5-Hexenyl bromide was purchased from Fluka AG, Buchs SG, Switzerland.

Reaction of Compound 1 with 5-Hexenylmagnesium Bromide. The Grignard reagent was prepared by the usual method starting from freshly distilled 5-hexenyl bromide (3 g in 10 mL of THF) and magnesium (0.45 g in 10 mL of THF), under nitrogen. The Grignard solution was added to a solution of 1 (3.3 g in 100 mL of THF) at room temperature under stirring. After 1 h, the reaction mixture was poured onto 10% aqueous NH_4Cl (300 mL) and extracted with benzene (150 mL). The separated benzene layer was washed with water (2 × 50 mL).

The benzene solution was dried with Na_2SO_4, 1 mL of it was evaporated to dryness, and the residue was kept under vacuum (0.5 mmHg) for 1 h at 70 °C. The small residue was dissolved in 5 mL of pure methanol, and the solution was injected into the HPLC apparatus (see below). The main benzene solution was treated with PbO_2 (1.5 g) under stirring. After 1 h, the mixture was filtered, and the filtrate, reduced to a small volume, was chromatographed on an SiO_2 column eluting with benzene. As the first eluate was obtained the fluorescent mixture of 4 and 5, after the yellow fraction of the mixture of 6 and 7, and finally the starting material. The yields are reported in Table I.

Compound 4: UV (EtOH) λ_{max} 225 nm (S), 255, 319; IR (Nujol) ν 3400, 1600, 1500 cm^{-1}; NMR ($CDCl_3$) δ 2.6–1.7 (8 H, cyclopentyl), 1.9–2.43 (1 H, m, cyclopentyl), 3.5 (2 H, d, CH_2-cyclopentyl), 6.7–7.7 (14 H, m, arom), 8.54 (1 H, br s, NH); mass spectrum calcd for $C_{26}H_{26}N_2$, m/e 366.48; found, m/e 366; m/e (relative intensity) 366 (M^+, 84), 297 (100), 284 (31).

Compound 5: for UV and IR spectra, see compound 4; NMR $CDCl_3$) δ 0.85–2.05 [6 H, m, $(CH_2)_3$], 3.6 [2 H, t, $CH_2(CH_3)_3$], 4.9 (1 H, s, CH=CH_2), 5.02 (2 H, d, CH=CH_2), 5.74 (1 H, m, CH=CH_2), 6.74–7.8 (14 H, m, arom), 8.37 (1 H, br s, NH); mass spectrum calcd for $C_{26}H_{26}N_2$, m/e 366.48; found, m/e 366; m/e (relative intensity) 366 (M^+, 3), 297 (87), 284 (67), recorded on the mixture 4 plus 5.

Compound 6: This is the only compound isolated in a crystalline state, mp 125 °C, from *n*-pentane, and its microanalysis was satisfactory: C, −0.03; H, +0.03; N, −0.12. UV (EtOH) λ_{max} max 250 nm, 270 (s), 380; IR (Nujol) ν 3420, 1650, 1610, 1595 cm^{-1}; NMR ($CDCl_3$) δ 1.0–2.0 (9 H, m, cyclopentyl), 2.46 (2 H, d, CH_2-cyclopentyl), 4.84 (1 H, br s, NH), 6.46 (2 H, d, arom), 6.8–7.0 (3 H, m, arom), 7.1–7.5 (7 H, m, arom), 7.68–7.91 (2 H, m, arom); mass spectrum calcd for $C_{26}H_{26}N_2$, m/e 366.48; found, m/e 366; m/e (relative intensity) 366 (M^+, 3), 297 (8), 284 (100).

Compound 7: for UV and IR spectra, see compound 6; NMR ($CDCl_3$) δ 1.23–1.7 [4 H, m, $CH_2(CH_2)_2CH_2$), 1.97–2.46 [4 H, m, $CH_2(CH_2)_2CH_2$), 4.9 (1 H, br s, NH), 4.97 [1 H, s, $(CH_2)_4CH$=CH_2], 5.1 [1 H, d, $(CH_2)_4CH$=CH_2], 5.9 [1 H, m, $(CH_2)_4CH$=CH_2], 6.48 (2 H, d, arom), 6.85–7.0 (3 H, m, arom), 7.17–7.57 (7 H, m, arom), 7.7–7.9 (2 H, m, arom); mass spectrum calcd for $C_{26}H_{26}N_2$, m/e 366.48; found, m/e 366; m/e (relative intensity), 366 (M^+, 15), 284 (100).

HPLC. The liquid chromatography carried out on the reaction mixture (see above) was performed on a Sephadex C-18 column, eluting with water/methanol (1:4) and working at λ 254 nm and at 55 °C with a flow rate of 1.2 mL/min. The elution order of the compounds was 1, 7, 6, 5, and 4.

