JOC Article

Enhanced Stereoselectivity in Internucleotidic Bond Formation by the Use of the Chiral Ribose Moiety of Thymidine

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This paper deals with the synthesis of new cyclic thymidine 3′-phosphoramidite building blocks having a covalent linker between the trityl type 5′-hydroxyl protecting group and the phosphorus atom attached to the 3′-hydroxyl group of thymidine. The ring structures were designed to reduce the conformational freedom around the phosphorus center so that the stereoselectivity in the internucleotide linkage formation would be improved. The linkers were also designed to be removed readily by treatment with aqueous ammonia. These building blocks were synthesized in good yield by one-pot cyclization of the diol precursors with dichloro(*N*,*N*-diisopropylamino)phosphine, despite their large-membered ring. Various activators having 1*H*-tetrazole, imidazole, and triazole structures were investigated to find the best selectivity in the synthesis of thymidylyl(5′-3′)thymidine phosphorothioate. It turned out that our cyclic phosphoramidites gave preferentially the R_p diastereoisomer in high coupling yield applicable to the solid-phase synthesis of oligodeoxynucleotides. It should be noted that high stereoselectivity was achieved without any chiral sources other than the 2′-deoxyribose moiety itself. The mechanistic studies revealed the importance of the steric bulk and the acidity of the activators. It was also found that the steric bulk of the alcoholic nucleophile was an important factor that determined the stereoselectivity in our systems.

Introduction

Most oligodeoxynucleotide derivatives modified at internucleotidic phosphates have chirality on their phosphorus atoms. Several approaches toward the stereoselective synthesis of such modified oligodeoxynucleotide derivatives have been reported, especially for the synthesis of methylphosphonate oligodeoxynucleotides[1] and phosphorothioate oligodeoxynucleotides.[2] These approaches have been developed on the basis of the preparation of the stereochemically pure 3′-nucleotidyl synthetic units and successive stereoselective alcoholysis. For example, Stec et al. reported an oxathiaphospholane approach,[3] and, thereafter, other chiral phosphoramidite approaches have been reported by several authors.[4]

Apart from these precedents, there is another strategy for the stereoselective formation of internucleotidic linkage utilizing the intrinsically chiral 2′-deoxyribose moiety of the nucleoside itself as a chiral auxiliary. The potential of the ribose moiety to induce the stereoselectivity was reported in the 1980s by Ohtsuka et al.[5] in natural DNA synthesis by use of phosphotriester chemistry and by Engels et al.[6] in the alkylphosphonate DNA synthesis by use of an alkyldichlorophosphine. The latter group also reported a study on the stereoselective dinucleoside methylphosphonate synthesis by using a combination of sterically hindered 2-trityl-4,5-dicyanoimidazole derivatives and a diastereomeric mixture of nucleoside 3′-(*N*,*N*-diisopropyl)methylphosphonamidite and accomplished the R_p:S_p stereoselectivity up to 89:11 without using any chiral source other than the 2′-deoxyribose moiety.[7] Although these results apparently show the potential of the ribose residue as a chiral auxiliary, such bulky activators are not suitable for the

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(3) (a) Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, *19*, 5883−5888. (b) Stec, W.; Wilk, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 709−722. (c) Stec, W. J.; Grajkowski, A.; Karwowski, B.; Kobylanska, A.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019−12029. (d) Uznanski, B.; Grajkowski, A.; Krzyzanowska, B.; Kazmierkowska, A.; Stec, W. J.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1992**, *114*, 10197−10202. (e) Stec, W. J.; Karwowski, B.; Boczkowska, M.; Guga, P.; Koziolkiewicz, M.; Sochacki, M.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1998**, *120*, 7156−7167. (f) Karwowski, B.; Guga, P.; Kobylanska, A.; Stec, W. J. *Nucleosides Nucleotides* **1998**, *17*, 1747−1759.

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(6) (a) Samstag, V. W.; Engels, L. W. *Angew. Chem.* **1992**, *104*, 1367−1369. (b) Löschner, T.; Engels, J. *Tetrahedron Lett.* **1989**, *30*, 5587−5590.

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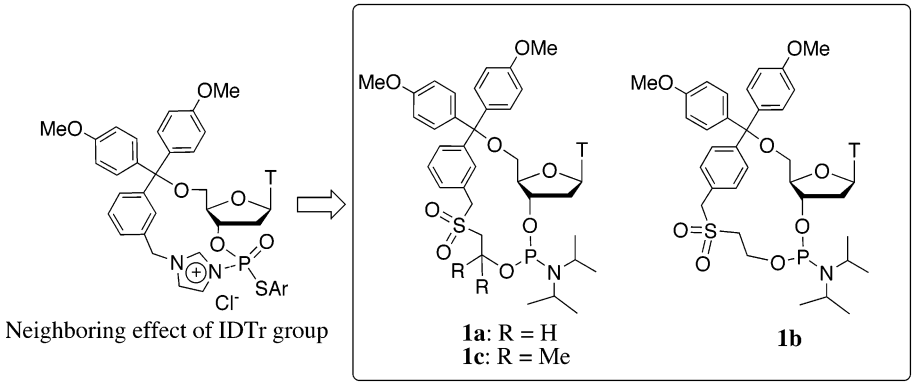
SCHEME 1



Neighboring effect of IDTr group

solid-phase oligodeoxynucleotide synthesis because of their reduced reactivity.

Instead of using such bulky activators, we assumed that higher stereoselectivity could be achieved by reducing the free rotation of the chemical bond connecting the phosphorus atom and the 2′-deoxyribose chiral auxiliary. Such conformational fixation would render the interaction between the reaction center and the 2′-deoxyribose moiety more effective and increase the stereoselectivity.

In this paper, we propose new phosphoramidite units (**1a**, **1b**, and **1c**) having a cyclic structure. These ring systems were introduced to reduce the motion of atoms around the phosphorus center and improve the stereoselectivity in the internucleotidic bond formation. The effectiveness of such cyclic structures for the stereocontrolled synthesis was originally shown in our previous studies[8] on 3-(imidazol-1-ylmethyl)-4′,4″-dimethoxytrityl (IDTr) group and its derivatives. The use of IDTr resulted in considerably high stereoselectivity (85:15) in the dithymidine phosphorothioate synthesis that was carried out in the phosphotriester approach.

We searched for the best combination from the ring structures (**1a**, **1b**, and **1c**) and the activators chosen from 1*H*-tetrazole-, imidazole-, and triazole-type compounds. In some cases, the use of our cyclic phosphoramidite achieved more than 80:20 stereoselectivity, maintaining high coupling yield applicable to solid-phase oligonucleotide synthesis even in the absence of any chiral sources other than the 2′-deoxyribose moiety.

Results and Discussion

Design of Cyclic Phosphoramidite Building Blocks. The chemical structures of our new cyclic phosphoramidite units (**1a**, **1b**, and **1c**) are shown in Scheme 1. The reaction intermediate involving the neighboring group participation of the IDTr group previously reported[8] is also shown. The methylsulfonylethoxy-type linkers of **1a**, **1b**, and **1c** were designed to connect a DMTr type 5′-protecting group and the 3′-phosphorus atom. At the same time, they act as phosphate protecting groups removable by treatment with aqueous ammonia like that of conventional alkylsulfonylethoxy protecting groups.[9] Compound **1a** bearing a linker from the meta position of one of the three phenyl rings was designed from the IDTr-mediated intermediate. Compound **1b** has almost the same structure as **1a** except that the linker binds to the para position of the phenyl group. Compound **1c**, a dimethylated derivative of **1a**, was designed to clarify the steric effect around the phosphorus center. It should be noted that in all compounds, no additional chiral sources were included on both the trityl and the linker parts.

