), 6.11 (1 H, d, $H_{1'}$(Ap)), 5.99 and 6.12 (1 H, 2 × d, $H_{1'}$(pU)), 6.82 (2 H, d, arom MMTr), 7.2–7.5 (16 H, m, arom Fmoc/arom MMTr), 7.52 (1 H, d, H_6(pU)), 7.70 (2 H, d, arom Fmoc), 7.78 (2 H, d, arom Fmoc), 8.17 (1 H, 2 × s, H_2(Ap)), 8.48 (1 H, bs, NH), 8.62 (1 H, 2 × s, H_8(Ap)), 9.06 (1 H, bs, NH); exact mass calcd 1291; FAB $(M + H)^+$ = 1292.

2′-*O*-Methyladenylyl-(3′→5′)-uridine *O*-(Methyl phosphate) (16). Compound **10** (300 mg, 0.232 mmol) was dissolved in 10 mL of a 0.05 M solution of K_2CO_3 in CH_3OH (0.50 mmol). Removal of the Lev groups required 10 min of stirring, while the Fmoc group was completely cleaved off after 2 h. Then the solution was neutralized (to pH ≈ 6) by addition of Dowex-H^+ resin. After filtration over a glass filter the solution was evaporated to yield a white solid: R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.39; ^{31}P NMR (CD_3OD) δ 0.31 (peaks overlap). The product was dissolved in 10 mL of 80% acetic acid and stirred for 14 h. The mixture was concentrated in vacuo and the last traces of acetic acid were removed by coevaporation (four times) with water. The resulting white residue was then partitioned between water (30 mL) and diethyl ether (15 mL). The aqueous layer was washed with 15 mL of diethyl ether, filtrated over a glass filter, and evaporated to afford 134 mg (96%) of **16** as a white solid: R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.16; exact mass calcd 601; FAB $(M + H)^+$ 602. **(S_P)-16**: ^{31}P NMR (D_2O) δ −0.31; ^1H NMR (D_2O) δ 3.40 (3 H, s, 2′-OCH_3), 3.78 (1 H, dd, $H_{5''}$(Ap)), 3.83 (1 H, dd, $H_{5'}$(Ap)), 3.85 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.22 (1 H, dd, $H_{3'}$(pU)), 4.24 (1 H, ddd, $H_{4'}$(pU)), 4.27 (1 H, dd, $H_{2'}$(pU)), 4.38 (1 H, ddd, $H_{5''}$(pU)), 4.45 (1 H, ddd, $H_{4'}$(Ap)), 4.47 (1 H, ddd, $H_{5'}$(pU)), 4.63 (1 H, ddd, $H_{2'}$(Ap)), 5.19 (1 H, ddd, $H_{3'}$(Ap)), 5.69 (1 H, d, H_5(pU)), 5.78 (1 H, d, $H_{1'}$(pU)), 6.04 (1 H, d, $H_{1'}$(Ap)), 7.59 (1 H, d, H_6(pU)), 8.15 (1 H, s, H_2(Ap)), 8.28 (1 H, s, H_8(Ap)); Ap residue $J_{1'2'}$ = 7.0 Hz, $J_{2'3'}$ = 4.7 Hz, $J_{3'4'}$ = 2.3 Hz, $J_{3'P}$ = 7.1 Hz, $J_{4'5'}$ = 3.1 Hz, $J_{4'5''}$ = 3.3 Hz, $J_{5'5''}$ = −13.0 Hz; pU residue $J_{1'2'}$ = 4.0 Hz, $J_{2'3'}$ = 5.2 Hz, $J_{3'4'}$ = 6.1 Hz, $J_{4'5'}$ = 2.2 Hz, $J_{4'5''}$ = 4.5 Hz, $J_{5'P}$ = 5.9 Hz, $J_{5''P}$ = 6.2 Hz, $J_{5'5''}$ = −11.7 Hz. **(R_P)-16**: ^{31}P NMR (D_2O) δ −0.35; ^1H NMR (D_2O) δ 3.38 (3 H, s, 2′-OCH_3), 3.78 (1 H, dd, $H_{5''}$(Ap)), 3.82 (1 H, dd, $H_{5'}$(Ap)), 3.88 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.23 (1 H, ddd, $H_{4'}$(pU)), 4.26 (1 H, dd, $H_{3'}$(pU)), 4.33 (1 H, dd, $H_{2'}$(pU)), 4.37 (1 H, ddd, $H_{5''}$(pU)), 