Merck silica gel, 230–400 mesh, was used for flash chromatography, and Whatman Magnum-9 Partisil-10 and ODS-3 columns were used for preparative HPLC.

Isolation and Extraction. Fresh leaves of *C. dichogamus* were collected in Kenya in mid-1987 (a voucher sample has been deposited at the herbarium in the Botany Dept., UBC). The air-dried leaves (225 g) were soaked in methanol (500 mL) overnight. The methanol extract was concentrated in vacuo to a gum, water was added, and the resulting suspension was exhaustively extracted with CH_2Cl_2. Evaporation of the combined organic extracts in vacuo gave a residue, which was fractionated via silica gel flash chromatography (step gradient: CH_2Cl_2 to EtOAc) to give crude crotoxides A (1) and B (2).

Crotoxide A (1). The flash fractions containing crude crotoxide A (1) were combined and further purified by silica gel preparative TLC (2:1 CH_2Cl_2/EtOAc). The preparative TLC fraction containing crotoxide A was dissolved in methanol (5 mL), palladium on charcoal was added (20 mg), and the suspension was stirred at room temperature under 1 atm of hydrogen for 2 days. Filtration of the reaction mixture, followed by vacuum evaporation of the filtrate, gave an oily residue, which was fractionated on preparative silica gel HPLC (9:1 CH_2Cl_2/EtOAc) to give pure crotoxide A (1) (yield 0.005% dry wt.): colorless needles (hexane), mp 149–152 °C; $[\alpha]^{25}_D$ +113° ($CHCl_3$, *c* 0.2); IR ($CHCl_3$) 3477 cm^{-1}; ^1H NMR see Table I; ^{13}C NMR see Table II; HREIMS M^+ 330.1837 ($C_{20}H_{26}O_4$) (ΔM + 0.6 mmu); LRMS *m/z* (relative intensity) 330 (62), 315 (20), 297 (9), 189 (42), 43 (100).

Crotoxide B (2). Crotoxide B (2) was partially purified by using the same flash, preparative TLC, and hydrogenation procedures described above for crotoxide A (1). Preparative reverse-phase HPLC (1:10 H_2O/CH_3CN) of the hydrogenation mixture residue gave pure crotoxide B (2) as an unstable white powder. 2: IR ($CHCl_3$) 3477, 1742, 1455, 1235 cm^{-1}; ^1H NMR see Table I; HREIMS M^+ 386.1742 ($C_{22}H_{26}O_6$) (ΔM + 1.3 mmu).

X-ray Analysis of Crotoxide A (1). The crystals grown from hexane were suitable for single-crystal X-ray analysis, and a specimen roughly 0.3 × 0.3 × 0.2 was mounted on a glass fiber. From preliminary X-ray photographs, the crystals were unambiguously assigned to space group $P2_12_12_1$ with cell constants of a = 6.402 (3) Å, b = 15.495 (6) Å, and c = 19.619 (6) Å determined from a least-squares fit of 25 2θ values. One molecule of composition $C_{20}H_{26}O_4$ formed the asymmetric unit giving a crystal density of 1.25 g/cm^3. All unique diffraction maxima with $2\theta \leq 114°$ were collected by using variable-width θ–2θ scans. Of the 1385 symmetry-unique reflections, 1293 (93%) were judged observed ($|F_o| > 3\sigma(F_o)$) after correction for Lorentz, background, and polarization effects. A phasing model was easily found by using the SHELX library of programs, and full-matrix least-squares refinements with anisotropic non-hydrogen atoms and riding hydrogen atoms smoothly converged to a conventional crystallographic residual of 0.050 for the observed reflections. Additional crystallographic details are available and are described in the paragraph entitled "Supplementary Material Available" at the end of this paper.

Acknowledgment. Financial support at UBC was provided by NSERC grants to R.J.A. and A.R.E.S. Financial support at Cornell University was provided by the NIH (CA24487) and the New York State Sea Grant. M.K.J. was on leave from the University of the South Pacific, Suva, Fiji, and L.P. was on leave from the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.

Supplementary Material Available: Tables 1–5 of fractional coordinates, thermal parameters, bond distances, and bond angles from the X-ray analysis of crotoxide A (1) (5 pages). Ordering information is given on any current masthead page.

Synthesis of Phosphate-Methylated DNA Fragments Using 9-Fluorenylmethoxycarbonyl as Transient Base Protecting Group

Leo H. Koole,*[†] Harold M. Moody,[†] Niek L. H. L. Broeders,[†] Peter J. L. M. Quaedflieg,[†] Will H. A. Kuijpers,[†] Marcel H. P. van Genderen,[†] Annie J. J. M. Coenen,[‡] Sjoerd van der Wal,[‡] and Henk M. Buck[†]

Department of Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands, and DSM Research Laboratories, Geleen, The Netherlands

Received October 4, 1988

Synthesis of the phosphate-methylated DNA dinucleotides d(CpG) (4), d(GpC) (5), d(ApC) (6), d(ApT) (7), d(ApA) (8), and d(CpC) (9) is described. The 9-fluorenylmethoxycarbonyl (Fmoc) group was used for the protection of the amino groups of the bases A, C, and G during the synthesis. In contrast to routinely used benzoyl or isobutyryl groups, Fmoc could be easily removed without cleavage of the methyl phosphotriester group. Initially, the systems 4–9 were obtained as mixtures of R_p and S_p diastereoisomers. These were separated on a milligram scale with reversed-phase HPLC. Use of Fmoc is regarded as the method of choice for the preparation of phosphate-methylated DNA fragments. Fmoc synthesis of longer phosphate-methylated DNA's will open the possibility to evaluate the utility of these neutral DNA analogues as antisense matagens for inhibition of DNA replication and transcription in vitro and in vivo.

Introduction

A survey of the current literature shows that there is a growing interest in nucleic acid analogues possessing modified internucleoside linkages. Two types of modifications can be distinguished: (i) oligonucleotide alkyl phosphotriesters[1] and (ii) oligonucleotide methyl phosphonates.[2] Both modifications correspond with a neutral backbone structure, which leads to interesting chemical and biological properties. These include formation of stable hydrogen-bonded complexes with complementary

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

compd	modifier	%	pH	K'_{R_p}	α
4	acetonitrile	6	7.1	8.6	1.14
5	acetonitrile	8	7.1	8.5	1.12
6	methanol	25	5.2	5.8	1.08
7	methanol	30	4.2	8.2	1.19
8	acetonitrile	17	5.0	8.0	1.10
9	acetonitrile	13	3.5	2.6	1.08

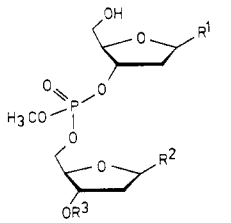
[†] Eindhoven University of Technology.
[‡] DSM Research.

0022-3263/89/1954-1657$01.50/0

polynucleotides, easy transport into bacterial and mammalian cells, and resistance to hydrolysis by cellular nucleases. It is recognized that sequence-specific neutral DNA fragments are potentially useful for blocking gene expression (i.e., DNA replication or m-RNA translation). This so-called matagen approach[2b] (*ma*sking *ta*pe for *gene* expression) provides a new way to design cytostatic or antiviral agents in a rational manner. Hitherto, methyl phosphonate DNA analogues have received most attention. These systems can now be synthesized automatically on a solid-state support,[2c] and detailed knowledge exists concerning the specific inhibitory effects on growth of living cells and cellular protein synthesis.[3,4]

Our interests primarily concern *methyl phosphotriester* analogues of DNA. Our previous work has shown that methyl phosphotriesters preferentially adopt a conformation which closely resembles B DNA,[1a-d] whereas this is not the case for methyl phosphonate systems. Consequently the hybrids of methyl phosphotriester DNA–natural DNA are substantially more stable than the hybrids methyl phosphonate DNA–natural DNA.[5] This difference becomes apparent especially for elongation of the hybridization site. We have also found that the methyl phosphotriester group is perfectly accommodated in the B DNA double helix, both for the R_p configuration (methyl oriented in the major groove of the duplex), and the S_p configuration (methyl protruding from the double helix into the solvent bulk).[1c,d] This was most evident for the phosphate-methylated trinucleotide d(ApApA), which consists of four diastereoisomers. Hybridization with poly(dT) leads to a single sharp melting transition at 41 °C, showing that all diastereoisomers form an equally stable duplex.[1b] It should be noted that ethyl, propyl, or isopropyl[1e-i] phosphotriester DNA's show unfavorable steric crowding for R_p, which substantially interferes with duplex formation. Thus, comparing methyl phosphotriester DNA fragments with methyl phosphonate and other alkyl phosphotriester systems, it seems to us that the methyl phosphotriester modification leads to the closest possible structural resemblance with respect to natural DNA.

In this paper, we report a novel synthetic approach to methyl phosphotriester DNA oligomers of an arbitrary nucleotide sequence. Initially,[1b] we have synthesized phosphate-methylated d(ApA), d(ApApA), and d(ApApApA) with the amidine 6-*N*-(1-(dimethylamino)ethylene) as protective group for the 6-NH_2 group of A.[6] However, workup of the final product proved to be troublesome because of a large excess of phenol which is necessary for the deprotection of the bases. Our present synthetic method is based on the use of 9-fluorenylmethoxycarbonyl (Fmoc)[7-9] for protection of the bases A, C, and G during the synthesis of phosphate-methylated DNA fragments. The advantage of Fmoc over the customary acyl blocking groups for A, C, and G is that its removal in the final stage of the synthesis can be accomplished *under conditions that leave methylated phosphate intact*. We describe the synthesis of the phosphate-methylated dinucleotides d(CpG) (**4**), d(GpC) (**5**), d(ApC) (**6**), d(ApT) (**7**), d(ApA) (**8**), and d(CpC) (**9**).[10] The R_p and S_p diastereoisomers of these systems were separated with reversed-phase HPLC.