Preparation of Trityl-Type Protecting Groups and Their Introduction into the 5′-Position of Thymidine. The preparation of trityl chloride derivatives **2a, 2b**, and **2c**, which are necessary for the synthesis of **1a**, **1b**, and **1c**, respectively, is shown in Scheme 2. In the case of **2a**, methyl *m*-toluate was chosen as the starting material. Condensation of methyl *m*-toluate with 4-methoxyphenylmagnesium bromide followed by hydrolysis gave **3a**, which, in turn, was converted to the isopropyl ether derivative **4a**. Compound **4a** was brominated by use of NBS in the presence of AIBN. Successively, the bromide **5a** was allowed to react with 2-mercaptoethanol to give the sulfide **6a**. The conversion of **6a** to the desired sulfone **7a** was carried out in 82% yield by treatment with *m*-chloroperbenzoic acid in the presence of $NaHCO_3$ which neutralizes the released *m*-chlorobenzoic acid to avoid the decomposition of the trityl ether. The hydroxyl group of **7a** was protected with the TBDMS group and the 2-propoxy group was removed by an acid treatment to give **9a**. The yield of **9a** from *m*-toluate was 33%.

The other two trityl compounds (**9b**, **9c**) were synthesized according to similar procedures. Compound **9b** was synthesized from methyl *p*-toluate in place of methyl *m*-toluate. Compound **9c** was synthesized by simply using 1,1-dimethyl-2-mecaptoethanol[10] in place of 2-mercaptoethanol. Those alcohols, thus obtained, were further converted to the corresponding trityl chloride derivative (**2a**−**c**) by refluxing with acetyl chloride. Compound **2a** and **2b** were used in the next procedure simply after removing the reagents by evaporation. Compound **2c** was isolated as crystals from hexane in 98% yield. These trityl groups were introduced to the 5′-hydroxyl group of thymidine in 95−98% yields, as shown in Scheme 3. The successive removal of the TBDMS group gave the diols **11a**, **11b**, and **11c** which were used in the next cyclization.

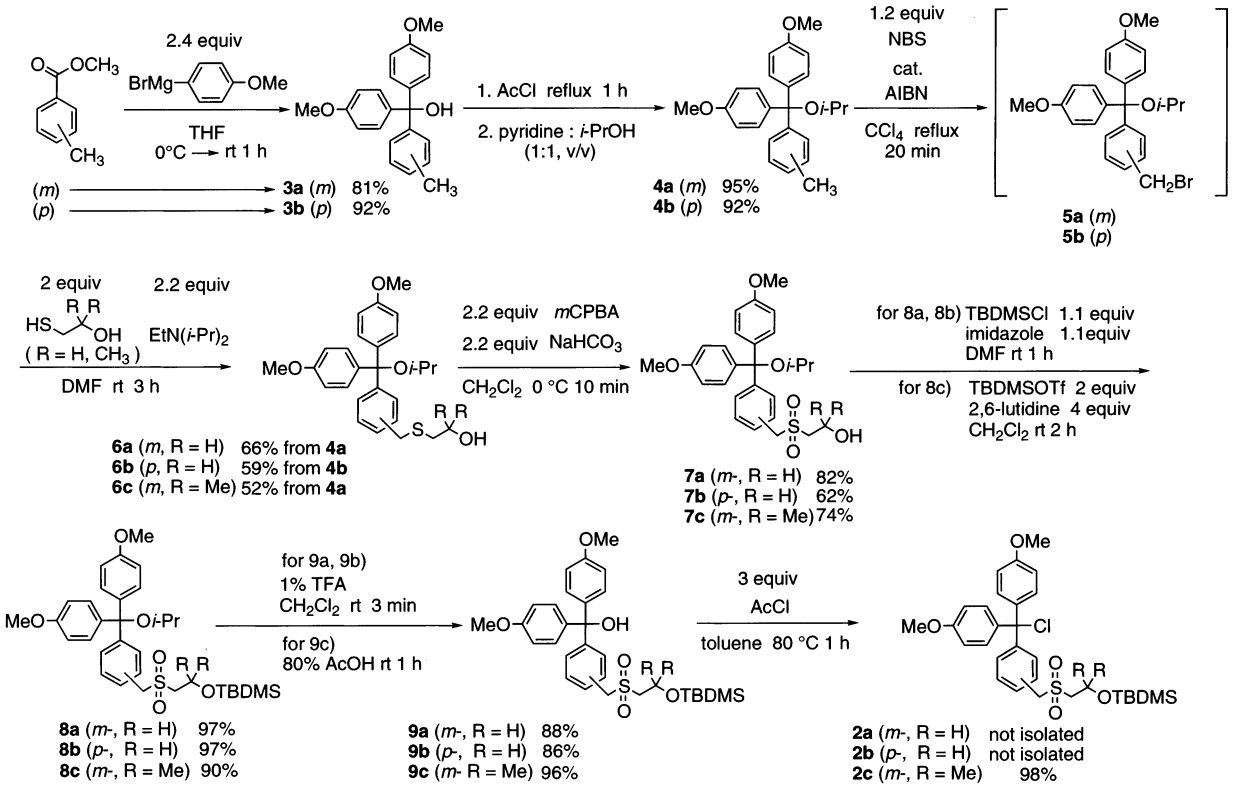
Optimized Conditions for the Cyclization of 11a−c. Several factors such as temperature and concen-

(8) Wada, T.; Kobayashi, N.; Mori, T.; Sekine, M. *Nucleosides Nucleotides* **1998**, *17*, 351−364.

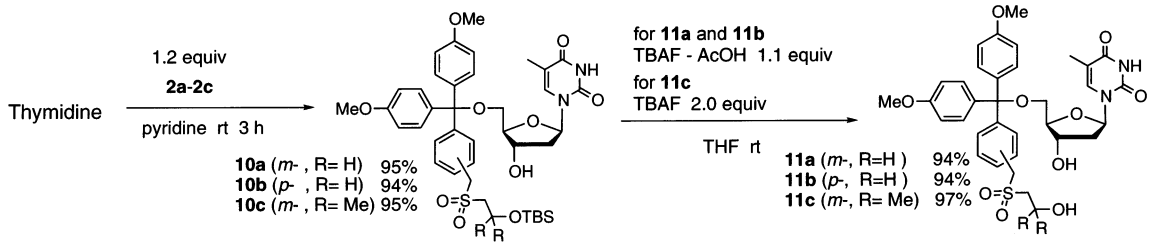
(9) (a) Claesen, C.; Tesser, G. I.; Dreef, C. E.; Marugg, J. E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1984**, *25*, 1307−1310. (b) Claesen, C. A. A.; Segers, R. P. A. M.; Tesser, G. I. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 209−214. (c) Claesen, C. A. A.; Segers, R. P. A. M.; Tesser, G. I. *Recl. Trav. Chim. Pays-Bas* **1985**, *104*, 119−122.

(10) Meyers, A. I. *J. Org. Chem.* **1960**, *25*, 1147−1151.

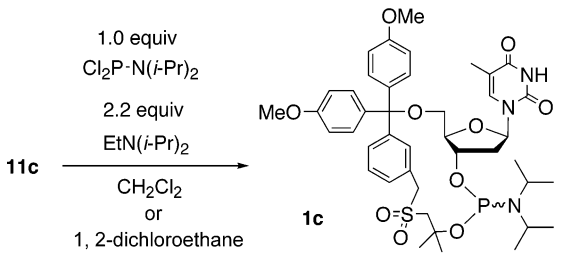
SCHEME 2



SCHEME 3



SCHEME 4



tration must be optimized to achieve efficient cyclization. The optimized conditions were studied by using **11c** as a model compound. A 15-membered ring must be formed to obtain cyclized product **1c**. We chose a one-pot cyclization procedure using dichloro(*N*,*N*-diisopropylamino)-phosphine[11] as the phosphitylating agent, as shown in Scheme 4.