4.46 (2 H, m, $H_{4'}$(Ap)/$H_{5'}$(pU)), 5.21 (1 H, ddd, $H_{3'}$(Ap)), 5.75 (1 H, d, H_5(pU)), 5.80 (1 H, d, $H_{1'}$(pU)), 6.06 (1 H, d, $H_{1'}$(Ap)), 7.62 (1 H, d, H_6(pU)), 8.21 (1 H, s, H_2(Ap)), 8.29 (1 H, s, H_8(Ap)), $H_{2'}$(Ap) resides under the HDO peak; Ap residue $J_{1'2'}$ = 7.1 Hz, $J_{2'3'}$ = 4.8 Hz, $J_{3'4'}$ = 2.1 Hz, $J_{3'P}$ = 7.1 Hz, $J_{4'5'}$ = 3.0 Hz, $J_{4'5''}$ = 3.3 Hz, $J_{5'5''}$ = −13.0 Hz; pU residue $J_{1'2'}$ = 3.9 Hz, $J_{2'3'}$ = 5.4 Hz, $J_{3'4'}$ = 6.2 Hz, $J_{4'5'}$ = 2.3 Hz, $J_{4'5''}$ = 4.7 Hz, $J_{5'P}$ = 6.3 Hz, $J_{5''P}$ = 6.4 Hz, $J_{5'5''}$ = −11.8 Hz.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidyl-(3′→5′)-2′,3′-di-*O*-levulinoyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidine *O*-(Methyl phosphate) (11). To a solution of compound **3a** (1.00 g, 1.33 mmol) in 5 mL of CH_2Cl_2 were added 1*H*-tetrazole (1.33 mL of a 0.50 M solution in CH_3CN, 0.67 mmol) and a solution of bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.38 g, 1.45 mmol) in 1 mL of CH_2Cl_2. After 30 min of stirring ^{31}P NMR spectroscopy revealed quantitative conversion into the phosphoramidite structure **4a** ($CDCl_3$: δ 151.0 and 150.9). Then a solution of compound **8b** (0.93 g, 1.40 mmol) in 3 mL of CH_2Cl_2 and 1*H*-tetrazole (5.30 mL of a 0.50 M solution in CH_3CN, 2.70 mmol) were added. After $1^1/_2$ h of stirring **4a** had been completely converted into the corresponding phosphite triester ($CDCl_3$: δ 141.6 and 140.8). Subsequently, tBuOOH (1.1 mL) was added to the reaction mixture, and after 15 min of stirring, ^{31}P NMR revealed the quantitative formation of the phosphate triester function. After evaporation of all volatiles (coevaporation with toluene (twice) and chloroform (twice)), the product was purified by column chromatography, using a mixture of ethyl acetate and CH_3OH (98:2 v/v) as eluent (R_f = 0.16). This afforded 0.81 g (41%) of pure **11** as a white solid. This product decomposed upon heating (approximately 148 °C): ^{31}P NMR ($CDCl_3$) δ 0.05 and −0.46; ^1H NMR ($CDCl_3$) δ 2.14 (6 H, 4 × s, 2 × CH_3 Lev), 2.5–2.8 (8 H, m, 2 × CH_2CH_2 Lev), 3.50 (1 H, dd, $H_{5''}$(Cp)), 3.69 (1 H, m, $H_{5'}$(Cp)), 3.72 (3 H, 2 × s, 2′-OCH_3), 3.78 (3 H, 2 × s, CH_3O MMTr), 3.59 and 3.82 (3 H, 2 × d, $POCH_3$, J = 11.4 Hz), 4.0–4.5 (11 H, m, $H_{2'}$(Cp)/$H_{4'}$(Cp)/$H_{4'}$(pC)/$H_{5'}$(pC)/$H_{5''}$(pC)/2 × CH_2 Fmoc/2 × CH Fmoc), 5.12 (1 H, m, $H_{3'}$(Cp)), 5.3–5.5 (2 H, m, $H_{2'}$(pC) and $H_{3'}$(pC)), 5.96 and 6.05 (1 H, 2 × d, $H_{1'}$(pC)), 6.07 (1 H, s, $H_{1'}$(Cp)), 6.83 (3 H, m, arom MMTr and H_5(Cp)), 7.18

(1 H, d, H_5(pC)), 7.1–7.8 (28 H, m, arom Fmoc/arom MMTr), 7.90 (1 H, d, H_6(pC)), 8.53 (1 H, d, H_6(Cp)).