4 : R^1 = 1-cytidyl ; R^2 = 9-guanyl ; R^3 = H
5 : R^1 = 9-guanyl ; R^2 = 1-cytidyl ; R^3 = H
6 : R^1 = 9-adenyl ; R^2 = 1-cytidyl ; R^3 = H
7 : R^1 = 9-adenyl ; R^2 = 1-thymyl ; R^3 = Ac
8 : R^1 = 9-adenyl ; R^2 = 9-adenyl ; R^3 = Ac
9 : R^1 = 1-cytidyl ; R^2 = 1-cytidyl ; R^3 = Ac

Synthesis

The synthesis of **4–9** in diastereoisomerically pure form comprises three essential steps, which can be summarized as follows: (1) protection of the base amino groups of dC, dG, or dA with Fmoc and tritylation of the 5′-OH groups; (2) coupling of two Fmoc protected nucleosides via an in situ generated phosphoramidite synthon; and (3) removal of Fmoc and 5′-trityl 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. The 3′,5′-bis(trimethylsilyl) derivatives of dC, dG, and dA[11] were reacted with 9-fluorenylmethoxycarbonyl chloride. Subsequent deprotection of the 3′- and 5′-OH groups readily furnished the Fmoc protected nucleosides as white amorphous solids (yields: **1a** (dC-Fmoc), 97%; **1b** (dG-Fmoc), 71%; **1c** (dA-Fmoc), 46%).[12] For the

(1) (a) Koole, L. H.; van Genderen, M. H. P.; Buck, H. M. *J. Am. Chem. Soc.* **1987**, *109*, 3916. (b) Koole, L. H.; van Genderen, M. H. P.; Reiniers, R. G.; Buck, H. M. *Proc. K. Ned. Akad. Wet., Ser. B* **1987**, *90*, 41. (c) Van Genderen, M. H. P.; Koole, L. H.; Aagaard, O. M.; van Lare, C. E. J.; Buck, H. M. *Biopolymers* **1987**, *26*, 1447. (d) Van Genderen, M. H. P.; Koole, L. H.; Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 28. (e) Gallo, K. A.; Shao, K.; Phillips, L. R.; Regan, J. B.; Koziolkiewicz, M.; Uzanski, B.; Stec, W. J.; Zon, G. *Nucl. Acids Res.* **1986**, *14*, 7405. (f) Pramanik, P.; Kan, L. *Biochemistry* **1987**, *26*, 3807. (g) Zon, G.; Summers, M. F.; Gallo, K. A.; Shao, K.-L.; Koziolkiewicz, M.; Uznanski, B.; Stec, W. J. In *Biophosphates and Their Analogues*; Elsevier: Amsterdam, 1987. (h) Miller, P. S.; Fang, K. N.; Kondo, N.-S.; Ts'o, P. O. P. *J. Am. Chem. Soc.* **1971**, *93*, 6657. (i) Miller, P. S.; Chandrasegaran, S.; Dow, D. L.; Pulford, S. M.; Kan, L. S. *Biochemistry* **1982**, *21*, 5468.

(2) (a) Lee, B. L.; Murakami, A.; Blake, K. R.; Lin, S.-B.; Miller, P. S. *Biochemistry* **1988**, *27*, 3197. (b) Miller, P. S.; Ts'o, P. O. P. *Anti-Cancer Drug Des.* **1987**, *2*, 117. (c) Miller, P. S.; Reddy, M. P.; Murakami, A.; Blake, K. R.; Lin, S.-B.; Agris, C. H. *Biochemistry* **1986**, *25*, 5092. (d) Blake, K. R.; Murakami, A.; Spitz, S. A.; Glave, S. A.; Reddy, M. P.; Ts'o, P. O. P.; Miller, P. S. *Biochemistry* **1985**, *24*, 6139. (e) Miller, P. S.; Agris, C. H.; Blake, K. R.; Murakami, A.; Spitz, S. A.; Reddy, M. P.; Ts'o, P. O. P. In *Nucleic Acids: The Vectors of Life*; Jerusalem Symp. Quantum Chem. *Biochem.* **1983**, *16*, 521.

(3) It has been shown that oligonucleotide methyl phosphonates can inhibit mRNA translation in mammalian cells and in certain bacterial cells. Furthermore, phosphonate systems complementary to the splice junctions of SV 40 and herpes simplex virus pre-mRNA are capable of selective inhibition of the synthesis of viral proteins in virus-infected cells. See ref 4.

(4) Smith, C. C.; Aurelian, L.; Reddy, M. P.; Miller, P. S.; Ts'o, P. O. P. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2787.

(5) Van Genderen, M. H. P.; Koole, L. H.; Merck, K. B.; Meijer, E. M.; Sluyterman, L. A. AE.; Buck, H. M. *Proc. K. Ned. Akad. Wet., Ser. B* **1988**, *90*, 155.

(6) McBride, L. J.; Kierzek, S. L.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040.

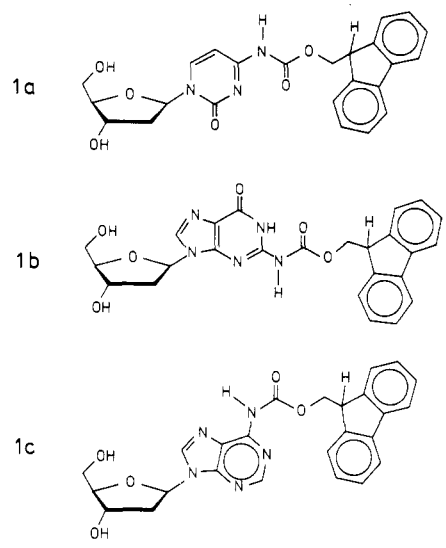
(7) It is noteworthy that examples of the use of Fmoc in nucleotide chemistry are only found in recent literature while application of Fmoc is quite common in peptide synthesis. See: Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748.

(8) (a) Gioeli, C.; Chattopadhyaya, J. *J. Chem. Soc., Chem. Commun.* **1982**, 672. (b) Heikkila, J.; Chattopadhyaya, J. *Acta Chem. Scand., Ser. B* **1983**, *37*, 263.

(9) (a) Webb, T. R.; Matteucci, M. D. *Nucl. Acids Res.* **1986**, *14*, 7661. (b) Happ, E.; Scalfi-Happ, C.; Chládek, S. *J. Org. Chem.* **1987**, *52*, 5387.

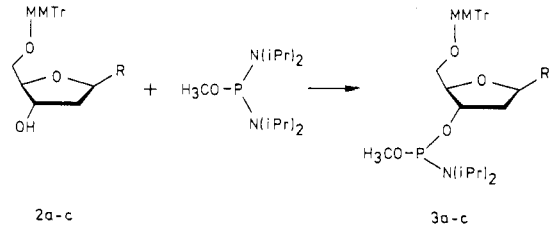
(10) To our knowledge, only **8** has been described before. See ref 1h. However, ref 1h refers to **8** as an unresolved mixture of R_p and S_p diastereoisomers.

(11) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.



subsequent protection of the 5′-OH group we used the 4-monomethoxytrityl (MMTr) group, which could be introduced under standard reaction conditions[13] (yields: **2a** (dC-Fmoc, 5′-MMTr), 70%; **2b** (dG-Fmoc, 5′-MMTr), 86%; **2c** (dA-Fmoc, 5′-MMTr), 50%).

Step 2. Our synthetic approach for the 3′–5′ coupling reactions is largely based on recent papers by Moore et al.[14] and Marugg et al.,[15] in which it is shown that bifunctional phosphitylating agents are very effective for the in situ preparation of nucleoside 3′-phosphoramidites. The underlying principle is that alkoxybis(dialkylamino)phosphines are selectively activated by 1*H*-tetrazole. The advantage of this procedure is that chromatographic isolation of the reactive 3′-phosphoramidites is circumvented. We have now reacted the compounds **2a–c** with methoxybis(diisopropylamino)phosphine in the presence of 0.5 equiv of 1*H*-tetrazole in dry pyridine. By use of ^{31}P NMR, it was concluded that quantitative formation of the desired **3a–c** in situ occurs within several minutes. Furthermore, it



appeared that the reaction proceeds equally well in various media; we have routinely used dry pyridine as the solvent. The diastereoisomeric nature of **3a–c** results in two distinct absorptions around δ 150 ppm in the ^{31}P NMR spectrum. The chemical shift difference between these lines amounts to approximately 0.3 ppm (vide infra). In the synthesis of **4–6** we coupled the in situ phosphoramidites **3a–c** directly with the corresponding Fmoc-protected nucleosides. Thus, **3a** was fused with **1b**, **3b** with **1a**, and **3c** with **1a**. These reactions required addition of an additional quantity

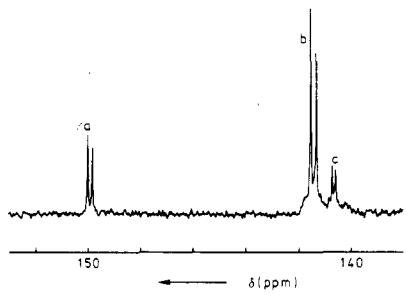


Figure 1. ^{31}P NMR spectrum of the reaction mixture of the coupling of the phosphoramidite synthon **3c** with **1a** (dC-Fmoc) in the synthesis of **6** (phosphate-methylated d(ApC)). Peaks a correspond to the R_p and S_p diastereoisomers of the phosphoramidite, peaks b denote the R_p and S_p 3′–5′ phosphite triester (desired structures), peaks c correspond to the wrongly coupled (i.e. 3′–3′) phosphite triester. The spectrum clearly shows that 3′–5′ coupling is favored over 3′–3′ coupling (ratio 5:1, see text).

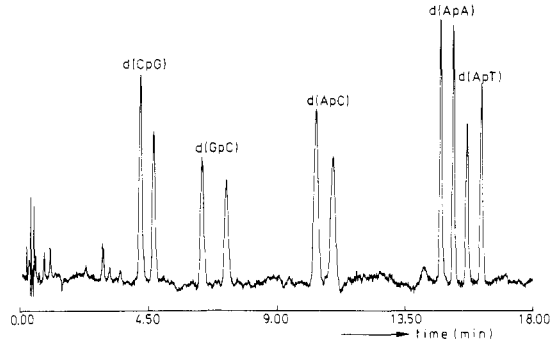


Figure 2. Chromatogram of the R_p and S_p diastereoisomers of the phosphate-methylated dimers **4–8** (simultaneous elution).[19] Detection: absorption at 260 nm.

of 1*H*-tetrazole. In all three cases, ^{31}P NMR spectroscopy showed the formation of the desired 3′–5′ phosphite triester as well as the undesired 3′–3′ phosphite triester in the ratio of ca. 5:1 (Figure 1). These observations are in accord with the work of Marugg et al.[15] who prepared a set of natural DNA dimers with an unprotected 3′-OH group according to the in situ phosphoramidite method. Complete specificity for 3′–5′ coupling is realized when a 3′-protected nucleoside is used. In the synthesis of **7–9**, we coupled phosphoramidites with the corresponding 3′-*O*-acetyl, Fmoc-protected nucleosides. The choice of the 3′-*O*-acetyl group was based on our experience that nucleotide structures with acetylated 3′- and/or 5′-OH groups may readily form single crystals suitable for X-ray diffraction.[16] Further work in this direction is currently in progress. It should be noted that the 3′-*O*-acetyl group is in fact incompatible with the methyl phosphotriester group, i.e. full deprotection of **7–9** cannot be executed. All phosphite triesters were oxidized through reaction with *tert*-butyl hydroperoxide. This reaction proceeded smoothly without byproducts within several minutes, as was evident from the ^{31}P NMR spectra.

