Nonoxidative Chlorination of Dialkyl Phosphonates to Dialkyl Phosphorochloridites. A New Approach to Oligonucleotide Synthesis

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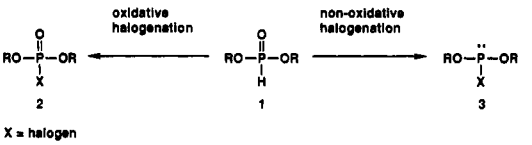
Several dialkyl phosphonates and alkyl nucleoside 3′-phosphonates were transformed into the corresponding highly reactive phosphorochloridites without oxidation of phosphorus by use of tris(2,4,6-tribromophenoxy)-dichlorophosphorane (BDCP) as a chlorinating reagent. The reaction was applied to internucleotidic bond formation. 2-Cyanoethyl and methyl nucleoside 3′-phosphonates were prepared in high yields and were stable enough to be used as starting materials in oligonucleotide synthesis. Examination of dodecathymidylate synthesis on a polymer support, using 2-cyanoethyl or methyl nucleoside 3′-phosphonate as building blocks, showed that the 2-cyanoethyl nucleoside 3′-phosphonate was more effective.

Introduction

Methods for internucleotidic bond formation via phosphorus(III) intermediates such as phosphoramidites[1] or *H*-phosphonates[2] are proving to be powerful tools for the synthesis of DNA and RNA fragments. In the phosphoramidite approach, the nucleoside phosphoramidite units are stable enough, but the method requires an excess of expensive 1*H*-tetrazole to activate them. In the *H*-phosphonate approach, the nucleoside phosphonate units are quite stable compounds compared with the phosphoramidite units, but the method requires a condensing reagent. Several side reactions caused by the condensing reagents during the activation of the phosphonate units have been reported.[3] These reactions are due to the dibasic acid character of the monoesters of phosphorous acid. Diesters of phosphorous acid were explored in order to avoid such reactions. Protected nucleoside 3′-phosphonates are alkyl nucleoside 3′-phosphonates that correspond to hydrolyzed products of the phosphoramidite units. However, these "monobasic phosphonates" cannot be activated by the condensing reagents ordinarily used in the phosphotriester approach.[2a] In oligonucleotide synthesis, alkyl nucleoside 3′-phosphonates have not been used as starting nucleotide units except for the first synthesis of thymidyl(3′→5′)thymidine achieved by Todd.[4] In this case, *N*-chlorosuccinimide (NCS) was employed as an oxidative chlorinating reagent for the transformation of benzyl nucleoside 3′-phosphonates into the corresponding phosphorochloridates. Generally, dialkyl phosphonates **1** react with certain "positive halogen" compounds to form the corresponding phosphoryl halides **2**, with oxidation of the phosphorus atom[5] (Scheme I). In a previous paper,[6] we described a novel strategy for the rapid internucleotidic bond formation by using 2-cyanoethyl nucleoside 3′-phosphonates as starting nucleotide units. Thus "nonoxidative" chlorination of dialkyl phosphonates **1** to dialkyl phosphorochloridites **3** was achieved by using tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP) as a chlorinating reagent.

Scheme I



In this paper, we report a further study of the nonoxidative chlorination of dialkyl phosphonates as a general reaction and its application to oligodeoxyribonucleotide synthesis.

Results and Discussion

Preparation of Alkyl Nucleoside 3′-Phosphonates. Protected nucleosides **4a–e** were allowed to react with 2-cyanoethyl phosphorobistriazolidite, prepared in situ from 2-cyanoethyl phosphorodichloridite and 1,2,4-triazole, to give the 2-cyanoethyl nucleoside 3′-phosphonates **5a–e** in 90–100% yields (Scheme II). Excess phosphitylating reagent and 1,2,4-triazole were removed by extraction. No contamination of the bis(3′-nucleoside) phosphites with the nucleoside 3′-phosphonate was detected by ^{31}P NMR, TLC, and ^{1}H NMR. Further purification was not necessary for practical use. This procedure is promising for large-scale synthesis of the 2-cyanoethyl nucleoside 3′-phosphonate units. A reference compound for **5b** was obtained from the corresponding commercially available phosphoramidite unit **6b** by hydrolysis.

Methyl nucleoside 3′-phosphonates **7a–d** were also synthesized. Methyl phosphorobistriazolidite is more reactive than 2-cyanoethyl phosphorobistriazolidite. In order to reduce the reactivity of the phosphitylating reagent to prevent the formation of bis(3′-nucleoside) phosphite, we chose the bisimidazolidite instead of the bistriazolidite. With the bisimidazolidite, the desired methyl nucleoside 3′-phosphonates **7a–d** were obtained in high purity in 90–99% yields as the 2-cyanoethyl derivatives. N^2-Propionyl-O^6-diphenylcarbamoyl protection could not be applied to the methyl nucleoside 3′-phosphonate unit in deoxyguanosine because the (diphenylcarbamoyl)oxy group at the C^6 position of the guanine would be displaced by the phenylthio group during deprotection of the methyl group by benzenethiolate ion.[7]

(1) (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron* Lett. **1981**, *22*, 1859. (b) Sinha, N. D.; Biernat, J.; Köster, H. *Ibid.* **1983**, *24*, 5843. (c) Usman, N.; Ogilvie, K. K.; Jiang, M.-Y.; Cedergren, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 7845. (d) Tanimura, H.; Fukazawa, T.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1988**, *29*, 577.

(2) (a) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chem. Scr.* **1985**, *25*, 280. (b) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R. *Tetrahedron Lett.* **1986**, *27*, 4055. (c) Froehler, B. C.; Matteucci, M. D. *Ibid.* **1986**, *27*, 469. (d) Tanaka, T.; Tamatsukri, S.; Ikehara, M. *Nucleic Acids Res.* **1987**, *15*, 7235.

(3) (a) Kuyl-Yehekiely, E.; Spierenburg, M.; van der Elst, H.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 505. (b) de Vroom, E.; Spierenburg, M. L.; Dreef, C. E.; van der Marel, G. A.; van Boom, J. H. *Ibid.* **1987**, *106*, 65. (c) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Nucleosides Nucleotides* **1987**, *6*, 655. (d) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *J. Chem. Soc., Perkin Trans. II* **1987**, 1209. (e) Garegg, P. J.; Stawinski, J.; Strömberg, R. *J. Org. Chem.* **1987**, *52*, 284.

(4) Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1955**, 2632.

(5) (a) Atherton, F. R.; Oppenshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1945**, 382. (b) Gerrard, W.; Jeacocke, G. J. *Ibid.* **1945**, 3647. (c) McCombie, H.; Saunders, B. C.; Stacey, G. J. *Ibid.* **1945**, 921. (d) Atherton, F. R.; Todd, A. R. *Ibid.* **1947**, 647. (e) Atherton, F. R.; Howard, H. T.; Todd, A. R. *Ibid.* **1948**, 1106.

(6) Wada, T.; Hotoda, H.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1988**, *29*, 4143.

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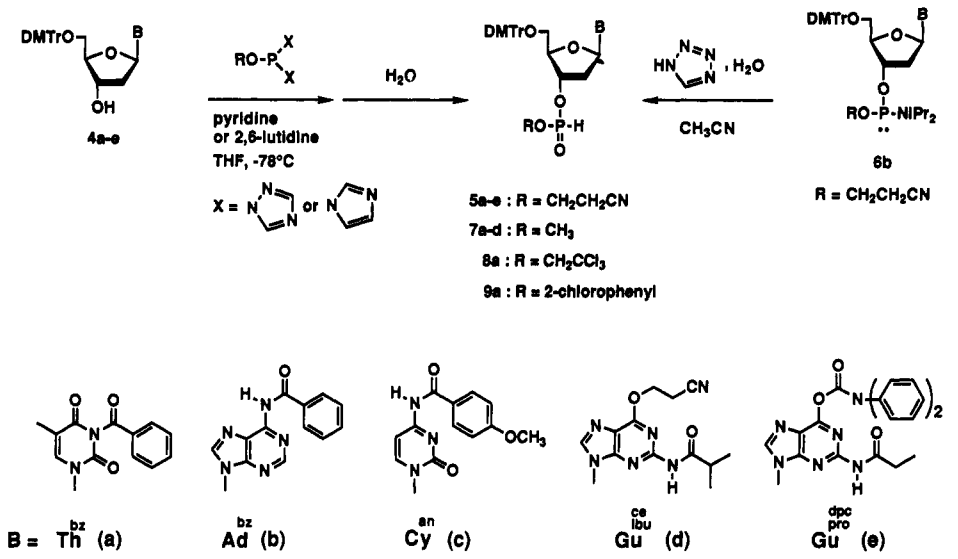
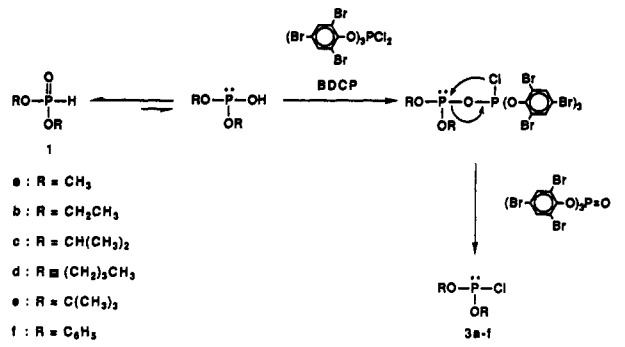
Scheme II



