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Acylphosphonates. 7.[1] A new method for Stereospecific and Stereoselective Generation of Dideoxyribonucleoside Phosphorothioates via the Acylphosphonate Intermediates

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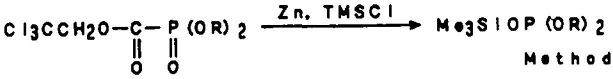
Summary: Dideoxyribonucleoside phosphorothioates were synthesized by a new method via dideoxyribonucleoside 2,2,2-trichloroethoxycarbonylphosphonates and aroylphosphonates. The conversion of dideoxyribonucleoside 2,2,2-trichloroethoxycarbonylphosphonates to dideoxyribonucleoside trimethylsilyl phosphites by treatment with Me_3SiCl-Zn-acetylacetone proceeded with retention configuration at phosphorus. The silyl phosphite intermediates were converted to the phosphorothioates by in situ treatment with sulfur. The aroyl groups were easily removed from 8-11 by the action of $n-BuNH_2$ and in situ converted to only one diastereomer (Rp-configuration) of dideoxyribonucleoside phosphorothioates by treatment with elemental sulfur.

Replacement of the P=S bond for the P=O bond in dideoxyribonucleoside phosphates produces a pair of two diastereomers due to the generation of a new chiral center at phosphorus. Optically active dideoxyribonucleoside phosphorothioates have been synthesized extensively and characterized by Eckstein[2-4] and Frey[5] since they could serve as useful substrates for elucidation of the mechanism of enzyme reaction. However, these approaches involve tedious chromatographic separations of diastereomers which were found in a ratio of about 1 : 1.

In this paper, we wish to report a highly stereospecific synthesis of Rp-dinucleoside phosphorothioates and a stereoselective synthesis of dideoxyribonucleoside phosphorothioates in detail.

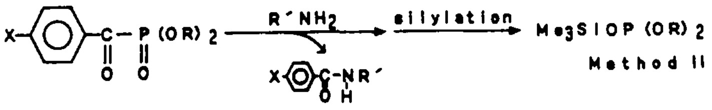
We have reported two methods for the conversion of five-valent dialkyl acylphosphonates to tervalent dialkyl trimethylsilyl phosphites.[6,7]

One of them involves the use of the 2,2,2-trichloroethoxycarbonyl groups as the P-H bond blockers which are removed by treatment with zinc powder and trimethylsilyl chloride.



$$Cl_3CCH_2O-\underset{\displaystyle O}{\overset{}{\underset{\|}{C}}}-\underset{\displaystyle O}{\underset{\|}{P}}(OR)_2 \xrightarrow{Zn,\ TMSCl} Me_3SiOP(OR)_2$$

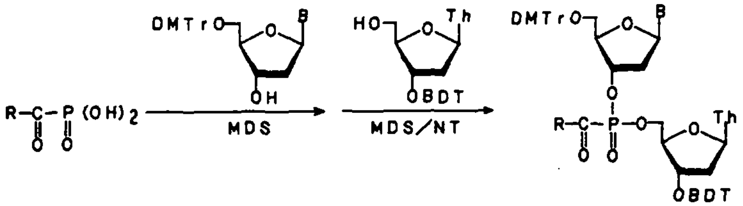
Method I



Method II

In this method, the resulting carboxylphosphonates were converted to the silyl phosphites (Method I).[6] The others involve deacylation of dialkyl aroylphosphonates with primary amines followed by silylation (Method II).[7]

In Method I, in order to examine stereochemistry of the transformation of acylphosphonates to silylphosphites, we synthesized dideoxyribonucleoside 2,2,2-trichloroethoxycarbonylphosphonates (1 and 2), which were protected with the 4,4'-dimethoxytrityl (DMTr) and 1,3-benzodithiol-2-yl (BDT)[8] groups at the 5' and 3'-positions, respectively, in a method similar to that described in the previous paper.[7] The 6-amino group of deoxyadenosine was protected with the DMTr group. The introduction of the aroylphosphonyl group into the 3'-hydroxyl at the first stage was best performance by condensation of nucleoside with the corresponding aroylphosphonic acid in the presence of a bifunctional condensing agent, i.e., mesitylene disulfonyl dichloride (MDS). All attempts to use 2,2,2-trichloroethoxycarbonylphosphonate as a phosphonyl agent have failed since the reaction was not complete in pyridine even in the presence of triazole and a 3'-3' linked product found to a considerable extent. The diastereomers (1a,b or 2a,b) derived from the chirality of the phosphorus atom were separated by preparative liquid chromatography. Although the isolated yields of the diastereomers were not satisfactory. The ratios of diastereomers were nearly 1 : 1. In the second condensation, an unidentical by-product appeared higher than the desired products on TLC. The ^1H and ^{31}P NMR spectra and elemental analysis of this by-product suggest the presence of the two different deoxyribonucleoside residue and the trichloroethyl group. However, the trichloroethyl group was resistance to the Zn-Me_3SiCl-acetylacetone (AcAc) treatment. We can not propose the clear structure of this by-product at this time. The by-product was always found when the condensation was carried out in various condition.