Step 3. Detritylation was accomplished through treatment with either acetic acid or zinc bromide.[17] Then, reaction with excess triethylamine in pyridine quantitatively removed the Fmoc groups. Reversed-phase HPLC was used to separate the R_p and S_p diastereoisomers of **4–9** on a milligram scale. For this, we optimized and modified

(12) The preparation of dA-Fmoc (**1c**) is slightly complicated since the (6-*N*,6-*N*) doubly protected derivative (dA-(Fmoc)$_2$) is formed as well. The yield of dA-Fmoc could be raised by treatment of the crude mixture of dA-Fmoc and dA-(Fmoc)$_2$ with 1:1 (v/v) water/pyridine at room temperature for 16 h. This resulted in partial conversion of dA-(Fmoc)$_2$ into dA-Fmoc. Column chromatography on silica gel using 2-butanone as eluent easily separated dA-(Fmoc)$_2$ (R_f 0.53) from dA-Fmoc (R_f 0.14).

(13) Gait, M. J. In *Oligonucleotide Synthesis, a Practical Approach*; IRL Press: Oxford–Washington, DC, 1984.

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

(15) Marugg, J. E.; Nielsen, J.; Dahl, O.; Burik, A.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1986**, *106*, 72.

(16) (a) Koole, L. H.; Buck, H. M.; Kanters, J. A.; Schouten, A. *Can. J. Chem.* **1987**, *65*, 326. (b) Koole, L. H.; Buck, H. M.; Kanters, J. A.; Schouten, A. *Can. J. Chem.* **1988**, *66*, 2634.

(17) Herdering, W.; Kehne, A.; Seela, F. *Helv. Chim. Acta* **1985**, *68*, 2119.

Table II. Vicinal Proton-Proton and Proton-Phosphorus Spin-Spin Coupling Constants, Measured in D_2O for the R_p and S_p Diastereoisomers of 4–9[a]

	d(CpG) (4)				d(GpC) (5)				d(ApC) (6)				d(ApT) (7)				d(ApA) (8)		d(CpC) (9)			
	S_p		R_p		S_p		R_p		S_p		R_p		S_p		R_p		S_p		S_p		R_p	
	dCp	pdG	dCp	pdG	dGp	pdC	dGp	pdC	dAp	pdC	dAp	pdC	dAp	pdT	dAp	pdT	dAp	pdA	dCp	pdC	dCp	pdC
$J_{1',2'}$	8.4	6.6	8.1	5.6	8.6	6.8	8.3	6.6	8.5	6.7	8.4	6.7	8.7	8.0	8.2	7.6	9.2	8.4	8.0	7.4	7.6	7.2
$J_{1',2''}$	5.8	6.6	5.9	6.9	5.6	6.8	5.6	6.6	5.8	6.7	5.8	6.7	5.6	6.2	5.8	6.4	5.6	6.0	6.0	6.2	6.0	6.4
$J_{2',3'}$	6.0	6.5	6.3	6.6	5.6	6.8		6.8	5.6	7.0	5.8	6.7	5.5	7.2	6.6	7.1	5.0	6.4	7.2	7.2	7.2	7.2
$J_{2'',3'}$	2.4	4.8	2.4	5.7	2.6	4.8	2.6	4.7	2.4	4.6	2.6	4.7	2.3	3.0	2.5	3.4	1.7	2.8	2.6	3.1	3.0	3.0
$J_{2',2''}$	14.5	14.1	14.2	14.2	14.4	14.2	14.6	14.2	14.8	14.4	14.7	4.4	14.4	14.6	14.2	14.6	14.4	14.8	14.8	14.6	14.4	14.8
$J_{3',P}$									7.2								4.8		4.8		4	
$J_{3',4'}$	2.5	6.5	2.5	6.9	2.2	2.0	2.1	2.0	2.8	4.4	2.0	4.8		3.6		3.7	1.5	3.6	3.2	2.3	4.0	3.0
$J_{4',5'}$	3.9	3.6	4.1	3.6	4.2	2.6	3.8	2.6	3.3	2.6	3.4	2.6	3.5	2.3	3.5	2.5	3.8	3.0	4.0	2.2	3.6	1.8
$J_{4',5''}$	4.6	3.6	4.5	3.6	4.2	5.2	4.2	4.7	3.3	5.2	3.8	4.6	3.8	4.7	3.7	4.3	3.8	3.0	4.0	3.0	3.6	4.2
$J_{5',P}$		5.6		5.4		6.4		5.4		6.0		5.6		5.7		5.3		4.8		5.0		6.3
$J_{5'',P}$		5.6		5.4		5.2		6.4		5.2		6.2		4.7		5.9		4.8		7.2		5.9
$^4J_{POCC2'}$											1.2				1.2							

[a] In some cases, a routine simulation–iteration algorithm was used to extract precise values for coupling constants.

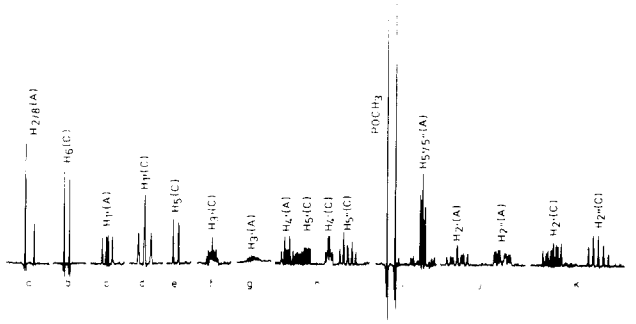


Figure 3. Expansions of the 600-MHz ^1H NMR spectrum of the S_p diastereoisomer of **6** (phosphate-methylated d(ApC)). Regions: a, 8.17–8.07 ppm; b, 7.57–7.49 ppm; c, 6.38–6.30 ppm; d, 6.15–6.09 ppm; e, 5.76–5.70 ppm; f, 5.30–5.24 ppm; g 5.17–5.09 ppm; h, 4.46–4.24 ppm; i, 3.84–3.70 ppm; j, 2.90–2.64 ppm; k, 2.38–2.16 ppm. The spectrum (32K data points, sweep width 6000 Hz) was resolution enhanced by an appropriate Gaussian multiplication.

the separation parameters as previously reported by Stec et al.[18] Table I summarizes our conditions for isocratic separation of the respective diastereoisomers. Figure 2 shows the chromatogram that was obtained upon simultaneous elution of the R_p and S_p diastereoisomers of **4–8**.

Structural Assignment

The phosphate-methylated dinucleotides **4–9** were studied with 200-, 300-, and 600-MHz ^1H NMR. As a representative example, the 600-MHz spectrum of S_p (**6**) (phosphate-methylated d(ApC)) is shown in Figure 3. Assignment of the configuration at phosphorus was performed according to the recent method of Zon et al.[20] For each of the compounds **4–9** 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 almost lacks such a contact. The structures with the strong NOE contact were assigned the R_p configuration. The set of experimental vicinal proton–proton and proton–phosphorus J-coupling constants are summarized in Table II. A detailed conformational analysis on the basis of these data will be published elsewhere.

Concluding Remarks

Considering the present results, we believe that Fmoc synthesis is the method of choice for the preparation of phosphate-methylated DNA fragments. We have already extended the method to the stepwise synthesis of trimer and tetramer phosphate-methylated DNA's. Furthermore, we have used the Fmoc method to convert longer strands of natural DNA (synthesized on 10-μmol scale on an automated DNA synthesizer) as a whole into their phosphate-methylated congeners. Full details of these results will be published. Work is in progress to study the detailed structural and biological effects of phosphate methylation in DNA. This information will be of great value for the design of new phosphate-methylated DNA fragments, which can ultimately be used as antisense matagens in rationalized biological experiments directed toward site-specific inhibition of DNA replication and/or transcription in vitro and in vivo.

Experimental Section

Materials and Methods. The ^1H NMR spectra were recorded on Bruker AM 600,[21] CXP 300,[22] and AC 200[22] NMR spectrometers. Tetramethylsilane (TMS) 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. ^{31}P NMR spectra were recorded at 81 MHz on the AC 200 instrument and referenced against 85% H_3PO_4 as external standard. For all column chromatographic separations, we used ICN Biochemicals Silica TSC 60A. Pyridine was distilled from KOH pellets and dried on 4-Å molecular sieves. Acetonitrile was distilled from CaH_2. Nitromethane was distilled and stored on $CaCl_2$. Methanol was distilled and stored on 4-Å molecular sieves. 2-Butanone was distilled prior to use as eluent in chromatographic separations. 1*H*-Tetrazole was purified through sublimation. *tert*-Butyl hydroperoxide was used as received (75% solution in di-*tert*-butyl peroxide). Reactions were routinely run in an inert atmosphere of dry nitrogen or dry argon. Prior to each reaction step, we removed last traces of water from unprotected and protected nucleosides via coevaporation with small portions of dry pyridine. Unless otherwise noted, reactions were run at ambient temperature. Fast atom bombardment (FAB) mass spectrometry was carried out using a VG Micromass ZAB-2HF mass spectrometer, an instrument with reverse geometry, fitted with a high-field magnet and coupled to a VG 11/250 data system. 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 diastereoisomers was developed on a HP 1090 gradient HPLC system. Preparative chromatography was executed on a HPLC system consisting of a Waters M 590 solvent delivery system equipped with a solvent select valve module, an

(18) Stec, W. J.; Zon, G.; Uznanski, B. *J. Chromatogr.* **1985**, *326*, 263.
(19) Column: 100 × 4.6 mm Microsper C18; flow 3 mL/min; ambient temperature. Solvents: A, 1 mL/L acetic acid, 0.2 mL/L triethylamine adjusted to pH 6.1 with 25% NH_4OH; B, acetonitrile; C, methanol. Gradient program: A/B/C for 0, 11, 12, and 18 min is 88/0/12; 88/0/20; 79/10/11; and 75/15/10.
(20) Summers, M. F.; Powell, C.; Egan, W.; Byrd, R. A.; Wilson, W. D.; Zon, G. *Nucl. Acids Res.* **1986**, *14*, 7421.
(21) Dutch National hf NMR facility at Nijmegen, The Netherlands.
(22) NMR facility at the Eindhoven University of Technology.