Scheme III



2,2,2-Trichloroethyl nucleoside 3′-phosphonate (**8a**) was obtained in 98% yield by the procedure used for the 2-cyanoethyl derivatives. 2-Cyanoethyl and methyl derivatives are stable at least 1 year at −20 °C, but **8a** was unstable and gradually decomposed during storage at the same temperature. In addition, synthesis of a 2-chlorophenyl nucleoside 3′-phosphonate (**9a**) was attempted, but the compound was quickly hydrolyzed to the nucleoside 3-phosphonate and 2-chlorophenol.

From the above experiments, the 2-cyanoethyl and methyl esters (**5** and **7**) were found to be stable as alkyl nucleoside phosphonates.[8]

Nonoxidative Chlorination of Dialkyl Phosphonates and Internucleotidic Bond Formation. We have reported that new types of condensing reagents i.e., bis(2,4,6-tribromophenoxy)trichlorophosphorane (BTCP)[9] and tris(2,4,6-tribromophenoxy)dichlorophosphorane (BDCP),[10] are effective in the phosphotriester approach to oligonucleotide synthesis. During the condensation reaction, the reagents act as chlorinating reagents for the transformation of *S*-phenyl nucleoside 3′-phosphorothioates into the corresponding phosphorochloridates; the P(O)*OH* function of the phosphodiester is converted into P(O)*Cl*.

Since dialkyl phosphonates **1** tautomerize to dialkyl phosphites, we examined whether BDCP could convert the P*OH* group of dialkyl phosphites into P*Cl* without oxidation of the phosphorus atom (Scheme III). It is apparent that the P*Cl* function is more reactive than P(O)*Cl*, and the former is advantageous for rapid ester bond formation. In order to test the conversion, an appropriate dialkyl or diphenyl phosphonate or alkyl nucleoside 3′-phosphonate (**1**, **5**, **7**, or **8**) was mixed with 1.5 equiv of BDCP in pyridine, and the reaction was monitored by ^{31}P NMR. In each case, the signal of the phosphonate disappeared within 5 min and a new signal was observed in the low-field region 160–170 ppm. The chemical shift showed the formation of the corresponding phosphorochloridite (**3**, **10**, **11**, or **12**) without oxidation of the phosphorus atom (Table I). The BDCP (−64.62 ppm) was completely converted into an inert species, tris(2,4,6-tribromophenyl) phosphate (−23.01 ppm). The reaction proceeded quantitatively when a slight excess of BDCP was used. Practically, 1.5 equiv of BDCP per phosphonate was required to compensate for the hydrolysis of BDCP. In all cases, no side reaction was observed. Typical ^{31}P

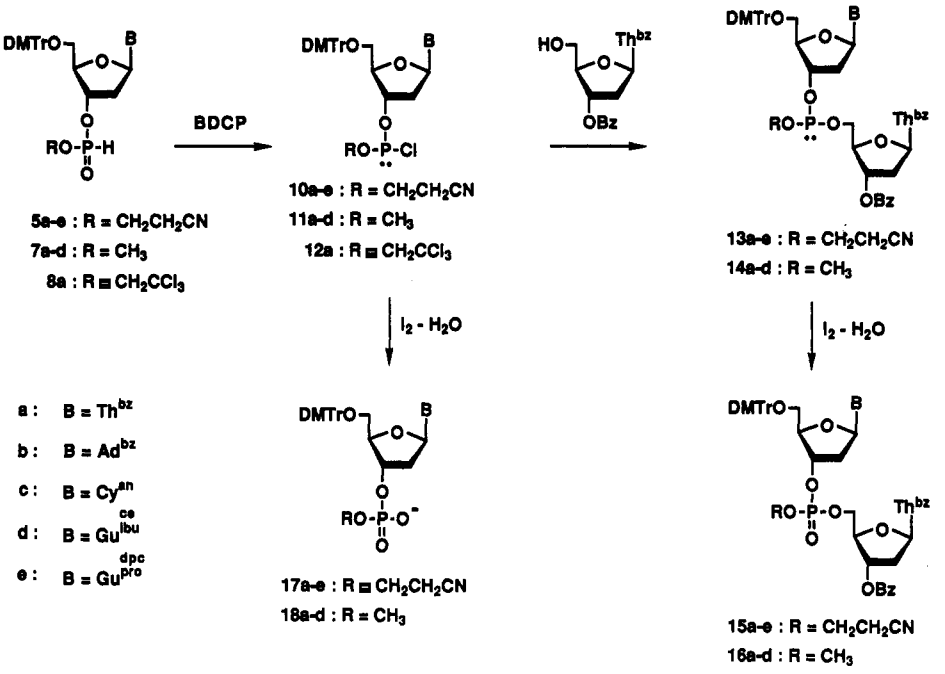
Table I. ^{31}P NMR Chemical Shifts in the BDCP-Promoted Chlorination

dialkyl phosphonate, δ^a		dialkyl phosphorochloridite, δ^a	
1a	10.95	**3a**	169.44
1b	7.27	**3b**	166.14
1c	4.35	**3c**	166.82
1d	8.14	**3d**	166.92
1e	−4.55	**3e**	173.89
1f	1.26	**3f**	158.59
5a	7.94	**10a**	165.27
5b	7.85	**10b**	165.27
5c	7.94	**10c**	165.27
5d	6.98	**10d**	163.63
5e	8.43	**10e**	165.27
7a	9.11	**11a**	167.02
7b	9.20	**11b**	167.31
7c	9.30	**11c**	167.50
7d	8.43, 8.23	**11d**	166.14
8a	6.20	**12a**	162.95

[a] Pyridine was used as a solvent and 85% H_3PO_4 was used as an external standard.

(7) Huss, S.; Gosselin, G.; Imbach, J. L. *J. Org. Chem.* **1988**, *53*, 499.
(8) About 30–40% of the alkyl nucleoside 3′-phosphonate was decomposed by being passed through the silical gel column, forming nucleoside 3′-phosphonates and parent 3′-hydroxy components. This fact may be attributed to the hydrolysis by nonselective silica gel catalyzed P–O bond fission. However, as shown in the above experiment, the pure alkyl nucleoside 3′-phosphonates **5** and **7** could be obtained without using silica gel column chromatography.
(9) Matsuzaki, J.; Hotoda, H.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1986**, *27*, 5645.
(10) (a) Hotoda, H.; Wada, T.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1987**, *28*, 1681; (b) *Nucleic Acids Res.* **1989**, *17*, 5291.

Scheme IV



NMR spectra of the reaction of dimethyl phosphonate (**1a**) are shown in Figure 1.

