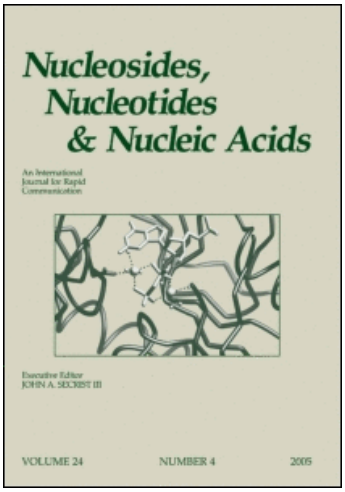

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STEREOCONTROLLED SYNTHESIS OF DITHYMIDINE PHOSPHOROTHIOATES BY USE OF A FUNCTIONALIZED 5'-PROTECTING GROUP BEARING AN IMIDAZOLE RESIDUE†

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ABSTRACT: Diastereoselective formation of internucleotidic phosphorothioate triester bonds was achieved by use of 3-(imidazol-1-ylmethyl)-4',4"-dimethoxytrityl (IDTr) as a 5'-hydroxyl protecting group in the phosphotriester approach. After removal of all the protecting groups, stereochemistry of the major product was determined as the Sp-configuration by enzymatic digestion.

Phosphorothioate analogs of oligodeoxyribonucleotides have been widely used as reliable antisense DNAs for selective inhibition of gene expression.[1] Phosphorothioate DNAs can be routinely synthesized by the automated solid-phase phosphoramidite[2] and *H*-phosphonate method[3] with sulfurization of the phosphite and *H*-phosphonate intermediates, respectively. However, in both methods a random mixture of *S*p and *R*p isomers is formed. It has been demonstrated that all-*S*p phosphorothioates have higher affinity for their complementary oligonucleotides than all-*R*p diastereoisomers.[4] Therefore, there is much current interest in developing improved methods for the stereocontrolled synthesis of phosphorothioate DNAs.[5]

On the other hand, we have reported that the use of 3-(imidazol-1-ylmethyl)-4',4"-dimethoxytrityl (IDTr) as a 5'-hydroxyl protecting group resulted in remarkable enhancement of the rate of internucleotidic bond formation.[6] Recently, it was found that the IDTr group-catalyzed condensation of 5'-hydroxyl components with P-prochiral *S*-phenyl deoxynucleoside 3'-phosphorothioates proceeded in a stereospecific manner to some extent.[7]

† This paper is dedicated to the memory of the late Professor Tsujiaki Hata.

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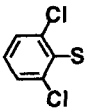
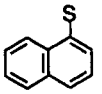
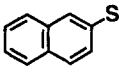
In this paper, we wish to describe a new approach to stereocontrolled formation of internucleotidic phosphorothioate triester bonds by use of the IDTr group as the 5'-hydroxyl protecting group in the phosphotriester approach.

RESULTS AND DISCUSSION

In a previous paper, we demonstrated that condensation of *S*-phenyl 5'-*O*-IDTr-thymidine 3'-phosphorothioate (**4a**) with 3'-*O*-benzoylthymidine (**7**) in the presence of isodurenedisulfonyl dichloride (DDS)[8] resulted in predominant formation of one of the diastereomers of the product **8a** in the ratio of 85:15.[7] In extension of our study, substituent effects of the *S*-phenyl group were examined. Phosphorylating reagents **2b-d** were synthesized in 56-86% yields by a procedure similar to that described in the synthesis of **2a**.[9] By use of these reagents, *S,S*-diaryl 5'-*O*-IDTr-thymidine 3'-phosphorodithioates **3c** and **3d** were synthesized in 90% and 93% yields, respectively. The resulting compounds were treated with H_2O-Et_3N-pyridine (1:2:2, v/v/v) to give the corresponding phosphodiester components **4c** and **4d** quantitatively. In the case of **3b**, one of the 2,6-dichlorophenylthio groups was lost during the aqueous workup, and **4b** obtained directly in 65% yield.

Next, we examined the condensation of **4b-d** with 1 equiv of 3'-*O*-benzoylthymidine (**7**) in the presence of 3 equiv of DDS as a condensing reagent. TLC monitoring of the reaction mixture indicated that the reactions were completed within 10 min in all cases and almost quantitative formation of the dimers **8b-d** was observed. After extraction, the crude products were dissolved in $CDCl_3$ and the ratios of the diastereomers were estimated by ^{31}P NMR analysis. The results are listed in TABLE 1. FIG. 1 shows the ^{31}P NMR spectrum of **8c**. The ratio of the diastereomers which appeared at 24.11 and 24.40 ppm is estimated to be 86:14. In the case of **8b**, partial elimination of the 2,6-dichlorophenylthio group from the product was observed during the aqueous treatment. Therefore, the diastereomeric ratio of the dimer **8b** did not show an accurate value for the internucleotidic bond formation. In spite of the substitution of the phenylthio group by larger arylthio groups, no significant difference in the ratio of the diastereomers was observed in these reactions. As a reference reaction, when *S*-phenyl 5'-*O*-DMTr-thymidine 3'-phosphorothioate was used in place of **4a** in the presence of 1 equiv of *N*-methylimidazole and 3 equiv of DDS, a 1:1 mixture of the corresponding diastereomers was obtained. These results suggested that the predominant formation of one of the diastereomers is apparently attributed to the intramolecular assistant of the imidazole residue as shown in SCHEME 1. When **4a** was activated with an excess amount of DDS in the absence of the 5'-hydroxyl component, formation of the relatively stable phosphorochloridate intermediate **5a** was observed as a nearly 1:1

TABLE 1. Condensation of *S*-aryl 5'-*O*-IDTr-thymidine 3'-phosphorothioates **4a-d** with **7** in the presence of DDS.