Alltech RSil C18 10-μm column (250 × 22 mm), and a Waters 480 detector.

Synthesis. Bis(*N*,*N*-diisopropylamino)methoxyphosphine. Phosphorus trichloride (275.0 g, 2.00 mol) was cooled to −10 °C, and dry methanol (81.1 mL, 2.00 mol) was added dropwise over 1.5 h with stirring. The reaction flask was kept at −10 °C, and the produced hydrochloric acid was absorbed in a gas trap containing water. After distillation at atmospheric pressure a 5:1 mixture (bp 81–84 °C) of methoxydichlorophosphine (^{31}P NMR ($CDCl_3$): δ 181.5) and unreacted phosphorus trichloride (^{31}P NMR ($CDCl_3$): δ 220.2) was obtained (36.2 g). This mixture was added dropwise during 1.5 h to a solution of *N*,*N*-diisopropylamine (118.5 g, 1.17 mol) in 400 mL of dry ether, which was kept at 0 °C. Then, the ammonium salt was removed by filtration, and the solution was concentrated in vacuo. Pure bis(*N*,*N*-diisopropylamino)methoxyphosphine was obtained by distillation of the residue at 0.04 mmHg (bp 66 °C) as a colorless liquid. Yield: 55.1 g (11%). ^{1}H NMR (acetone-d_6): δ 1.26 (24 H, m, CH_3), 3.49 (3 H, d, $POCH_3$, J = 11.2 Hz), 3.67 (4 H, m, CH). ^{31}P NMR ($CDCl_3$): δ 131.6.

4-*N*-(9-Fluorenylmethoxycarbonyl)-2′-deoxycytidine (1a). 2′-Deoxycytidine (3.85 g, 16.92 mmol) was suspended in 100 mL of dry pyridine. During 5 min, chlorotrimethylsilane (10.8 mL, 84.6 mmol) was added dropwise, and the reaction mixture was stirred for 15 min.[11] Then, 9-fluorenylmethoxycarbonyl chloride (5.25 g, 20.92 mmol) was added, and the mixture was stirred for 1.5 h. Hydrolysis of the trimethylsilyl groups and excess chlorides was effected by addition of water (40 mL). After stirring overnight, a yellow solution was obtained, which was evaporated to near dryness. Upon addition of water (250 mL) a white precipitate appeared. The mixture was shaken vigorously until no more yellow oil was visible. After addition of ethyl acetate (150 mL) and shaking, a precipitate was formed on the separation layer. This solid was isolated by filtration and washed with ethyl acetate. After drying in vacuo, **1a** was obtained as a white solid. Yield: 7.38 g (97%). Mp: 132 °C. ^{1}H NMR (acetone-d_6): δ 2.40 (1 H, m, $H_{2'}$), 2.59 (1 H, m, $H_{2''}$), 3.77 (2 H, m, $H_{5'}/H_{5''}$), 4.03 (1 H, m, $H_{4'}$), 4.33 (1 H, t, CH Fmoc), 4.49 (3 H, m, $H_{3'}/CH_2$ Fmoc), 6.29 (1 H, dd, $H_{1'}$), 7.12 (1 H, d, H_5), 7.37 (4 H, m, aromatic Fmoc), 7.85 (4 H, m, aromatic Fmoc), 8.50 (1 H, d, H_6). Calculated mass: 449. FAB: $(M + H)^+$ = 450, $(M + Na)^+$ = 472.

2-*N*-(9-Fluorenylmethoxycarbonyl)-2′-deoxyguanosine (1b). Chlorotrimethylsilane (10.85 g, 100 mmol) was added dropwise to a suspension of 2′-deoxyguanosine (4.00 g, 15.0 mmol) in 150 mL of dry pyridine, and the reaction mixture was stirred for 1 h.[11] During this time the 2′-deoxyguanosine dissolved completely, and a precipitate of pyridinium hydrochloride appeared. 9-Fluorenylmethoxycarbonyl chloride (5.15 g, 20.0 mmol) was transferred into the reaction flask, and stirring was continued for 1 h. Hydrolysis of the trimethylsilyl groups and excess chlorides was effected by addition of water (15 mL), and the solution was stirred for 20 min. Then, the solution was poured into saturated aqueous sodium bicarbonate (500 mL) and extracted with three 500-mL portions of dichloromethane. The collected organic layers were concentrated in vacuo, and the last traces of pyridine were removed by coevaporation with three 250-mL portions of toluene. Dichloromethane (200 mL) was added to this residue, and after filtration, washing with dichloromethane, and drying in vacuo, **1b** was obtained as a slightly colored solid (4.88 g). The mother liquor was concentrated in vacuo, and dichloromethane (200 mL) and diethyl ether (600 mL) were added to the residue. After filtration and drying in vacuo another 0.33 g of **1b** was obtained. Total yield: 5.21 g (71%). ^{1}H NMR (DMSO-d_6): δ 2.50–2.70 (2 H, m, $H_{2'}/H_{2''}$), 3.55 (2 H, m, $H_{5'}/H_{5''}$), 3.86 (1 H, m, $H_{4'}$), 4.37 (1 H, t, CH Fmoc), 4.49 (2 H, d, CH_2 Fmoc), 4.95 (1 H, m, $H_{3'}$), 6.22 (1 H, dd, $H_{1'}$), 7.29–7.52 (4 H, m, aromatic Fmoc), 7.87 (4 H, m, aromatic Fmoc). Calculated mass: 489. FAB: $(M + H)^+$ = 490, $(M + Na)^+$ = 512.

6-*N*-(9-Fluorenylmethoxycarbonyl)-2′-deoxyadenosine (1c). 2′-Deoxyadenosine (5.0 g, 20 mmol) was suspended in 100 mL of dry pyridine. Chlorotrimethylsilane (12.8 mL, 100 mmol) was added, and the mixture was stirred for 15 min.[11] Then, 9-fluorenylmethoxycarbonyl chloride (6.2 g, 24 mmol) was added, and the reaction mixture was stirred for 2 h. Hydrolysis of the trimethylsilyl groups and excess chlorides was effected by addition of water (60 mL) at 0 °C. After stirring for 18 h, the mixture was evaporated to near dryness and coevaporated with water to remove the last traces of pyridine. Subsequently, 300 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 100-mL and 50-mL ethyl acetate portions successively. The collected organic layers were evaporated and the residue dried in vacuo, affording a white solid. NMR analysis indicated the presence of two products, namely the desired 6-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyadenosine, and 6-*N*-bis(9-fluorenylmethoxycarbonyl)-2′-deoxyadenosine (dA-$(Fmoc)_2$). In order to remove one Fmoc group from dA-$(Fmoc)_2$, the white solid was treated with a 1:1 v/v mixture of water and pyridine for 16 h. Evaporation of all volatile components yielded a white foam, which was chromatographed on a silica gel column with 2-butanone as eluent. This afforded 4.4 g (46%) of **1c** (R_f 0.14), along with 1.1 g (8%) of dA-$(Fmoc)_2$ (R_f 0.53) as white solids. ^{1}H NMR of **1c** (acetone-d_6): δ 2.59 (1 H, m, $H_{2''}$), 3.03 (1 H, m, $H_{2'}$), 3.90 (2 H, m, $H_{5'}/H_{5''}$), 4.21 (1 H, m, $H_{4'}$), 4.48 (1 H, t, CH Fmoc), 4.66 (2 H, d, CH_2 Fmoc), 4.79 (1 H, m, $H_{3'}$), 6.66 (1 H, dd, $H_{1'}$), 7.35–7.7 (4 H, m, aromatic Fmoc), 7.9–8.1 (4 H, m, aromatic Fmoc), 8.62 (1 H, s, H_2), 8.73 (1 H, s, H_8). ^{1}H NMR of dA-$(Fmoc)_2$ ($CDCl_3$): δ 2.42 (1 H, m, $H_{2''}$), 3.07 (1 H, m, $H_{2'}$), 3.93 (2 H, m, $H_{5'}/H_{5''}$), 4.08 (2 H, t, CH Fmoc), 4.34 (1 H, m, $H_{4'}$), 4.55 (4 H, d, CH_2 Fmoc), 4.85 (1 H, m, $H_{3'}$), 6.46 (1 H, dd, $H_{1'}$), 7.15–7.35 (12 H, m, aromatic Fmoc), 7.55–7.65 (4 H, m, aromatic Fmoc), 8.01 (1 H, s, H_2), 8.66 (1 H, s, H_8). Calculated mass (**1c**): 473. FAB: $(M + H)^+$ = 474, $(M + Na)^+$ = 496.

5′-*O*-(4-Monomethoxytrityl)-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxycytidine (2a). Compound **1a** (2.00 g, 4.45 mmol) was suspended in 200 mL of dry pyridine. After addition of 4-monomethoxytrityl chloride (1.51 g, 4.89 mmol), the solution was stirred for 15 h in darkness. The mixture was then poured into saturated aqueous sodium bicarbonate (60 mL) and extracted three times with dichloromethane. The combined organic layers were washed with saturated aqueous sodium bicarbonate and dried on magnesium sulfate. After filtration, the solution was concentrated in vacuo. Removal of all pyridine from the brownish oil was accomplished by coevaporation with toluene (twice) and 2-butanone (once). The resulting brown foam was purified by column chromatography on silica gel with 2-butanone as eluent (R_f 0.40), yielding 2.24 g (70%) of **2a** as a white solid. Mp: 109 °C. ^{1}H NMR ($CDCl_3$): δ 2.27 (1 H, m, $H_{2'}$), 2.71 (1 H, m, $H_{2''}$), 3.48 (2 H, m, $H_{5'}/H_{5''}$), 3.80 (3 H, s, OCH_3 MMTr), 4.10 (1 H, m, $H_{4'}$), 4.28 (1 H, t, CH Fmoc), 4.48 (3 H, m, $H_{3'}/CH_2$ Fmoc), 6.26 (1 H, dd, $H_{1'}$), 6.85 (2 H, m, aromatic MMTr), 6.98 (1 H, d, H_5), 7.20–7.48 (16 H, m, aromatic MMTr/aromatic Fmoc), 7.69 (4 H, m, aromatic Fmoc), 8.22 (1 H, d, H_6). Calculated mass: 721. FAB: $(M + H)^+$ = 722, $(M + Na)^+$ = 744.