2′-*O*-Methylcytidyl-(3′→5′)-cytidine *O*-(Methyl phosphate) (17). Compound **11** (228 mg, 0.153 mmol) was dissolved in 6.0 mL of a 0.05 M solution of K_2CO_3 in CH_3OH (0.30 mmol). Cleavage of both Lev groups appeared to be complete within 5 min, whereas the removal of both Fmoc groups was accomplished after $2^1/_2$ h. The clear solution was neutralized (to pH ≈ 6) by addition of several small portions of Dowex-H^+, filtered over a glass filter, and evaporated to dryness: R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.10; ^{31}P NMR (CD_3OD) δ 0.24 and 0.18. The product was detritylated as described in the synthesis of **16**, which afforded **17** as a colorless film (73 mg, 83%); R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.02. (***S*$_P$)-17**: ^{31}P NMR (D_2O) δ −0.23; ^{1}H NMR (D_2O) δ 3.42 (3 H, s, 2′-OCH_3), 3.71 (1 H, dd, $H_{5''}$(Cp)), 3.79 (3 H, d, $POCH_3$, J = 11.4 Hz), 3.81 (1 H, dd, $H_{5'}$(Cp)), 4.13 (1 H, dd, $H_{2'}$(Cp)), 4.15 (1 H, dd, $H_{3'}$(pC)), 4.19 (1 H, dd, $H_{2'}$(pC)), 4.21 (1 H, m, $H_{4'}$(pC)), 4.25 (1 H, m, $H_{4'}$(Cp)), 4.32 (1 H, ddd, $H_{5''}$(pC)), 4.42 (1 H, ddd, $H_{5'}$(pC)), 4.86 (1 H, ddd, $H_{3'}$(Cp)), 5.78 (1 H, d, $H_{1'}$(pC)), 5.90 (1 H, d, $H_{1'}$(Cp)), 5.96 and 5.97 (2 H, 2 × d, H_5(Cp)/H_5(pC)), 7.65 and 7.78 (2 H, 2 × d, H_6(Cp)/H_6(pC)); Cp residue $J_{1'2'}$ = 4.7 Hz, $J_{2'3'}$ = 4.8 Hz, $J_{3'4'}$ = 5.3 Hz, $J_{3'P}$ = 7.4 Hz, $J_{4'5'}$ = 3.0 Hz, $J_{4'5''}$ 3.7 Hz, $J_{5'5''}$ = −12.9 Hz; pC residue $J_{1'2'}$ = 3.4 Hz, $J_{2'3'}$ = 5.3 Hz, $J_{3'4'}$ = 6.7 Hz, $J_{4'5'}$ = 2.3 Hz, $J_{4'5''}$ = 4.6 Hz, $J_{5'P}$ = 5.9 Hz, $J_{5''P}$ = 5.9 Hz, $J_{5'5''}$ = −11.8 Hz. **(*R*$_P$)-17**: ^{31}P NMR (D_2O) δ −0.43; ^{1}H NMR (D_2O) δ 3.43 (3 H, s, 2′-OCH_3), 3.70 (1 H, dd, $H_{5''}$(Cp)), 3.78 (1 H, dd, $H_{5'}$(Cp)), 3.81 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.14 (1 H, ddd, $H_{2'}$(Cp)), 4.18 (1 H, m, $H_{4'}$(pC)), 4.19 (1 H, dd, $H_{3'}$(pC)), 4.21 (1 H, dd, $H_{2'}$(pC)), 4.22 (1 H, m, $H_{4'}$(Cp)), 4.31 (1 H, ddd, $H_{5''}$(pC)), 4.41 (1 H, ddd, $H_{5'}$(pC)), 4.88 (1 H, ddd, $H_{3'}$(Cp)), 5.79 (1 H, d, $H_{1'}$(pC)), 5.91 (1 H, d, $H_{1'}$(Cp)), 5.94 and 5.95 (2 H, bm, H_5-(Cp)/H_5(pC)), 7.59 and 7.72 (2 H, 2 × d, H_6(Cp)/H_6(pC)); Cp residue $J_{1'2'}$ = 5.1 Hz, $J_{2'3'}$ = 5.1 Hz, $J_{3'4'}$ = 4.6 Hz, $J_{3'P}$ = 7.2 Hz, $J_{4'5'}$ = 3.1 Hz, $J_{4'5''}$ = 3.7 Hz, $J_{5'5''}$ = −12.9 Hz; pC residue $J_{1'2'}$ = 3.3 Hz, $J_{2'3'}$ = 5.4 Hz, $J_{3'4'}$ = 6.5 Hz, $J_{4'5'}$ = 1.7 Hz, $J_{4'5''}$ = 4.0 Hz, $J_{5'P}$ = 5.6 Hz, $J_{5''P}$ = 5.9 Hz, $J_{5'5''}$ = −12.0 Hz; exact mass calcd 576; FAB $(M + H)^+$ = 577.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-6-*N*-(9-fluorenylmethoxycarbonyl)adenylyl-(3′→5′)-2′,3′-di-*O*-levulinoyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidine *O*-(Methyl phosphate) (12). 