Reaction of Compound 1 with (Cyclopentylmethyl)magnesium Bromide. The Grignard reagent, prepared starting from cyclopentylmethyl bromide (1.6 g in 10 mL of THF) and magnesium (0.24 g in 5 mL of THF), was added to the solution of 1 (0.8 g in 30 mL of THF). The products shown in eq 1 were isolated by working up the reaction as described above.

HMO Calculations. The calculations were carried out with the aid of a computer program, using the following parameter set:[29,30] h_N = 0.5, K_{CC} = 1.0, K_{C-C} = 0.9, $K_{C=N}$ = 1.06, K_{C-N} = 0.8. Calculated electron densities for N (endocyclic), C-2, C-3, and N (exocyclic) were 1.263, 0.882, 0.889, and 1.089, respectively.

Reaction in the ESR Cavity. Acetonitrile solutions of 4 plus 5 or 4 (10^{-1} M) and the corresponding quantities of I_2 and $AgClO_4$ in acetonitrile solution were placed in one of the two arms of an inverted cell similar to that described by Russell[31] and degassed with nitrogen at room temperature. The mixed materials were transferred into ESR cavity.

Registry No. 1, 23073-34-9; 2 (alkyl bromide), 2695-47-8; 4, 89031-91-4; 5, 89031-92-5; 6, 89031-93-6; 7, 89031-94-7; cyclopentylmethyl bromide, 3814-30-0.

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Acylphosphonates. 4.[1] Synthesis of Dithymidine Phosphonate: A New Method for Generation of Phosphonate Function via Aroylphosphonate Intermediates

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Dithymidine phosphonate (10) was synthesized by a new method via dithymidine aroylphosphonates (3) in which the aroyl group served as a protecting group for O=P—H functions. In a way analogous to the phosphotriester method, 3 was prepared by successive condensation of an aroylphosphonic acid with two kinds of thymidine derivatives. In the last step, the aroyl group was easily removed from 3 by *n*-butylamine in the presence of a catalytic amount of DBU to generate the O=P—H function. In enzymatic hydrolysis dithymidine phosphonate (10) was resistant to phosphodiesterases. On oxidation and sulfurization of 10 via silylation 10 was successfully converted to the corresponding phosphate and phosphorothioate derivatives, respectively.

During the past 15 years a number of oligonucleotide analogues in which the internal phosphate oxygen (O=P—OH) was altered by other elements have been synthesized and their physical properties and biological activities have been studied.

They are classified in two groups according to their replacement modes: (i) replacement of the internucleotidic P=O by P=S,[2-4] P=Se[3], and P=NR;[3] (ii) replacement

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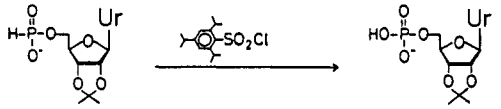
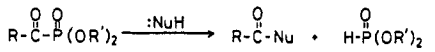
0022-3263/84/1949-2139$01.50/0

Scheme I



Scheme II



of the internucleotidic P—OH by P—NH_2,[3,5] P—NRR'[6] and P—R.[7] Eckstein has synthesized a number of phosphorothioate analogues belonging to the former class and utilized them as substrates for elucidation of the mechanism of enzyme reactions.[4] On the other hand, nonionic analogues such as methylphosphonates have been synthesized by Miller and Ts'o[8] in connection with their biological interest.

Dinucleoside phosphonates with a simple nonionic backbone structure (P–H) are expected to serve as models for the conformational and biochemical studies of nucleic acids. Dinucleoside phosphonates will also be used as synthetic intermediates in nucleotide chemistry, since dialkyl ester types of phosphonates can be converted to phosphate, phosphorothioate, and phosphoramidate derivatives. Although there have been many papers published on the synthesis of mononucleoside phosphonates,[9] to date only one example has been shown of the synthesis of a dinucleoside phosphonate, i.e., Up(H)U via a phosphite triester intermediate.[10] However this method cannot be applied to the synthesis of mixed oligonucleotide analogues which have both phosphate and phosphonate functions because of the difficulty in oxidizing the specific phosphite functions.

Independently we have developed other synthetic approaches to obtain oligonucleotide analogues containing P–H groups in a way analogous to the phosphotriester method.[11]

In this paper, we report a new method for the synthesis of dinucleoside phosphonates by use of aroylphosphonate derivatives in which the aroyl group served as a protecting group for the P–H function.

Results and Discussion

Previously we have attempted to synthesize dinucleoside phosphonates by the condensation of a mononucleoside phosphonate with another nucleoside. However, the use of arenesulfonyl chlorides or azolides such as 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) as the condensing agent gave the corresponding nucleoside phosphate as the main product unexpectedly[12] (Scheme I).