	ArS	monomer unit 4 ^{31}P NMR ($CDCl_3$)	dimer 8 ^{31}P NMR ($CDCl_3$)	ratio of diastereomers
a		12.52	24.77, 25.09	85 : 15
b		11.97	21.11, 21.51	77 : 23
c		13.75	24.11, 24.40	86 : 14
d		13.77	23.99, 24.41	86 : 14

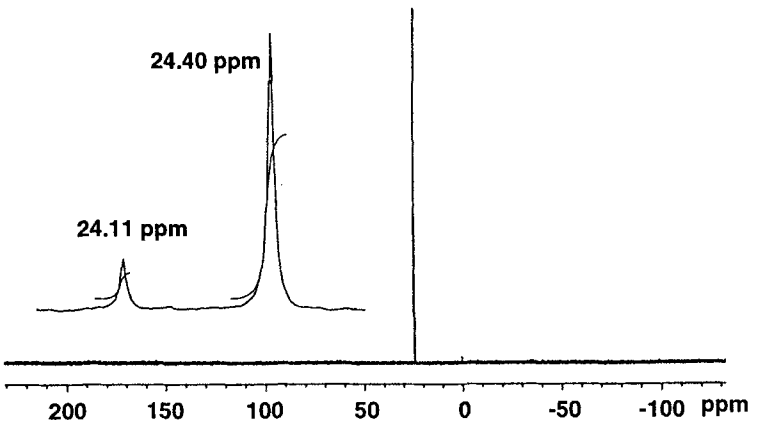
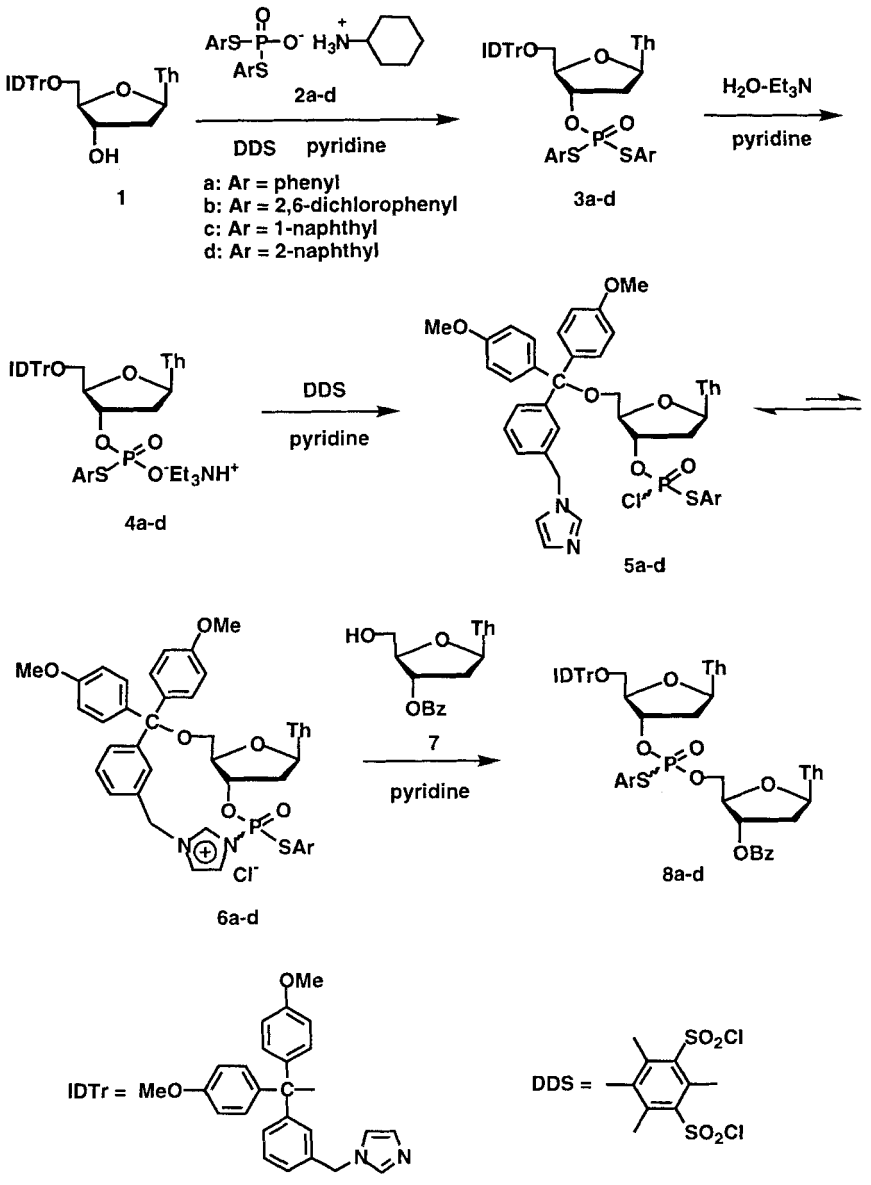


FIG. 1. ^{31}P NMR spectrum of **8c** in $CDCl_3$ after aqueous workup.