5′-*O*-(4-Monomethoxytrityl)-2-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyguanosine (2b). 4-Monomethoxytrityl chloride (2.10 g, 6.80 mmol) was added to a solution of compound **1b** in 30 mL of dry pyridine, and the reaction mixture was stirred for 15 h in darkness. Then, the mixture was poured into aqueous saturated sodium bicarbonate (200 mL) and extracted with three 150-mL portions of dichloromethane. After drying on magnesium sulfate and filtration, the organic phase was concentrated in vacuo. The last traces of pyridine were coevaporated with toluene (three times) and 2-butanone (once). The resulting brown foam was purified by column chromatography on silica gel with a gradient of methanol in dichloromethane as eluent (1 → 8 vol % methanol). R_f 0.30 (2-butanone) and R_f 0.47 (dichloromethane/methanol, 9:1 v/v). Compound **2b** was obtained as a brownish solid. Yield: 3.60 g (86%). ^{1}H NMR ($CDCl_3$): δ 2.66 (2 H, m, $H_{2'}/H_{2''}$), 3.44 (2 H, m, $H_{5'}/H_{5''}$), 3.61 (3 H, s, OCH_3 MMTr), 3.92 (1 H, t, CH Fmoc), 4.24 (2 H, d, CH_2 Fmoc), 4.37 (1 H, m, $H_{4'}$), 5.20 (1 H, m, $H_{3'}$), 5.66 (1 H, b s, 3′-OH), 6.21 (1 H, dd, $H_{1'}$), 6.62 (2 H, d, aromatic MMTr), 6.96–7.49 (20 H, m, aromatic MMTr/aromatic Fmoc), 7.67 (1 H, s, H_8).

5′-*O*-(4-Monomethoxytrityl)-6-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyadenosine (2c). Compound **1c** (1.0 g, 2.11 mmol) was dissolved in 10 mL of dry pyridine. After addition of 4-monomethoxytrityl chloride (0.72 g, 2.32 mmol), the solution was stirred for 18 h in darkness. The mixture was then poured into saturated aqueous sodium bicarbonate (50 mL) and extracted with 50 mL of dichloromethane (three times). During the extraction the organic layer turned purple. The collected layers of

dichloromethane were washed with 50 mL of saturated aqueous sodium bicarbonate solution and dried on magnesium sulfate. After filtration, the solution was concentrated in vacuo. Removal of all pyridine was accomplished by coevaporation with toluene and dichloromethane. The resulting purple foam was purified by column chromatography on silica gel with 2-butanone as eluent (R_f 0.52), yielding 0.78 g (50%) of **2c** as a white solid. ^{1}H NMR ($CDCl_3$): δ 2.55 (1 H, m, $H_{2''}$), 2.88 (1 H, m, $H_{2'}$), 3.41 (2 H, m, $H_{5'}/H_{5''}$), 3.79 (3 H, s, OCH_3 MMTr), 4.16 (1 H, m, $H_{4'}$), 4.33 (1 H, t, CH Fmoc), 4.62 (2 H, d, CH_2 Fmoc), 4.72 (1 H, m, $H_{3'}$), 6.48 (1 H, dd, $H_{1'}$), 6.7–6.85 (2 H, d, aromatic MMTr), 7.1–7.5 (16 H, m, aromatic Fmoc and MMTr), 7.6–7.8 (4 H, m, aromatic Fmoc), 8.11 (1 H, s, H_2), 8.37 (1 H, b s, NH Fmoc), 8.70 (1 H, s, H_8). Calculated mass: 745. FAB: $(M + H)^+ = 746$, $(M + Na)^+ = 768$.

3′-*O*-Acetyl-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxycytidine. A solution of compound **2a** (1.04 g, 1.44 mmol) and acetic anhydride (1.0 mL) in 10 mL of dry pyridine was stirred for 15 h and then poured into aqueous sodium bicarbonate. Extraction with dichloromethane afforded an oil. Chromatography using silica gel as the stationary phase and 2-butanone as eluent yielded 5′-(4-monomethoxytrityl)-3′-*O*-acetyl-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxycytidine (R_f 0.57) as a white solid (0.88 g, 80%) with mp 110 °C. ^{1}H NMR ($CDCl_3$): δ 2.09 (3 H, s, CH_3 of acetyl), 2.29 (1 H, m, $H_{2'}$), 2.76 (1 H, m, $H_{2''}$), 3.47 (2 H, m, $H_{5'}/H_{5''}$), 3.78 (3 H, s, OCH_3 MMTr), 4.24 (1 H, m, $H_{4'}$), 4.28 (1 H, t, CH Fmoc), 4.50 (2 H, d, CH_2 Fmoc), 5.37 (1 H, m, $H_{3'}$), 6.29 (1 H, t, $H_{1'}$), 6.85 (2 H, m, MMTr), 6.94 (1 H, d, H_5), 7.20–7.47 (16 H, m, aromatic MMTr and Fmoc), 7.68 (4 H, m, aromatic Fmoc), 8.09 (1 H, d, H_6). The solid was dissolved in 50 mL of 80% acetic acid and stirred for 15 h; 30 mL of water was added, and all volatiles were evaporated. The last traces of acetic acid were removed by coevaporation with 2-butanone. Crystallization from 2-butanone yielded the title compound in 56% yield, mp 148 °C. ^{1}H NMR ($CDCl_3$): δ 2.12 (3 H, s, CH_3 of acetyl), 2.40 (1 H, m, $H_{2'}$), 2.67 (1 H, m, $H_{2''}$), 3.95 (2 H, m, $H_{5'}/H_{5''}$), 4.19 (1 H, m, $H_{4'}$), 4.29 (1 H, t, CH Fmoc), 4.51 (2 H, d, CH_2 Fmoc), 5.35 (1 H, m, $H_{3'}$), 6.25 (1 H, t, $H_{1'}$), 7.13–7.48 (5 H, m, H_5/aromatic Fmoc), 7.68 (4 H, m, aromatic Fmoc), 8.19 (1 H, d, H_6). Calculated mass: 491. FAB: $(M + H)^+ = 492$.

5′-*O*-(4-Monomethoxytrityl)-2′-deoxyadenosine. Compound **2c** (4.24 g, 5.7 mmol) was dissolved in 50 mL of 2-butanone, and triethylamine (0.5 mL) was added. After the solution was stirred for 3 days, the mixture was concentrated by evaporation of 2-butanone. Column chromatography using silica gel as the stationary phase and 2-butanone as eluent afforded 1.7 g (60%) of the title compound as a white solid (R_f 0.13). ^{1}H NMR ($CDCl_3$): δ 2.53 (1 H, m, $H_{2''}$), 2.74 (1 H, m, $H_{2'}$), 3.39 (2 H, m, $H_{5'}/H_{5''}$), 3.71 (3 H, s, OCH_3 MMTr), 4.22 (1 H, m, $H_{4'}$), 4.68 (1 H, m, $H_{3'}$), 6.34 (2 H, b s, NH_2), 6.46 (1 H, dd, $H_{1'}$), 6.70–6.80 (2 H, d, aromatic MMTr), 7.05–7.55 (12 H, m, aromatic MMTr), 7.98 (1 H, s, H_2), 8.25 (1 H, s, H_8).

3′-*O*-Acetyl-6-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyadenosine. Compound **2c** (2.24 g, 3.0 mmol) was dissolved in 22 mL of dry pyridine. After addition of acetic anhydride (2.0 mL), the solution was stirred overnight. The pyridine was evaporated and coevaporated three times with toluene to dryness. A mixture of dry nitromethane and dry methanol (19 mL, 95:5 v/v) was added. Addition of anhydrous zinc bromide (5.0 g) gave an orange suspension, which was stirred for 3 h. Then, the mixture was washed with 100 mL of 5% aqueous ammonium acetate and subsequently extracted with 45 mL of dichloromethane (three times). The combined organic layers were dried on magnesium sulfate. After filtration, the solution was concentrated in vacuo, and purification occurred by column chromatography on silica gel with ethyl acetate as eluent (R_f 0.18), yielding 1.0 g (65%) of the title compound as a white solid. ^{1}H NMR ($CDCl_3$): δ 2.14 (3 H, s, CH_3 of acetyl), 2.48 (1 H, m, $H_{2''}$), 3.11 (1 H, m, $H_{2'}$), 3.93 (2 H, m, $H_{5'}/H_{5''}$), 4.29 (3 H, m, $H_{4'}$/CH Fmoc), 4.63 (2 H, d, CH_2 Fmoc), 5.55 (1 H, d, 5′-OH), 6.34 (1 H, dd, $H_{1'}$), 7.23–7.46 (4 H, m, aromatic Fmoc), 7.60–7.81 (4 H, m, aromatic Fmoc), 8.05 (1 H, s, H_2), 8.75 (1 H, s, H_8), 9.08 (1 H, b s, NH Fmoc). Calculated mass: 515. FAB: $(M + H)^+ = 516$, $(M + Na)^+ = 538$.