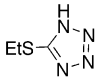
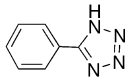
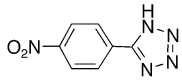
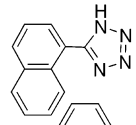
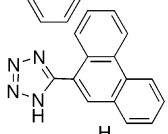
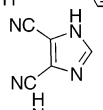
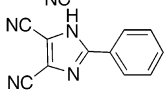
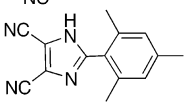
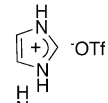
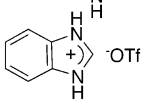
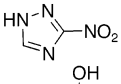
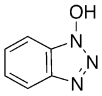
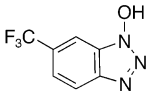
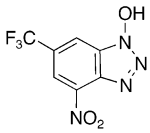
The effect of the concentration on the cyclization efficiency was examined at room temperature. When the reaction was carried out at 10 mM, the cyclized product **1c** could be isolated in 18% yield (entry 1 of Table 1) from the complex mixture with the various side products. Removal of a trace amount of water by adding molecular sieves 4 Å proved to be effective to reduce the amount of side products generated by the hydrolysis of the reaction intermediates (entry 2 of Table 1). In this case **1c** was obtained in 42% yield by column chromatography. The use of a more concentrated 100 mM solution did not improve the cyclization yield significantly (entry 3), and

TABLE 1. Influence of the Temperature and Concentration on the Cyclization of 11c into 1c in Dichloromethane (entries 1−7) or 1,2-Dichloroethane (entry 8)

entry	temp	concn of **11c**, mM	MS 4A	time (h)	yield (%)
1	rt	10	−	6	18
2	rt	10	+	6	42
3	rt	100	+	3	35
4	rt	1	+	6	−
5	reflux	10	+	2	36
6	reflux	10	+	4	43
7	reflux	10	+	6	49
8	reflux	10	+	3	38

(11) (a) Shimidzu, T.; Yamana, K.; Maikuma, S. *Tetrahedron Lett.* **1984**, *25*, 4237−4240. (b) Nurminen, E. J.; Mattinen, J. K.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1621−1628.

TABLE 2. Structures of the Activators and the Stereoselectivity in the Internucleotide Bond Formation[a]

enry		1a → 13a *Rp*:*Sp**	1b → 13b *Rp*:*Sp**	1c → 13c *Rp*:*Sp**, **
1		70 : 30 (85)	72 : 28 (92)	47 : 53 (76)
2		75 : 25 (91)	79 : 21 (93)	47 : 53 (89)
3		81 : 19 (85)	81 : 19 (91)	52 : 48 (65)
4		77 : 23 (88)	80 : 20 (91)	43 : 57 (86)
5		81 : 19 (87)	86 : 14 (85)	52 : 48 (47)
6		82 : 18 (77)	85 : 15 (33)	52 : 48 (4)
7		69 : 31 (96)	40 : 60 (85)	63 : 37 (62)
8		78 : 22 (21)	54 : 46 (20)	47 : 53 (5)
9		66: 34 (71)	37: 63 (54)	49: 51 (7)
10		69: 31 (93)	50: 50 (82)	52: 48 (40)
11		79: 21 (99<)	46: 54 (85)	66: 34 (71)
12		83: 17 (60)	44: 56 (91)	46: 54 (49)
13		63: 37 (45)	74: 26 (52)	31: 69 (39)
14		66: 34 (83)	82: 18 (92)	29: 71 (73)
15		62: 38 (87)	82: 18 (92)	23: 77 (70)

[a] (*) R_p/S_p ratio and yield (numbers in the parenthesis) were determined from the ^{31}P NMR spectra of the reaction mixture after sulfurization (see Experimental Section). (**) One of the diastereoisomers of **13** that gives R_p-14 (S_p-14) after deprotection is denoted by $R_p(S_p)$.

a more diluted concentration of 1.0 mM (entry 4), which had been expected to reduce the rate of the intermolecular side reaction, was not effective probably because the reaction rate of the phosphitylation became much slower than the decomposition of dichloro(*N*,*N*-diisopropylamino)phosphine.

The ^{31}P NMR spectra of the mixtures showed the presence of the two diastereomers of **1c** in an almost 1:1 ratio in all cases. This is not surprising because this cyclization begins with the nonstereoselective substitution of one of the two chlorine atoms by a hydroxyl oxygen and is completed by the second substitution with inversion of the stereochemistry of the phosphorus atom. Thus, as a whole, the cyclization is nonstereoselective.

We next studied the temperature dependence of the cyclization and found interesting phenomena. In general, intramolecular cyclization is thought to be more favorable at lower temperatures because intermolecular collision of the reactants occurs less frequently at lower temperatures. However, when a reaction mixture at a concentration of 10 mM was heated to the refluxing temperature of dichloromethane (bp = 40 °C), the yield of the cyclized product increased significantly up to 49%. This temperature dependence suggests that the major conformers of **11c** at room temperature are unsuitable for the cyclization: It is likely that the primary hydroxyl group of the sulfonylethanol linker is distant from the 3′-hydroxyl group. The population of the conformers, which have higher potential energies but are suitable for cyclization, must increase as the temperature is elevated. However, elevating the reaction temperature by carrying out the reaction in refluxing 1,2-dichloroethane (bp = 83 °C) reduced the yield to 38%.

On the basis of the above-mentioned results of **11c**, we postulated that the cyclization of **11a** and **11b** must proceed similarly when they were carried out in a 10 mM solution in dichloromethane at its refluxing temperature. Indeed, we applied these conditions to **11a** and **11b** and obtained the cyclized products **1a** and **1b** in 45% and 41% yields, respectively. These yields seem to be high enough for such large-ring formation.

Synthesis of Dithymidine Phosphorothioate Derivatives and Their Stereoselectivity. Condensation of the cyclic phosphoramidites **1a**−**c** and 3′-TBDMS-protected thymidine was conducted in the presence of one of the activators listed in Table 2, as shown in Scheme 5. The notation of R_p and S_p in Table 2 refers to one of the diastreomers of **13a**−**c** that gives R_p-**14** and S_p-**14**, respectively, after the deprotection. Such notation was employed because it is convenient to compare the data in Table 2 and the reaction mechanisms although in the case of **13c** the notation is not in agreement with the IUPAC rule.

Among the activators listed in Table 2, 1*H*-tetrazole,[12] 5-ethylthio-1*H*-tetrazole,[13] 5-phenyl-1*H*-tetrazole,[14] 5-(4-nitrophenyl)-1*H*-tetrazole,[14] 4, 5-dicyanoimidazole,[16] 2-(2,4,6-trimethylphenyl)-4, 5-dicyanoimidazole,[18] imidazolium triflate,[19] benzimidazolium triflate,[20] 3-nitrotriazole,[21] and 1-hydroxybenzotriazole[8a] were previously reported as activators in the phosphoramidite method, and

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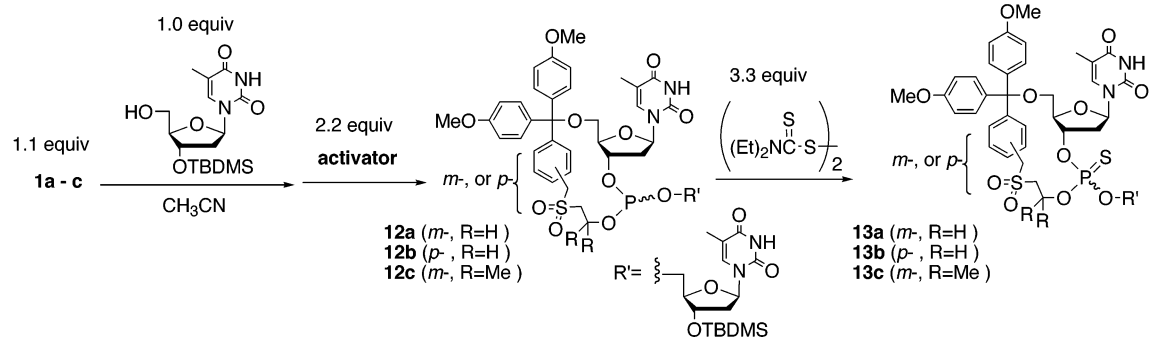
(13) (a) Sproat, B.; Colonna, F.; Mullah, B.; Tsou, D.; Andrus, A.; Hampel, A.; Vinayak, R. *Nucleosides Nucleotides* **1995**, *14*, 255−273. (b) Wincott, F.; DiRenzo, A.; Schaffer, C.; Grimm, S.; Tracz, D.; Workman, C.; Sweedler, D.; Gonzalez, C.; Scaringe, S.; Usman, N. *Nucleic Acids Res.* **1995**, *23*, 2677−2684

(14) Froehler, B.; Matteeucci, M. D. *Tetrahedron Lett.* **1983**, *24*, 3171−3174.