This method for quantitative transformation of dialkyl phosphonates to dialkyl phosphorochloridites was successfully applied to internucleotidic bond formation. An in situ prepared nucleoside 3′-phosphorochloridite (**10** or **11**, 0.15 mmol) was treated with N^3,3′-*O*-dibenzoylthymidine (0.1 mmol) in pyridine. TLC monitoring indicated that the reaction was almost complete within 1 min. The ^{31}P NMR spectra showed a new signal of the dinucleoside phosphite (**13** or **14**) in the region 138–140 ppm. The phosphite intermediate was oxidized with I_2–H_2O for 10 min to give the dinucleoside phosphate (**15** or **16**), while excess alkyl nucleoside 3′-phosphorochloridite was oxidized and hydrolyzed to the corresponding phosphodiester (**17** or **18**). The ^{31}P NMR chemical shifts of the all nucleotide derivatives observed in a series of reactions (Scheme IV) are summarized in Table II. Typical ^{31}P NMR spectra of these reactions are shown in Figure 2. During the oxidation of I_2–H_2O, tris(2,4,6-tribromophenyl) phosphate was hydrolyzed to bis(2,4,6-tribromophenyl) phosphate (−12.79 ppm) and 2,4,6-tribromophenol. In some cases, bis(2,4,6-tribromophenyl) phosphate or 2,4,6-tribromophenol could not be removed and contaminated the desired dimer during silica gel column chromatography. In order to avoid hydrolysis of tris(2,4,6-tribromophenyl) phosphate, *tert*-butyl hydroperoxide[11] was used as an oxidizing reagent in a dry system. Tris(2,4,6-tribromophenyl) phosphate formed in the reaction crystallized during concentration of the reaction mixture and was easily removed by filtration and silica gel column chromatography. The procedure was effective for obtaining pure nucleotides. The protected TpT derivatives **15a** and **16a**, obtained by *tert*-butyl hydroperoxide oxidation, were isolated in 95% and 98% yields, respectively. These results indicate that the present approach should be superior to the phosphoramidite approach, especially in large-scale liquid-phase synthesis.[12]

Solid-Phase Synthesis of Oligomers. The above condensation reaction was used in the solid-phase synthesis. 2-Cyanoethyl nucleoside 3′-phosphonates **5a–e** were preactivated with BDCP in pyridine for 5 min. The reaction mixture was added to N^4-anisoyldeoxycytidine 3′-*O*-succinate bound to controlled pore glass (CPG). The phosphonate units **5a–e** were used in 30-fold excess relative to the 5′-hydroxy component on the CPG. The reaction mixture was quenched at 1, 3, 5, or 10 min and oxidized with I_2–H_2O to give the fully protected dimers **19a–e** on the CPG (Scheme V). Then the dimethoxytrityl group was removed by 1% TFA in dichloromethane, and the yield of the condensation was estimated by colorimetry of released dimethoxytrityl cation (Figure 3). In each case, except for the N^2-propionyl-O^6-diphenylcarbamoyl-protected deoxyguanosine unit **5e**, the condensation was complete within 3–5 min and the yield was higher than 98%. With **5e**, the coupling yield was lower (94% for 10-min condensation). This result suggests that the bulkiness of the protecting groups, especially at O^6, affects the reactivity of the unit. In consideration of this fact, we chose N^2-isobutyryl-O^6-(2-cyanoethyl) protection[13] for the deoxyguanosine unit **5d**.

Table II. ^{31}P NMR Chemical Shifts of the Nucleotide Derivatives after Condensation and Oxidation

phosphite triester, δ^a		phosphate triester, δ^a		phosphate diester, δ^a	
13a	139.21	**15a**	−2.13	**17a**	−0.97
13b	139.41	**15b**	−1.84	**17b**	−0.87
13c	139.50	**15c**	−2.03	**17c**	−0.87
13d	138.44	**15d**	−3.39	**17d**	−2.23
13e	139.79	**15e**	−2.13	**17e**	−0.97
14a	140.18, 139.50	**16a**	−0.48, −0.68	**18a**	0.76
14b	140.76, 139.86	**16b**	−0.10, −0.39	**18b**	0.97
14c	140.67, 140.18	**16c**	−0.19	**18c**	0.87
14d	139.68, 139.02	**16d**	−1.74, −1.94	**18d**	−0.48

[a] Pyridine was used as a solvent and 85% H_3PO_4 was used as an external standard.

(11) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191.
(12) Beiter, A. H.; Pfleiderer, W. *Synthesis* **1989**, 497.
(13) (1) Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2257. (b) Jones, R. A. In *Oligonucleotide Synthesis*; Gait, M. J., Ed.; IRL Press; Oxford, 1984; pp 23–34. (c) Hagen, M.; Chládek, S. *J. Org. Chem.* **1989**, *54*, 3189.

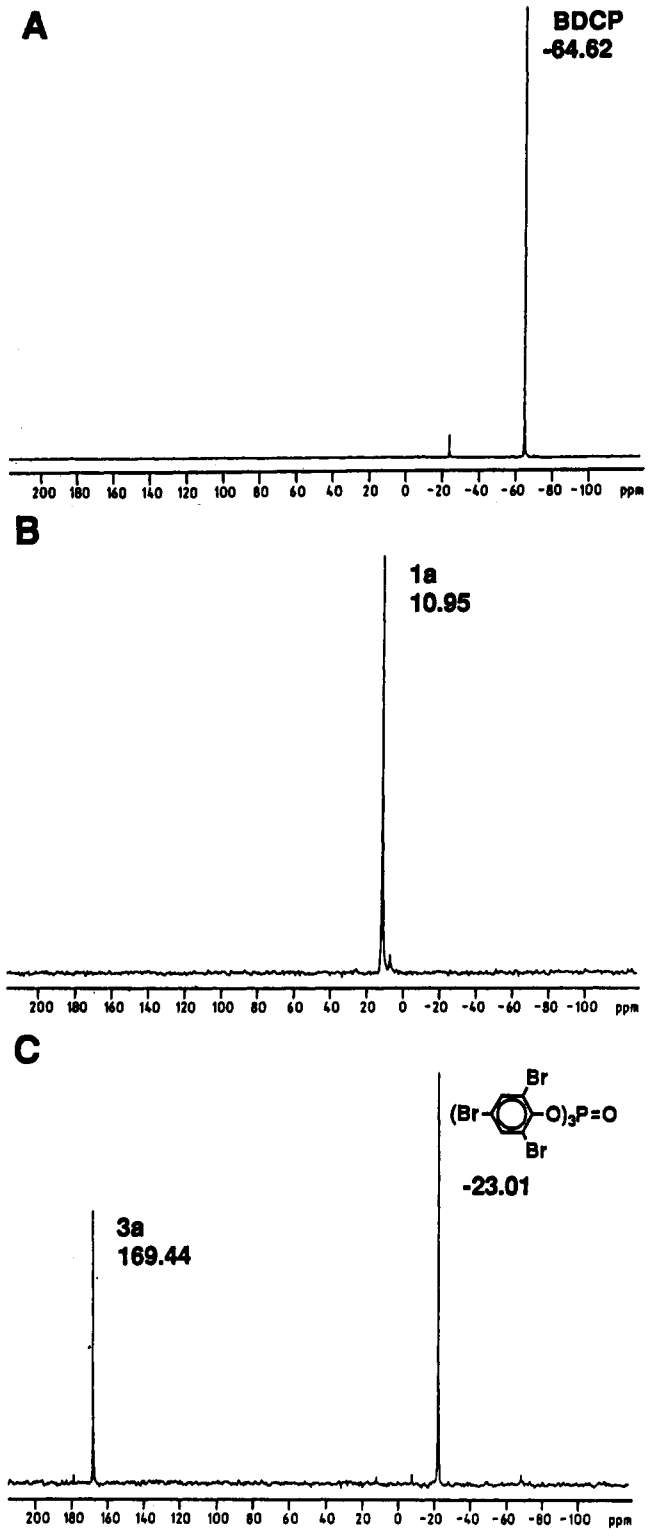


Figure 1. ^{31}P NMR spectra: A, BDCP in pyridine; B, **1a** in pyridine; C, reaction mixture obtained by the reaction of **1a** with 1.5 equiv of BDCP in pyridine for 5 min.