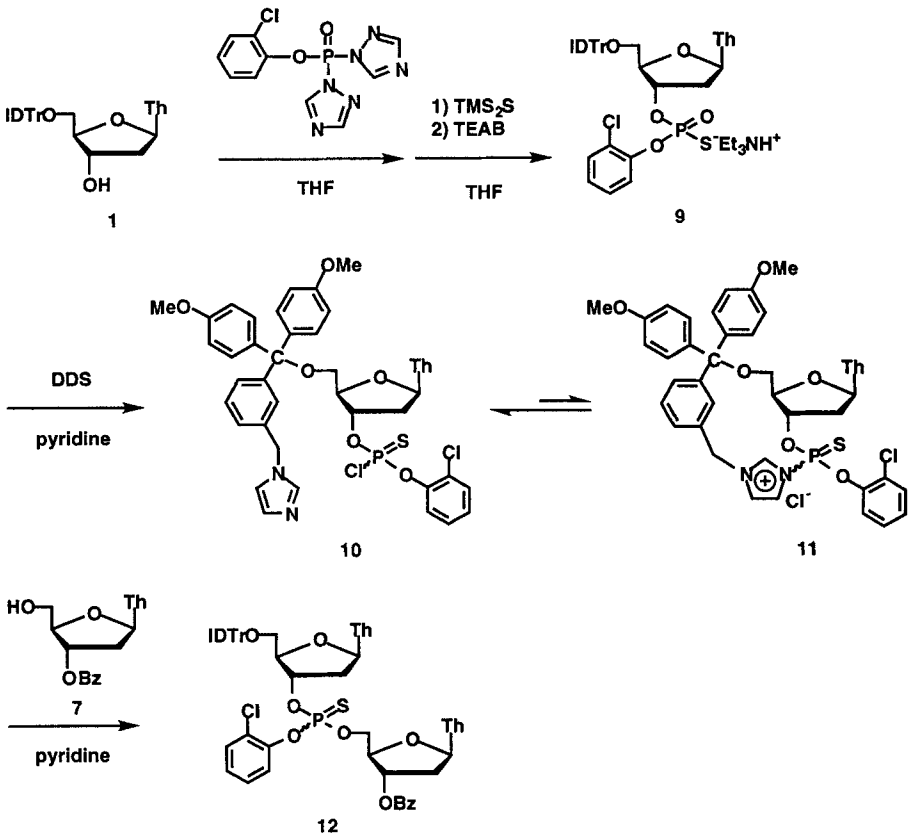


SCHEME 1

mixture of the diastereomers by ^{31}P NMR spectroscopy.[6,10] According to the remarkable acceleration of the rate of condensation and enrichment of the stereoselectivity caused by the IDTr group, the existence of a cyclic intermediate **6** is highly plausible. It is likely that steric bulk resulted from formation of an intramolecular cyclic structure restricts the direction of the nucleophilic attack of the nucleophile at the phosphorus center.

These results prompted us to study stereocontrolled synthesis of dinucleoside phosphorothioate diesters. The IDTr-assisted condensation system requires neither optically pure starting materials nor optically pure reactive intermediates. Therefore, we synthesized racemic 5'-*O*-IDTr-thymidine 3'-*O*-(2-chlorophenyl)phosphorothioate (**9**) as the starting material. 5'-*O*-IDTr-thymidine (**1**) was allowed to react with *in situ* prepared (2-chlorophenyl)phosphorobis(1,2,4-triazolide)[11] in THF. The resulting phosphorotriazolide intermediate was further treated with hexamethyldisilathiane as an anhydrous H_2S equivalent.[12,13] After the usual workup, a diastereomixture of **9** (53.51, 54.27 ppm) was obtained in 58% yield. Next, condensation of **9** with 1 equiv of the 5'-hydroxyl component (**7**) in the presence of 3 equiv of DDS was elucidated by means of ^{31}P NMR (FIG. 2). After 1 h, the yield of the dimer **12** (62.66, 63.11 ppm) was estimated to be 92% and the ratio of the diastereomers was 18:82. It was noteworthy that formation of the phosphotriester resulting from *S*-activation was less than 1%. Activation of **9** with 3 equiv of DDS in the absence of **7** gave a nearly 1:1 diastereomeric mixture of the phosphorochloridate intermediates **10** (62.70, 63.00 pm). In addition, small signals of pyrophosphorothioate derivatives (less than 5%) were observed in the region around 45 to 50 ppm.