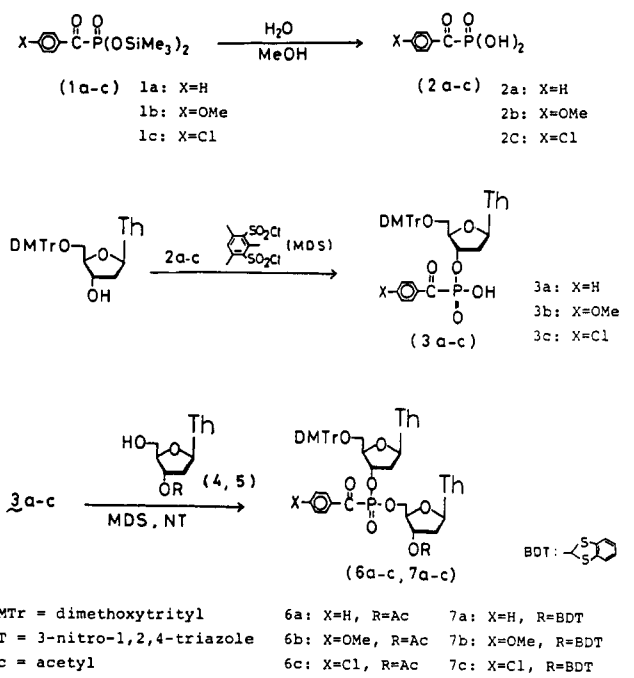
This result suggests that the oxidation by TPS proceeded via the phosphonate → phosphite isomerization. Consequently, an appropriate masking group for the P–H function is required for our purpose. We have searched for a suitable protecting group of the P–H function not in reactive phosphite form but in unreactive phosphonate form.

It is generally recognized that phosphorus–carbon (P–C) bonds of organophosphorus compounds are quite stable.[13] On the contrary, those of dialkyl acylphosphonates are known to be labile. A variety of nucleophiles attack selectively on the carbonyl carbon of acylphosphonates to give acylated products and diakyl phosphonates[14] (Scheme II).

We have recently studied the P–C bond cleavage of dialkyl acylphosphonates in detail and utilized them as acylating agents for various nucleophiles, i.e., amines,[14] alcohols,[15] and enolates.[1] These findings led us to study a new method for the synthesis of dinucleoside phosphonates by means of acylphosphonates as generators of P–H functions.

For this project aroylphosphonic acids were chosen as phosphorylating agents because their diesters were stable

Scheme III



DMTr = dimethoxytrityl
NT = 3-nitro-1,2,4-triazole
Ac = acetyl

6a: X=H, R=Ac 7a: X=H, R=BDT
6b: X=OMe, R=Ac 7b: X=OMe, R=BDT
6c: X=Cl, R=Ac 7c: X=Cl, R=BDT

Table I. Preparation of Mononucleoside Aroylphosphonates (3a–c)

compd 1, mmol	DMTrT, mmol	MDS, mmol	time, min	product yield, %
1a, 2.0	1.0	3.0	10	**3a**, 92
1b, 3.0	1.5	3.0	10	**3b**, 79
1c, 3.1	1.4	5.3	120	**3c**, 81

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Table II. Preparation of Dithymidine Aroylphosphonates (6a–c, 7a–b)

compd 3, mg, mmol	nucleoside, mg, mmol	MDS, mg, mmol	NT, mg, mmol	time, min	product, mg	yield, %
3a, 179, 0.22	**4**, 45, 0.16	162, 0.51	59, 0.51	60	**6a**, 150	96
3a, 685, 0.84	**5**, 231, 0.48	787, 2.48	283, 2.48	30	**7a**, 518	92
3b, 135, 0.16	**4**, 31, 0.11	158, 0.50	57, 0.50	60	**6b**, 97	92
3b, 532, 0.63	**5**, 240, 0.50	508, 1.60	180, 1.58	60	**7b**, 468	78
3c, 602, 0.71	**4**, 141, 0.50	432, 1.55	177, 1.45	60	**6c**, 335	66
3c, 424, 0.50	**5**, 193, 0.40	392, 1.23	140, 1.23	60	**7c**, 288	60

during chromatography compared with the diesters of aliphatic acylphosphonates.

Therefore, we tried to synthesize several appropriately protected dithymidine aroylphosphonates in order to examine their reactivity and stability.

Aroylphosphonic acids (**2a–c**) were easily prepared by the methanolysis of bis(trimethylsilyl) aroylphosphonates (**1a–c**).[16] After hexamethyldisiloxane and methanol were removed in vacuo, the acids (**2a–c**) were used without further purification for the subsequent reaction (Scheme III).

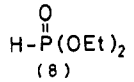
5′-*O*-(Dimethoxytrityl)thymidine (DMTrT) was allowed to react with an excess of **2a** in the presence of a bifunctional condensing agent, mesitylene-1,3-disulfonyl chloride (MDS),[17] in pyridine at room temperature. Monoesterification of **2a** was complete in 10 min and no diesterified product was detected by TLC analysis. 5′-*O*-(Dimethoxytrityl)thymidine 3′-aroylphosphonate (**3a**) were easily separated from the excess of **2a** and mesitylenedisulfonic acid by extraction and obtained in 92% yield as an analytically pure triethylammonium salt which was identified by ^{1}H NMR spectrum.