1*H*-Tetrazole (0.90 mL of a 0.50 M solution in CH_3CN, 0.45 mmol) and a solution of bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.33 g, 1.26 mmol) in 1.5 mL of CH_2Cl_2 were added to a solution of compound **3b** (0.79 g, 1.02 mmol) in 4 mL of CH_2Cl_2 and the reaction mixture was stirred for 40 min. Formation of the phosphoramidite coupling synthon in situ **4b** was evident from the ^{31}P NMR spectrum ($CDCl_3$: δ 152.0 and 151.1). Then a solution of compound **8b** (0.73 g, 1.10 mmol) in 6 mL of CH_2Cl_2 and 1*H*-tetrazole (6.00 mL of a 0.50 M solution in CH_3CN, 3.00 mmol) were added to the reaction mixture, and stirring was continued for $1^1/_2$ h. ^{31}P NMR showed complete conversion into the phosphite triester ($CDCl_3$: δ 142.0 and 141.6). One peak (3-fold lower intensity, ($CDCl_3$) δ 142.0) of a side product was visible as well. Then tBuOOH (1.0 mL) was added, and the reaction mixture was stirred for another 25 min. ^{31}P NMR data showed that all phosphite triesters had been converted into phosphate triesters (**12**, ($CDCl_3$) δ 0.2 and 0.0; side product, ($CDCl_3$) δ 0.5). The mixture was concentrated to near dryness and coevaporated with toluene (three times) and $CHCl_3$ (twice). The resulting yellow foam was purified by column chromatography, using a mixture of ethyl acetate and CH_3OH (96:4 v/v) as eluent. This afforded 0.94 g (61%) of pure **12** as a white solid, R_f 0.32. This product decomposed upon heating (approximately 110 °C), ^{31}P NMR ($CDCl_3$) δ 0.36 and 0.11. The side product was obtained as a white solid (0.21 g) by further elution with a gradient of CH_3OH (4 → 15 vol %) in ethyl acetate: R_f (ethyl acetate/CH_3OH 96:4 v/v) = 0.08; ^{31}P NMR ($CDCl_3$) δ 0.55. ^{1}H NMR analysis showed this compound to be the symmetric (5′-5′) coupled phosphate triester of **8b**. ^{1}H NMR analysis of **12** ($CDCl_3$): δ 2.18 (6 H, 4 × s, 2 × CH_3 Lev), 2.5–2.9 (8 H, m, 2 × CH_2CH_2 Lev), 3.47 (3 H, 2 × s, 2′-OCH_3), 3.55 (1 H, dd, $H_{5''}$(Ap)), 3.63 (1 H, dd, $H_{5'}$(Ap)), 3.78 (3 H, 2 × s, CH_3O MMTr), 3.77 and 3.86 (3 H, 2 × d, $POCH_3$, J = 11.4 Hz), 4.2–4.7 (11 H, m, $H_{2'}$(Ap)/$H_{4'}$(Ap)/$H_{4'}$(pC)/$H_{5'}$(pC)/$H_{5''}$(pC)/2 × CH_2 Fmoc/2 × CH Fmoc), 5.00 (1 H, 2 × dd, $H_{3'}$(Ap)), 5.2–5.5 (2 H, m, $H_{2'}$-(pC)/$H_{3'}$(pC)), 6.0–6.3 (2 H, 4 × d, $H_{1'}$(Ap)/$H_{1'}$(pC)), 6.83 (3 H, m, arom MMTr/H_5(pC)), 7.1–7.8 (28 H, m, arom Fmoc/arom MMTr), 7.91 (1 H, 2 × d, H_6(pC)), 8.20 (1 H, 2 × s, H_2(Ap)), 8.58 (1 H, 2 × s, H_8(Ap)).

2′-*O*-Methyladenylyl-(3′→5′)-cytidine *O*-(Methyl phosphate) (18). Compound **12** (260 mg, 0.172 mmol) was suspended in 10 mL of a 0.05 M solution of K_2CO_3 in CH_3OH (0.50 mmol) and stirred. Cleavage of both Lev groups appeared to be complete within 3 min, whereas the removal of both Fmoc groups was accomplished after 3 h. The clear yellow solution was neutralized (to pH ≈ 6) by addition of small portions of Dowex-H^+ resin. After filtration over a glass filter the solution was evaporated to afford a white solid (240 mg): ^{31}P NMR (CD_3OD) δ 0.29 and 0.27; R_f ($CHCl_3/CH_3OH$ 8:2 v/v) = 0.16. The product was detritylated as described for **16**, affording **18** as a colorless film (95 mg, 92%), R_f = 0.03 ($CHCl_3/CH_3OH$ 8:2 v/v). **(*S*$_P$)-18**: ^{31}P NMR (D_2O) δ −0.38; ^{1}H NMR (D_2O) δ 3.36 (3 H, s, 2′-OCH_3), 3.73 (1 H, dd, $H_{5''}$(Ap)), 3.77 (1 H, dd, $H_{5'}$(Ap)), 3.80 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.12 (1 H, dd, $H_{3'}$(pC)), 4.14 (1 H, dd, $H_{2'}$(pC)), 4.20 (1 H, m, $H_{4'}$(pC)), 4.35 (1 H, ddd, $H_{5''}$(pC)), 4.40 (1 H, m, $H_{4'}$(Ap)), 4.44 (1 H, ddd, $H_{5'}$(pC)), 4.59 (1 H, ddd, $H_{2'}$(Ap)), 5.14 (1 H, ddd, $H_{3'}$(Ap)), 5.74 (1 H, d, $H_{1'}$(pC)), 5.98 (1 H, d, $H_{1'}$(Ap)), 7.53 (1 H, d, H_6(pC)), 8.08 (1 H, s, H_2(Ap)), 8.23 (1 H, s, H_8(Ap)); Ap residue $J_{1'2'}$ = 7.0 Hz, $J_{2'3'}$ = 4.7 Hz, $J_{3'4'}$ = 2.3 Hz, $J_{3'P}$ = 7.0 Hz, $J_{4'5'}$ = 3.1 Hz, $J_{4'5''}$ = 3.3 Hz, $J_{5'5''}$ = −13.0 Hz; pC residue $J_{1'2'}$ = 3.3 Hz, $J_{2'3'}$ = 5.3 Hz, $J_{3'4'}$ = 6.6 Hz, $J_{4'5'}$ = 2.3 Hz, $J_{4'5''}$ = 5.0 Hz, $J_{5'P}$ = 5.7 Hz, $J_{5''P}$ = 6.0 Hz, $J_{5'5''}$ = −11.8 Hz. **(*R*$_P$)-18**: ^{31}P NMR (D_2O) δ −0.43; ^{1}H NMR (D_2O) δ 3.26 (1 H, s, 2′-OCH_3), 3.74 (1 H, dd, $H_{5''}$(Ap)), 3.76 (1 H, dd, $H_{5'}$(Ap)), 3.84 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.20 (2 H, m, $H_{3'}$(pC)/$H_{4'}$(pC)), 4.22 (1 H, dd, $H_{2'}$(pC)), 4.35 (1 H, ddd, $H_{5''}$(pC)), 4.43 (1 H, m, $H_{4'}$(Ap)), 4.44 (1 H, ddd, $H_{5'}$(pC)), 5.17 (1 H, ddd, $H_{3'}$(Ap)), 5.77 (1 H, d, $H_{1'}$(pC)), 5.83 (1 H, d, H_5(pC)), 6.01 (1 H, d, $H_{1'}$(Ap)), 7.55 (1 H, d, H_6(pC)), 8.03 (1 H, s, H_2(Ap)), 8.12 (1 H, s, H_8(Ap)), $H_{2'}$(Ap) resides under the HDO peak; Ap residue $J_{1'2'}$ = 7.0 Hz, $J_{2'3'}$ = 4.7 Hz, $J_{3'4'}$ = 2.2 Hz, $J_{3'P}$ = 7.0 Hz, $J_{4'5'}$ = 2.9 Hz, $J_{4'5''}$ = 3.1 Hz, $J_{5'5''}$ = −12.9 Hz; pC residue $J_{1'2'}$ = 3.3 Hz, $J_{2'3'}$ = 5.2 Hz, $J_{3'4'}$ = 6.6 Hz, $J_{4'5'}$ = 1.7 Hz, $J_{4'5''}$ = 4.4 Hz, $J_{5'P}$ = 5.9 Hz, $J_{5''P}$ = 6.3 Hz, $J_{5'5''}$ = −11.8 Hz.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-4-*N*-(9-fluorenylmethoxycarbonyl)cytidyl-(3′→5′)-2′,3′-di-*O*-levulinoyl-2-*N*-(9-fluorenylmethoxycarbonyl)guanosine *O*-(Methyl phosphate) (13). To a solution of compound **3a** (0.44 g, 0.59 mmol) in 3.0 mL of CH_2Cl_2 were added 1*H*-tetrazole (0.59 mL of a 0.50 M solution in CH_3CN, 0.29 mmol) and a solution of bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.19 g, 0.72 mmol) in 0.5 mL of CH_2Cl_2, and the reaction mixture was stirred for 1 h. ^{31}P NMR spectroscopy revealed quantitative conversion into **4a** ($CDCl_3$: δ 151.0 and 150.8). Then a solution of **8c** (0.43 g, 0.61 mmol) in 4 mL of CH_2Cl_2 and an extra portion of 1*H*-tetrazole (3.20 mL of a 0.5 M solution in CH_3CN, 1.60 mmol) were transferred into the reaction vessel and the mixture was stirred for another $^1/_2$ h. ^{31}P NMR showed the complete conversion into the corresponding phosphite triester ($CDCl_3$: δ 141.1 and 141.0). After addition of tBuOOH (2.0 mL) and 10 min of stirring, ^{31}P NMR showed complete conversion into the phosphate triester function. All volatiles were removed by coevaporation with toluene (four times) and chloroform (three times), and the crude product was purified by column chromatography, using a mixture of CH_2Cl_2 and CH_3OH ($5^1/_2$ vol %) as eluent (R_f = 0.20). This afforded 0.46 g (51%) of pure **13** as a white solid, which decomposed upon heating (approximately 94 °C): ^{31}P NMR ($CDCl_3$) δ −1.2 and −1.4; ^{1}H NMR ($CDCl_3$) δ 2.20 (6 H, 4 × s, 2 × CH_3 Lev), 2.4–2.9 (8 H, m, 2 × CH_2CH_2 Lev), 3.53 (2 H, m, $H_{5'}/H_{5''}$(Cp)), 3.63 (3 H, 2 × s, 2′-OCH_3), 3.72 (3 H, 2 × s, CH_3O MMTr), 3.75 and 3.83 (3 H, 2 × d, $POCH_3$, J = 11.4 Hz), 3.7–3.9 (11 H, $H_{2'}$-(Cp)/$H_{4'}$(Cp)/$H_{4'}$(pG)/$H_{5'}$(Cp)/$H_{5''}$(Cp)/2 × CH_2 Fmoc/2 × CH Fmoc), 5.03 (1 H, 2 × dd, $H_{3'}$(Cp)), 5.55 (1 H, dd, $H_{3'}$(pG)), 5.68 (1 H, dd, $H_{2'}$(pG)), 5.95 (2 H, 4 × d, $H_{1'}$(Cp)/$H_{1'}$(pG)), 6.64 (1 H, d, H_5(Cp)), 6.73 and 6.82 (2 H, 2 × d, arom MMTr), 7.2–7.9 (28 H, m, arom Fmoc/arom MMTr), 8.32 (1 H, 2 × d, H_6(Cp)), 8.61 (1 H, s, H_8(pG)).

2′-*O*-Methylcytidyl-(3′→5′)-guanosine *O*-(Methyl phosphate) (19). Compound **13** (0.20 g, 0.13 mmol) was stirred in 20.0 mL of a 0.05 M solution of K_2CO_3 in CH_3OH (1.00 mmol). After cleavage of both Lev and Fmoc groups, which required 2 h of stirring, the clear solution was neutralized (to pH ≈ 6) by careful addition of small portions of Dowex-H^+, filtered over a

glass filter, and evaporated to dryness: R_f ($CHCl_3/CH_3OH$ 3:2 v/v) = 0.21; ^{31}P NMR (CD_3OD) δ 0.13 and 0.03. Detritylation as described for **16** yielded **19** as a white solid (60 mg, 74%), R_f = 0.09 ($CHCl_3/CH_3OH$ 1:1 v/v). **(S_P)-19**: ^{31}P NMR (D_2O) δ −0.25; ^1H NMR (D_2O) δ 3.38 (3 H, s, 2′-OCH_3), 3.64 (1 H, dd, $H_{5''}$(Cp)), 3.71 (3 H, d, $POCH_3$, J = 11.4 Hz), 3.72 (1 H, dd, $H_{5'}$(Cp)), 4.04 (1 H, t, $H_{2'}$(Cp)), 4.13 (1 H, m, $H_{4'}$(Cp)), 4.28 (1 H, m, $H_{4'}$(pG)), 4.34 (1 H, ddd, $H_{5'}$(pG)), 4.38 (1 H, ddd, $H_{5''}$(pG)), 4.42 (1 H, t, $H_{3'}$(pG)), 5.80 (1 H, d, $H_{1'}$(pG)), 5.84 (1 H, d, $H_{1'}$(Cp)), 7.65 (1 H, d, H_6(Cp)), 7.84 (1 H, s, H_8(pG)); the $H_{2'}$(pG) and $H_{3'}$(Cp) signals reside under the HDO peak; Cp residue $J_{1'2'}$ = 4.5 Hz, $J_{2'3'}$ = 5.2 Hz, $J_{3'4'}$ = 5.1 Hz, $J_{4'5'}$ = 3.0 Hz, $J_{4'5''}$ = 3.7 Hz, $J_{5'5''}$ = −12.9 Hz; pG residue $J_{1'2'}$ = 4.9 Hz, $J_{2'3'}$ = 5.0 Hz, $J_{3'4'}$ = 5.0 Hz, $J_{4'5'}$ = 2.6 Hz, $J_{4'5''}$ = 5.4 Hz, $J_{5'P}$ = 5.7 Hz, $J_{5''P}$ = 5.4 Hz, $J_{5'5''}$ = −11.5 Hz. **(R_P)-19**: ^{31}P NMR (D_2O) δ −0.46; ^1H NMR (D_2O) δ 3.37 (3 H, s, 2′-OCH_3), 3.57 (1 H, dd, $H_{5''}$(Cp)), 3.60 (1 H, dd, $H_{5'}$(Cp)), 3.74 (3 H, d, $POCH_3$, J = 11.4 Hz), 3.99 (1 H, m, $H_{4'}$(Cp)), 4.06 (1 H, t, $H_{2'}$(Cp)), 4.25 (1 H, m, $H_{4'}$(pG)), 4.34 (2 H, m, $H_{5'}/H_{5''}$(pG)), 4.52 (1 H, t, $H_{3'}$(pG)), 5.82 (1 H, d, $H_{1'}$(pG)), 5.84 (1 H, d, $H_{1'}$(Cp)), 7.65 (1 H, d, H_6(Cp)), 7.85 (1 H, s, H_8(pG)); the $H_{2'}$(pG) and $H_{3'}$(Cp) signals reside under the HDO peak; Cp residue $J_{1'2'}$ = 4.5 Hz, $J_{2'3'}$ = 5.3 Hz, $J_{3'4'}$ = 4.0 Hz, $J_{4'5'}$ = 3.3 Hz, $J_{4'5''}$ = 3.6 Hz, $J_{5'5''}$ = −12.8 Hz; pG residue $J_{1'2'}$ = 5.3 Hz, $J_{2'3'}$ = 5.5 Hz, $J_{3'4'}$ = 5.5 Hz, $J_{4'5'}$ = 2.6 Hz, $J_{4'5''}$ = 5.4 Hz, $J_{5'P}$ = 5.7 Hz, $J_{5''P}$ = 5.4 Hz.