The diastereomeric mixture of **12** was treated with 1% TFA followed by tetramethylguanidinium 2-pyridinaldoximate to remove the 5'-*O*-IDTr and 2-chlorophenyl groups. Finally, the 3'-benzoyl group was removed by aqueous ammonia treatment and the fully deprotected dinucleoside phosphorothioate **13** was obtained. After purification by paper chromatography, dithymidine phosphorothioate **13** was obtained in 42% yield. Reversed phase HPLC analysis of the purified product indicated that the ratio of the diastereomers was 23:77. Difference in the diastereomeric ratio between **12** and **13** might be due to the racemization of **12** during the oximate treatment or a partial loss of the one of the diastereomers of **13** during purification. The major isomer was separated by HPLC and treated with snake venom phosphodiesterase and nuclease P1. The major isomer was resistant to snake venom phosphodiesterase (FIG. 3B) and was completely digested by nuclease P1 (FIG. 3C). These results clearly indicated the major product had



SCHEME 2

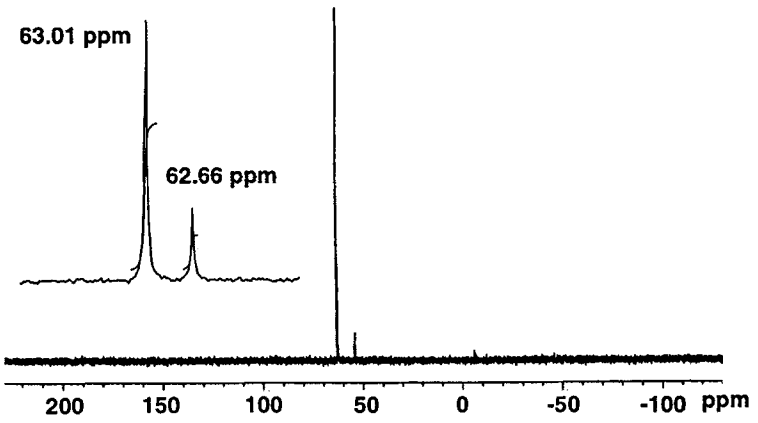
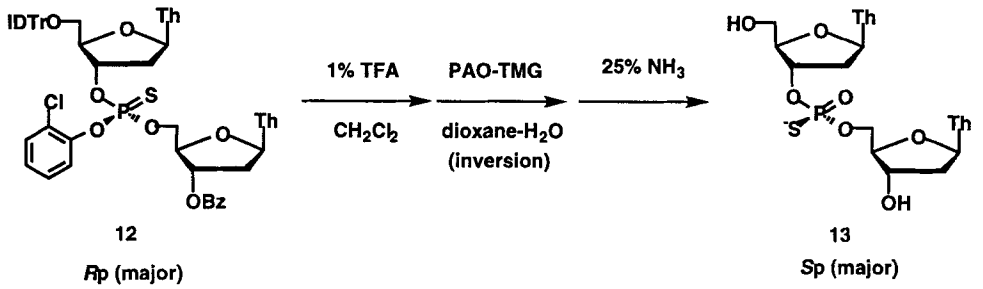


FIG. 2. ^{31}P NMR spectrum of the mixture obtained by the reaction of **9** with **7** in the presence of DDS in pyridine-C_5D_5N (9:1, v/v).



SCHEME 3

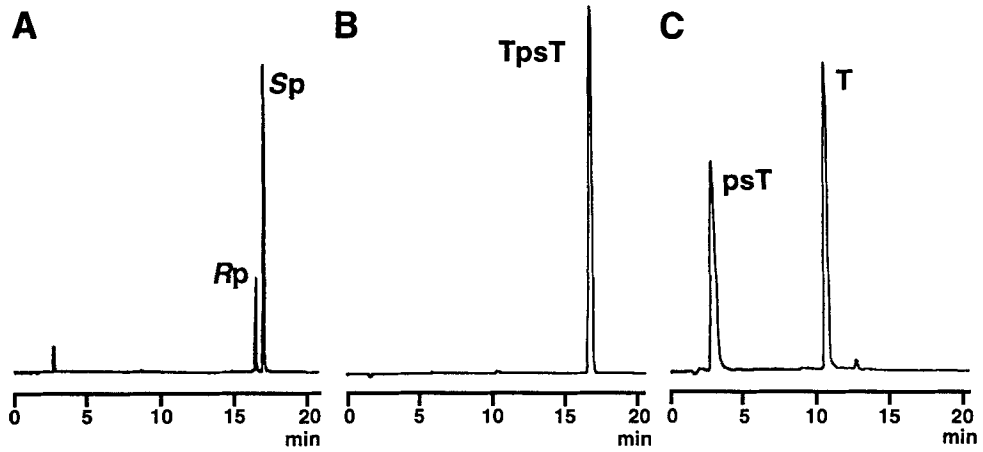


FIG. 3. (A) Reversed phase HPLC profile of the diastereomeric mixture of TpsT. (B) Snake venom phosphodiester digestion of the major isomer. (C) Nuclease P1 digestion of the major isomer.

the *Sp* configuration.[14,15] During removal of the phosphate protecting group of **12**, attack of the oximate anion at the phosphorus center caused inversion of the configuration. Therefore, absolute configuration of the major isomer of **12** was determined to be *Rp*.