(1, 2, 8−11)

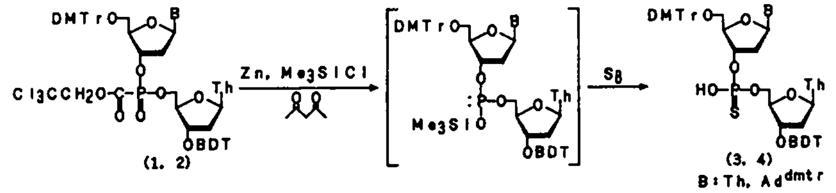
	1	2	8	9	10	11
B	Th	Ad^{dmtr}	Th	Th	Ad^{dmtr}	Ad^{dmtr}
R	Cl_3CCH_2O	Cl_3CCH_2O	Ph	p-ClPh	Ph	p-ClPh

Table 1 The synthesis of dideoxyribonucleoside acylphosphonates (1,2,8-11)

compd	yield (%)	^{31}P NMR (ppm)
1a	18	7.29
1b	15	8.26
1a+1b	8	
2a	18	7.31
2b	16	8.28
2a+2b	9	
8	92	7.54, 8.31
9	60	7.31, 8.45
10	71	7.25, 8.21
11	61	7.27, 8.23

Sulfurization of optical active trialkyl phosphites with elemental sulfur is well known to proceed with retention configuration.[9] The initial products obtained by treatment of 1 and 2 with Zn-Me_3SiCl-AcAc[10] were further in situ converted to the phosphorothioates (3,4) by addition of sulfur to make sure the configuration of the tervalent phosphorus intermediates.

Treatment of 2a with zinc powder (10 equiv.) in pyridine in the presence of trimethylsilyl chloride (10 equiv.) and acetylacetone (10 equiv.) for 1 h followed by reaction with elemental sulfur (10 equiv.) for 1 h gave the sole product in 90 % yield. This product was identified with Sp-Ap(s)T (4a) by comparison with their ^{31}P NMR spectra.[2,11,12] On the other hand, the other isomer, 2b was similarly converted to Rp-Ap(s)T (4b) in 92 % yield as shown in Figure 1.



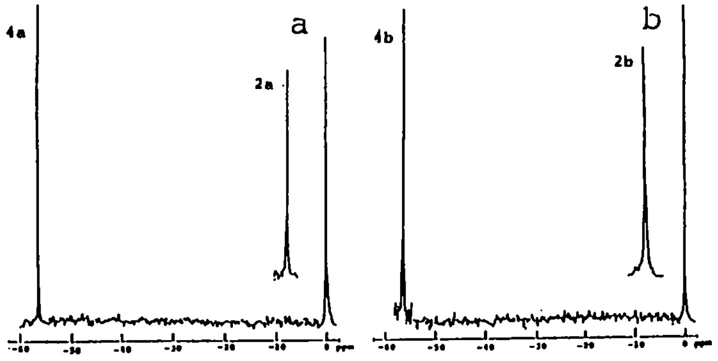


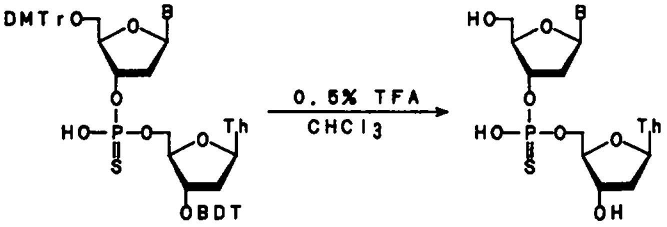
Figure 1 ^{31}P NMR spectra ($CDCl_3$/Py,3:1,v/v) of 4 obtained by treatment of 2 with Zn-Me_3SiCl-AcAc followed elemental sulfur. The chemical shifts were relative to an external standard of 85 % H_3PO_4 (aq.).

These results suggest that the conversion of 2 to 4 proceeded without racemization. No racemization was observed also in the case of the transformation of 1a and 1b to Sp-Tp(S)T (3a) and Rp-Tp(S)T (3b) which was obtained in 88 % and 94 % yields, respectively.

The reductive removal of the 2,2,2-trichloroethyl group proceeded more rapidly in the presence of trimethylsilyl chloride than in the absence of it.

On subsequent treatment of 3 and 4 with 0.5 % TFA in $CHCl_3$, dideoxyribonucleoside phosphorothioates (5,6) were obtained.

During the acid hydrolysis of the protecting groups, the phosphorothioates was decomposed to some extent to the natural phosphates. Therefore, a limited amount of TFA was used for removal of the DMTr and BDT groups.



3 4
B Th Ad^{dmtr}

5 6
B Th Ad

Table 2 The yields of dideoxyribonucleoside phosphorothioates (5,6) from 1 and 2.

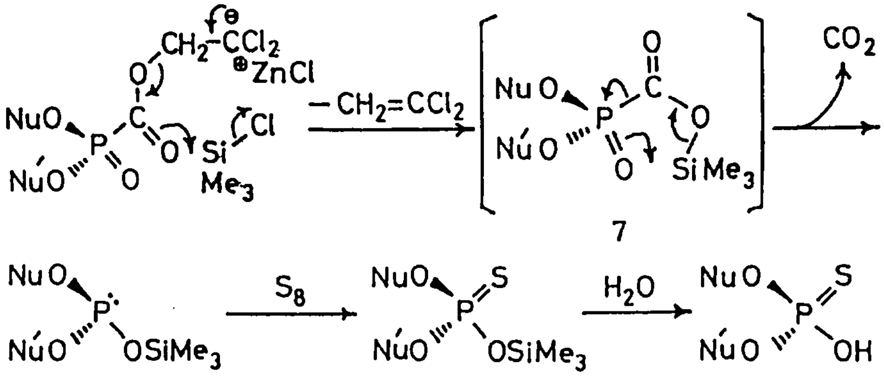
	5a	5b	6a	6b
B	Th	Th	Ad	Ad
yield (%)	57	63	53	58

Table 3 ^{31}P NMR spectra of 3-6

compd	^{31}P NMR (ppm)	compd	^{31}P NMR (ppm)
3a,b	56.22, 56.98	5a,b	56.51, 57.17*
4a,b	56.73, 57.38	6a,b	56.62, 57.31*

The chemical shifts of compounds in $CDCl_3$/Py(3:1,v/v) were relative to internal standard of 85 % H_3PO_4 (aq.).
*The chemical shifts were measured in D_2O/Py (3:1,v/v).