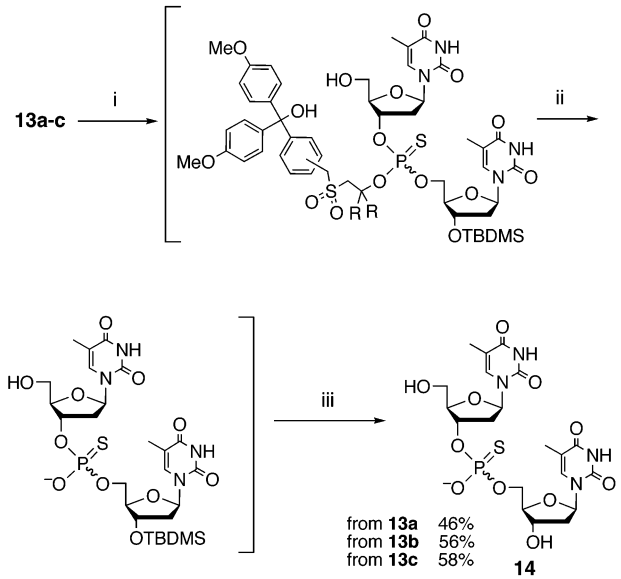
SCHEME 5



the others such as 5-(1-naphthyl)-1*H*-tetrazole,[15] 5-(9-phenanthryl)-1*H*-tetrazole, 2-phenyl-4,5-dicyanoimidazole,[17] 1-hydroxy-6-trifluoromethylbenzotriazole,[22] and 4-nitro-1-hydroxy-6-trifluoromethylbenzotriazole[23] were chosen in this study in consideration of their acidity, steric bulk, and nucleophilicity. The reaction mixtures were analyzed by ^{31}P NMR spectroscopy after the sulfurization of the resulting intermediates **12a**−**c** with *N*,*N*,*N*′,*N*′-tetramethylthiuram disulfide,[24] as shown in Scheme 5. The resonance signals of the diastereomers of **13a**−**c** were integrated, and the ratios were used to determine the stereoselectivity. The results are listed in Table 2. The absolute configuration was unambiguously determined by enzymatic digestion[25] after the fully protected dimers **13a** (entry 1), **13b** (entry 5), and **13c** (entry 15) were converted to thymidyl(5′-3′)thymidine phosphorothioate **14**, as shown in Scheme 6.

In all cases, one of the diastereoisomers of **14**, which has a longer retention time in the reversed-phase HPLC chart (21.1 min, slow isomer), was digested by nuclease P1 but was tolerant to snake venom phosphodiesterase. The other having a shorter retention time (19.7 min, fast isomer) had the opposite sensitivity. These enzymatic properties clearly showed that the fast isomer is the R_p isomer of **14** and the slow isomer is the S_p species. It should be noted that in all cases the diastereomer ratios determined by ^{31}P NMR spectra of **13a**−**c** in the reaction mixtures were identical with those determined by HPLC profiles after the deprotection. Therefore, we could

SCHEME 6[a]



[a] (i) 80% AcOH, rt, 3 h; (ii) concd NH_3−EtOH (2:1), v/v), rt, 1 h; (iii) TFA−H_2O (1:1), v/v), rt, 1 h.

confirm that one of the diastereoisomers of **13** showing a ^{31}P NMR signal in higher magnetic field is the S_p isomer. The cells in the dotted boxes in Table 2 show the combination of phosphoramidites (**1a**−**c**) and the activating agents which gave significant stereoselectivity (more than 60:40). The data of Table 2 can be summarized as follows. (1) Phosphoramidite **1a** gave the R_p isomer in a significant stereoselective manner independent of the activating agents tested in this study. (2) Phosphoramidite **1b** also gave the R_p isomer preferentially in many cases but gave the S_p isomer in entries 7 and 9. Compound **1b** did not show stereoselectivity when activated by some of the imidazole and triazole derivatives (entries 8, 10, 11, and 12). (3) Phosphoroamidite **1c** showed little selectivity when activated by most of the tetrazole, imidazole, and triazole derivatives (entries 1−12 except for entry 7). Interestingly, it gave the S_p isomer as a major product when 1-hydroxybenzotriazole, 1-hydroxy-6-trifluoromethylbenzotriazole, and 4-nitro-1-hydroxy-6-trifluoromethylbenzotriazole (entries 13−15) were used as the activators.

A conventional 5′-*O*-DMTr-thymidine 3′-(cyanoethyl *N*,*N*-diisopropylphosphoramidite) showed little selectivity, R_p:S_p = 46:52−58:42, except when activated by 5-(1-naphthyl)-1*H*-tetrazaole, R_p:S_p = 60:40 (data not shown).

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(19) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395−12401.
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(21) Zhang, Z.; Tang, J. Y. *Tetrahedron Lett.* **1996**, *37*, 331−334.
(22) (a) Roelen, H. C. P. F.; de Vroom, E.; Wang, A. H. J.; van der Marel, G. A.; van Boom, J. H. *Nucleosides Nucleotides* **1992**, *11*, 141−156. (b) Roelen, H. C. P. F.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1988**, *16*, 7633−7645. (c) de Vroom, E.; Marugg, J. E.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1986**, *14*, 5885−5900.
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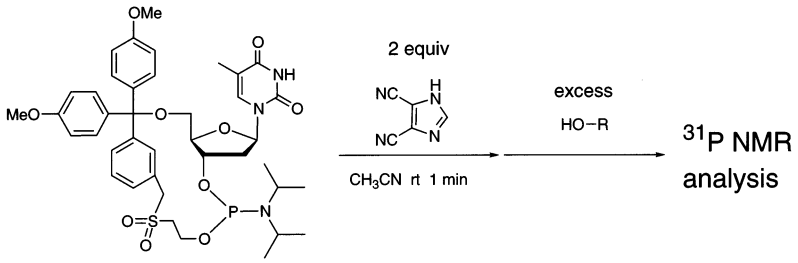
(25) (a) Bryant, F. R.; Benkovic, S. J. *Biochemistry* **1979**, *18*, 2825−2828. (b) Potter, B. V. L.; Connonly, B. A.; Eckstein, F. *Ibid.* **1983**, *22*, 1369−1377.

SCHEME 7



These results support our concept that reduction of the conformational freedom can increase the stereoselectivity in the internucleotidic bond formation. The combinations such as entries 3 and 5 of phosphoramidite **1a** or entries 3, 4, and 5 of phosphoramidite **1b** seem to be a good choice in terms of the coupling yield and the stereoselectivity of more than 80:20, and the combination of phosphoramidite **1a** and benzimidazole triflate (entry 11) gave both higher stereoselectity (79:21) and similar coupling yield (<99%) compared with the conventional phosphoramidite unit.

In contrast, 5-(9-phenanthryl)-1*H*-tetrazole (enty 6) gave good stereoselectivity for phosphoramidite **1a**, but the coupling yield was relatively low because of its low solubility in organic solvents.

Steric Factors and the Acidity of the Activators. Because the observed R_p/S_p ratios depend on the structures of both the activators and the phosophoramidites, we cannot explain the selectivity by simply considering the structure of either the phosphoramidites or the activators. Many properties such as the conformation and reactivity of the reaction intermediates or the transition states must affect the stereoselectivity. However, in some cases, our data indicate some important factors that might determine, at least to some extent, the observed R_p/S_p ratios.

For example, when we compare the results of phosphoramidite **1a** activated by the tetrazole-type activators (entries 1,2, 3, 5, and 6) having similar pK_a values,[26] the order of the selectivity is in good correlation with the bulk of the substituent at position 5 of the tetrazole ring, phenanthryl (pK_a 4.4) > naphthyl (pK_a 4.4) > phenyl (pK_a 4.5) > ethylthio (pK_a 3.9) > H (pK_a 4.96). Therefore, in these combinations, the steric hindrance of the activators plays an essential role.