In conclusion, the present IDTr-catalyzed condensation enabled us to synthesize dinucleoside phosphorothioate bearing the *Sp* configuration as the major product. The method requires neither diasteromerically pure starting materials nor reactive intermediates.

EXPERIMENTAL

General. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from CaH_2, and stored over molecular sieves 4A. ^1H NMR spectra were obtained at 270 MHz on a JEOL-EX-270 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$. ^{13}C NMR spectra were obtained at 67.8 MHz on a JEOL-EX-270 spectrometer with tetramethylsilane as an internal standard. ^{31}P NMR spectra were obtained at 109.25 MHz on a JEOL-EX-270 spectrometer using 85% H_3PO_4 as an external standard. UV spectra were recorded on a Hitachi 220A spectrophotometer. Thin layer chromatography was performed on precoated glass plates of Kieselgel 60 F_{254} (Merck, No. 5715). Silica gel column chromatography was carried out using Wakogel C-200. Reversed phase HPLC was performed on a column of μBondasphere 5-μm C18 100 Å, 3.9 mm x 15 cm (Nihon Waters Ltd.) with a linear gradient of 0-60% acetonitrile in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C for 60 min at a rate of 1.0 mL/min.

Cyclohexylammonium *S,S'*-Bis(2,6-dichlorophenyl) Phosphorodithioate (2b). To dry pyridine (16 mL) cooled at 0 °C was added dropwise methyl phosphorodichloridate (1.11 mL, 11.2 mmol) under argon. The mixture was stirred at 0 °C for 15 min. A mixture of 2,6-dichlorobenzenethiol (4.0 g, 22.3 mmol) and triethylamine (3.11 mL, 22.3 mmol) in dry pyridine (5 mL) was added dropwise to the above solution. After being stirred at rt for 3 h, the mixture was treated with water (20 mL), diluted with $CHCl_3$, and transferred into a separating funnel. The organic layer was washed three times with water, and the aqueous layer was back-extracted three times with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was coevaporated three times with toluene and dissolved in $CHCl_3$ (20 mL). Cyclohexylamine (2.55 mL, 22.3 mmol) was added dropwise to the above solution. After the mixture was stirred at rt for 12 h, the resulting white solid was collected by filtration and washed with ether. Recrystallization of the white powder from ethanol gave **2b** (4.83 g, 83%) as a white needle: mp 185-186 °C; ^{31}P NMR (C_5D_5N) δ 19.64; ^1H NMR (C_5D_5N) δ 1.17-1.85 (10H, m, 2,3,4,5,6-H of cyclohexylamine), 2.83 (1H, m, 1-H of cyclohexylamine), 7.25 (2H, t, J = 6.9 Hz, 4-H of dichlorophenyl), 7.42 (4H, d J = 6.9 Hz, 3,5-H of dichlorophenyl), 7.79 (2H, bs, NH_2); ^{13}C NMR (C_5D_5N) δ 23.89, 24.53, 49.49, 128.18, 129.00, 129.54, 129.58, 132.20, 132.31, 141.04, 141.12. Anal. Calcd for $C_{18}H_{20}Cl_4NO_2PS_2$: C, 41.63; H, 3.88; N, 2.70; S, 12.35. Found: C, 41.22; H, 3.95; N, 2.62; S, 12.52.

Cyclohexylammonium *S,S'*-Bis(1-naphthyl) Phosphorodithioate (2c). The same procedure as described in the synthesis of **2b** by use of 1-naphthalenethiol (2.12 g, 13.2

mmol) gave **2c** (1.78 g, 56%) as a white needle: mp 184-187 °C; ^{31}P NMR (C_5D_5N) δ 27.56; ^1H NMR (C_5D_5N) δ 0.92-2.07 (10H, m, 2,3,4,5,6-H of cyclohexylamine), 2.91 (1H, m, 1-H of cyclohexylamine), 7.31-7.45 (6H, 3,6,7-H of 1-naphthyl), 7.85 (4H, t, J = 14.4 Hz, 5,8-H of 1-naphthyl), 8.58 (2H, d, J = 5.4 Hz, 2-H of 1-naphthyl), 8.68 (2H, d, J = 5.4 Hz, 4-H of 1-naphthyl), 9.36 (2H, bs, NH_2); ^{13}C NMR (C_5D_5N) δ 22.23, 22.62, 28.84, 47.85, 123.65, 123.77, 123.94, 124.60, 125.95, 125.98, 126.13, 129.04, 129.13, 131.21, 131.29, 132.11, 132,49. Anal. Calcd for $C_{26}H_{28}NO_2PS_2$: C, 64.84; H, 5.86; N, 2.91; S, 13.32. Found: C, 65.43; H, 5.85; N, 2.65; S, 13.15.