5′-*O*-(4-Monomethoxytrityl)-2′-*O*-methyl-6-*N*-(9-fluorenylmethoxycarbonyl)adenylyl-(3′→5′)-2-*N*-(9-fluorenylmethoxycarbonyl)-2′,3′-di-*O*-levulinoylguanosine *O*-(Methyl phosphate) (14). 1*H*-Tetrazole (0.60 mL of a 0.50 M solution in CH_3CN, 0.30 mmol) and a solution of bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.17 g, 0.65 mmol) in 0.5 mL of CH_2Cl_2 were added to a solution of compound **3b** (0.44 g, 0.57 mmol) in 4.0 mL of CH_2Cl_2 and the reaction mixture was stirred for $1^1/_2$ h. Formation of the phosphoramidite coupling synthon **4b** was evident from the ^{31}P NMR spectrum ($CDCl_3$: δ 152.0 and 151.1). Then a solution of compound **8c** (0.41 g, 0.58 mmol) in 5.0 mL of CH_2Cl_2 and 1*H*-tetrazole (2.50 mL of a 0.50 M solution in CH_3CN, 1.25 mmol) were added to the reaction mixture and stirring was continued for 40 min. ^{31}P NMR analysis showed the complete conversion of **4b** into the corresponding phosphite triester ($CDCl_3$: δ 142.0 and 141.2), which was readily oxidized through the addition of tBuOOH (2.0 mL) and 10 min of stirring. The mixture was evaporated to dryness and coevaporated with toluene (four times) and CH_2Cl_2 (three times). The crude product was purified by column chromatography using a gradient of CH_3OH (4 → 6 vol %) in CH_2Cl_2 as eluent, R_f = 0.31 (CH_2Cl_2/CH_3OH 96:4 v/v). Pure **14** (0.62 g, 70%) was obtained as a white solid, which decomposed upon heating (approximately 115 °C): ^{31}P NMR ($CDCl_3$) δ −0.8 and −1.4; ^1H NMR ($CDCl_3$) δ 2.14 (6 H, 4 × s, 2 × CH_3 Lev), 2.4–2.7 (2 H, m, $H_{2'}$(Ap)/$H_{2'}$(pG)), 2.7–2.9 (8 H, m, 2 × CH_2CH_2 Lev), 3.36 (3 H, 2 × s, 2′-OCH_3), 3.49 (2 H, m, $H_{5'}$(Ap)/$H_{5''}$(Ap)), 3.69 (3 H, 2 × s, CH_3O MMTr), 3.82 and 3.88 (3 H, 2 × d, $POCH_3$, J = 11.4 Hz), 4.00 (1 H, m, $H_{4'}$(Ap)), 4.3–4.7 (9 H, m, $H_{4'}$(pG)/$H_{5'}$(pG)/$H_{5''}$(pG)/2 × CH_2 Fmoc/2 × CH Fmoc), 4.88 (1 H, dd, $H_{2'}$(Ap)), 5.02 (1 H, m, $H_{3'}$(Ap)), 5.19 (1 H, m, $H_{2'}$(pG)), 5.68 (1 H, 2 × dd, $H_{3'}$(pG)), 5.9–6.2 (2 H, 4 × d, $H_{1'}$(Ap)/$H_{1'}$(pG)), 6.70 (2 H, 2 × d, arom MMTr), 7.1–7.8 (28 H, m, arom Fmoc/arom MMTr), 8.0–8.1 (2 H, 2 × s, H_2(Ap)/H_8(Ap)), 8.54 (1 H, 2 × s, H_8(pG)).