2′-Deoxycytidyl-(3′→5′)-2′-deoxyguanosine *O*-(Methyl phosphate) (4). Bis(*N*,*N*-diisopropylamino)methoxyphosphine (1.66 g, 6.34 mmol) and 1*H*-tetrazole (0.19 g, 2.71 mmol) were added to a solution of compound **2a** (3.80 g, 5.26 mmol) in 25 mL of dry pyridine, and the reaction mixture was stirred for 10 min. Formation of the phosphoramidite coupling synthon in situ **3a** was evident from the ^{31}P NMR spectrum (($CDCl_3$): δ 150.0 and 149.4). Then a solution of compound **1a** (3.07 g, 6.28 mmol) and 1*H*-tetrazole (1.10 g, 15.71 mmol) in 60 mL of dry pyridine was added to the reaction mixture, and stirring was continued for 2 h. ^{31}P NMR analysis showed that all phosphoramidite **3a** had been converted into the phosphite triester (2 diastereoisomers, ($CDCl_3$): δ 140.8 and 140.1). The two peaks of the 3′–3′ coupled phosphite triester were visible as well (($CDCl_3$): δ 141.0 and 140.4) but with an approximately 5-fold lower intensity. Then *tert*-butyl hydroperoxide (8.0 mL) was added, and the reaction mixture was stirred for another 15 min. ^{31}P NMR data showed that all phosphite triester had been converted into phosphate triester (two diastereoisomers, ($CDCl_3$): δ −0.3 and −0.5). The mixture was concentrated in vacuo (at room temperature) to near dryness and coevaporated with toluene (three times), chloroform (twice), and 2-butanone (once). The product was purified by column chromatography on silica gel. First, impurities were eluted with 2-butanone/methanol (95:5 v/v); then, with a gradient of 2-butanone/methanol (95:5 → 75:25 v/v) as eluent, the product was obtained as a white solid. Yield: 2.40 g (35.5%). R_f 0.10 (2-butanone/methanol, 95:5 v/v) and R_f 0.37 (dichloromethane/methanol, 9:1 v/v). The product decomposed upon heating (ca. 134 °C). ^{31}P NMR ($CDCl_3$): δ −0.5 and −1.1. The solid was dissolved in a mixture of 5 mL of dry nitromethane and 1 mL of methanol (p.a.). Then anhydrous zinc bromide (2.30 g, 10.1 mmol) was added, turning the solution into a red color. The reaction mixture was stirred for 1.5 h and subsequently poured into a solution of ammonium acetate (2.0 g) in 40 mL of water and extracted four times with 15-mL portions of dichloromethane. The organic phase was washed with 25 mL of water, and the water layer was extracted with two 15-mL portions of dichloromethane. The collected organic phase was concentrated in vacuo. TLC experiments showed the detritylation reaction to be complete (R_f 0 (dichloromethane/methanol, 95:5 v/v), R_f 0.20 (dichloromethane/methanol, 9:1 v/v)). ^{31}P NMR (pyridine-d_5): δ 0.6 and 0.5. The residue was dried by coevaporation with dry pyridine (twice) and subsequently dissolved in a mixture of dry pyridine (4.0 mL) and triethylamine (0.8 mL). The reaction mixture was stirred for 6 h and subsequently concentrated in vacuo and coevaporated with toluene (twice), 2-butanone (twice), and water (twice). TLC experiments showed the deprotection of the bases to be complete (R_f 0 (dichloromethane/methanol, 9:1 v/v)). Purification of the methylated and fully deprotected dinucleotides and separation of the diastereoisomers was accomplished by means of HPLC. S_p-(**4**). ^{1}H NMR (D_2O): δ 1.92 (1 H, m, $H_{2'}$ of dCp), 2.28 (1 H, m, $H_{2''}$ of dCp), 2.48 (1 H, m, $H_{2''}$ of pdG), 2.81 (1 H, m, $H_{2'}$ of pdG), 3.58 (1 H, dd, $H_{5''}$ of dCp), 3.61 (1 H, dd, $H_{5'}$ of dCp), 3.69 (3 H, d, OCH_3, J = 11.2 Hz), 4.08 (1 H, m, $H_{4'}$ of dCp), 4.16 (1 H, m, $H_{4'}$ of pdG), 4.29 (2 H, dd, $H_{5'}/H_{5''}$ of pdG), 5.92 (1 H, d, H_5), 6.05 (1 H, dd, $H_{1'}$ of dCp), 6.20 (1 H, dd, $H_{1'}$ of pdG), 7.56 (1 H, d, H_6), 7.90 (1 H, s, H_8). $H_{3'}$ of dCp and $H_{3'}$ of pdG are located under the HDO peak. ^{31}P NMR (D_2O): δ 1.99. R_p-(**4**). ^{1}H NMR (D_2O): δ 1.99 (1 H, m, $H_{2'}$ of dCp), 2.40 (1 H, m, $H_{2''}$ of dCp), 2.52 (1 H, m, $H_{2''}$ of pdG), 2.84 (1 H, m, $H_{2'}$ of pdG), 3.56 (1 H, dd, $H_{5''}$ of dCp), 3.59 (1 H, dd, $H_{5'}$ of dCp), 3.72 (3 H, d, OCH_3, J = 11.6 Hz), 3.99 (1 H, m, $H_{4'}$ of dCp), 4.16 (1 H, m, $H_{4'}$ of pdG), 4.27 (2 H, dd, $H_{5'}/H_{5''}$ of pdG), 5.94 (1 H, d, H_5), 6.01 (1 H, dd, $H_{1'}$ of dCp), 6.21 (1 H, dd, $H_{1'}$ of pdG), 7.60 (1 H, d, H_6), 7.89 (1 H, s, H_8). $H_{3'}$ of dCp and $H_{3'}$ of pdG are located under the HDO peak. ^{31}P NMR (D_2O): δ 1.98.

2′-Deoxyguanylyl-(3′→5′)-2′-deoxycytidine *O*-(Methyl phosphate) (5). Bis(*N*,*N*-diisopropylamino)methoxyphosphine (1.07 g, 4.08 mmol) and 1*H*-tetrazole (0.105 g, 1.50 mmol) were added to a solution of compound **2b** (2.28 g, 3.00 mmol) in 8.0 mL of dry pyridine, and the reaction mixture was stirred for 10 min. Formation of the phosphoramidite coupling synthon in situ **3b** was evident from the ^{31}P NMR data ($CDCl_3$: δ 149.6 and 149.5). Then a solution of compound **1a** (1.62 g, 3.60 mmol) and of 1*H*-tetrazole (0.75 g, 10.71 mmol) in 12 mL of dry pyridine was added to the reaction mixture, and stirring was continued for 1.5 h. ^{31}P NMR spectroscopy showed that all phosphoramidite had been converted into the phosphite triester (2 diastereoisomers, ($CDCl_3$): δ 141.4 and 141.1). Then *tert*-butyl hydroperoxide (5.5 mL) was added, and the reaction mixture was stirred for another

15 min. ^{31}P NMR showed that all phosphite triester had been converted into phosphate triester (two diastereoisomers, ($CDCl_3$): δ 0.7 and −0.8). The mixture was concentrated in vacuo to near dryness and coevaporated with toluene (three times) and dichloromethane (twice). The product was purified by column chromatography on silica gel. First, impurities were eluted with 2-butanone/methanol (95:5 v/v); then with a gradient of 2-butanone/methanol (95:5 v/v → 75:25 v/v) as eluent, we obtained a white solid. Yield: 0.72 g (19%). R_f 0.14 (2-butanone/methanol, 95:5 v/v) and R_f 0.50 (dichloromethane/methanol, 9:1 v/v). The solid decomposed upon heating (ca. 140 °C). ^{31}P NMR ($CDCl_3/CD_3OD$, 1:1 v/v): δ 2.8 and 2.7. The solid was dissolved in a mixture of 2.0 mL of dry nitromethane and 0.2 mL of methanol. Then, anhydrous zinc bromide (0.75 g, 3.29 mmol) was added, turning the solution into a red color. The reaction mixture was stirred for 1 h and subsequently poured into 20 mL of a 5% aqueous ammonium acetate solution and extracted with four 10-mL portions of dichloromethane. The organic phase was washed with 10 mL of water, and the water layer was extracted with two 10-mL portions of dichloromethane. The collected organic phase was concentrated in vacuo. TLC experiments showed the detritylation reaction to be complete (R_f 0 (dichloromethane/methanol, 9:1 v/v)). ^{31}P NMR (pyridine-d_5): δ 0.5 (peaks overlap). The residue was dried by coevaporation (twice) with dry pyridine and dissolved in a mixture of dry pyridine (2.0 mL) and triethylamine (0.55 mL). The reaction mixture was stirred for 6 h and subsequently concentrated in vacuo and coevaporated with toluene (three times), 2-butanone (twice), and water (twice). TLC experiments showed the deprotection of the bases to be complete (R_f 0 (dichloromethane/methanol, 9:1 v/v)). Purification of the methylated and fully deprotected dinucleotides and separation of the diastereoisomers was accomplished by means of HPLC. S_p-(**5**). ^1H NMR (D_2O): δ 2.24 (1 H, m, $H_{2'}$ of pdC), 2.35 (1 H, m, $H_{2''}$ of pdC), 2.64 (1 H, m, $H_{2''}$ of dGp), 2.80 (1 H, m, $H_{2'}$ of dGp), 3.72 (2 H, d, $H_{5'}/H_{5''}$ of dGp), 3.82 (3 H, d, OCH_3, J = 11.3 Hz), 4.12 (1 H, m, $H_{4'}$ of pdC), 4.30 (1 H, m, $H_{4'}$ of dGp), 4.32 (1 H, m, $H_{5''}$ of pdC), 4.40 (1 H, m, $H_{5'}$ of pdC), 5.10 (1 H, m, $H_{3'}$ of pdC), 5.77 (1 H, d, H_5), 6.15 (1 H, dd, $H_{1'}$ of dGp), 6.17 (1 H, dd, $H_{1'}$ of pdC), 7.57 (1 H, d, H_6), 7.90 (1 H, s, H_8). $H_{3'}$ of dGp was located under the HDO peak. ^{31}P NMR (D_2O): δ 2.10. R_p-(**5**). ^1H NMR (D_2O): δ 2.27 (1 H, m, $H_{2'}$ of pdC), 2.41 (1 H, m, $H_{2''}$ of pdC), 2.68 (1 H, m, $H_{2''}$ of dGp), 2.83 (1 H, m, $H_{2'}$ of dGp), 3.73 (2 H, dd, $H_{5'}/H_{5''}$ of dGp), 4.16 (1 H, m, $H_{4'}$ of pdC), 4.29 (1 H, m, $H_{4'}$ of dGp), 4.27 (1 H, m, $H_{5''}$ of pdC), 4.38 (1 H, m, $H_{5'}$ of pdC), 5.12 (1 H, m, $H_{3'}$ of pdC), 5.86 (1 H, d, H_5), 6.17 (1 H, dd, $H_{1'}$ of pdC), 6.19 (1 H, dd, $H_{1'}$ of dGp), 7.63 (1 H, d, H_6), 7.90 (1 H, s, H_8). $H_{3'}$ of dGp was located under the HDO peak. ^{31}P NMR (D_2O): δ 1.97.