Cyclohexylammonium *S,S'*-Bis(2-naphthyl) Phophorodithioate (2d). The same procedure as described in the synthesis of **2b** by use of 2-naphthalenethiol (10.76 g, 67.2 mmol) gave **2d** (11.20 g, 86%) as a white needle: mp 179-181 °C; ^{31}P NMR (C_5D_5N) δ 27.30; ^1H NMR (C_5D_5N) δ 0.92-2.18 (10H, m, 2,3,4,5,6-H of cyclohexylamine), 3.07 (1H, m, 1-H of cyclohexylamine), 7.34-7.44 (4H, 6,7-H of 2-naphthyl), 7.74-7.80 (6H, m, 4,5,8-H of 2-naphthyl), 8.18, (2H, d, J = 5.4 Hz, 3-H of 2-naphthyl), 8.49 (2H, s, 1-H of 2-naphthyl); ^{13}C NMR (C_5D_5N) δ 22.27, 22.68, 29.04, 48.09, 121.47, 121.64, 125.95, 127.15, 129.20, 129.56, 129.61, 130.51, 130.87, 131.78. Anal. Calcd for $C_{26}H_{28}NO_2PS_2$: C, 64.84; H, 5.86; N, 2.91; S, 13.32. Found: C, 65.36; H, 6.09; N, 2.81; S, 13.03.

***S,S'*-Bis(1-naphthyl) 5'-*O*-[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]trityl-thymidine 3'-Phosphorodithioate (3c).** 5'-*O* -[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]trityl-thymidine **1** (0.10 g, 0.16 mmol) and **2c** (0.12 g, 0.24 mmol) were dried by repeated coevaporation with dry pyridine and dissolved in dry pyridine (1.6 ml). To the above solution was added isodurenedisulfonyl dichloride (0.15 g, 0.48 mmol). The mixture was stirred at rt for 30 min. The reaction was quenched by addition of 5% $NaHCO_3$ and the mixture was extracted three times with $CHCl_3$. The aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a column of silica gel (4 g). Chromatography eluted with $CHCl_3$ containing 0.5% of methanol gave **3c** (0.14 g, 90%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 49.55; ^1H NMR ($CDCl_3$) δ 1.34 (3H, s, 5-CH_3), 1.99-2.03 (2H, m, 2',2"-H), 3.14 (2H, m, 5',5"-H), 3.70-3.76 (7H, m, 4'-H, OCH_3 of IDTr), 4.92 (2H, s, CH_2 of IDTr), 5.17-5.20 (1H, m, 3'-H), 6.07 (1H, dd J = 6.3 Hz, J = 7.3 Hz, 1'-H), 6.77-8.24 (31H, m, 6-H, ArH of IDTr, 1-naphthyl), 8.96 (1H, s, 3-H); ^{13}C NMR ($CDCl_3$) δ 11.65, 24.94, 38.78, 38.85, 50.69, 55.22, 63.00, 77.20, 78.96, 79.08, 84.04, 84.35, 84.40, 86.87, 111.34, 113.39, 119.17, 122.73, 122.84, 122.95, 123.07, 125.61, 125.66, 125.77, 125.95, 126.69, 126.85, 127.37, 127.92, 128.46, 128.55, 128.66, 129.20, 129.78, 130.12, 130.98, 131.03, 131.09, 131.16, 134.23, 134.30, 134.54, 134.59, 134.63, 134.68, 135.06, 135.09, 135.98, 136.05,

136.14, 137.14, 145.09, 150.10, 158.83, 158.89, 163.45. Anal. Calcd for $C_{55}H_{49}N_4O_8PS_2 \cdot 2H_2O$: C, 64.44; H, 4.82; N, 5.47; S, 6.26. Found: C, 64.77; H, 5.20; N, 5.25; S, 7.01.

S,S'-Bis(2-naphthyl) 5'-*O*-[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]trityl-thymidine 3'-Phosphorodithioate (3d). The same procedure as described in the synthesis of **3c** by use of **2d** (0.12 g, 0.24 mmol) gave **3d** (0.14 g, 90%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 50.85; ^1H NMR ($CDCl_3$) δ 1.41 (3H, s, 5-CH_3), 2.25-2.36 (1H, m, 2'-H), 2.50-2.57 (1H, m, 2"-H), 3.30, 3.31 (2H, m, 5',5"-H), 3.76 (OCH_3 of IDTr), 4.17 (1H, m, 4'-H), 4.99 (2H, s, CH_2 of IDTr), 5.44-5.50 (1H, m, 3'-H), 6.40 (1H, dd J = 5.3 Hz, J = 8.8 Hz, 1'-H), 6.79-8.01 (31H, m, 6-H, ArH of IDTr, 2-naphthyl), 8.79 (1H, s, 3-H); ^{13}C NMR ($CDCl_3$) δ 8.48, 11.72, 24.82, 29.17, 33.89, 46.04, 51.23, 55.15, 62.91, 77.20, 84.34, 86.83, 111.32, 113.35, 119.59, 122.28, 122.59, 122.70, 126.24, 126.54, 126.92, 127.46, 127.69, 128.21, 128.73, 129.17, 129.24, 129.74, 130.08, 131.03, 131.09, 131.16, 131.21, 133.15, 133.19, 133.23, 133.33, 133.39, 133.44, 134.07, 134.95, 135.24, 135.35, 135.45, 135.54, 135.65, 136.44, 138.69, 145.30, 147.69, 149.02, 150.15, 158.80, 158.83, 153.49. Anal. Calcd for $C_{55}H_{49}N_4O_8PS_2 \cdot 2/3H_2O$: C, 65.01; H, 4.86; N, 5.51; S, 6.31. Found: C, 65.13; H, 4.77; N, 5.31; S, 6.70.