It is clearly shown from the ^{31}P NMR analysis that the configuration at phosphorus was maintained in the conversion of 3 or 4 to 5 or 6 (Table 3). These results indicate that the chirality of 1 or 2 at phosphorus was preserved during the whole reaction process. Therefore, we proposed the following mechanism for conversion of acylphosphonates to phosphorothioates. The conversion of 1 or 2 to 3 or 4 proceeds via a five-membered ring transition state as depicted in 7.



In Method II, dideoxyribonucleoside aroylphosphonates (8-11) were synthesized in a similar manner.

Removal of the aroyl groups from 8 by the action of n-$BuNH_2$ (10 equiv.) in the presence of DBU (0.6 equiv.)[13] at room temperature for 1 h gave 12 in 72 % yield. In a similar manner, compounds 9-11 were converted to the dideoxyribonucleoside phosphonates (12,13). These reaction conditions and results are summarized in Table 4. Deacylation of 10 and 11 were slower than 8 and 9, respectively.

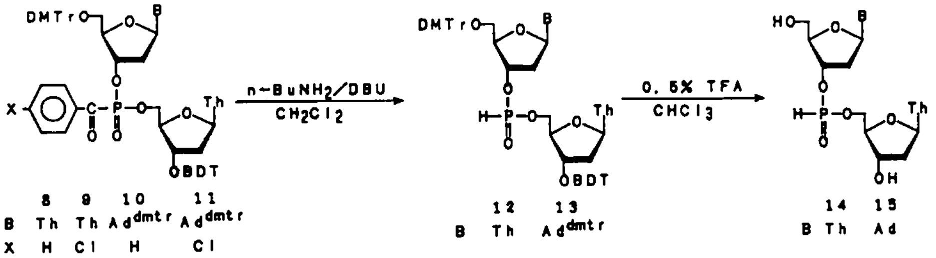
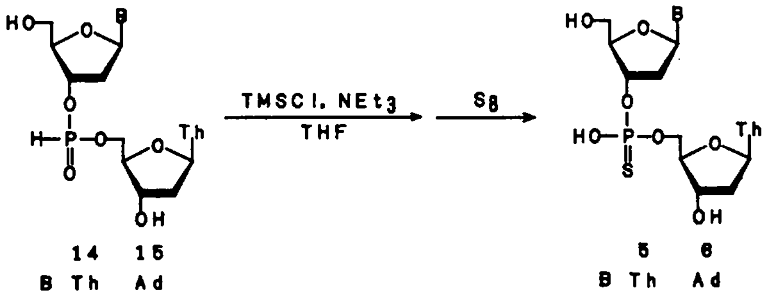


Table 4 Deacylation of Compounds 8-11.

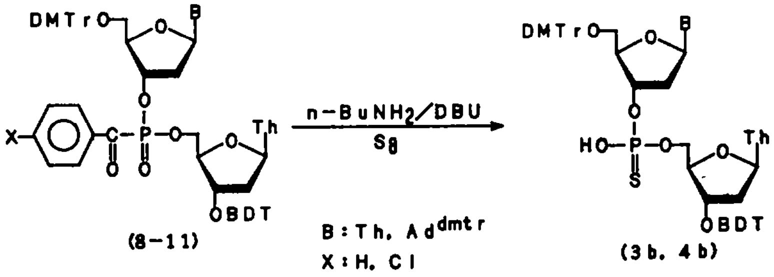
compd	DBU (equiv.)	n-$BuNH_2$ (equiv.)	time (h)	product	yield (%)
8	0.6	10	1	12	72
9	0.6	10	0.5	12	76
10	1.2	20	2	13	49
11	1.2	20	1.5	13	72

Treatment of 12 and 13 with 0.5 % TFA at 0 °C for 1 h gave compounds 14 and 15 in 53 % and 51 % yields, respectively.

Sulfurization of 14 and 15 in the presence of trimethylsilyl chloride and triethylamine in pyridine at room temperature for 10 min followed by treatment with elemental sulfur for 2 h resulted in the direct formation of phosphorothioates 5 and 6 in 73 % and 71 % yields, respectively. The ^{31}P NMR analysis showed that the ratios of the Sp/Rp-isomers were 2 : 1 and 2.5 : 1 for compounds 5 and 6, respectively (Figure 2a). The deprotection procedure described above provided predominantly the Sp-isomers over the Rp-isomers.



Contrary to this fact, it was found that treatment of 8 with n-BuNH$_2$ (10 equiv.) in the presence of DBU (0.5 equiv.) and elemental sulfur (15 equiv.) for 1 h prior to removal of the DMTr and BDT groups gave exclusively the Rp-isomer (3b). In a similar manner, compounds 9-10 were converted stereospecifically to the Rp-isomer (4b, Figure 2b).