2′-Deoxyadenylyl-(3′→5′)-2′-deoxycytidine *O*-(Methyl phosphate) (6). 5′-*O*-(4-Monomethoxytrityl)-2′-deoxyadenosine (0.50 g, 0.96 mmol) and 1*H*-tetrazole (33 mg, 0.48 mmol) were dissolved in 4 mL of dry pyridine. Bis(*N*,*N*-diisopropylamino)-methoxyphosphine (302 mg, 1.14 mmol) was added, and the reaction mixture was stirred for 30 min. ^{31}P NMR analysis showed complete conversion into the corresponding phosphoramidite coupling synthon as an approximately 1:1 mixture of two diastereoisomers (^{31}P NMR: δ 149.5 and 149.4). A solution of compound **1a** (431 mg, 0.96 mmol) and 1*H*-tetrazole (168 mg, 2.4 mmol) in 7 mL of dry pyridine was transferred into the reaction vessel, and the mixture was stirred for 1.5 h. ^{31}P NMR showed the formation of two couples of diastereoisomeric phosphites, namely the desired 3′–5′ coupling product (ca. 80%) and the undesired 3′–3′ coupling product (ca. 20%). ^{31}P NMR ($CDCl_3$) (3′–5′): δ 140.8 and 140.6. ^{31}P NMR ($CDCl_3$) (3′–3′): δ 140.0 and 139.8. The phosphites were readily oxidized through the addition of *tert*-butyl hydroperoxide (1 mL).[13] After evaporation of all volatiles (coevaporation with toluene), the residue was chromatographed on a silica gel column with dichloromethane/methanol (9:1 v/v) as eluent (R_f 0.30). This afforded a white solid. (Yield: 0.5 g (50%). ^{31}P NMR ($CDCl_3$): δ 0.3 and −0.4). The solid was dissolved in 3 mL of dry nitromethane. After addition of anhydrous zinc bromide (1.71 g), the solution, which colored red, was stirred for 3 h. The mixture was poured into 16 mL of a 5% aqueous ammonium acetate solution and subsequently extracted with 8 mL of dichloromethane (four times). The combined organic layers were dried on magnesium sulfate. After filtration, the solution was concentrated in vacuo and purified on a silica gel column with dichloromethane/methanol (9:1 v/v) as eluent (R_f 0.32). Again, this yielded a white solid. (Yield: 160 mg (50%). ^{31}P NMR ($CDCl_3$): δ 0.2 and −0.6). This solid was dissolved in 1.5 mL of dry pyridine, and triethylamine (0.43 mL, 3.1 mmol) was added. After being stirred for 6 h the mixture was evaporated, and the residue was coevaporated with toluene and dichloromethane, yielding a yellow oil. The two diastereoisomers (S_p and R_p) of **6** were separated with reversed-phase HPLC. S_p-(**6**). ^1H NMR (D_2O): δ 2.20 (1 H, m, $H_{2'}$ of pdC), 2.32 (1 H, m, $H_{2''}$ of pdC), 2.70 (1 H, m, $H_{2''}$ of dAp), 2.88 (1 H, m, $H_{2'}$ of dAp), 3.76 (2 H, m, $H_{5'}/H_{5''}$ of dAp), 3.82 (3 H, d, OCH_3, J = 11.4 Hz), 4.12 (1 H, m, $H_{4'}$ of pdC), 4.31 (1 H, m, $H_{5''}$ of pdC), 4.44 (1 H, m, $H_{5'}$ of pdC), 4.78 (1 H, m, $H_{3'}$ of dAp), 5.16 (1 H, m, $H_{3'}$ of pdC), 5.78 (1 H, d, H_5), 6.18 (1 H, dd, $H_{1'}$ of pdC), 6.42 (1 H, dd, $H_{1'}$ of dAp), 7.72 (1 H, d, H_6), 8.10 and 8.18 (2 H, s, H_2 and H_8). ^{31}P NMR (D_2O): δ 2.06. R_p-(**6**) ^1H NMR (D_2O): δ 2.26 (1 H, m, $H_{2'}$ of pdC), 2.38 (1 H, m, $H_{2''}$ of pdC), 2.72 (1 H, m, $H_{2''}$ of dAp), 2.87 (1 H, m, $H_{2'}$ of dAp), 3.74 (1 H, m, $H_{5''}$ of dAp), 3.76 (1 H, m, $H_{5'}$ of dAp), 3.82 (3 H, d, OCH_3, J = 11.4 Hz), 4.14 (1 H, m, $H_{4'}$ of pdC), 4.30 (1 H, m, $H_{5''}$ of pdC), 4.33 (1 H, m, $H_{4'}$ of dAp), 4.40 (1 H, m, $H_{5'}$ of pdC), 5.14 (1 H, m, $H_{3'}$ of pdC), 5.86 (1 H, d, H_5), 6.14 (1 H, dd, $H_{1'}$ of pdC), 6.38 (1 H, dd, $H_{1'}$ of dAp), 7.60 (1 H, d, H_6), 8.12 and 8.15 (2 H, s, H_2 and H_8), $H_{3'}$ of dAp was located under the HDO peak. ^{31}P NMR (D_2O): δ 1.91.

2′-Deoxyadenylyl-(3′→5′)-3′-*O*-acetylthymidine *O*-(Methyl phosphate) (7). Compound **2c** (494 mg, 0.663 mmol) was dissolved in 3 mL of dry pyridine. 1*H*-Tetrazole (23.2 mg, 0.332 mmol) and bis-(*N*,*N*-diisopropylamino)methoxyphosphine (204 mg, 0.78 mmol) were added, and the reaction mixture was stirred for 1.5 h. ^{31}P NMR data indicated complete conversion into the phosphoramidite coupling synthon **3c** as an approximately 1:1 mixture of two diastereoisomers (^{31}P NMR ($CDCl_3$): δ 149.7 and 149.5). A solution of 3′-*O*-acetylthymidine (224 mg, 0.79 mmol) and 1*H*-tetrazole (113 mg, 1.61 mmol) in 3 mL of dry pyridine was transferred into the reaction vessel, and the mixture was stirred for 30 min. ^{31}P NMR data indicated the formation of two diastereoisomeric phosphites (^{31}P NMR ($CDCl_3$): δ 141.3 and 140.4), which were readily oxidized through the addition of *tert*-butyl hydroperoxide (0.5 mL).[13] After evaporation of all volatiles (coevaporation with toluene), the residue was purified on a silica gel column with 2-butanone as eluent (R_f 0.28). This afforded a slightly colored oil, which was dissolved in an acetic acid/water mixture (4:1 v/v) and stirred overnight. After evaporation of the mixture to near dryness, the residue was coevaporated with water. The residue was dissolved in 2.5 mL of dry pyridine, and triethylamine (0.6 mL, 4.34 mmol) was added. After being stirred for 4 h, the mixture was evaporated, and the residue was coevaporated with chloroform. ^{31}P NMR ($CDCl_3$): δ −0.4 and −0.7. The two diastereoisomers (S_p and R_p) of **7** were separated with reversed-phase HPLC. S_p-(**7**). ^1H NMR (D_2O): δ 1.63 (3 H, d, CH_3 of pdT), 2.07 (3 H, s, CH_3 of acetyl), 2.34 (1 H, m, $H_{2'}$ of pdT), 2.40 (1 H, m, $H_{2''}$ of pdT), 2.73 (1 H, m, $H_{2''}$ of dAp), 2.88 (1 H, m, $H_{2'}$ of dAp), 3.76 (2 H, m, $H_{5'}/H_{5''}$ of dAp), 3.83 (3 H, d, OCH_3, J = 11.3 Hz), 4.29 (1 H, m, $H_{4'}$ of pdT), 4.36 (2 H, m, $H_{4'}$ of dAP/$H_{5''}$ of pdT), 4.43 (1 H, m, $H_{5'}$ of pdT), 5.15 (1 H, m, $H_{3'}$ of dAp), 5.27 (1 H, m, $H_{3'}$ of pdT), 6.14 (1 H, dd, $H_{1'}$ of pdT), 6.35 (1 H, dd, $H_{1'}$ of dAp), 7.35 (1 H, d, H_6), 8.12 and 8.21 (2 H, s, H_2 and H_8). ^{31}P NMR (D_2O): δ 1.93. R_p-(**7**). ^1H NMR (D_2O): δ 1.70 (3 H, d, CH_3 of pdT), 2.06 (3 H, s, CH_3 of acetyl), 2.39 (1 H, m, $H_{2'}$ of pdT), 2.47 (1 H, m, $H_{2''}$ of pdT), 2.76 (1 H, m, $H_{2''}$ of dAp), 2.88 (1 H, m, $H_{2'}$ of dAp), 3.77 (2 H, m, $H_{5'}/H_{5''}$ of dAp), 3.85 (3 H, d, OCH_3, J = 11.3 Hz), 4.34 (3 H, m, $H_{4'}$ of dAp, $H_{4'}$ of pdT and $H_{5''}$ of pdT), 4.41 (1 H, m, $H_{5'}$ of pdT), 5.15 (1 H, m, $H_{3'}$ of dAp), 5.31 (1 H, m, $H_{3'}$ of pdT), 6.17 (1 H, dd, $H_{1'}$ of pdT), 6.36 (1 H, dd, $H_{1'}$ of dAp), 7.40 (1 H, d, H_6), 8.13 and 8.21 (2 H, s, H_2 and H_8). ^{31}P NMR (D_2O): δ 1.80.