For a comparative study of 2-cyanoethyl and methyl nucleoside 3′-phosphonates (**5a** and **7a**) as building blocks, dodecathymidylate synthesis was examined. In each case, 0.70 μmol of the 5′-*O*-dimethoxytrityl-N^3-benzoylthymidine 3′-*O*-succinate bound to CPG (0.05 g) was used as an anchored nucleoside. The fully protected dodecamer was synthesized by repetition of the chain elongation cycle (Table III). In the case of the 2-cyanoethyl unit **5a**, the fully protected dodecamer was synthesized in 88% yield and the average yield of each cycle was 99%. After ammonia treatment, C_{18} reversed phase HPLC of the crude mixture showed a very simple profile (Figure 4A). The crude product (50 A_{260} units, 0.56 μmol) was obtained in 80% yield on the basis of the anchored nucleoside. The crude product was further purified by HPLC to obtain 33 A_{260} units (0.37 μmol) of the pure dodecathymidylate in 53% yield. In the case of the methyl unit **7a**, the fully protected dodecamer was synthesized in 92% yield, with 99% average yield at each cycle. The fully protected dodecamer on the CPG was treated successively with benzenethiol–triethylamine–dioxane (1:1:2, v/v/v)[14] for 30 min at room temperature and with ammonia. The crude mixture was analyzed by C_{18} reversed phase HPLC. However, the HPLC profile of the crude mixture did not reflect the average yield of the synthetic cycles, and some shorter oligomers were detected (Figure 4B). These oligomers may be produced during removal of the methyl group. Benzenethiolate ion may attack the 5′-carbon atom to cleave internucleotidic bonds. However, products with phenylthio groups were not detected. After the C_{18} reversed phase HPLC purification, 16 A_{260} units (0.18 μmol) of pure dodecathymidylate was obtained in 26% yield from the anchored nucleoside. The result indicates that the 2-cyanoethyl-protected unit **5** is more suitable than the methyl protected unit **7**.

Table III. Chain Elongation Cycle

step	manipulation[a]	reagent and solvent	time
1	detritylation	1% TFA in CH_2Cl_2	30 s × 3
2	wash	i. CH_2Cl_2; ii. pyridine	
3	dry		
4	preactivation	**5** or **7** (30 equiv), BDCP (45 equiv) in pyridine	5 min
5	condensation	**10** or **11** (30 equiv) in pyridine	5 min
6	wash	pyridine	
7	oxidation	0.1 M I_2 in THF–2,6-lutidine–H_2O (2:2:1, v/v/v)	1 min
8	wash	pyridine	
9	capping	Ac_2O–0.12 M DMAP in pyridine (1:9 v/v)	1 min
10	wash	i. pyridine; ii. CH_2Cl_2	

[a] Solid-phase synthesis was performed manually.

Experimental Section

General Remarks. THF was distilled from sodium and benzophenone on a continuous reflux apparatus. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from calcium hydride after several hours of refluxing, and stored over 4A molecular sieves. Dimethyl phosphonate, diethyl phosphonate, diisopropyl phosphonate, and diphenyl phosphonate (Tokyo Kasei Kogyo Co., Ltd.) were purified by distillation. Dibutyl phosphonate[15] and di-*tert*-butyl phosphonate[16] were prepared according to the literature procedures. Tris(2,4,6-tribromophenoxy)dichlorophosphorane was prepared by the procedure reported previously.[9] 2-Cyanoethyl phosphorodichloridite,[17] methyl phosphorodichloridite,[18] 2,2,2-trichloroethyl phosphorodichloridite,[19] and 2-chlorophenyl phosphorodichloridite[20] were prepared by slightly modified procedures of the literature. *tert*-Butyl hydroperoxide (3.0 M solution in 2,2,4-trimethylpentane) was purchased from Aldrich Chemical Co., Inc. Deoxyribonucleosides were purchansed from

(14) Daub, G. W.; van Tamelen, E. E. *J. Am. Chem. Soc.* **1977**, *99*, 3526.
(15) Pudovik, A. N. *Zh. Obshch. Khim.* **1957**, *27*, 2755.
(16) Goldwhite, H.; Saunders, B. C. *J. Chem. Soc.* **1957**, 2409.
(17) Nagai, H.; Fujiwara, T.; Fujii, M.; Sekine, M.; Hata, T. *Nucleic Acids Res.* **1989**, *17*, 8581.
(18) Stawinski, J.; Hozumi, T.; Narang, S. A.; Bahl, C. P.; Wu, R. *Nucleic Acids Res.* **1972**, *4*, 353.
(19) Tolkmith, J. *J. Org. Chem.* **1952**, *23*, 1682.
(20) Martin, D. R.; Pizzolato, P. J. *J. Am. Chem. Soc.* **1950**, *72*, 4584.

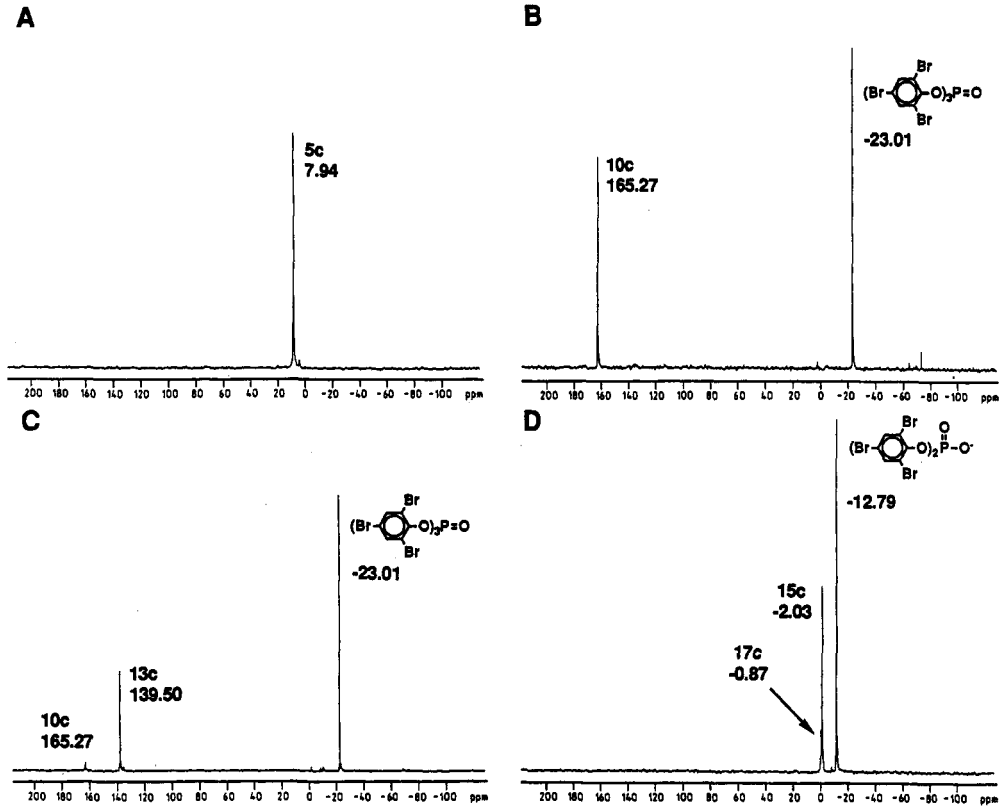


Figure 2. ^{31}P NMR spectra of the BDCP-promoted condensation: A, **5c** in pyridine; B, reaction mixture obtained by the reaction of **5c** with 1.5 equiv of BDCP in pyridine for 5 min; C, reaction mixture after addition of N^3,3′-*O*-dibenzoylthymidine in pyridine for 5 min; D, reaction mixture after oxidation with I_2–H_2O in pyridine for 10 min.