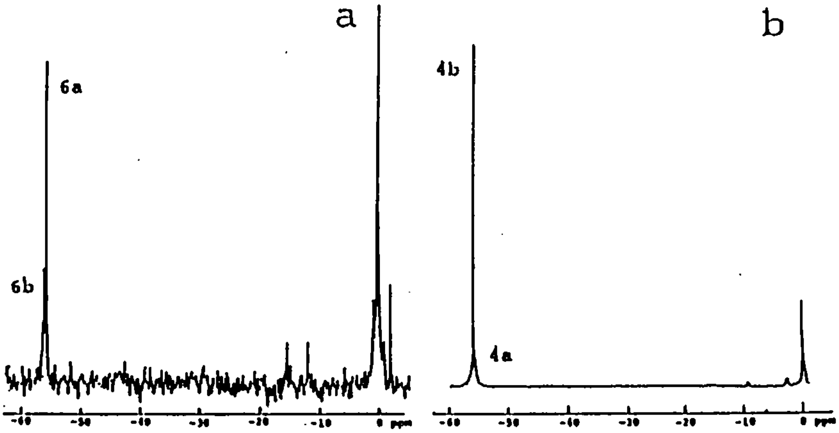


Figure 2 ^{31}P NMR spectra of compounds 4 and 6

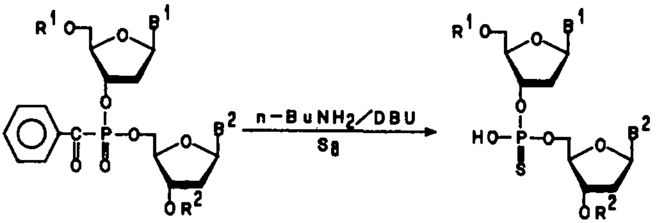
Table 5 Conversion of compounds 8-11 to phosphorothioates

	8	9	10	11
product	3b	3b	4b	4b
yield (%)	86	90	92	93

On subsequent treatment of 3b and 4b with 0.5 % TFA in $CHCl_3$ at 0°C for 1 h, dideoxyribonucleoside phosphorothioates 5b and 6b were obtained.

The stereochemistry of 5 and 6 obtained in these experiments was also confirmed by enzyme assay reported by Eckstein[4] and Stec.[11,14] Nuclease P1 digested 5b and 6b but did not interact with 5a and 6a. On the contrary, snake venom phosphodiesterase lead to digestion of 5a and 6a but did not digest 5b and 6b.

The exclusive formation of the Rp-isomer in the latter process may be explained in terms of a strong interaction between the two nucleoside residues having the DMTr and BDT groups. Therefore, we tried to synthesize a number of dideoxyribonucleoside benzoylphosphonates (16-26) which had other protecting groups and nucleoside bases. These compounds (16-26) were allowed to react with n-$BuNH_2$, DBU, and elemental sulfur in a similar manner. Unfortunately, in all cases, the mixture of Rp-isomer and Sp-isomers were obtained.



(16-26) (27-37)

Table 6 Conversion of compounds 16-26 to phosphorothioates

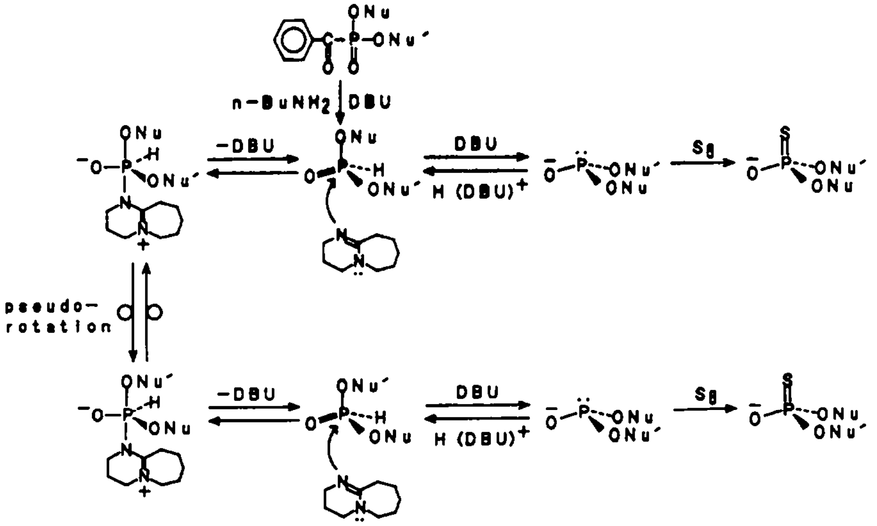
compd	B^1	B^2	R^1	R^2	yield (%)	product	^{31}P NMR (ppm)	ratio
16	Th	Th	DMTr	Bz	90	27	57.11, 57.21	1 : 1
17	Th	Th	DMTr	TBDMS	91	28	56.53, 56.82	1 : 1
18	Th	Th	Bz	BDT	88	29	56.82, 56.97	1 : 1
19	Th	Th	DMTr	DMTr	95	30	56.89, 56.99	1 : 1.5
20	Th	Th	BDT	BDT	91	31	56.46, 56.63	1.2 : 1
21	Th	Th	BDT	DMTr	98	32	56.31, 56.46	1 : 1.3
22	Cy^{dmtr}	Th	DMTr	BDT	95	33	56.99, 57.45	1.1 : 1
23	Gu^{dmtr}	Th	DMTr	BDT	93	34	56.75, 57.04	1 : 1
24	Gu^{ibu}	Th	DMTr	BDT	92	35	57.16, 57.28	1.2 : 1
25	Ad^{dmtr}	Ad^{bz}	DMTr	BDT	78	36	57.02, 57.31	1 : 1
26	Th	Ad^{bz}	DMTr	BDT	73	37	57.02, 57.35	1 : 1

Only compounds 8-11 were capable of the stereospecific fixation of a DBU-catalyzed reaction intermediate. We suggest the following mechanism which has intermediate of pentavalent phosphorus atom.