2′-Deoxyadenylyl-(3′→5′)-3′-*O*-acetyl-2′-deoxyadenosine *O*-(Methyl phosphate) (8). Compound **2c** (2.00 g, 2.68 mmol) and 1*H*-tetrazole (93.8 mg, 1.34 mmol) were dissolved in 12 mL of dry pyridine. After addition of bis(*N*,*N*-diisopropylamino)-methoxyphosphine, the mixture was stirred for 20 min. ^{31}P NMR indicated complete conversion into the phosphoramidite coupling synthon **3c** as an approximately 1:1 mixture of two diastereoisomers (^{31}P NMR ($CDCl_3$): δ 149.7 and 149.5). A solution of 3′-*O*-acetyl-6-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxyadenosine

(1.00 g, 1.94 mmol) and 1*H*-tetrazole (0.47 g, 6.7 mmol) in 12 mL of dry pyridine was transferred into the reaction vessel, and the mixture was stirred for 3.5 h. ^{31}P NMR indicated the formation of two diastereoisomeric phosphites (^{31}P NMR ($CDCl_3$): δ 140.8 and 140.4), which were readily oxidized through the addition of *tert*-butyl hydroperoxide (2 mL).[13] After evaporation of all volatiles (coevaporation with toluene), the residue was dissolved in 18 mL of dry nitromethane and 0.5 mL of dry methanol. After addition of anhydrous zinc bromide (7 g) the orange suspension was stirred for 4 h. To this suspension 100 mL of a 5% aqueous ammonium acetate solution was added, and subsequently the mixture was extracted with 50 mL of dichloromethane (four times). The combined organic layers were dried on magnesium sulfate. After filtration, the filtrate was concentrated in vacuo, and purification was effected by column chromatography on silica gel with dichloromethane/methanol (92:8 v/v) as eluent (R_f 0.36). This afforded a white solid. (Yield: 1.0 g (48%). ^{31}P NMR ($CDCl_3$): δ −0.1 and −0.2), which was dissolved in 1 mL of dry pyridine. Triethylamine (0.25 mL, 1.8 mmol) was added. After being stirred for 5 h the mixture was evaporated, and the residue was coevaporated with toluene and chloroform, yielding a yellow oil containing **8**. ^{31}P NMR: δ −0.7 and −0.8. The two diastereoisomers (S_p and R_p) of **8** were separated with reversed-phase HPLC. S_p-(**8**). ^1H NMR (D_2O): δ 2.10 (3 H, s, CH_3 of acetyl), 2.36 (1 H, m, $H_{2''}$ of dAp), 2.46 (1 H, m, $H_{2'}$ of dAp), 2.64 (1 H, m, $H_{2''}$ of pdA), 2.89 (1 H, m, $H_{2'}$ of pdA), 3.64 (2 H, d, $H_{5'}/H_{5''}$ of dAp), 3.77 (3 H, d, OCH_3, J = 11.2 Hz) 4.24 (1 H, m, $H_{4'}$ of dAp), 4.40 2 H, m, $H_{5'}/H_{5''}$ of pdA), 4.42 (1 H, m, $H_{4'}$ of pdA), 5.03 (1 H, m, $H_{3'}$ of dAp), 5.47 (1 H, m, $H_{3'}$ of pdA), 6.05 (1 H, dd, $H_{1'}$ of dAp), 6.28 (1 H, dd, $H_{1'}$ of pdA), 7.88 and 8.02 (2 H, s, H_2 of dAp and H_2 of pdA), 8.00 (1 H, s, H_8 of dAp),[19] 8.24 (1 H, s, H_8 of pdA).[19] ^{31}P NMR (D_2O): δ 1.66. Because of the partially demethylated R_p-(**8**) diastereoisomer, it was impossible to determine the complete ^1H NMR spectrum. R_p-(**8**) ^{31}P NMR (D_2O): δ 1.74.

2′-Deoxycytidyl-(3′→5′)-3′-*O*-acetyl-2′-deoxycytidine *O*-(Methyl phosphate) (9). Compound **2a** (1.38 g, 1.91 mmol) was dissolved in 11 mL of dry pyridine. To this solution were added bis(*N*,*N*-diisopropylamino)methoxyphosphine (0.546 g, 2.08 mmol) and 1*H*-tetrazole (0.07 g, 1.02 mmol). After 10 min ^{31}P NMR spectroscopy revealed quantitative conversion into the phosphoramidite structure (δ ^{31}P ($CDCl_3$): 150.0 150.0 and 149.4). A solution of 3′-*O*-acetyl-4-*N*-(9-fluorenylmethoxycarbonyl)-2′-deoxycytidine (0.98 g, 1.99 mmol) and 1*H*-tetrazole (0.29 g, 4.12 mmol) in 12 mL of dry pyridine was added, and the mixture was stirred for 2.5 h. Then, ^{31}P NMR showed complete formation of the 3′–5′ phosphite triester (δ ^{31}P ($CDCl_3$): 140.8 140.8 and 140.5). Subsequently, *tert*-butyl hydroperoxide (1.5 mL) was added. After the mixture was stirred for 15 min, ^{31}P NMR revealed formation of the phosphate triester structure. After thorough evaporation of all volatiles, the residue was taken up in 20 mL of 80% acetic acid and stirred for 15 h. Evaporation of all acetic acid afforded a yellowish viscous substance, which was chromatographed on a silica gel column with 2-butanone as eluent. This yielded a white solid (1.09 g, 56%. Mp: 141 °C. ^{31}P NMR ($CDCl_3$): δ 0.07 and −0.35. Calculated mass: 1016. FAB: $(M + H)^+$ = 1017, $(M + Na)^+$ = 1039). This compound was dissolved in a mixture of chloroform (5.0 mL) and triethylamine (5.0 mL) and stirred for 15 h. During stirring, a white precipitate was formed, which proved to be the base-deprotected compound **9**. The R_p and S_p diastereoisomers of **9** were separated with reversed-phase HPLC. S_p-(**9**). ^1H NMR (D_2O): δ 2.06 (3 H, s, CH_3 of acetyl), 2.28–2.36 (2 H, m, $H_{2'}$ of dCp and pdC), 2.54 (1 H, m, $H_{2''}$ of pdC), 2.59 (1 H, m, $H_{2''}$ of dCp), 3.67–3.77 (2 H, m, $H_{5'}/H_{5''}$ of pdC), 3.80 (3 H, d, OCH_3, J = 11.4 Hz), 4.20 (1 H, m, $H_{4'}$ of dCp), 4.27–4.37 (3 H, m, $H_{4'}/H_{5'}/H_{5''}$ of pdC), 4.97 (1 H, m, $H_{3'}$ of dCp), 5.29 (1 H, m, $H_{3'}$ of pdC), 5.99 (1 H, d, H_5 of dCp), 6.00 (1 H, d, H_5 of pdC), 6.14 (1 H, dd, $H_{1'}$ of dCp), 6.20 (1 H, dd, $H_{1'}$ of pdC), 7.70 (1 H, d, H_6 of dCp), 7.75 (1 H, d, H_6 of pdC). ^{31}P NMR (D_2O): δ 2.03. R_p-(**9**). ^1H NMR (D_2O): δ 2.07 (3 H, s, CH_3 of acetyl), 2.31–2.39 (2 H, m, $H_{2'}$ of dCp and pdC), 2.55 (1 H, m, $H_{2''}$ of pdC), 2.60 (1 H, m, $H_{2''}$ of dCp), 3.67–3.77 (2 H, m, $H_{5'}/H_{5''}$ of dCp), 3.80 (3 H, d, OCH_3, J = 11.4 Hz), 4.23 (1 H, m, $H_{4'}$ of dCp), 4.30–4.41 (3 H, m, $H_{4'}/H_{5'}/H_{5''}$ of pdC), 4.98 (1 H, m, $H_{3'}$ of dCp), 5.30 (1 H, m, $H_{3'}$ of pdC), 5.99 (1 H, d, H_5 of dCp), 6.00 (1 H, d, H_5 of pdC), 6.16 (1 H, dd, $H_{1'}$ of dCp), 6.20 (1 H, dd, $H_{1'}$ of pdC), 7.72 (1 H, d, H_6 of dCp), 7.77 (1 H, d, H_6 of pdC). ^{31}P NMR (D_2O): δ 2.13.

Registry No. **1a**, 87424-19-9; **1b**, 87424-21-3; **1c**, 87424-20-2; **2a**, 119184-91-7; **2b**, 119184-92-8; **2c**, 119184-93-9; (S_p)-**4**, 119241-83-7; (R_p)-**4**, 119241-84-8; (S_p)-**5**, 119184-97-3; (R_p)-**5**, 119241-85-9; (S_p)-**6**, 119241-86-0; (R_p)-**6**, 119241-87-1; (S_p)-**7**, 119184-98-4; (R_p)-**7**, 119241-88-2; (S_p)-**8**, 119184-99-5; (R_p)-**8**, 119241-89-3; (S_p)-**9**, 119185-00-1; (R_p)-**9**, 119241-90-6; Fmoc-Cl, 28920-43-6; $MeOPCl_2$, 3279-26-3; PCl_3, 7719-12-2; MeOP[N(*i*-Pr)$_2$]$_2$, 92611-10-4; H-dCyd-H, 951-77-9; H-dGuo-H, 961-07-9; H-dAdo-H, 958-09-8; MMTr-dCyd(Fmoc)-Ac, 119184-94-0; H-dCyd(Fmoc)-Ac, 119184-95-1; MMtr-dAdo-H, 51600-10-3; H-dAdo(Fmoc)-Ac, 119184-96-2; H-dThd-Ac, 21090-30-2.

Synthesis of N(1)-Phosphorylated Tryptophan Derivatives[1,2]

Helen A. Guillaume,† John W. Perich,† R. B. Johns,*,† and Geoffrey W. Tregear‡

Department of Organic Chemistry, University of Melbourne, Parkville 3052, Victoria, Australia, and Howard Florey Institute of Experimental Physiology and Medicine, Parkville 3052, Victoria, Australia

Received October 4, 1988

The first synthesis of the *N*(1)-(dimethylphosphono)tryptophan derivatives Z-Trp(PO_3Me_2)-OBzl and Boc-Trp(PO_3Me_2)-ONBzl by reaction of the lithium indolate of protected tryptophan derivatives Z-Trp-OBzl and Boc-Trp-ONBzl with dimethyl phosphorochloridate is described. The *N*(1)-(dimethylphosphono)tryptophan or Trp(Dmop) derivatives are stable to hydrogenation, and to TFA and high HF treatment, and can be fully deprotected with TFMSA/TFA/*m*-cresol/dimethyl sulfide or TFMSA/TFA/*m*-cresol/thioanisole to yield the novel hydrophilic amino acid *N*(1)-phosphonotryptophan quantitatively. Weak base treatment of Trp(Dmop) compounds yields *N*(1)-(methylphosphono)tryptophan derivatives.

The phosphorylation of serine,[3] tyrosine,[4] and threonine[5] residues in biological peptides is well documented, and N_ϵ-phosphoarginine[6] is the only amino acid with an N–P bond that has so far been isolated from biological material. The phosphorylation of these amino acids is known to

† Melbourne University.
‡ Howard Florey Institute.

(1) Preliminary communication: Guillaume, H. A.; Perich, J. W.; Johns, R. B.; Tregear, G. W. *Chem. Commun.* **1988**, 970.

0022-3263/89/1954-1664$01.50/0