Moreover, the results of the tetrazole derivatives indicate the importance of the acidity of the activators. 5-Phenyl-1*H*-tetrazole and 5-(4-nitrophenyl)-1*H*-tetrazole, (entries 3 and 4, respectively) are considered to give phosphorazolidite intermediates[27] that have similar steric hindrance around the phosphorus center. However, the addition of the nitro group to 5-(4-nitrophenyl)-1*H*-tetrazole resulted in decrease of the R_p/S_p ratio significantly from 81:19 to 77:23 in the case of the phosphoramidite **1a**. We also observed a similar effect of the nitro group when **1a** was activated by 4-nitrobenzimidazole (R_p:S_p = 72:18, data not shown in Table 2) in place of benzimidazole (R_p:S_p = 79:21). These effects of the nitro group might be the consequence of the increased acidity of the 1*H*-tetrazole and benzimidazole derivatives that might weaken the P−N bond of the intermediates and lengthen the distance between the phosphorus and the azole ring. The fact that the effect due to such a nitro group was not seen in the case of the phosphoramidite derivatives **1b** and **1c** indicates a difference in structure between the phosphorazolidite intermediates derived from the phosphoramidites. A 1-hydroxybenzotriazole series (entries 13, 14, and 15), which give phosphite intermediates where the distance between the phosphorus atom and the azole ring is intrinsically long because of the inserted oxygen atom, no longer shows a similar trend with the increased acidity for any of these three phosphoramidite units.

TABLE 3. Stereoselectivity in the Alcoholysis of 1a Catalyzed by 4,5-Dicyanoimidazole

R	observed peaks (δ P in CD_3CN)	ratio[a]
H	8.2, 7.8	44:56
Me	141.8, 140.6	60:40
Et	141.2, 139.3	64:36
i-Pr	141.3, 138.9	70:30
t-Bu	139.3, 135.7	70:30

[a] Determined by ^{31}P NMR spectroscopy.

Effects of the Structure of Alcoholic Nucleophiles. In the experiments described above, we used the 3′-*O*-TBDMS protected thymidine derivative as an alcoholic nucleophile. However, the structure of the nucleophile might be an important factor that determines the R_p/S_p ratio. Therefore, the condensation of phosphoramidite **1a** with several simple alcohols was carried out by use of 4,5-dicyanoimidazole as an activator (Scheme 7), and the diastereomeric ratios were determined by ^{31}P NMR spectroscopy. Interestingly, the stereoselectivity was enhanced as the length of the alkyl chains attached to the oxygen atom are increased from methyl to isopropyl. The effect of the chain length, however, seemed to be saturated at the length of isopropyl, and the use of the *tert*-butyl group no longer enhanced the stereoselectivity.

On the assumption that the diastereoisomers of phosphite triesters showing their ^{31}P NMR chemical shifts in lower magnetic field are the R_p isomers as in the case of the fully protected dimer **13a**, the major products in all cases are R_p. The highest stereoselectivity was obtained when *i*-PrOH and *t*-BuOH were used as nucleophiles (Table 3). The stereoselectivity (70:30) shown in the case of *i*-PrOH and *t*-BuOH was almost identical to that of **13a** obtained by the combination of the phosphoramidite **1a** and 4,5-dicyanoimidazole (entry 7 in Table 2, 69:31).

In contrast, the smallest ROH nucleophile, H_2O, gave the *H*-phosphonate derivative as an almost 1:1 diaster-

(26) All of the pK_a values were calculated using Soralis v 4.67 software (Advanced Chemistry Development Inc.).

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eomeric mixture. From these data it is suggested that the steric interaction between the alcohol nucleophiles and the substituents around the phosphorus center of **1a** might be the most important factor that determines the stereoselectivity in this reaction system. If the size of a water molecule is small enough to avoid any steric interactions, it can attack equally both the R_p and the S_p diastereoisomers of the phosphorazolidite intermediate. If so, the disappearance of the stereoselectivity in the case of water might be explained by the observed 1:1 diastereomeric ratio of the phosphorazolidite intermediate. It should be noted, however, to confirm our discussion here, the absolute configuration must be determined unambiguously. An idea to determine the stereochemistry after converting the phosphite derivatives to corresponding phosphorothioates by the above-mentioned enzymatic analysis could not be used because snake venom phosphodiesterase does not cleave these alkyl nucleoside 3′-phosphate derivatives. The unambiguous stereochemistry can be determined by using X-ray crystallography, but the analysis is still under way.

From these data, we can hypothesize the mechanism of the stereoselective condensation of **1a** as follows. The cyclic phosphoramidite is activated by an acidic azole to give the phosphorazolidite intermediate which is in nonstereoselective equilibrium with the diastereomer having the opposite configuration at the phosphorus center by rapid exchange of the azole substituent via substitution with another azole molecule.[28] The stereoselectivity appears in the subsequent displacement of the azolyl group by an alcoholic nucleophile in which one of the diastereoisomers is consumed faster than the other probably because the latter is protected from the nucleophilic attack of an alcoholic nucleophile by the steric hindrance arising from the interaction between the 2′-deoxyribose moiety of the phosphoramidite itself and the substituents on the phosphorus atom. Such steric interaction must work more effectively than usual when the conformation around the phosphorus center is restricted by a cyclic structure like our systems, but is less effective when the nucleophile is sterically very small like a water molecule. The reason the different activators gave different stereoselectivities might be explained by the conformational difference of the macrocyclic ring structure in each phosphorazolidite intermediate. Indeed, our preliminary molecular mechanics calculation revealed considerable flexibility of the ring conformation of **1a**. A number of different conformations of the phosphorazolidite compounds could be formed depending on the structure of the activators. The flexibility could be a factor that reduces the stereoselectivity in our system by diminishing the effective steric interaction between the alcoholic nucleophile and the cyclic phosphoramidite. So, although more precise molecular simulation must be needed, we can predict qualitatively that higher stereoselectivity than that observed in this study can be achieved by designing more rigid cyclic structures.

Conclusions

In this paper we have described our attempt to achieve stereoselective internucleotidic bond formation by using the new cyclic phosphoramidite derivatives **1a**, **1b**, and **1c**. The most salient feature of our system is the absence of any other chirality source than the intrinsic chirality of the 2′-deoxyribose moiety of the phosphoramidites themselves. Among the new phosphoramidites, **1a** is the most promising for further development, because it showed the widest spectrum of activators that gave significant stereoselectivity maintaining high coupling efficiency. The stereoselectivity might be due to the different reactivity of alcoholic nucleophiles to each diastereoisomer of the phosphorazolidite intermediates because of the steric interaction between them. As far as the cyclic phosphoramidites reported in this study are used, we were able to obtain at most marginal selectivity of R_p:S_p = 86:14. This selectivity is very high for the phosphoramidite method without an additional chirality source other than the 2′-deoxyribose moiety, but it is not enough for the stereoselective oligodeoxynucleotide phosphorothioate synthesis. The difficulty to form a large-membered ring in **1a**−**c** is another problem shown in this paper. Further studies in the design and synthesis of new cyclic phosphoramidites having a more rigid cyclic structure are under way to improve both the stereoselectivity and the cyclization yield.

Experimental Section

General Methods. CH_2Cl_2 and MeCN were distilled from CaH_2 after being refluxed for several hours and stored over molecular sieves 4 Å. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from CaH_2, and stored over molecular sieves 4 Å. ^{1}H NMR spectra were obtained at 270 and 400 MHz with tetramethylsilane (TMS) as an internal standard in $CDCl_3$ and with sodium 3-(trimethylsilyl)propanesulfonate (DSS) as an external standard in D_2O. ^{13}C NMR spectra were obtained at 67.8 MHz with TMS as an internal standard and with DSS as an external standard in D_2O. ^{31}P NMR spectra were obtained at 109.25 MHz using 85% H_3PO_4 as an external standard. ESI-TOF mass spectra were obtained in the positive ion mode. The calibration was performed with reserpine and dioctyl phthalate. HPLC analysis was performed with a μBondapak column (Waters, C18−100 Å) using a linear gradient of 0−30% acetonitrile in 0.1 M NH_4OAc (pH 7.0) for 30 min at a flow rate of 1.0 mL/min at 50 °C.