Triethylammonium *S*-(2,6-Dichlorophenyl) 5'-*O*-[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]tritylthymidine 3'-Phosphorothioate (4b). 5'-*O* -[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]tritylthymidine **1** (0.10 g, 0.16 mmol) and **2c** (0.12 g, 0.24 mmol) were dried by repeated coevaporation with dry pyridine and dissolved in dry pyridine (1.6 ml). To the above solution was added isodurenedisulfonyl dichloride (0.15 g, 0.48 mmol). The mixture was stirred at rt for 30 min. To the above solution, 2 M TEAB buffer (2 mL) was added. After being stirred at rt for 2 h, the mixture was diluted with $CHCl_3$ and washed three times with 2 M TEAB buffer. The aqueous layer was back-extracted three times with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a column of silica gel (4 g). Chromatography eluted with $CHCl_3$ containing 5% of methanol gave **4b** (0.10 g, 65%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 11.97; ^1H NMR ($CDCl_3$) δ 1.28 (9H, t, J = 7.3 Hz, CH_3 of Et_3N), 1.37 (3H, s, 5-CH_3), 2.29-2.72 (2H, m, 2',2"-H), 3.01 (6H, q, J = 7.3 Hz, CH_2 of Et_3N), 3.30 (1H, m, 5'-H), 3.52 (1H, m, 5"-H), 3.77, 3.79 (6H, 2s, OCH_3 of IDTr), 4.47 (1H, m, 4'-H), 5.17 (2H, m, CH_2 of IDTr), 5.42 (1H, m, 3'-H), 6.39 (1H, dd J = 5.8 Hz, J = 7.3 Hz, 1'-H), 6.80-8.70 (20H, m, 6-H, ArH of IDTr and 2,6-dichlorophenyl), 8.70 (1H, bs, 3-H); ^{13}C NMR ($CDCl_3$) δ 8.88, 11.50, 29.60, 39.84, 45.79, 50.77, 55.19, 64.24, 76.80, 77.21, 84.46, 85.19, 85.27, 86.79, 110.98, 113.32, 119.17, 125.70, 126.79, 128.10, 128.23, 128.46, 128.63, 129.20, 129.52, 129.78, 130.05, 130.30, 131.30, 131.32, 134.39, 135.65,

135.69, 136.26, 137.30, 141.56, 141.62, 145.28, 150.51, 158.67, 158.76, 163.79. Anal. Calcd for $C_{47}H_{54}Cl_2N_5O_9PS$: C, 58.38; H, 6.53; N, 7.24; S, 3.32. Found: C, 58.14; H, 5.98; N, 6.52; S, 4.14.

Triethylammonium *S*-(1-Naphthyl) 5'-*O*-[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]tritylthymidine 3'-Phosphorothioate (4c). *S,S'*-bis(1-naphthyl) 5'-*O*-[3-(imidazol-1-ylmethyl)-4',4"-dimethoxy]tritylthymidine 3'-phosphorodithioate (**3c**). (0.069 g, 0.070 mmol) was dissolved in H_2O-Et_3N-pyridine (1:2:2, v/v/v, 5 mL) and the mixture was stirred at rt for 10 min. The mixture was diluted with $CHCl_3$ and washed three times with 2 M TEAB buffer. The aqueous layer was back-extracted three times with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure to give **4c** (0.067 g, quant) as a foam. This compound was used for the condensation with 3'-*O*-benzoylthymidine without further purification: ^{31}P NMR ($CDCl_3$) δ 13.75.

Triethylammonium 5'-*O*-[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]trityl-thymidine 3'-[*S*-(2-Naphthyl)] Phosphorothioate (4d). The same procedure with **3d** (0.129 g, 0.130 mmol) gave **4d** (0.124 g, quant) as a foam: ^{31}P NMR ($CDCl_3$) δ 13.77.