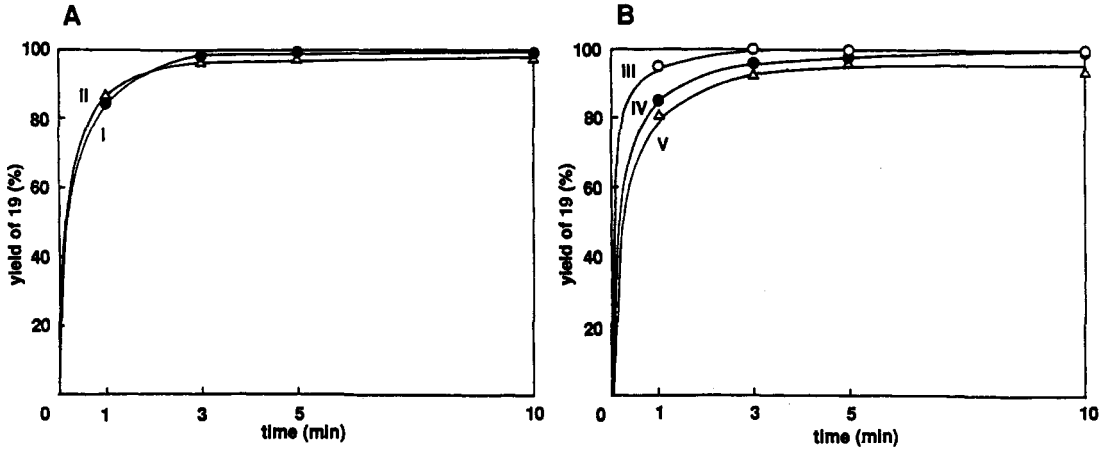
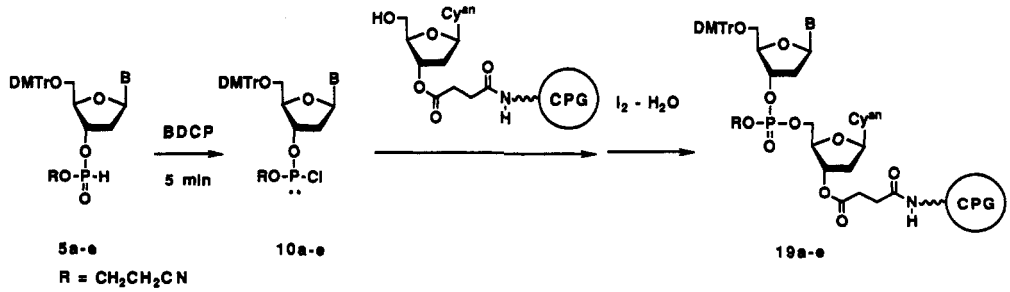


Figure 3. Rate of formation of **19a–e** using 30 equiv of **10a–e**: A, curves I (●) and II (△) correspond to **19a** and **19b**, respectively; B, curves III (○), IV (●), and V (△) correspond to **19c**, **19d**, and **19e**, respectively.

Scheme V



Yoshitomi Co., Ltd. 2-Cyanoethyl 5′-*O*-dimethoxytrityl-N^6-benzoyldeoxyadenosin-3′-yl *N*,*N*-diisopropylphosphoramidite (**6b**) was purchased from Applied Biosystems Inc. Aminopropyl CPG (BIO-500B, 515 Å, 110 μmol/g of amino group, particle size 120–200 μm), was purchased from Electro Nucleonics Inc. Solid-phase synthesis was performed manually by using a small glass filter with a stopper at the top and a stopcock at the bottom as a reaction vessel. Thin-layer chromatography was performed on precoated glass plates of kieselgel 60 F_{254} (Merck, No. 5715) and developed with dichloromethane–methanol (12:1, v/v). Column

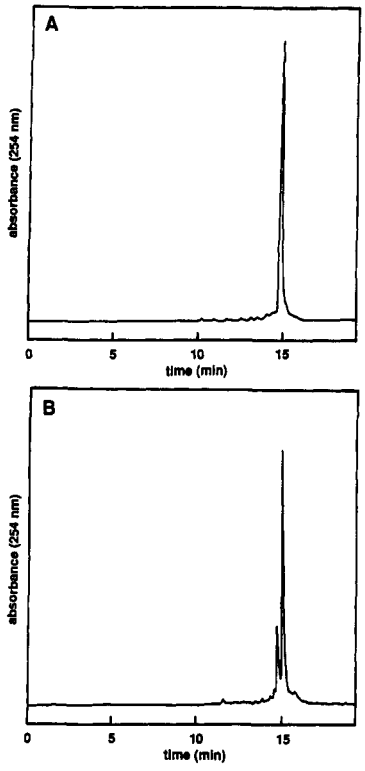


Figure 4. A: reversed phase HPLC profile of the crude dodecathymidylate obtained by using **5a** as a building block. B: reversed phase HPLC profile of the crude dodecathymidylate obtained by using **7a** as a building block.

chromatography was carried out on Wakogel C-200 silica gel. Reversed phase HPLC was performed on a column of μBondasphere 5-μm C18 100 Å, 3.9 mm × 15 cm (Nihon Waters Ltd., No. 10066) with a linear gradient of 0–60% acetonitrile in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C for 40 min at a rate of 1.0 mL/min.

2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl Phosphonate (5a). Typical Procedure. To a solution of 2-cyanoethyl phosphorodichloridite (0.247 mL, 2.0 mmol) in dry THF (5 mL) at −78 °C was added dropwise a solution of pyridine (0.25 mL) and 1,2,4-triazole (0.276 g, 4.0 mmol, dried by repeated coevaporation with dry pyridine) in dry THF (5 mL) under dry argon. The reaction mixture was stirred for 30 min at −78 °C. Compound **4a**[21] (0.648 g, 1.0 mmol, dried by coevaporation with dry pyridine) was dissolved in dry THF (5 mL) and added dropwise to the above solution over 30 min. After being stirred at −78 °C for 2 h, the mixture was treated with THF containing a small amount of water, diluted and ether, and transferred into a separating funnel. The organic layer was washed three times with 5% $NaHCO_3$, and the aqueous layer was back extracted three times with ether. The organic layer and washings were combined and dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **5a** (0.758 g, 99%) as a pale yellow foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 6.88 (J_{PH} = 726.6 Hz); ^1H NMR (270 MHz) ($CDCl_3$) δ 1.43 (3 H, s, 5-CH_3), 2.53 (1 H, m, 2′-H), 2.62 (1 H, m, 2″-H), 2.71 (2 H, t, J = 6.1 Hz, CH_2CN), 3.43 (1 H, m, 5′-H), 3.57 (1 H, m, 5″-H), 3.80 (6 H, s, OCH_3 of DMTr), 4.21 (2 H, m, $POCH_2$), 4.28 (1 H, m, 4′-H), 5.28 (1 H, m, 3′-H), 6.43 (1 H, dd, $J_{1'2'}$ = 6.0 Hz, $J_{1'2''}$ = 7.8 Hz, 1′-H), 6.87 (4 H, d, J = 8.6 Hz, 3,5-H of DMTr), 6.88, 6.90 (1 H, 2 d, J_{PH} = 724.1 Hz and 723.1 Hz, PH, diastereomers), 7.15–7.69 (15 H, m, 6-H and ArH); FAB MS m/z 765 (M^+).

2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyldeoxyadenosin-3′-yl Phosphonate (5b). The above procedure with **4b**[22] (0.658 g, 1.0 mmol) gave **5b** (0.759 g, 98%) as a colorless foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 7.05 (J_{PH} = 718.7 Hz); ^1H NMR (100 MHz) ($CDCl_3$) δ 2.62 (2 H, m, 2′-H), 2.75 (2 H, t, J = 6.8 Hz, CH_2CN), 3.46 (1 H, m, 5′-H), 3.51 (1 H, m, 5″-H), 3.78 (6 H, s, OCH_3 of DMTr), 4.23 (2 H, m, $POCH_2$), 4.37 (1 H, m, 4′-H), 5.40 (1 H, m, 3′-H), 6.52 (1 H, t, J = 7.6 Hz, 1′-H), 6.87 (4 H, d, J = 9.0 Hz, 3,5-H of DMTr), 6.99 (1 H, d, J_{PH} = 720.6 Hz, PH), 7.40–8.16 (14 H, m, ArH), 8.18 (1 H, s, 2-H), 8.71 (1 H, s, 8-H).

Synthesis of the Reference Compound. To a solution of 2-cyanoethyl 5′-*O*-dimethoxytrityl-*N*^{6}-benzoyldeoxyadenosin-3′-yl *N*,*N*-diisopropylphosphoramidite (**6b**, 0.086 g, 0.1 mmol) in acetonitrile (2 mL) was added 1*H*-tetrazole (0.007 g, 0.1 mmol). The solution was stirred for 10 min and then water (0.1 mL) was added. After being stirred for 30 min, the mixture was diluted with $CHCl_3$ and transferred into a separating funnel. The organic layer was washed three times with 5% $NaHCO_3$, and the aqueous layer was back extracted with $CHCl_3$. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **5b** (0.078 g, quantitative) as a colorless foam.