4,4′-Dimethoxy-3″-methyltrityl Alcohol (3a). Magnesium (9.74 g, 401 mmol) dried over P_2O_5 in vacuo was placed in a 1000 mL three-necked reaction flask. A THF (200 mL) solution of 4-bromoanisole (75.6 g, 401 mmol) was added over the period of 30 min, and the reaction was completed by stirring the resulting mixture for 1 h at room temperature. To this solution was added dropwise a THF solution (200 mL) of methyl *m*-toluate (27.4 g, 182 mmol) over the period of 30 min at 0 °C. After being kept for 1 h at room temperature, the mixture was poured to 10% ammonium chloride (w/v, 500 mL). The product was extracted three times by ethyl acetate (500 mL). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (9:1, v/v). The fractions containing the desired product were concentrated under reduced pressure. Trituation of the residue with isopropyl ether and hexane gave **3a**. The further purification by recrystallization from isopropyl ether and hexane gave **3a** (49.3 g, 81%): mp 72−76 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.31 (3H, s), 2.71 (1H, s,), 3.80 (6H, s), 6.81−7.18 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.7, 55.3, 81.4, 113.1, 124.9, 127.6, 127.7, 128.2, 129.0, 137.4, 139.4, 147.1, 158.4. Anal. Calcd for $C_{22}H_{22}O_3$: C, 79.02; H, 6.63. Found: C, 79.16; H; 6.70.

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4,4′-Dimethoxy-4″-methyltrityl Alcohol (3b). A procedure similar to that described in the case of **3a** using methyl *p*-toluate in place of methyl *m*-toluate gave **3b** in 92% yield: mp 67−70 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.32 (3H, s), 2.74 (1H, s), 3.77 (6H, s), 6.78−7.83, 7.07−7.18 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.1, 55.2, 81.2, 113.0, 127.5, 128.4, 129.0, 136.5, 139.5, 144.4, 158.4. Anal. Calcd for $C_{22}H_{22}O_3 \cdot 0.3H_2O$: C, 77.76; H, 6.70. Found: C, 77.74; H; 6.51.

4,4′-Dimethoxy-3″-methyltrityl 1-Methylethyl Ether (4a). Acetyl chloride (23.0 mL, 324 mmol) was added to **3a** (40.5 g, 108 mmol), and the resulting mixture was heated under reflux for 1 h. The resulting solution was added dropwise to a mixure of pyridine-2-propanol (1:1, v/v) 200 mL at 0 °C. After the removal of 2-propanol under reduced pressure, water (150 mL) was added to the residue, and the desired compound was extracted three times with ethyl acetate (250 mL). The organic layer was dried over sodium sulfate. The solvents were removed under reduced pressure, and the residue was chromatographed on a silica gel with hexane−ethyl acetate−pyridine (100:1:0.5, v/v/v) gave **4a** (43.2 g, 95%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.84 (6H, d, J = 5.9 Hz), 2.30 (3H, s), 3.75 (1H, m), 3.77 (6H, s), 6.78−7.42 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.8, 24.1, 55.2, 66.1, 85.9, 112.8, 125.5, 127.1, 127.3, 128.9, 130.2, 136.9, 137.7,146.3, 158.1. Anal. Calcd for $C_{25}H_{28}O_3 \cdot 0.5H_2O$: C, 77.89; H, 7.58. Found: C, 77.82; H; 7.45.

4,4′-Dimethoxy-4″-methyltrityl 1-Methylethyl Ether (4b). Compound **4b** was obtained from **3b** in 92% yield according to a procedure similar to that described in the case of **4a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.83 (6H, d, J = 5.9 Hz), 2.30 (3H, s), 3.72 (1H, m), 3.77 (6H, s), 6.77−6.83, 7.05−7.08, 7.36−7.42 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.1, 24.2, 55.2, 66.1, 85.8, 112.8, 128.2, 128.4, 130.1, 136.1, 137.9, 143.2, 158.1. Anal. Calcd for $C_{25}H_{28}O_3 \cdot 0.2H_2O$: C, 79.00; H, 7.53. Found: C, 79.08; H; 7.14.

3-[(2-Hydroxyethyl)thiomethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (6a). To a solution of **4a** (27.2 g, 72.3 mmol) in carbon tetrachloride (150 mL) were added *N*-bromosuccinimide (15.4 g, 86.8 mmol) and a catalytic amount of *N*,*N*′-azobisisobutylonitrile (1.4 g, 8.68 mmol), and the resulting mixture was heated under reflux for 20 min. After being cooled to room temperature, the solution was washed three times with 10% sodium thiosulfate (150 mL) and three times with saturated sodium bicarbonate (150 mL). The organic layer was collected, dried over sodium sulfate, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (50:1, v/v) to obtain an oily crude product that contains both the starting material **4a** and 3-[bis(4-methoxyphenyl)isopropoxymethyl]benzyl bromide. The yield of the later bromide was estimated to be 77% from the ^{1}H NMR spectrum of the crude product. The oily material was redissolved in *N*,*N*-dimethylformamide (100 mL), and *N*-ethyldiisopropylamine (19.0 mL, 110 mmol) and 2-mercaptoethanol (7.10 mL, 100 mmol) were added to the mixture. After the mixture was stirred for 3 h, ethyl acetate (500 mL) was added. The solution was washed three times with brine. The organic layer was collected and dried over sodium sulfate. The solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (5:1, v/v) containing 0.5% pyridine to give **6a** (21.5 g, 66% yield from **4a**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.85 (6H, d, J = 5.9 Hz), 2.08 (1H, t, J = 5.9 Hz), 2.53 (2H, t, J = 5.9 Hz), 3.55 (2H, m), 3.65 (2H, s), 3.73 (1H, m), 3.77 (6H, s), 6.79−7.42 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 24.1, 34.2, 35.8, 55.2, 60.1, 66.2, 85.8, 112.8, 126.8, 127.0, 127.8, 128.8, 130.2, 137.1, 137.3,146.9, 158.2. Anal. Calcd for $C_{27}H_{32}O_4S \cdot 0.5H_2O$: C, 70.25; H, 7.21; S, 6.94. Found: C, 69.99; H; 7.15; S; 6.63.

4-[(2-Hydroxyethyl)thiomethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (6b). Compound **6b** (24 g, 59%) was obtained from **4b** (33 g, 90 mmol) according to a procedure similar to that described in the case of **6a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89 (6H, d, J = 5.9 Hz), 2.61 (2H, m), 2.84 (1H, br), 3.65 (2H, m), 3.67 (2H, s), 3.72 (6H, s), 3.77 (1H, m), 6.81−6.84, 7.22−7.25, 7.42−7.53 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 24.1, 34.2, 35.8, 55.2, 60.1, 66.2, 85.8, 112.8, 126.8, 127.0, 127.8, 128.8, 130.2, 137.1, 137.3,146.9, 158.2. Anal. Calcd for $C_{27}H_{32}O_4S \cdot 0.5H_2O$: C, 70.25; H, 7.21; S, 6.94. Found: C, 70.35; H; 6.88; S; 6.59.

3-[(2-Hydroxy-2-methylpropyl)thiomethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (6c). Compound **6c** (32 g, 52%) was obtained from **4c** (49 g, 13 mmol) according to a procedure similar to that described in the case of **6a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86 (6H, d, J = 5.9 Hz), 1.21 (6H, s), 2.11 (2H, s), 3.72 (2H, s), 3.74 (1H, m), 3.80 (6H, s), 6.80−7.44 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 24.2, 28.7, 38.3, 45.3, 55.2, 66.2, 70.5, 85.8, 112.8, 127.0, 127.1, 127.8, 128.9, 129.0, 130.2, 137.4, 146.8, 158.2. Anal. Calcd for $C_{25}H_{28}O_3 \cdot 0.4H_2O$: C, 71.40; H, 7.60; S,6.57. Found: C, 71.21; H; 7.47; S, 6.54.