Triethylammonium *O*-(2-Chlorophenyl) 5'-*O*-[3-(Imidazol-1-ylmethyl)-4',4"-dimethoxy]tritylthymidine 3'-Phosphorothioate (9). To a solution of 2-chlorophenyl phosphorodichloridate (0.07 g, 0.29 mmol) in dry THF (2.5 mL) were added 1*H*-1,2,4-triazole (0.06 g, 0.86 mmol) and Et_3N (120 μL). The mixture was stirred at rt for 40 min. Compound **1** (0.12 g, 0.19 mmol, dried by repeated coevaporation with dry pyridine) in dry THF (2 mL) was added to the above mixture. After being stirred at rt for 2 h, the mixture was treated with hexamethyldisilathian (700 mL). The mixture was diluted with $CHCl_3$ and washed three times with 2 M TEAB buffer. The aqueous layer was back-extracted three times with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a column of silica gel (3 g). Chromatography eluted with $CHCl_3$ containing 3% of methanol gave **9** (0.10 g, 58%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 53.51, 54.27; ^1H NMR ($CDCl_3$) δ 1.28 (9H, t, J = 7.3 Hz, CH_3 of Et_3N), 1.36 (3H, s, 5-CH_3), 2.28-2.42 (1H, m, 2'-H), 2.61-2.72 (1H, m, 2"-H), 3.06 (6H, q, J = 7.3 Hz, CH_2 of Et_3N), 3.15-3.38 (1H, m, 5'-H), 3.48-3.66 (1H, m, 5"-H), 3.76, 3.78 (6H, 2s, OCH_3 of IDTr), 4.43 (1H, m, 4'-H), 5.18 (2H, m, CH_2 of IDTr), 5.63 (1H, m, 3'-H), 6..48 (1H, m, 1'-H), 6..69-7.63 (20H, m, 6-H, ArH of IDTr and 2-chlorophenyl), 9.98 (1H, bs, 3-H); ^{13}C NMR ($CDCl_3$) δ 8.41, 10.77, 11.43, 22.75, 23.52, 28.70, 39.19, 45.64, 50.59, 50.64, 50.73, 53.34, 55.06, 63.95, 74.88, 84.22, 84.37, 84.57, 84.62, 85.34, 85.43, 86.38, 86.61, 86.81, 110.96, 113.17, 118.89, 119.16, 119.25, 121.85, 121.91, 121,94, 122.00, 123.83, 123.95, 125.25, 125.34, 125.45, 125.50, 125.61, 125.77, 126.47, 126.63, 127.06, 127.19,

127.37, 127.44, 128.10, 128.16, 128.45, 128.57, 128.66, 128.82, 129.09, 129.27, 129.38, 129.56, 129.76, 129.83, 129.90, 130.01, 130.26, 130.42, 130.71, 134.02, 134.14, 134.93, 135.40, 135.72, 135.81, 136.01, 136.12, 136.26, 137.05, 137.13, 145.01, 145.16, 146.61, 146.58, 148.57, 148.61, 148.72, 150.51, 150.58, 158.47, 158.51, 158.54, 158.63, 158.71, 163.81, 163.92.

^{31}P NMR Study of Internucleotidic Bond Formation. A triethylammonium 5'-*O*-[3-(imidazol-1-ylmethyl)-4',4"-dimethoxy]tritylthymidine 3'-phosphorothioate derivative (**4b-d** or **9**, 0.025 mmol) and 3'-*O*-benzoylthymidine (0.009 g, 0.025 mmol) were dried by repeated coevaporation with dry pyridine and dissolved in pyridine (250 μL). To the solution was added isodurenedisulfonyl dichloride (0.025 g, 0.075 mmol). After being stirred for 10 min, the mixture was quenched by addition of 5% $NaHCO_3$ (1 mL). The mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The aqueous layer was back-extracted three times with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude dimer (**8b-d** or **12**) was dissolved in $CDCl_3$ (500 μL) and its ^{31}P NMR spectrum was recorded. The ratio of the diastereomers was estimated on the basis of integration of the resonance signals.

Dithymidine Phosphorothioate (13). A crude mixture of the dimer **12** (0.049 g, 0.043 mmol) was treated with 1% TFA in CH_2Cl_2 (500 μL) at rt for 30 min. To the mixture was added pyridine (100 μL) and the solution was concentrated to dryness. The residue was dissolved in dioxane-H_2O (1:1, v/v, 500 μL) and a mixture of 2-pyridinealdoxime (0.053 g, 0.43 mmol) and *N,N,N',N'*-tertramethylguanidine (51 μL, 0.41 mmol) in dioxane-H_2O (1:1, v/v, 500 μL) was added. After the mixture was stirred at rt for 24 h, 25% NH_3 was added and the mixture was stirred at rt for 24 h. The mixture was evaporated to dryness and dissolved in water. The solution was washed three times with ether and concentrated to a small volume. The crude product was purified by paper chromatography (Whatmann 3MM) eluted with 2-PrOH-25% NH_3-H_2O (7:1:2, v/v/v) to give dithymidine phosphorothioate **13** (302 A_{260} units, 42%).

Enzymatic Characterization of the Major Isomer. The major isomer of dithymidine phosphorothioate **13** was separated by reversed phase HPLC. This isomer (0.5 A_{260} units) was treated with snake venom phosphodiesterase (4 μL) in 0.5 M Tris•HCl buffer (pH 8.0, 40 μL) at 37 °C for 3.5 h and the mixture was analyzed by reversed phase HPLC. In a similar manner, the major isomer (0.5 A260 units) was treated with nuclease P1 (10 μL) in 50 mM sodium acetate buffer (pH 5.3, 50 μL) at 37 °C for 3.5 h and the mixture was analyzed by reversed phase HPLC.

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