2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{4}-anisoyldeoxycytidin-3′-yl Phosphonate (5c). The same procedure with **4c**[22] (0.664 g, 1.0 mmol) gave **5c** (0.781 g, quantitative) as a colorless foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 7.07 (J_{PH} = 726.6 Hz); ^1H NMR (100 MHz) ($CDCl_3$) δ 2.60 (2 H, m, 2′-H), 2.76 (2 H, t, J = 6.0 Hz, CH_2CN), 3.52 (2 H, m, 5′-H), 3.86 (6 H, s, OCH_3 of DMTr), 3.89 (3 H, s, OCH_3 of anisoyl), 4.20 (2 H, m, $POCH_2$), 4.36 (1 H, m, 4′-H), 5.28 (1 H, m, 3′-H), 6.30 (1 H, t, J = 6.0 Hz, 1′-H), 6.87 (4 H, d, J = 8.9 Hz, 3,5-H of DMTr), 6.94 (2 H, d, J = 8.1 Hz, 3,5-H of anisoyl), 6.90, 6.93 (1 H, 2 d, J_{PH} = 718.8 Hz and 716.4 Hz, PH, diastereomers), 7.20–2.50 (12 H, m, H-5 and ArH), 7.88 (2 H, d, J = 8.1 Hz, 2,6-H of anisoyl), 8.11 (1, H, d, J = 7.2 Hz, 6-H).

2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryl-*O*^{6}-(2-cyanoethyl)deoxyguanosin-3′-yl Phosphonate (5d). The same procedure with **4d**[13] (0.693 g, 0.1 mmol) gave **5d** (0.728 g, 90%) as a colorless foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 7.56, 5.36 (J_{PH} = 722.7 Hz); ^1H NMR (100 MHz) ($CDCl_3$) δ 1.16 (3 H, d, J = 6.7 Hz, CH_3 of isobutyryl), 1.19 (3 H, d, J = 7.0 Hz, CH_3 of isobutyryl), 2.50–2.85 (3 H, m, 2′-H and CH of isobutyryl), 2.75 (2 H, t, J = 6.0 Hz, $POCH_2CH_2$), 3.01 (2 H, t, J = 6.4 Hz, O^6–CH_2), 3.38 (2 H, m, 5′-H), 3.75 (6 H, s, OCH_3 of DMTr), 4.12–4.38 (3 H, m, 4′-H and $POCH_2$), 4.76 (2 H, t, J = 6.4 Hz, O^6-CH_2CH_2), 5.62 (1 H, m, 3′-H), 6.36 (1 H, t, J = 6.0 Hz, 1′-H), 6.75 (4 H, d, J = 8.6 Hz, 3,5-H of DMTr), 7.00 (1 H, d, J_{PH} = 721.7 Hz, PH), 7.04–7.44 (9 H, m, ArH), 7.98 (1 H, s, 8-H), 8.17 (1 H, br s, 2-NH).

2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{2}-propionyl-*O*^{6}-diphenylcarbamoyldeoxyguanosin-3′-yl Phosphonate (5e). The same procedure with **4e**[23] (0.829 g, 0.1 mmol) gave **5e** (0.938 g, quantitative) as a pale yellow foam; ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 7.46 (J_{PH} = 722.7 Hz); ^1H NMR (100 MHz) ($CDCl_3$) δ 1.14 (3 H, t, J = 7.4 Hz, CH_3 of propionyl), 2.43–2.88 (4 H, m, 2′-H and CH_2 of propionyl), 2.66 (2 H, t, J = 6.0 Hz, CH_2CN), 3.45 (2 H, m, 5′-H), 3.78 (6 H, s, OCH_3 of DMTr), 4.13–4.35 (3 H, m, 4′-H and $POCH_2$), 5.58 (1 H, m, 3′-H), 6.36 (1 H, t, J = 6.0 Hz, 1′-H), 6.76 (4 H, d, J = 8.7 Hz, 3,5-H of DMTr), 6.93 (1 H, d, J_{PH} = 710.7 Hz, PH), 7.14–7.44 (19 H, m, ArH), 8.01 (1 H s, 8-H), 8.30 (1 H, br s, 2-NH).

Methyl 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl Phosphonate (7a). Typical Procedure. To a solution of methyl phosphorodichloridite (0.189 mL, 2.0 mmol) in dry THF (5 mL) at −78 °C was added dropwise a mixture of imidazole (0.272 g, 4.0 mmol) and 2,6-lutidine (0.58 mL, 5 mmol) in dry THF (5 mL) under dry argon. The reaction mixture was stirred for 30 min at −78 °C. Compound **4a** (0.648 g, 1.0 mmol, dried by coevaporation with dry pyridine) was dissolved in dry THF (5 mL) and added dropwise to the above solution over 30 min. After being stirred at −78 °C for 2 h, the mixture was treated with THF containing a small amount of water, diluted with ether, and transferred into a separating funnel. The organic layer was washed three times with 5% $NaHCO_3$, and the aqueous layer was back extracted three times with ether. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting foam was dissolved in

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dichloromethane (2 mL) and precipitated with *n*-hexane (500 mL) to remove 2,6-lutidine. The precipitate was filtered, washed with *n*-hexane, and dried in vacuo to give **7a** (0.661 g, 91%) as a white powder: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 8.43 (J_{PH} = 699.2 Hz); ^{1}H NMR (270 MHz) ($CDCl_3$) δ 1.43 (3 H, s, 5-CH_3), 2.51 (1 H, m, 2′-H), 2.63 (1 H, m, 2″-H), 3.43 (1 H, m, 5′-H), 3.57 (1 H, m, 5″-H), 3.69, 3.75 (3 H, 2 d, J_{POCH} = 12.1 Hz and 12.1 Hz, $POCH_3$, diastereomers), 8.31 (6 H, s, OCH_3 of DMTr), 4.27 (1 H, m, 4′-H), 5.27 (1 H, m, 3′-H), 6.44 (1 H, dd, $J_{1'2'}$ = 8.2 Hz, $J_{1'2''}$ = 5.7 Hz, 1′-H), 6.78, 6.81 (1 H, J_{PH} = 710.0 Hz and 708.9 Hz, PH, diastereomers), 6.86 (4 H, d, J = 9.5 Hz, 3,5-H of DMTr), 7.27–7.94 (15 H, m, 6-H and ArH); FAB MS m/z 726 (M^+).

Methyl 5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyldeoxyadenosin-3′-yl Phosphonate (7b). In a similar procedure, using pyridine instead of 2,6-lutidine, **4b** (0.658 g, 1.0 mmol) gave **7b** (0.728 g, 99%) as a colorless foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 9.01, 8.72 (J_{PH} = 707.0 Hz and 710.9 Hz); ^{1}H NMR (100 MHz) ($CDCl_3$) δ 2.80 (2 H, m, 2′-H), 3.35 (1 H, m, 5′-H), 3.40 (1 H, m, 5″-H), 3.70, 3.70 (3 H, 2 d, J_{POCH} = 11.4 Hz and 11.7 Hz, $POCH_3$), 3.72 (6 H, s, OCH_3 of DMTr), 4.31 (1 H, m, 4″-H), 5.22 (1 H, m, 1′-H), 6.73 (1 H, d, J_{PH} = 694.5 Hz, PH), 6.81 (4 H, d, J = 8.8 Hz, 3,5-H of DMTr), 7.06–7.91 (14 H, m, ArH), 8.01 (1 H, s, 2-H), 8.92 (1 H, s, 8-H).

Methyl 5′-*O*-Dimethoxytrityl-*N*^{4}-anisoyldeoxycytidin-3′-yl Phosphonate (7c). With use of the same procedure as for **7b**, **4c** (0.664 g, 1.0 mmol) gave **7c** (0.727 g, 98%) as a colorless foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 8.43 (J_{PH} = 709.0 Hz); ^{1}H NMR (100 MHz) ($CDCl_3$) δ 2.81 (2 H, m, 2′-H), 3.45 (2 H, m, 5′-H), 3.70, 3.75 (3 H, 2 d, J_{POCH} = 13.0 Hz and 14.7 Hz, $POCH_3$, diastereomers), 3.79 (6 H, s, OCH_3 of DMTr), 3.85 (3 H, OCH_3 of anisoyl), 6.69, 6.73 (1 H, 2 d, J_{PH} = 696.6 Hz and 698.7 Hz, PH, diastereomers), 6.80 (4 H, d, J = 8.9 Hz, 3,5-H of DMTr), 6.87 (2 H, d, J = 8.9 Hz, 3,5-H of anisoyl), 7.0–7.4 (12 H, m, 5-H and ArH), 7.78 (2 H, d, J = 8.4 Hz, 2,6-H of anisoyl), 8.03 (1, H, d, J = 7.8 Hz, 6-H).