3-[(2-Hydroxyethyl)sulfonylmethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (7a). *m*-Chloroperbenzoic acid (22.7 g, 131 mmol) was dissolved in dichloromethane (300 mL). Sodium bicarbonate (11.4 g, 131 mmol) was added, and the reaction mixture was cooled to 0 °C. To this mixture was added dropwise **6a** (27.0 g, 59.7 mmol) in dichloromethane (300 mL) over a period of 15 min. Chloroform (200 mL) was added, and the solution was washed three times with saturated sodium biocarbonate (500 mL). The organic layer was collected and dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (3:2, v/v) containing 0.5% pyridine to give **7a** (23.7 g, 82%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.85 (6H, d, J = 5.9 Hz), 2.65 (1H, m), 2.93 (2H, m), 3.73 (1H, m), 3.76 (6H, s), 3.92 (2H, q, J = 5.0 Hz), 4.25 (2H, s), 6.79−7.42 (12H, m,); ^{13}C NMR (68 MHz, $CDCl_3$) δ 24.1, 52.8, 55.2, 56.2, 61.2, 66.3, 85.7, 112.9, 127.0, 128.2, 128.6, 128.8, 130.2, 130.8, 136.9, 147.8, 158.3. Anal. Calcd for $C_{27}H_{32}O_6S$: C, 66.92; H, 6.66; S, 6.62. Found: C, 66.86; H, 6.61; S, 6.67.

4-[(2-Hydroxyethyl)sulfonylmethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (7b). Compound **7b** (15.2 g, 62%) was obtained from **6a** (23 g, 51 mmol) according to a procedure similar procedure to that described in the case of **7a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.84 (6H, d, J = 6.2 Hz), 2.42 (1H, m), 3.01 (2H, m), 3.72 (1H, m), 3.80 (6H, s), 4.08 (2H, m), 4.29 (2H, s), 6.79−6.85, 7.26−7.39, 7.55−7.58 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 24.1, 53.0, 55.1, 56.3, 60.6, 66.2, 85.7, 112.9, 125.8, 128.5, 130.4, 130.3, 136.8, 147.9, 158.3. Anal. Calcd for $C_{27}H_{32}O_6S$: C, 66.92; H, 6.66; S, 6.62. Found: C, 67.06; H, 6.47; S, 6.44.

3-[(2-Hydroxy-2-methylpropyl)sulfonylmethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (7c). Compound **7c** (26 g, 74%) was obtained from **6c** (29 g, 60 mmol) according to a procedure similar to that described in the case of **7a**: mp 98−99 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.84 (6H, d, J = 5.9 Hz), 1.34 (6H, s), 2.92 (2H, s), 3.65−3.78 (7H, m), 4.20 (2H, s), 6.78−7.59 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 23.9, 26.7, 54.9, 60.1, 62.1, 66.1, 69.3, 85.4, 112.7, 127.0, 128.0, 128.4, 128.6, 130.0, 130.4, 136.7, 147.5, 158.1. Anal. Calcd for $C_{29}H_{36}O_4S \cdot 0.4H_2O$: C, 71.40; H, 7.60; S, 6.57. Found: C, 71.21; H, 7.47; S, 6.54.

3-[[2-(*tert*-Butyldimethylsilyloxy)ethyl]sulfonylmethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (8a). To a solution of **7a** (19.0 g, 39.2 mmol) in *N*,*N*-dimethylformamide (100 mL) were added imidazole (2.94 g, 43.2 mmol) and chloro-*tert*-butyldimethylsilane (6.50 g, 43.2 mmol), and the resulting solution was stirred at room temperature for 1 h. Ethyl acetate (300 mL) was added, and the solution was washed three times with brine (300 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column using hexane−ethyl acetate (5:1, v/v) containing 0.5% pyrdine to give **8a** (22.8 g, 97%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.09 (6H, s), 0.85 (6H, d, J = 5.9 Hz), 0.90 (9H, s), 2.98 (2H, t, J = 5.3 Hz), 3.72 (1H, m), 3.78

(6H, s), 4.04 (2H, t, J = 5.3 Hz), 4.29 (2H, s), 6.77−7.60 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.5, 18.4, 24.1, 26.0, 53.1, 55.2, 57.8, 61.6, 66.3, 85.7, 112.9, 127.3, 128.1, 128.5, 129.1, 130.2, 131.1, 137.2, 147.5, 158.3. Anal. Calcd for $C_{33}H_{46}O_6SSi\cdot 0.5H_2O$: C, 65.20; H, 7.73; S, 5.28. Found: C, 65.20; H, 7.73; S, 5.35.

4-[[2-(*tert*-Butyldimethylsilyloxy)ethyl]sulfonylmethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (8b). Compound **8b** (10.7 g, 97%) was obtained from **7b** (9.0 g, 19 mmol) according to a procedure similar to that described in the case of **8a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.13 (6H, s), 0.84 (6H, d, J = 5.9 Hz), 0.92 (9H, s), 3.07 (2H, m), 3.74 (1H, m), 3.79 (6H, s), 4.08 (2H, m), 4.30 (2H, s), 6.79−6.83, 7.32−7.40, 7.53−7.56 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.5, 18.2, 24.1, 25.8, 53.4, 54.9, 57.6, 60.7, 66.0, 85.5, 112.7, 125.9, 128.3, 130.0, 130.1, 136.7, 147.5, 158.1. Anal. Calcd for $C_{33}H_{46}O_6SSi\cdot 0.4H_2O$: C, 65.40; H, 7.78; S, 5.30. Found: C, 65.33; H, 7.39; S, 5.30.

3-[[2-(*tert*-Butyldimethylsilyloxy)-2-methylpropyl]sulfonylmethyl]-4′,4″-dimethoxytrityl 1-Methylethyl Ether (8c). To a solution of **7c** (20.0 g, 39.0 mmol) in dichloromethane (150 mL) were added 2,6-lutidine (16.7 g, 156 mmol) and *tert*-butyldimethylsilyl triflate (20.6 g, 78 mmol). After being stirred for 2 h, the solution was washed three times with brine, dried over sodium sulfate, and evaporated to dryness under reduced pressure. The residue was chromatograhed on silica gel column using hexane−ethyl acetate (5:1, v/v) containing 0.5% pyridine. The appropriate fractions were concentrated under reduced pressure, and the resulting solid was recrystallized from ethyl acetate to give **8c** (21.9 g, 90%): mp 100−104 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.12 (6H, s), 0.84 (6H, d, J = 5.9 Hz), 0.86 (9H, s), 1.46 (6H, s), 2.98 (2H, s), 3.72 (1H, m), 3.78 (6H, s), 4.26 (2H, s,), 6.79−7.56 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.5, 18.2, 24.1, 25.8, 53.4, 54.9, 57.6, 60.7, 66.0, 85.5, 112.7, 125.9, 128.3, 130.0, 130.1, 136.7, 147.5, 158.1. Anal. Calcd for $C_{33}H_{46}O_6SSi\cdot 0.4H_2O$: C, 65.40; H, 7.78; S, 5.30. Found: C, 65.33; H, 7.39; S, 5.30.

3-[[2-(*tert*-Butyldimethylsilyloxy)ethyl]sulfonylmethyl]-4′,4″-dimethoxytrityl Alcohol (9a). Compound **8a** (23.1 g, 38.6 mmol) was treated with trifluoroacetic acid (1% in dichloromethane, v/v) at room temperature for 10 min. The mixture was poured into pyridine−water (1:1, v/v, 1000 mL), and the separated organic layer was collected. The aqueous layer was extracted twice with chloroform (300 mL). The organic layer was combined, dried over sodium sulfate, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (5:1, v/v) containing 0.5% pyridine to give **7a** (19.0 g, 88%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.09 (6H, s), 0.89 (9H, s), 3.02 (2H, t, J = 5.3 Hz), 3.79 (6H, s), 4.04 (2H, t, J = 5.3 Hz), 4.30 (2H, s), 6.81−6.84, 7.16−7.41 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.6, 18.2, 25.8, 53.0, 55.0, 57.5, 61.1, 80.8, 112.9, 127.9, 128.8, 129.3, 130.5, 138.9, 148.0, 158.2. Anal. Calcd for $C_{30}H_{40}O_6SSi\cdot 0.3H_2O$: C, 64.09; H, 7.28; S, 5.70. Found: C, 64.07; H, 7.52; S, 5.51.