In a similar manner, **3b** and **3c** were synthesized from **2b** and **2c**, respectively. In the case of **2c**, the reaction was slower than those of **2a** and **2b** and was not complete within 2 h. These reaction conditions and results are summarized in Table I.

All the reactions proceeded like the usual phosphorylation without damage of the P–C bonds. In contrast with dialkyl aroylphosphonates, mononucleoside aroylphosphonates (**3**) proved to be very stable and the P–C bonds of **3a–c** were not cleaved under strongly basic conditions such as 1 M NaOH–pyridine (1:1, v/v).

Mononucleoside phosphonate **3a–c** were further condensed with 3′-*O*-acetylthymidine (**4**) of 3′-*O*-(1,3-benzodithiol-2-yl)thymidine (**5**)[18] in the presence of MDS and 3-nitro-1,2,4-triazole (NT)[19] in pyridine at room temperature. All the second esterifications of **3a–c** proceeded smoothly and there was no difference in reactivity among them.[20] After the usual workup, **6** or **7** was readily isolated by silica gel column chromatography. Though two diastereoisomers due to the chirality of the phosphorus were not separated on TLC, their presence was confirmed by ^{31}P NMR spectroscopy (Table IV). The reaction conditions and yields are shown in Table II.

Prior to the deprotection of **6** and **7**, we examined the stability of diethyl phosphonate (**8**) as a model compound similar to dinucleoside phosphonates.



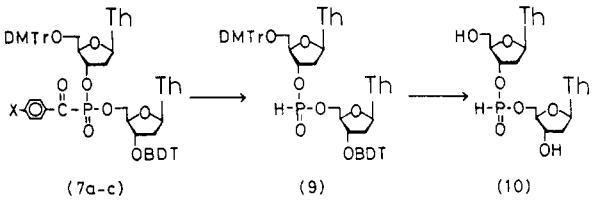
(8)

Table III. Debenzoylation of 7[a]

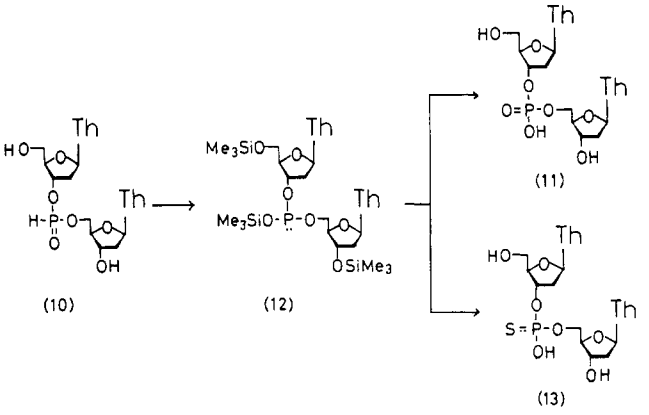
compd 7, mmol	DBU, equiv	*n*-BuNH$_2$, equiv	time, h	yield, %
7a, 0.22	0.4	5	8.5	51
0.22	0.6	10	1	72
7b, 0.21	0.4	5	45	40
0.21	0.6	10	4	73
7c, 0.22	0.4	5	6	70
0.22	0.6	10	0.5	76

[a] Dichloromethane was used as solvent.

Scheme IV



Scheme V



Compound **8** was stable under mild acidic conditions such as 0.5% trifluoroacetic acid (TFA) in dichloromethane at 0 °C for 1 h. Upon treatment with concentrated ammonium hydroxide, **8** was decomposed rapidly to ammonium ethyl phosphonate.[21] However, **8** was found to be stable to *n*-butylamine in dioxane or in dichloromethane, the conditions of which we have previously used for the C–P bond cleavage of acylphosphonates.[14] These findings suggested that TFA or *n*-butylamine could be used for the deprotection of **6** and **7**.

Unexpectedly, the C–P bond cleavage did not occur on treatment of **7** with 5 equiv of *n*-butylamine in dichloromethane or dioxane even after a week. This was probably due to great steric hindrance of the two nucleoside residues. The addition of a catalytic amount of 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) accelerated the reaction and the debenzoyled product (**9**) was obtained by chromatography on silica gel. The conditions of the deben-

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Table IV. ^{31}P NMR Spectra

	7a	9	10	12	13
^{31}P NMR, ppm	−8.45	−8.55[a]	−8.50[a]	−117.47	−57.17
	−7.31			−116.64	−56.51
solvent	$CDCl_3$	$CDCl_3$	D_2O	C_5D_5N	D_2O/py 1/3

[a] This was observed as a broad peak.

zoylation of **7a–c** and the yield of **9** is shown in Table III. Under these conditions the cleavage of the diester linkage was observed to a negligible extent.

On the acid treatment of **9**, the desired product (**10**) was obtained in 53% yield by reverse phase column chromatography (Scheme IV).