Methyl 5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryl-*O*^{6}-(2-cyanoethyl)deoxyguanosin-3′-yl Phosphonate (7d). With use of the procedure for **7a**, **4d** (0.693 g, 0.1 mmol) gave **7d** (0.694 g, 90%) as a white powder: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 8.91, 8.62 (J_{PH} = 707.0 Hz); ^{1}H NMR (100 MHz) ($CDCl_3$) δ 1.17 (3 H, d, J = 6.6 Hz, CH_3 of isobutyryl), 1.21 (3 H, d, J = 6.6 Hz, CH_3 of isobutyryl), 2.62–2.82 (3 H, m, 2′-H and CH of isobutyryl), 3.01 (2 H, t, J = 6.3 Hz, O^6-CH_2), 3.38 (2 H, m, 5′-H), 3.71, 3.76 (3 H, 2 d, J_{POCH} m 13.5 Hz and 13.5 Hz, $POCH_3$, diastereomers), 3.78 (6 H, s, OCH_3 of DMTr), 4.29 (1 H, m, 4′-H), 4.74 (2 H, t, J = 6.3 Hz, O^6-CH_2CH_2), 5.52 (1 H, m, 3′-H), 6.27 (1 H, t, J = 6.0 Hz, 1′-H), 6.69 (4 H, d, J = 11.4 Hz, 3,5-H of DMTr), 6.78, 6.80 (1 H, 2 d, J_{PH} = 697.0 Hz and 698.9 Hz, PH, diastereomers), 7.04–7.34 (9 H, m, ArH), 7.86 (1 H, s, 8-H), 7.99 (1 H, br s, 2-NH).

2,2,2-Trichloroethyl 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl Phosphonate (8a). To a solution of 2,2,2-trichloroethyl phosphorodichloridite (0.311 mL, 2.0 mmol) in dry THF (5 mL) at −78 °C was added dropwise a mixture of 1,2,4-triazole (0.276 g, 4.0 mmol) and pyridine (0.25 mL) in dry THF (5 mL) under dry argon. The mixture was stirred for 30 min at −78 °C. Compound **4a** (0.648 g, 1.0 mmol, dried by coevaporation with dry pyridine) was dissolved in dry THF (5 mL) and added dropwise to the above solution over 30 min. After being stirred at −78 °C for 2 h, the reaction was quenched with THF containing a small amount of water, and the mixture was diluted with ether and then transferred into a separating funnel. The organic layer was washed three times with 5% $NaHCO_3$, and the aqueous layer was back extracted three times with ether. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **8a** (0.827 g, 98%) as a pale yellow foam: ^{31}P NMR ($CDCl_3$–$CHCl_3$, 1:3, v/v) δ 6.68 (J_{PH} = 738.3 Hz); ^{1}H NMR (270 MHz) ($CDCl_3$) δ 1.43 (3 H, s, 5-CH_3), 2.53 (1 H, m, 2′-H), 2.67 (1 H, m, 2″-H), 3.43 (1 H, m, 5′-H), 3.62 (1 H, m, 5″-H), 3.80 (6 H, s, OCH_3 of DMTr), 4.32 (1 H, m, 4′-H), 4.63, 4.66 (2 H, 2 d, J_{POCH} = 14.7 Hz and 14.7 Hz, $POCH_2$), 5.33 (1 H, m, 3′-H), 6.43 (1 H, dd, $J_{1'2'}$ = 6.0 Hz, $J_{1'2''}$ = 7.8 Hz, 1′-H), 6.87 (4 H, d, J = 8.4 Hz, 3,5-H of DMTr), 7.06 (1 H, d, J_{PH} = 738.9 Hz, PH), 7.15–7.95 (15 H, m, 6-H and ArH); FAB MS m/z 844 (M^+).

^{31}P NMR Study of the Reaction of Dialkyl Phosphonates with BDCP. The dialkyl phosphonate (**1a–f**, 0.2 mmol) was dissolved in a mixture of dry pyridine (1.5 mL) and pyridine-d_5 (0.2 mL), and the solution was transferred into an NMR sample tube (10 mm × 180 mm). BDCP (0.327 g, 0.3 mmol) was dissolved in hot dry pyridine (1.5 mL), and the solution was cooled to room temperature and added to the dialkyl phosphonate solution. A spectrum was recorded during 0–5 min.

^{31}P NMR Study of the Formation of Alkyl Nucleoside 3′-Phosphorochloridites and Internucleotidic Bond Formation. An alkyl nucleoside 3′-phosphonate (**5a–e**, **7a–d**, or **8a**, 0.15 mmol) was dried by repeated coevaporation with dry pyridine and dissolved in mixture of dry pyridine (1.2 mL) and pyridine-d_5 (0.3 mL). The solution was transferred into an NMR sample tube (10 mm × 180 mm). BDCP (0.246 g, 0.225 mmol) was dissolved in hot dry pyridine (1.0 mL), and the solution was cooled to room temperature and added to the alkyl nucleoside 3′-phosphonate solution. A spectrum was recorded during 0–5 min. To the reaction mixture was added a solution of N^3,3′-*O*-dibenzoylthymidine (0.045 g, 0.1 mmol, dried by repeated coevaporation with dry pyridine) in dry pyridine (0.3 mL), and another spectrum was recorded during 0–5 min. To the mixture was added a solution of iodine (0.076 g, 0.3 mmol) in pyridine–water (8:2, v/v, 0.3 mL), and a spectrum was recorded during 0–10 min.

2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl *N*^{3},3′-*O*-Dibenzoylthymidin-5′-yl Phosphate (15a). A solution of BDCP (0.246 g, 0.225 mmol) in dry pyridine (1.5 mL) was added to **5a** (0.115 g, 0.15 mmol, dried by repeated coevaporation with dry pyridine). After 5 min, the mixture was added to N^3,3′-*O*-dibenzoylthymidine (0.045 g, 0.1 mmol, dried by repeated coevaporation with dry pyridine). The mixture was stirred for 10 min, and a 3.0 M solution of *tert*-butyl hydroperoxide in 2,2,4-trimethylpentane (0.2 mL, 0.6 mmol) was added. After being stirred for 15 min, the mixture was concentrated and coevaporated with toluene. Tris(2,4,6-tribromophenyl) phosphate was removed by filtration, and the filtrate was concentrated to dryness. The residue was applied to a column of silica gel (10 g). Chromatography was performed with dichloromethane containing 0.5% pyridine, applying a methanol gradient (0–1%) to give **15a** (0.115 g, 95%) as a colorless foam: ^{31}P NMR (pyridine-d_5) δ −2.13; ^{1}H NMR (270 MHz) ($CDCl_3$) δ 1.44 (3 H, s, 5-CH_3 of Tp), 1.95, 1.97 (3 H, 2s, 5-CH_3 of pT, diastereomers), 2.52–2.78 (4 H, m, 2′-H of Tp, 2″-H of Tp, 2′-H of pT, and 2″-H of pT), 2.72 (2 H, t, J = 5.8 Hz, CH_2CN), 3.45 (1 H, m, 5′-H of Tp), 3.61 (1 H, m, H-5″ of Tp), 3.78 (6 H, s, OCH_3 of DMTr), 4.10–4.45 (6 H, m, 4′-H of Tp, 4′-H of pT, 5′-H of pT, 5″-H of pT, and $POCH_2$), 5.25 (1 H, m, 3′-H of Tp), 5.48 (1 H, m, 3′-H of pT), 6.33–6.47 (2 H, m, 1′-H of Tp and 1′-H of pT), 6.86 (4 H, d, J = 7.9 Hz, 3,5-H of DMTr), 7.18–7.99 (26 H, m, ArH).