In the case of the T-T sequence, the use of the benzoyl (Bz) or t-butyldimethylsilyl (TBDMS) group in place of the BDT as the 3'-terminal protecting group gave nonstereospecific conversion to phosphorothioates. A similar result was obtained when the 5'-DMTr group was changed to the benzoyl group. When the positions of the DMTr and BDT were changed with each other, an isomer showing a ^{31}P NMR peak at the lower field was formed somewhat predominately. Such a predominant formation of one isomer was also observed when the DMTr group was employed both as the 5'- and 3'-terminal protecting groups.

It is now clear that only the combination of the 5'-DMTr and 3'-BDT groups can provided a stereospecific synthesis of the Rp phosphorothioate. Based on this fact, the base sequence was varied by using N^4-dimethoxytrityldeoxycytidine (dC^{dmtr}), N^2-dimethoxytritylguanosine (dG^{dmtr}), or N^2-isobutyryldeoxyguanosine (dG^{ibu}) as the 5'-terminalnucleoside component. However, the stereospecificity was also lost in these cases. In a series of A-T sequence, similar results were obtained.

In conclusion, the choice of the hydroxyl protecting groups as well as the base sequence has influence dramatically on the stereospecific formation of the two diastereomers. We suggest a reasonable mechanism for the reason why on 8-11 were converted to phosphorothioates in a stereospecific manner. As showing in the following scheme, DBU may catalyze isomerization of dideoxyribonucleoside phosphonates by the addition of DBU molecule to the P=O bond. The resulting pentavalent intermediate can be isomerized by pseudorotation to give dideoxyribonucleoside phosphonates with eliminations of DBU. These reactions may be in equilibrium so that the equilibrium mainly depends on all kinds of interactions among the protecting groups, the base residues, and the DBU moiety at the stage of the pentavalent phosphorus intermediate.



Although further studies are required for elucidation of the mechanism of the present reaction, the very restricted interactions in the case of 8-11 in the intermediate may be expected so that a more stable pentavalent phosphorous intermediate can be effectively fixed.

EXPERIMENTAL

Melting points and boiling points are uncorrected. ^{1}H NMR spectra were recorded at 100 MHz on a JEOL INM 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-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. A mixture in water was applied to the column. Elusion was performed with water. Thin-layer chromatography was performed on Pre-coated TLC plates silica gel 60 F-254 (Merck, Art. No. 5717). The Rf values of the protected nucleoside derivatives were measured after development with CH_2Cl_2-MeOH (9:1, v/v) unless others noted. Preparative liquid chromatography was Waters Prep System 500 A (Silica gel, EtOAc). Pyridine was distilled twice from p-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves (4 A). CH_2Cl_2 was dried over P_4O_{10} overnight, decanted, distilled over K_2CO_3, and stored over molecular sieves (4 A). Triethylamine and n-butylamine were distilled and stored over calcium hydride and molecular sieves (4 A), respectively. 1,5-Diazabicyalo[5,4,0]undec-5-ene (DBU) was purchased from Tokyo Kasei Co. and used without purification. Snake venom phosphodiesterase was purchased from Böehringer Mannheim GmbH. Nuclease P1 was purchased from Yamasa Co.

General Procedure for the Preparation of dideoxyribonucleoside acylphosphonates (1,2,8-11, and 16-26). Methanol was added to tris(trimethylsilyl) acylphosphonate (1.5 equiv.). After 5 min, pyridine was added to the mixture, and then the solvent and hexamethyldisiloxane were removed in vacuo. To the residue was added 5'-O,N-protected nucleoside (1.2 equiv.) and the mixture was coevaporated three times with pyridine and dissolved in dry pyridine (10ml/mmol of acylphosphonate). Mesitylene disulfonyl dichloride (MDS, 2.3 equiv.) was added and the mixture was stirred for 10 min. After being quenched with 0.3 M triethylammonium bicarbonate solution (TEAB) the mixture was extracted with CH_2Cl_2. 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 layer were dried over Na_2SO_4, filtrated, and evaporated. A mixture of the residue, 3-nitro-1,2,4-triazole (1.9 equiv.) and 3'-O,N-protected nucleoside was coevaporated three times with dry pyridine. To the stirred solution was added MDS (1.9 equiv.) at room temperature. After 60 min, crashed ice was added. 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). In the case of 1 and 2, the diastereomers were separated by preparative liquid chromatography. The product was purified by reprecipitation from its CH_2Cl_2 solution to hexane. The results and ^{31}P NMR spectra are listed in Table 1. Compounds were characterized by their ^{1}H NMR spectra (Table 7) and elemental analysis (Table 8).