Structural proof of the dithymidine phosphonate **10** was accomplished by the following experiments: (i) After **10** was silylated with *N,O*-bis(trimethylsilyl)acetamide (BSA) in pyridine for 10 min at room temperature, iodine and triethylamine were added. After the usual workup, dithymidine phosphate (**11**) was obtained in 79% yield by paper chromatography. In this reaction, **10** was activated by simple trimethylsilylation to produce a silyl phosphite intermediate (**12**).[22] (ii) In a similar manner, treatment of **12** with elemental sulfur and triethylamine gave the phosphorothioate derivative (**13**) in 75% yield (Scheme V). (iii) The ^{13}P NMR chemical shift for **10** (−8.50 ppm) was nearly identical with that for Up(H)U (−6.85 ppm) as previously reported.[10] ^{31}P NMR spectra of **10**, **12**, and **13** are shown in Table IV. (iv) Dithymidine phosphonate **10** was degraded with spleen phosphodiesterase under the conditions where **11** was completely degraded and this result was consistent with that of Up(H)U.[10] Moreover **10** was resistant to snake venom phosphodiesterase and nuclease P1.

In conclusion the benzoyl group is the suitable protecting group for the O═P—H function to prepare dinucleoside phosphonate, because the synthetic intermediate, dinucleoside benzoylphosphonate, is obtained in good yield and the benzoyl group is easily removed at the last stage. Since the benzoylphosphonate derivatives are stable in the subsequent condensation, the new method described in this paper would be used for the synthesis of mixed oligonucleotide analogues containing both phosphate and phosphonate function. In addition nucleoside phosphonates would be useful intermediates which could be converted via silyl phosphites to biologically important nucleotide analogues in which the phosphate oxygen is altered.[23]

Experimental Section

Melting points and boiling points are uncorrected. ^1H NMR spectra were recorded at 100 MHz on a JEOL JNM PS-100 spectrometer using tetramethylsilane (Me_4Si) as an internal standard in $CDCl_3$. ^{31}P NMR spectra were obtained on a JEOL PS-100 FT spectrometer at 40.50 MHz using 85% H_3PO_4 as an external standard. UV spectra were obtained on a Hitachi 124 spectrophotometer. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta. Paper chromatography was performed by use of a descending technique with Whatman 3 MM papers using Solvent I (2-propanol-1-concentrated ammonia–water, 7:1:2, v/v/v). Column chromatography was performed using silica gel C-200 purchased from Wako Co. Ltd. and minipump for a goldfish basin was conveniently used to gain a medium pressure for rapid chromatographic separation. For reverse-phase column chromatography, C_{18} silica gel, used for Waters Prep LC/System 500A, was packed with acetone and equilibrated with water. Then a mixture dissolved in water was applied to the column. Elution was performed with water. Thin-layer chromatography was performed on Pre-coated TLC plates silica gel 60 F-254 (Merck, Art. No. 5715). The R_f values of the protected nucleoside derivatives were measured after development with CH_2Cl_2–MeOH (9:1, v/v) unless otherwise noted. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves (4 Å). CH_2Cl_2 was dried over P_4O_{10} overnight, decanted, distilled over K_2CO_3, and stored over molecular sieves (4 Å). Dioxane was distilled from sodium wire after being refluxed for 5 h and stored over molecular sieves 4 Å). *N,O*-Bis(trimethylsilyl)acetamide (BSA) was purchased from Tokyo Kasei Co. and distilled before use. Triethylamine and *n*-butylamine were distilled and stored over calcium hydride and molecular sieves (4 Å), respectively. 1,5-Diazabicyclo[5.4.0]undec-5-ene (DBU) was purchased from Tokyo Kasei Co. and used without purification. Spleen phosphodiesterase and snake venom phosphodiesterase were purchased from Böehringer Mannheim GmbH. Nuclease P1 was purchased from Yamasa Co.

Triethylammonium 5′-*O*-(Dimethoxytrityl)thymidine 3′-Benzoylphosphonate (3a). Methanol (2 mL) was added to 661 mg (2.0 mmol) of bis(trimethylsilyl) benzoylphosphonate (**1a**).[16] After 5 min, pyridine (1 mL) was added to the mixture, and then the solvent and hexamethyldisiloxane were removed in vacuo. To the residue was added 550 mg (1.0 mmol) of DMTrT, and the mixture was coevaporated three times with pyridine and dissolved in dry pyridine (20 mL). MDS (841 mg, 3.0 mmol) was added and the solution was stirred for 10 min. After being quenched with 0.3 M triethylammonium bicarbonate solution (TEAB, 20 mL) the mixture was extracted with CH_2Cl_2 (20 mL). The organic layer was washed three times with 0.3 M TEAB and the washings were further extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The residue was dissolved in CH_2Cl_2 (3 mL) and added dropwise to vigorously stirred ether (100 mL). White precipitate was collected by filtration and dried in vacuo to give analytically pure **3a** (750 mg, 92%): ^1H NMR ($CDCl_3$) δ 7.6–7.1 (15 H, m, PhH, ═CH), 6.9–6.7 (4 H, m, MeOC═C*H*), 6.42 (1 H, m, 1′-CH), 5.13 (1 H, m, 3′-CH), 4.37 (1 H, m, 4′-CH), 3.78 (6 H, s, CH_3O), 3.43 (2 H, m, 5′-CH_2), 3.1–2.9 (6 H, md, N–CH_2), 2.80–2.05 (2 H, m, 2′-CH), 1.4–1.2 (12 H, m, CH_3). Anal. Calcd for $C_{44}H_{52}O_{10}N_3P\cdot H_2O$: C, 63.53; H, 6.54; N, 5.05. Found: C, 63.50; H, 6.71; N, 4.62.