Methyl 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl *N*^{3},3′-*O*-Dibenzoylthymidin-5′-yl Phosphate (16a). Compound **16a** was synthesized by the procedure described above. Compound **7a** (0.109 g, 1.5 mmol) gave **16a** (0.115 g, 98%) as a colorless foam: ^{31}P NMR (pyridine-d_5) δ −0.48, −0.68; ^{1}H NMR (270 MHz) ($CDCl_3$) δ 1.43, 1.44 (3 H, 2 s, 5-CH_3 of Tp, diastereomers), 1.96, 1.98 (3 H, 2 s, 5-CH_3 of pT, diastereomers), 2.49–2.75 (4 H, m, 2′-H of Tp, 2″-H of Tp, 2′-H of pT, and 2″-H of pT), 3.45 (1 H, m, 5′-H of Tp), 3.60 (1 H, m, H-5″ of Tp), 3.76, 3.81 (3 H, 2 d, J_{POCH} = 12.1 Hz and 14.7 Hz, $POCH_3$, diastereomers), 3.80 (6 H, s, OCH_3 of DMTr), 4.24–4.32 (2 H, m, 4′-H of Tp and 5′-H of pT), 4.36–4.44 (2 H, m, 4′-H of pT and 5″-H of pT), 5.23 (1 H, m, 3′-H of Tp), 5.46 (1 H, m, 3′-H of pT), 6.39–6.48 (2 H, m, 1′-H of Tp and 1′-H of pT), 6.87 (4 H, d, J = 8.1 Hz, 3,5-H of DMTr), 7.29–7.99 (26 H, m, ArH).

Solid-Phase Synthesis of Dimers. An aminopropyl CPG was functionalized with 5′-*O*-dimethoxytrityl-*N*^{4}-anisoyldeoxycytidine 3′-*O*-succinate (18 μmol/g) by a literature procedure.[24] The functionalized CPG (0.05 g, 0.90 μmol) was treated with 1% TFA in dichloromethane (1 mL) for 30 s three times and washed and dichloromethane followed dry pyridine and then dried in vacuo. A nucleotide unit (**5a–e**) (27 μmol) was preactivated by BDCP (0.044 g, 40.5 μmol) in pyridine (0.2 mL) for 5 min as described for **15a**. The preactivated mixture was added to the CPG. The condensation reaction was stopped at 1, 3, 5, or 10 min by washing with pyridine, and each CPG was treated with

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a 0.1 M solution of iodine in THF-2,6-lutidine-water (2:2:1, v/v/v, 1 mL) for 1 min. Then the CPG was washed with pyridine followed by dichloromethane and further treated with a solution of 1% TFA. The condensation yield was estimated by colorimetry of released dimethoxytrityl cation in 60% $HClO_4$-ethanol (6:4, v/v) at 498 nm.

Solid-Phase Synthesis of Dodecathymidylate: Using 5a as a Building Block. An aminopropyl CPG was functionalized with 5'-dimethoxytrityl-N^3-benzoylthymidine 3'-*O*-succinate (14 μmol/g) by a literature procedure.[24] The fully protected dodecathymidylate was synthesized in 88% yield from the anchored N^3-benzoylthymidine on the CPG (0.05 g, 0.7 μmol) by repetition of the chain elongation cycle (Table III). Compound **5a** (0.016 g, 21 mmol) and BDCP (0.034 g, 31.5 μmol) were used for each cycle. The fully protected dodecamer on the CPG was treated with concentrated NH_3-pyridine (9:1, v/v, 20 mL) at room temperature for 12 h and then at 50 °C for 6 h. The CPG gel was filtered off and the filtrate was concentrated to a small volume. The aqueous solution was washed with ether three times, concentrated to a small volume (2 mL), and lyophilized to obtain 50 A_{260} units (0.56 μmol) of the crude product. It was further chromatographed by C_{18} reversed phase HPLC to obtain pure dodecathymidylate (33 A_{260} units, 0.37 μmol, 53% based on N^3-benzoylthymidine on the CPG). The chromatographic behavior and the UV spectrum of the product were identical with that of the authentic sample.[9b] UV: λ_{max} 265 nm (pH 7.0), λ_{min} 234 nm (pH 7.0).

Using 7a as a Building Block. The fully protected dodecathymidylate was synthesized in 92% yield from the anchored N^3-benzoylthymidine on the CPG (0.05 g, 0.7 μmol) by the procedure described above. Compound **7a** (0.015 g, 21 μmol) and BDCP (0.034 g, 31.5 μmol) were used for each cycle. After chain elongation, the CPG was treated with benzenethiol-triethylamine-dioxane (1:1:2, v/v/v, 0.5 mL) at room temperature for 30 min, washed with methanol, and finally treated with concentrated NH_3-pyridine (9:1, v/v, 20 mL) at room temperature for 12 h and then at 50 °C for 6 h. The CPG gel was filtered off and the filtrate was concentrated to a small volume. The aqueous solution was washed with ether three times and concentrated to a small volume (2 mL). The crude mixture was chromatographed by C_{18} reversed phase HPLC to obtain pure dodecathymidylate (16 A_{260} units, 0.18 μmol, 26% based on N^3-benzoylthymidine on the CPG). The chromatographic behavior and the UV spectrum of the product were identical with that of the authentic sample.[9b] UV: λ_{max} 265 nm (pH 7.0), λ_{min} 234 nm (pH 7.0).

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Registry No. **1a**, 868-85-9; **1b**, 762-04-9; **1c**, 1809-20-7; **1d**, 1809-19-4; **1e**, 13086-84-5; **1f**, 4712-55-4; **3a**, 3743-07-5; **3b**, 589-57-1; **3c**, 41662-51-5; **3d**, 4124-92-9; **3e**, 78543-77-8; **3f**, 5382-00-3; **4a**, 102573-69-3; **4b**, 64325-78-6; **4c**, 68892-40-0; **4d**, 90662-86-5; **4e**, 87036-65-5; **5a**, 130983-89-0; **5b**, 119484-01-4; **5c**, 130954-58-4; **5d**, 130954-59-5; **5e**, 130983-90-3; **6b**, 98796-53-3; **7a**, 130954-60-8; **7b**, 130249-64-8; **7c**, 130954-61-9; **7d**, 130954-62-0; **8a**, 130954-63-1; **9a**, 130954-64-2; **10a**, 130954-65-3; **10b**, 119484-02-5; **10c**, 130954-66-4; **10d**, 130954-67-5; **10e**, 130983-91-4; **11a**, 130954-68-6; **11b**, 78635-96-8; **11c**, 130954-69-7; **11d**, 130954-70-0; **12a**, 130954-71-1; **13a**, 131041-15-1; **13b**, 119484-03-6; **13c**, 130954-72-2; **13d**, 130954-73-3; **13e**, 130983-92-5; **14a**, 130983-93-6; **14b**, 130954-74-4; **14c**, 130983-94-7; **14d**, 130983-95-8; **15a**, 109946-76-1; **15b**, 119484-04-7; **15c**, 130983-96-9; **15d**, 130983-97-0; **15e**, 130983-98-1; **16a**, 102538-11-4; **16b**, 126733-74-2; **16c**, 130954-75-5; **16d**, 130954-76-6; **17a**, 130954-77-7; **17b**, 71100-65-7; **17c**, 130954-78-8; **17d**, 130954-79-9; **17e**, 130954-80-2; **18a**, 130954-81-3; **18b**, 130983-99-2; **18c**, 130954-82-4; **18d**, 130954-83-5; BDCP, 15493-07-9; H-dThd(*N*-Bz)-Bz, 94189-81-8.

New Spongian Diterpenoids from a Great Barrier Reef Sponge, *Spongia* sp.

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Four new spongian diterpenes, **4-6** and **8**, have been isolated from a *Spongia* sp. of sponge collected on the Great Barrier Reef. Two of the diterpenes have ring-A lactones instead of the conventional cyclohexane rings. Structures were determined primarily by proton and carbon-13 NMR analyses. One of the metabolites is slightly cytotoxic to murine leukemia cells.

A variety of spongian diterpenoids typified by epispongiadiol (**1**)[1] with a ring D furan and functionalized ring A have been isolated from sponges of the genus *Spongia*, order Dictyoceratida, collected from widely diverse geographical sites such as Australia, the Mediterranean, the Red Sea, and the Caribbean.[2,3] Additional relatives of **1** with the intact furan ring have been isolated from other Dictyoceratid sponges[3,4] and also nudibranchs,[3] although the ultimate source of the nudibranch diterpenes is considered most likely to be a sponge in the mollusk's diet. Another set of diterpenoids with the spongian carbon skeleton, but with saturated A rings and functionalization in ring D or even more extensively cleaved versions of this skeleton, e.g. **2**[5] and **3**,[6] have been reported from sponges from the Pacific, the Mediterranean, and the Caribbean, as well as from nudibranchs.[3] We report here additional members of the former group of spongians, including the first example of a nor-spongian with a ring-A γ-lactone.

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