Table 7. ^{1}H NMR Spectra of Dideoxyribonucleoside Acylphosphonates (1, 2, and 8-11)

compd	^{1}H NMR ($CDCl_3$)
1a	7.61-6.86 (15 H, m, ArH and =CH), 6.87 (1 H, s, SCHS), 6.82 (4 H, d, J = 8 Hz, CH_3OC=CH), 6.54-6.26 (1 H, m, 1'-CaH), 6.02 (1 H, t, J = 6 Hz, 1'-CbH), 5.47-5.13 (1 H, m, 3'-CaH), 4.61-3.94 (7 H, m , Cl_3CCH_2O, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.81 (6 H, s, OCH_3), 3.56-3.26 (2 H , m, 5'-CaH_2), 2.81-1.95 (4 H, m, 2'-CH_2), 1.85 (3 H, s, $CbCH_3$), 1.46 (3 H, s, CaH_3)
1b	7.64-6.86 (15 H, m, ArH and =CH), 6.91 (1 H, s, SCHS), 6.84 (4 H, d, J = 8 Hz, CH_3OC=CH), 6.56-6.28 (1 H, m, 1'-CaH), 6.12 (1 H, t, J = 6 Hz, 1'-CbH), 5.48-5.12 (1 H, m, 3'-CaH), 4.60-3.96 (7 H, m , Cl_3CCH_2O, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.80 (6 H, s, OCH_3), 3.56-3.28 (2 H, m, 5'-CaH_2), 2.80-1.96 (4 H, m, 2'-CH_2), 1.83 (3 H, s, $CbCH_3$), 1.40 (3 H, s, CaH_3)
2a	8.05 (1 H, s, 8-CaH), 7.92 (1 H, s, 2-CaH), 7.48-6.96 (23 H, m, ArH), 6.92-6.72 (9 H, m, CH_3OC=CH and SCHS), 6.52-6.30 (1 H, m, 1'-CaH), 6.01 (1 H, t, J = 6 Hz, 1'-CbH), 5.40-5.16 (1 H, m, 3'-CaH), 4.40-4.02 (7 H, m, Cl_3CCH_2O, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.78 (12 H, s, OCH_3), 3.54-3.32 (2 H , m, 5'-CaH_2), 3.06-2.68 (2 H, m, 2'-CaH_2), 2.52-2.20 (2 H, m, 2'-CbH_2), 1.82 (3 H, s, CH_3)
2b	7.92 (1 H, s, 8-CaH), 7.82 (1 H, s, 2-CaH), 7.40-6.88 (23 H, m, ArH), 6.88-6.60 (9 H, m, CH_3OC=CH and SCHS), 6.42-6.28 (1 H, m, 1'-CaH), 6.03 (1 H, t, J = 6 Hz, 1'-CbH), 5.40-5.12 (1 H, m, 3'-CaH), 4.40-3.92 (7 H, m , Cl_3CCH_2O, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.68 (12 H, s, OCH_3), 3.44-3.28 (2 H , m, 5'-CaH_2), 3.06-2.76 (2 H, m, 2'-CaH_2), 2.76-2.20 (2 H, m, 2'-CbH_2), 1.72 (3 H, s, CH_3)
3a	7.70-7.00 (19 H, m, ArH and =CH), 6.98 (1 H, s, SCHS), 6.80 (4 H, d, J = 9 Hz, CH_3OC=CH), 6.60-6.20 (2 H, m, 1'-CH), 5.64-5.08 (1 H, m, 3'-CaH), 4.68-4.00 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.65 (6 H, s, OCH_3), 3.48-3.20 (2 H , m, 5'-CaH_2), 3.20-2.84 (6 H, m, N-CH_2) 2.80-2.16 (4 H, m, 2'-CH_2), 1.90 (3 H, s, CbH_3), 1.55 (3 H, s, CaH_3), 1.24 (9 H, t, J=6 Hz)
3b	7.80-7.00 (19 H, m, ArH and =CH), 6.99 (1 H, s, SCHS), 6.84 (4 H, d, J = 9 Hz, CH_3OC=CH), 6.76-6.30 (2 H, m, 1'-CH), 5.76-4.80 (1 H, m, 3'-CaH), 4.72-4.02 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.68 (6 H, s, OCH_3), 3.48-3.20 (2 H , m, 5'-CaH_2), 3.20-2.88 (6 H, m, N-CH_2) 2.85-2.20 (4 H, m, 2'-CH $_2$), 1.94 (3 H, s, CbH_3), 1.56 (3 H, s, CaH_3), 1.26 (9 H, t, J=6 Hz)
4a	7.99 (1 H, s, 8-CaH), 7.84 (1 H, s, 2-CaH), 7.59-7.04 (28 H, m, ArH), 7.00-6.60 (9 H, m, CH_3OC=CH and SCHS), 6.28-5.64 (2 H, m, 1'-CH), 5.40-5.20 (1 H, m, 3'-CaH), 4.64-4.16 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.60 (12 H, s, OCH_3), 3.32-3.04 (2 H , m, 5'-CaH_2), 2.84-2.10 (10 H, m, 2'-CH_2 and N-CH_2), 1.88 (3 H, s, CH_3)
4b	8.02 (1 H, s, 8-CaH), 7.84 (1 H, s, 2-CaH), 7.59-7.00 (28 H, m,ArH),6.96-6.60 (9 H, m, CH_3OC=CH and SCHS), 6.20-5.64 (2 H, m, 1'-CH), 5.40-5.20 (1 H, m, 3'-CaH), 4.60-4.12 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.62 (12 H, s, OCH_3), 3.32-3.10 (2 H , m, 5'-CaH_2), 2.72-2.14 (10 H, m, 2'-CH_2 and N-CH_2), 1.84 (3 H, s, CH_3)
8	7.74-7.04 (20 H, m, ArH and =CH), 6.88 (4 H, d, J = 9 Hz, CH_3OC=CH), 6.80, (1 H, s, SCHS), 6.28 (1 H, t, J = 7 Hz, 1'-CaH), 6.10 (1 H, t, J = 7 Hz, 1'-CbH), 5.40-5.08 (1 H, m, 3'-CaH), 4.40-4.03 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.80 (6 H, s, OCH_3), 3.56-3.32 (2 H , m, 5'-CaH_2), 2.70-2.03 (4 H, m, 2'-CH_2), 1.92 (3 H, s, CbH_3), 1.44 (3 H, s, CaH_3)