Triethylammonium 5′-*O*-(Dimethoxytrityl)thymidine 3′-Anisoylphosphonate (3b). Compound **3b** (1.00 g, 79%) was similarly obtained by using 1.08 g (3.0 mmol) of **1b**, 817 mg (1.5 mmol) of DMTrT, and 1.26 g (3.0 mmol) of MDS in dry pyridine (20 mL): ^1H MMR ($CDCl_3$) δ 7.60–7.00 (12 H, m, ArH, ═CH), 7.00–6.40 (6 H, m, CH_3OC═C*H*), 6.39 (1 H, m, 1′-CH), 5.13 (1 H, m, 3′-CH), 4.38 (1 H, m, 4′-CH), 3.82 (3 H, s, OCH_3), 3.78 (6 H, s, OCH_3), 3.44 (2 H, m, 5′-CH), 3.28–2.84 (6 H, m, N–CH_2), 2.76–2.16 (2 H, m, 2′-CH), 1.60–1.00 (12 H, m, CH_3).

Triethylammonium 5′-*O*-(Dimethoxytrityl)thymidine (*p*-Chlorobenzoyl)phosphonate (3c). Compound **3c** (976 mg, 81%) was similarly obtained by using 1.66 g (3.1 mmol) of **1c**, 768 mg (1.4 mml) of DMTrT, and 1.49 g (5.3 mmol) of MDS in dry pyridine (14 mL): ^1H NMR ($CDCl_3$) δ 7.60–7.00 (14 H, m, ArH, ═CH), 6.80 (4 H, d, J = 9 Hz, CH_3OC═C*H*), 6.38 (1 H, t, J = 7 Hz), 1′-CH), 5.08 (1 H, m, 3′-CH), 4.22 (1 H, m, 4′-CH), 3.76 (6 H, s, OCH_3), 3.44 (2 H, m, 5′-CH), 3.26–2.80 (6 H, m, N–CH_2), 2.78–2.10 (2 H, m, 2′-CH) 1.80–1.00 (12 H, m, CH_3).

General Procedure for the Preparation of Dithymidine Aroylphosphonates (6a–c and 7a–c). A mixture of **3**, 3-nitro-1,2,4-triazole (NT), and a 3′-*O*-protected thymidine (**4** or **5**) was coevaporated three times with dry pyridine and dissolved in dry pyridine (10 mL/L mmol of **3**). To the stirred solution was added MDS at room temperature. After 60 min, crashed ice was added, and the mixture was stirred for 5 min and then extracted with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, evaporated, and chromatographed on silica gel (0.5–1% MeOH/CH_2Cl_2). The product was purified by reprecipitation from its CH_2Cl_2 solution to hexane. The detailed conditions and results are listed in Table II. All new compounds

(22) Sekine, M.; Yamagata, H.; Hata, T. *J. Chem. Soc., Chem. Commun.* **1981**, 971.
(23) Sekine, M.; Mori, H.; Hata, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 239.

Table V. ^{1}H NMR Spectra of Dinucleoside Phosphonates (6a–c, 7a–c)