2′-*O*-Methyladenylyl-(3′→5′)-guanosine *O*-(Methyl phosphate) (20). Compound **14** (226 mg, 0.15 mmol) was stirred in 12 mL of a 0.05 M solution of K_2CO_3 in CH_3OH (0.60 mmol). It was seen that cleavage of both Lev groups required 10 min of stirring, while both Fmoc groups were removed after $2^1/_2$ h. After neutralization (to pH ≈ 6) by addition of small portions of Dowex-H^+ resin and filtration over a glass filter, the solution was evaporated to yield a white solid: ^{31}P NMR (CD_3OD) δ 0.37 (peaks overlap). Detritylation as described for **16** afforded **20** as a white solid (65 mg, 70%), R_f = 0.14 (CH_2Cl_2/CH_3OH 2:1 v/v). **(S_P)-20**: ^{31}P NMR (D_2O) δ −0.26; ^1H NMR (D_2O) δ 3.27 (3 H, s, 2′-OCH_3), 3.59 (2 H, d, $H_{5'}$(Ap)/$H_{5''}$(Ap)), 3.76 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.04 (1 H, m, $H_{4'}$(Ap)), 4.24 (1 H, m, $H_{4'}$(pG)), 4.31 (1 H, ddd, $H_{5''}$(pG)), 4.34 (1 H, ddd, $H_{5'}$(pG)), 4.44 (1 H, ddd, $H_{2'}$(Ap)), 4.53 (1 H, dd, $H_{3'}$(pG)), 5.02 (1 H, ddd, $H_{3'}$(Ap)), 5.74 (1 H, d, $H_{1'}$(Ap)), 5.79 (1 H, d, $H_{1'}$(pG)), 7.82 (1 H, s, H_8(pG)), 8.03 (1 H, s, H_2(Ap)), 8.12 (1 H, s, H_8(Ap)); $H_{2'}$(pG) resides under the HDO peak; Ap residue $J_{1'2'}$ = 7.4 Hz, $J_{2'3'}$ = 4.7 Hz, $J_{3'4'}$ = 1.7 Hz, $J_{3'P}$ = 7.0 Hz, $J_{4'5'}$ = 2.9 Hz, $J_{4'5''}$ = 2.9 Hz; pG residue $J_{1'2'}$ = 3.7 Hz, $J_{2'3'}$ = 5.7 Hz, $J_{3'4'}$ = 5.7 Hz, $J_{4'5'}$ = 2.9 Hz, $J_{4'5''}$ = 4.0 Hz, $J_{5'P}$ = 5.8 Hz, $J_{5''P}$ = 5.8 Hz, $J_{5'5''}$ = −11.7 Hz. **(R_P)-20**: ^{31}P NMR (D_2O) δ −0.38; ^1H NMR (D_2O) δ 3.34 (3 H, s, 2′-OCH_3), 3.65 (1 H, dd, $H_{5''}$(Ap)), 3.68 (1 H, dd, $H_{5'}$(Ap)), 3.75 (3 H, d, $POCH_3$, J = 11.4 Hz), 4.27 (1 H, m, $H_{4'}$(pG)), 4.30 (1 H, m, $H_{4'}$(Ap)), 4.40 (2 H, m, $H_{5'}$-(pG)/$H_{5''}$(pG)), 4.42 (1 H, dd, $H_{3'}$(pG)), 4.52 (1 H, ddd, $H_{2'}$(Ap)), 5.09 (1 H, ddd, $H_{3'}$(Ap)), 5.78 (1 H, d, $H_{1'}$(pG)), 5.95 (1 H, d, $H_{1'}$(Ap)), 7.81 (1 H, s, H_8(pG)), 8.11 (1 H, s, H_2(Ap)), 8.23 (1 H, s, H_8(Ap)); $H_{2'}$(pG) resides under the HDO peak; Ap residue $J_{1'2'}$ = 6.6 Hz, $J_{2'3'}$ = 4.6 Hz, $J_{3'4'}$ = 2.7 Hz, $J_{3'P}$ = 7.2 Hz, $J_{4'5'}$ = 3.1 Hz, $J_{4'5''}$ = 3.3 Hz, $J_{5'5''}$ = −13.0 Hz; pG residue $J_{1'2'}$ = 4.6 Hz, $J_{2'3'}$ = 5.3 Hz, $J_{3'4'}$ = 5.3 Hz, $J_{4'5'}$ = 2.9 Hz, $J_{4'5''}$ = 4.0 Hz, $J_{5'P}$ = 5.8 Hz, $J_{5''P}$ = 5.8 Hz.

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Supplementary Material Available: ^1H NMR spectra of all title compounds, CD spectra of **16–20**, and HPLC diagrams of **15–20** (61 pages). Ordering information is given on any current masthead page.