9	7.88-7.00 (19 H, m, ArH and =CH), 6.88 (4 H, d, J = 9 Hz, $CH_3OC=CH$), 6.76, (1 H, s, SCHS), 6.44 (1 H, t, J = 7 Hz, 1'-CaH), 6.12 (1 H, t, J = 7 Hz, 1'-CbH), 5.40-4.96 (1 H, m, 3'-CaH), 4.45-4.05 (5 H, m, 3'-CbH,4'-CH, and 5'-CbH_2), 3.80 (6 H, s, OCH_3), 3.56-3.32 (2 H , m, 5'-CaH_2), 2.70-2.05 (4 H, m, 2'-CH_2), 1.81 (3 H, s, CbH_3), 1.43 (3 H, s, CaH_3)
10	8.12, 8.01 (1 H, s, 8-CaH), 7.89 (1 H, s, 2-CaH), 7.48-7.00 (28 H, m, ArH), 6.94-6.68 (9 H, m, $CH_3OC=CH$ and SCHS), 6.60-6.32 (1 H, m, 1'-CaH), 6.18 (1 H, t, J = 6 Hz, 1'-CbH), 5.48-5.16 (1 H, m, 3'-CaH), 4.48-3.96 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.82 (12 H, s, OCH_3), 3.56-3.32 (2 H , m, 5'-CaH_2), 2.88-2.00 (4 H, m, 2'-CH_2), 1.85 (3 H, s, CH_3)
11	8.06, 8.01 (1 H, s, 8-CaH), 7.90 (1 H, s, 2-CaH), 7.60-7.00 (27 H, m, ArH), 6.98-6.60 (9 H, m, $CH_3OC=CH$ and SCHS), 6.58-6.28 (1 H, m, 1'-CaH), 6.05(1 H, t, J = 6 Hz, 1'-CbH), 5.40-5.10 (1 H, m, 3'-CaH), 4.42-4.00 (5 H, m, 2'-CbH, 4'-CH, and 5'-CbH_2), 3.78 (12 H, s, OCH_3), 3.48-3.32 (2 H , m, 5'-CaH_2), 3.00-2.08 (4 H, m, 2'-CH_2), 1.82 (3 H, s, CH_3)
12	7.78-7.08 (15 H, m, ArH and =CH), 6.92 (4 H, d, J = 9 Hz, $CH_3OC=CH$), 6.80, (1 H, s, SCHS), 6.45 (1 H, t, J = 7 Hz, 1'-CaH), 6.16 (1 H, t, J = 7 Hz, 1'-CbH), 5.32-4.92 (1 H, m, 3'-CaH), 4.72-4.04 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.80 (6 H, s, OCH_3), 3.58-3.18 (2 H , m, 5'-CaH_2), 2.72-2.02 (4 H, m, 2'-CH_2), 1.84 (3 H, s, CbH_3), 1.48 (3 H, s, CaH_3)
13	8.02, 7.98 (1 H, s, 8-CaH), 7.88 (1 H, s, 2-CaH), 7.60-6.98 (23 H, m, ArH), 6.98-6.58 (9 H, m, $CH_3OC=CH$ and SCHS), 6.52-6.24 (1 H, m, 1'-CaH), 6.24-5.80 (1 H, m, 1'-CbH), 5.44-4.92 (1 H, m, 3'-CaH), 4.48-3.88 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH_2), 3.78 (12 H, s, OCH_3), 3.52-3.20 (2 H ,m, 5'-CaH_2), 3.08-2.00 (4 H, m, 2'-CH_2), 1.81 (3 H, s, CH_3)

Table 8. Elemental analysis of compounds 2, 8, 10, and 11.

compd	anal.
2	Calcd for $C_{72}H_{65}O_{15}N_7PS_2Cl_3$.H2O: C, 58.05; H, 4.67; N, 6.58. Found: C, 58.07; H, 4.97; N, 6.83.
8	Calcd for $C_{55}H_{53}O_{14}N_4PS_2.H_2O$: C, 59.67; H, 5.01; N, 5.06. Found: C, 59.76; H, 5.36; N, 5.46.
10	Calcd for $C_{76}H_{70}O_{14}N_7PS_2$: C, 65.18; H, 5.04; N, 7.00. Found: C, 65.80; H, 5.19; N, 7.36.
11	Calcd for $C_{76}H_{69}O_{14}N_7PS_2Cl.2H_2O$: C, 62.06; H, 5.00; N, 6.67. Found: C, 61.51; H, 5.00; N, 6.27.