compd	^{1}H NMR ($CDCl_3$)
6a	7.50–7.10 (16 H, m, Ar*H* and =CH), 6.92 (4 H, d, CH_3OC=C*H*), 6.30 (2 H, m, 1′-CH), 5.16 (1 H, m, 3′-C_aH), 4.54–4.00 (5 H, m, C′-C_bH_2, 3′-C_bH, and 4′-CH), 3.84 (6 H, s, OCH_3), 3.48 (2 H, m, 5′-C_aH_2), 2.78–2.22 (4 H, m, 2′-CH_2), 2.10 (3 H, s, CH_3CO), 1.90 (3 H, s, CH_3), 1.44 (3 H, s, CH_3)
6b	7.64–7.20 (15 H, m, Ar*H* and =CH), 6.90 (4 H, d, CH_3OC=C*H*), 6.33 (2 H, m, 1′-CH), 5.30 (1 H, m, 3′-C_aH), 4.50–4.05 (5 H, m, 5′-C_bH_2, 3′-C_bH, and 4′-CH), 3.76 (9 H, s, CH_3O), 3.44 (2 H, m, 5′-C_aH_2), 2.70–2.20 (4 H, m, 2MHCH_2), 2.06 (3 H, s, CH_3CO)8 1.88 (3 H, s, CH_3), 1.39 (3 H, s, CH_3)
6c	7.60–7.15 (15 H, m, ArH and =CH), 6.88 (4 H, d, CH_3OC=C*H*), 6.28 (2 H, m, 1′-CH), 5.14 (1 H, m, 3′-C_aH), 4.45–4.04 (5 H, m, 5′-C_bH_2, 3′-C_bH and 4′-CH), 3.80 (6 H, s, OCH_3), 3.48 (2 H, m, 5′-C_aH_2), 2.75–2.20 (4 H, m, 2′-CH_2), 2.08 (3 H, s, CH_3CO), 1.90 (3 H, s, CH_3), 1.42 (3 H, s, CH_3)
7a	7.74–7.04 (20 H, m, ArH and =CH), 6.88 (4 H, d, J = 9 Hz, CH_3OC=C*H*), 6.80 (1 H, s, SCHS), 6.28 (1 H, t, J = 7 Hz, 1′-C_aH), 6.10 (1 H, t, J = 7 Hz, 1′-C_bH), 5.16 (1 H, m, 3′-C_aH), 4.40–4.03 (5 H, m, 5′-C_bH_2, 3′-C_bH, and 4′-CH), 3.80 (6 H, s, OCH_3), 3.48 (2 H, m, 5′-C_aH_2-), 2. H, m, 2′-CH_2), 1.92 (3 H, s, CH_3), 1.44 (3 H, s, CH_3)
7b	7.61–6.98 (19 H, m, ArH and =CH), 6.87 (4 H, d, J = 9 Hz, CH_3OC=C*H*), 6.73 (1 H, s, SCHS), 6.44 (1 H, m, 1′-C_aH), 6.12 (1 H, m, 1′-C_bH), 5.32 (1 H, m, 3′-C_aH), 4.35–4.00 (5 H, m, 5′-C_bH_2, 3′-C_bH, and 4′-CH), 3.80 (9 H, s, OCH_3), 3.48 (2 H, m, 5′-C_aH_2, 2.65–2.10 (4 H, m, 2′-CH(3 H, s, CH_3), 1.40 (3 H, s, CH_3)
7c	7.88–700 (19 H, m, ArH and =CH), 6.88 (4 H, d, J = 9 Hz, CH_3OC=C*H*), 6.76 (1 H, s, SCHS), 6.44 (1 H, m, 1′-C_aH), 6.12 (1 H, m, 1′-C_bH), 5.18 (1 H, m, 3′-C_aH), 4.45–4.05 (5 H, m, 5′-C_bH_2, 3′-C_bH, and 4′-CH), 3.80 (6 H, d, OCH_3), 3.52 (2 H, m, 5′-C_aH_2), 2.70–2.05 (4 H, m, 2′-CH(3 H, s, CH_3), 1.43 (3 H, s, CH_3)

were characterized by means of their ^{1}H NMR spectra (Table V).

Acid–Base Treatments of Diethyl Phosphonate (8). The stabilities of **8** were examined under the following conditions by monitoring on TLC. The results of these experiments are described in the text.

(a) To a solution of **8** (20 mg, 0.14 mmol) in CH_2Cl_2 (0.7 mL) was added 1% trifluoroacetic acid in CH_2Cl_2 (0.7 mL) at 0 °C for 1 h. (b) Concentrated ammonia solution (1 mL) was added to **8** (20 mg, 0.14 mmol) at room temperature. (c) Compound **8** (20 mg, 0.14 mmol) was treated with *n*-butylamine (30 μL, 0.30 mmol) in CH_2Cl_2 (1 mL) at room temperature for 1 h.

5′-*O*-(Dimethoxytrityl)-3′-thymidine 3′-*O*-(1,3-Benzodithiol-2-yl)-5′-thymidine Phosphonate (9). To a solution of **7a** (256 mg, 0.22 mmol) in dry CH_2Cl_2 (10 mL) was added *n*-butylamine (0.125 mL, 1.25 mmol) and successively DBU (0.013 mL, 0.09 mmol). After 1 h, the mixture was quenched with 0.2 M phosphate buffer (pH 6, 10 mL) and the organic layer was washed three times with phosphate buffer and the washings were further extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, evaporated and chromatographed on silica gel to give **9** (119 mg, 0.219 mmol, 72%): ^{1}H NMR ($CDCl_3$) δ 7.36 (15 H, m, Ph*H*, =CH), 6.90 (4 H, d, J = 9 Hz, MeOC=C*H*), 6.80 (1 H, s, 1′-CH), 6.29 (1 H, t, J = 6 Hz, 1′-C_aH), 6.16 (1 H, t, J = 6 Hz, 1′-C_bH), 5.12 (1 H, m, 3′-C_aH), 4.14 (5 H, m, 5′-C_bH_2, 3′-C_bH, and 4-CH), 3.84 (6 H, s, CH_3O), 3.48 (2 H, m, 5′-CH_2), 2.40 (4 H, m, 2′-CH_2), 1.84 (3 H, s, CH_3), 1.48 (3 H, s, CH_3).

3′-Thymidine 5′-Thymidine Phosphonate (10). To a stirred solution of **9** (0.23 g, 0.22 mmol) in $CHCl_3$ (11 mL) was added 1% trifluoroacetic acid in $CHCl_3$ (11 mL) at 0 °C. After 1 h, pyridine (1 mL) and water (1 mL) were added and the mixture was extracted three times with water. The combined aqueous layers were condensed under reduced pressure, dissolved in water (1 mL), and applied to a reverse-phase C_{18} column. Elution with water gave **10** (61 mg, 0.12 mmol, 53%): ^{1}H NMR (CD_3OD) δ 7.77 (1 H, s, 6-CH), 7.48 (1 H, s, 6-CH), 6.22 (2 H, m, 1′-CH), 5.12 (1 H, m, 3′-C_aH), 4.20 (5 H, m, 5′-C_bH_2, 4′-CH, 3′-C_bH), 3.76 (2 H, m, 5′-C_aH_2), 2.44 (2 H, m, 2′-CH_2) 2.26 (2 H, m, 2′-CH_2) 1.88 (6 H, s, CH_3); UV (H_2O) λ_{max} 265, λ_{min} 234 nm.

dTpT (11). To a solution of **10** (13 mg, 24 μmol, 456 OD) in dry THF (0.5 mL) was added BSA (60 μL, 240 μmol) and successively triethylamine (10 μL). After 10 min I_2 (42 mg, 163 mmol) was added to the mixture. After 4 h, the mixture was quenched with crushed ice and washed three times with benzene. One-tenth of the aqueous layer was evaporated, applied to Whatman 3 MM papers, and then developed with Solvent I. The desired band was cut and eluted with water to give **11** (36 OD, 79%). The product was identified with an authentic sample of dTpT: UV (H_2O) λ_{max} 268, λ_{min} 240 nm. The yield was estimated by using the ε value (18 500) of thymidylyl (3′ → 5′) thymidine (dTpT).

3′-Thymidine 5′-Thymidine Phosphorothioate (13). To a solution of **10** (11 mg, 21 μmol, 384 OD) in pyridine (0.5 mL) was added BSA (0.1 mL, 0.4 mmol) and successively triethylamine (0.05 mL). After 10 min elemental sulfur (23 mg, 0.72 mmol) was added to the mixture. After 8 h the mixture was filtered and one-tenth of the filtrate was evaporated, applied to Whatman 3 MM papers, and developed with Solvent I. The desired band was cut and eluted with water to give **13** (30.4 OD, 75%): λ_{max} 268, λ_{min} 240 nm. The product was identified by its ^{31}P NMR spectra (Table IV).

Enzyme Treatment. All the reactions were monitored by TLC developed with CH_2Cl_2–MeOH (4:1, v/v) or 2-propanol–concentrated ammonia–water (7:1:2, v/v/v). The results are described in the text. (A) Treatment of **10** and **11** with spleen phosphodiesterase. To the solution of **10** (13.2 OD_{267}) of **11** (18.0 OD_{267}) in 50 mM ammonium acetate buffer (0.5 mL, pH 6.3) was added spleen phosphodiesterase solution (10 μL, 2 mg/mL). The mixture was incubated at 37 °C for 14 h. (B) Treatment of **10** with snake venom phosphodiesterase. Snake venom phosphodiesterase solution (20 μL, 1 mg/mL) was added to the solution of **10** (10.2 OD_{267}) in 0.1 M Tris-HCl buffer (0.5 mL, pH 8.7). The mixture was incubated at 37 °C for 12 h. (C) Treatment of **10** with nuclease P_1. To the solution of **10** (10.2 OD_{267}) in 50 mM acetate buffer (0.5 mL, pH 5.4) was added nuclease P1 solution (10 μL, 2 mg/mL). The mixture was incubated at 37 °C for 12 h.

Registry No. **1a**, 33876-85-6; **1b**, 66190-98-5; **1c**, 66190-99-6; **2a**, 6881-61-4; **2b**, 66191-02-4; **2c**, 66191-03-5; **3a**, 89849-82-1; **3b**, 89849-84-3; **3c**, 89849-86-5; **4**, 21090-30-2; **5**, 84752-64-7; **6a**, 89849-87-6; **6b**, 89849-88-7; **6c**, 89849-89-8; **7a**, 89849-90-1; **7b**, 89849-91-2; **7c**, 89849-92-3; **8**, 762-04-9; **9**, 89849-93-4; **10**, 89849-94-5; **11**, 1969-54-6; **12**, 89849-95-6; **13**, 41548-34-9; DMTrT, 40615-39-2.