General Procedure for Conversion of Dideoxyribonucleoside 2,2,2,-trichloroethoxycarbonylphosphonates (1,2) to Dideoxyribonucleoside Phosphorothioates (3,4). To a solution of 1 or 2 in dry CH_2Cl_2 (10 mL/mmol of 1 or 2) was added Zn (10 equiv.), TMSCl (10 equiv.), and acetylacetone (10 equiv.). After 1 h, elemental sulfur was added to the solution and the solution was stirred for 1 h. The mixture was extracted with CH_2Cl_2. 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, filtrated, and evaporated. The residue was dissolved in CH_2Cl_2 and added dropwise to vigorously stirred hexane. White precipitate was collected by filtration and dried in vacuo. The results and ^{31}P NMR are described in the text and Table 3, respectively. The products were characterized by their ^1H NMR spectra (Table 7)

General Procedure for Acid Treatment of Dideoxyribonucleoside Phosphorothioates (3,4). To a stirred solution of 3 or 4 in $CHCl_3$ (50 mL/mmol of 3 or 4) was added 1 % trifluoroacetic acid (TFA) in $CHCl_3$ (50 mL/mmol of 3 or 4) at 0 °C. After 1 h, pyridine (2 mL/mmol of 3 or 4) and water (2 mL/mmol of 3 or 4) were added and the mixture was extracted three times with water. The combined aqueous layers were condensed under reduced pressure, dissolved in water, applied to Whatman 3 MM papers, and developed with Solvent I. The desired band was cut and eluted with water to give 5 or 6. The product was identified by its ^{31}P NMR spectra (Table 5).

General Procedure for Deacylation of Compounds 8-11. To a solution of 8-11 in dry CH_2Cl_2 (10 mL/mmol of 8-11) were added n-butylamine and successively DBU (0.013 mL, 0.09 mmol). After 1 h, the mixture was quenched with 0.2 M phosphate buffer (pH 6) and the organic layer was washed three times with phosphate buffer. 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 12-13. The detailed conditions and results are listed in Table 4. The products were characterized by their ^1H NMR spectra (Table 7)

3'-Thymidine 5'-Thymidine Phosphonate (14). To a stirred solution of 12 (0.23 g, 0.22 mmol) in $CHCl_3$ (11 mL) was added 1 % TFA 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 reverced-phase C_{18} column. Elusion with water-acetone (8:2) gave 14 (61 mg, 0.12 mmol, 53 %): ^1H NMR 7.77 (1 H, s, 6-CaH), 7.48 (1 H, s, 6-CbH), 6.38-6.00 (2 H, m, 1'-CH), 5.24-4.95 (1 H, m, 3'-CaH), 4.52-3.85 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH$_2$), 3.85-3.40 (2 H , m, 5'-CaH$_2$), 2.68-1.98 (4 H, m, 2'-CH$_2$), 1.88 (6 H, s, CH_3)

3'-Adenosine 5'-Thymidine Phosphonate (15). Compound 15 (36 mg, 61 µmol, 51 %) was similarly obtained by using 154 mg (120 µmol) of 13 and 6 mL of 1 % TFA in $CHCl_3$ (6 mL).: ^1H NMR 8.42 (1 H, s, 8-CaH), 8.08 (1 H, s, 2-CaH), 7.55 (1 H, S, 6-CH), 7.02-6.82 (1 H, m, 1'-CH), 5.20-4.76 (1 H, m, 3'-CaH), 4.48-3.80 (5 H, m, 3'-CbH, 4'-CH, and 5'-CbH$_2$), 3.80-3.40 (2 H , m, 5'-CaH$_2$), 2.70-2.40 (4 H, m, 2'-CH$_2$), 1.85 (3 H, s, CH_3)

Sulfurization of 14. To a solution of 14 (58 mg, 114 µmol, 1484 OD) in pyridine (1.1 mL) were added trimethylsilyl chloride (98 µL, 770 µmol) and triethylamine (120 µL, 880 µmol). After 10 min elemental sulfur (110 mg, 330 µmol) was added to the mixture. After 2 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 5 (135 OD, 73 %). The product was identified by its ^{31}P NMR spectrum (Table 3).

Sulfurization of 15. Compound 6 (147 OD, 71 %) was similarly obtained by using 52 mg (91 µmol, 2074 OD) of 15, 74 µL (637 µmol) of trimethylsilyl chloride, 100 µL (728 µmol) of triethylamine, and 8.8 mg (273 µmol) of elemental sulfur in dry pyridine (910 µL).

General Procedure for Conversion of Dideoxyribonucleoside aroylphosphonates (8-11 and 16-28) to Dideoxyribonucleoside Phosphorothioates (3,4). To a solution of 8-11 or 16-28 in dry pyridine (10 mL/mmol of 8-11 or 16-28) were added n-butylamine (10 equiv.), DBU (0.5 equiv.), and elemental sulfur (15 equiv.). After 1 h, the mixture was extracted with CH_2Cl_2. 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, filtrated, and evaporated. The residue was dissolved in CH_2Cl_2 and added dropwise to vigorously stirred hexane. White precipitate was collected by filtration and dried in vacuo. The results and ^{31}P NMR are described in Table 3, 5, 6. The products were characterized by mean of their ^1H NMR spectra (Table 7).

Enzymatic Digestion. All the reactions were monitored by TLC developed with Solvent I. The results are described in the text. (A) Treatment of 5 and 6 with snake venom phosphodiesterase: Snake venom phosphodiesterase solution (20 μL, 1 mg/mL) was added to a solution of 5 and 6 (ca. 10 OD_{256}) in 0.1 M Tris-HCl buffer (0.5 mL, pH 8.7). The mixture was incubated at 37 °C for 12 h. (B) Treatment of 5 and 6 with nuclease P1: To a solution of 5 and 6 (ca. 10 OD_{256}) 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.

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