4-[[2-(*tert*-Butyldimethylsilyloxy)ethyl]sulfonylmethyl]-4′,4″-dimethoxytrityl Alcohol (9b). Compound **9b** (7.6 g, 86%) was synthesized from **8b** (9.5 g, 16 mmol) according to a procedure similar to that described in the case of **9a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.14 (6H, s), 0.94 (9H, s), 3.07 (2H, t, J = 5.4 Hz), 3.79 (6H, s), 4.09 (2H, m), 4.32 (2H, s), 6.79−7.39 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.6, 18.2, 25.8, 53.3, 54.9, 57.6, 60.6, 80.8, 112.8, 126.3, 127.9, 128.8, 130.2, 138.9, 147.9, 158.1. Anal. Calcd for $C_{30}H_{40}O_6SSi\cdot 0.4H_2O$: C, 63.89; H, 7.29; S, 5.69. Found: C, 63.81; H, 6.93; S, 5.51.

3-[[2-(*tert*-Butyldimethylsilyloxy)-2-methylpropyl]sulfonylmethyl]-4′,4″-dimethoxytrityl Alcohol (9c). 8c (15.0 g, 23.9 mmol) was dissolved in 80% acetic acid, and the solution was stirred at room temperature for 1 h. Ethyl acetate (500 mL) was added, and the excess acetic acid was removed by extraction three times with water (500 mL) and then three times with saturated sodium bicarbonate (500 mL). The solvent was removed by evaporation under reduced pressure, and the residue was treated with ethyl acetate to give **9c** (13.5 g, 96%) as crystals: mp 132−135 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.12 (6H, s), 0.85 (9H, s), 1.44 (6H, s) 2.97 (2H, s), 3.78 (6H, s), 4.25 (2H, s), 6.80−7.36 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −1.9, 18.1, 26.0, 30.2, 55.2, 61.6, 63.0, 72.3, 81.1, 113.2, 127.7, 128.2, 128.3, 129.0, 129.6, 130.2, 139.0, 148.0, 158.5. Anal. Calcd for $C_{32}H_{44}O_6SSi\cdot 0.2H_2O$: C, 65.32; H, 7.61; S, 5.45. Found: C, 65.15; H, 7.50; S, 5.43.

3-[[2-(*tert*-Butyldimethylsilyloxy)-2-methylpropyl]sulfonylmethyl]-4′,4″-dimethoxytrityl Chloride (2c). To a solution of **9c** (12.0 g, 20.6 mmol) in toluene (100 mL) was added acetyl chloriride (4.83 g, 61.6 mmol). The resulting solution was stirred under reflux for 1 h. After cooling to room temperature, hexane was added to the mixture. The precipitation was collected by filtration to give **2c** (12.1 g, 98%): mp 144−145 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.12 (6H, s), 0.85 (9H, s), 1.44 (6H, s), 2.97 (2H, s), 3.78 (6H, s), 4.25 (2H, s), 6.80−7.36 (12H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −1.9, 18.1, 26.0, 30.2, 55.2, 61.6, 63.0, 72.3, 81.1, 113.2, 127.7, 128.2, 128.3, 129.0, 129.6, 130.2, 139.0, 148.0, 158.5. Anal. Calcd for $C_{32}H_{43}ClO_5SSi$: C, 63.71; H, 7.18; Cl, 5.88; S, 5.32. Found: C, 63.94; H, 6.81; Cl, 5.86; S, 5.57.

5′-O-[3-[2-(*tert*-Butyldimethylsilyloxy)ethyl]sulfonylmethyl-4′,4″-dimethoxytrityl]thymidine (10a). To a solution of **9a** (976 mg, 1.74 mmol) in toluene (20 mL) was added acetyl chloride (0.38 mL, 5.2 mmol), and the resulting solution was heated under reflux for 1 h. The solvent was removed completely under reduced pressure and by the successive coevaporation three times with anhydrous pyridine. The residue was dissolved in pyridine (100 mL), and to this solution was added thymidine (378 mg, 1.43 mmol). After being stirred for 3 h, the mixture was diluted with ethyl acetate (100 mL) and then washed three times with saturated sodium bicarbonate (100 mL). The organic layer was collected, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (3:1, v/v) containing 0.5% pyridine gave **10a** (1.06 g, 95%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.12 (6H, d, J = 1.1 Hz), 0.91 (9H, s), 1.60 (3H, d, $J_{5-Me,\ 6}$ = 1.1 Hz), 2.28 (1H, m), 2.41 (1H, m), 2.70 (1H, br), 3.08 (2H, m), 3.34 (1H, m), 3.47 (1H, dd, $J_{5',5''}$ = 10.6, $J_{4',5''}$ = 3.3 Hz), 3.78, 3.80 (6H, 2s), 4.00 (1H, m), 4.08 (1H, t, J = 5.3), 4.33 (2H, m), 4.57 (1H, m), 6.32 (1H, dd, $J_{1',\ 2'}$ = 6.6, $J_{1',\ 2''}$ = 6.6), 6.80−7.57 (13H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.6, 12.0, 18.3, 25.9, 40.6, 53.8, 55.1, 57.7, 61.3, 63.4, 71.7, 84.7, 86.0, 86.4, 110.8, 113.2, 127.3, 128.3, 128.5, 129.5, 129.6, 129.9, 131.2, 134.6, 135.6, 135.7, 144.7, 150.4, 158.4, 158.5, 163.9. Anal. Calcd for $C_{40}H_{52}N_2O_{10}SSi\cdot H_2O$: C, 60.13; H, 6.81; N, 3.51; S, 4.01. Found: C, 60.63; H, 6.71; N, 3.29; S, 4.06.

5′-*O*-[4-[2-(*tert*-Butyldimethylsilyloxy)ethyl]sulfonylmethyl-4′,4″-dimethoxy trityl]thymidine (10b). 10b (7.9 g, 98%) was synthesized from **9b** (6.9 g, 12 mmol) according to a procedure similar to that described in the case of **10a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.14 (6H, s), 0.93 (9H, s), 1.53 (3H, d, $J_{5-Me,\ 6}$ = 1.0 Hz), 2.27−2.47 (2H, m), 3.11 (2H, m), 3.35 (1H, dd, $J_{5',\ 5''}$ = 7.3, $J_{5',\ 4'}$ = 3.0), 3.78 (6H, m), 4.02 (1H, m), 4.11 (2H, m), 4.32 (2H, s), 4.50 (1H, m), 6.38 (1H, m), 6.82−7.52 (13H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −5.7, 11.6, 18.1, 25.7, 40.5, 53.7, 54.9, 57.5, 60.4, 63.4, 71.6, 84.5, 85.9, 86.3, 110.7, 112.9, 126.2, 128.1, 129.6, 129.7, 130.4, 134.5, 134.8, 135.4, 144.9, 150.3, 158.3, 163.9. Anal. Calcd for $C_{40}H_{52}N_2O_{10}SSi$: C, 60.85; H, 6.16; N, 3.59; S, 4.11. Found: C, 60.63; H, 6.71; N, 3.29; S, 3.98.

5′-O-[3-[2-(*tert*-Butyldimethylsilyloxy)-2-methylpropyl]-sulfonylmethyl- 4′,4″-dimethoxytrityl]thymidine (10c). To a solution of **2c** (11.0 g, 18.2 mmol) in pyridine (75 mL) was added thymidine (3.68 g, 15.2 mmol), and the resulting solution was stirred at room temperature for 3 h. The mixture was diluted with chloroform (300 mL) and then washed three times with saturated sodium bicarbonate (300 mL). The organic layer was dried over sodium sulfate, filtered, and

evaporated under reduced pressure. The residue was chromatographed on silica gel column with hexane−ethyl acetate (3:1, v/v) containing 0.5% pyridine to give **10c** (11.7 g, 95%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.14 (6H, d, J = 1.7 Hz), 0.87 (9H, s), 1.49 (6H, s), 1.59 (3H, d, $J_{5-Me,6}$ = 1.0 Hz), 2.21−2.41 (2H, m), 2.80 (1H, br), 3.08 (2H, s), 3.33 (1H, dd, $J_{5',5''}$ = 10.6, $J_{5',4'}$ = 3.6), 3.45 (1H, dd, $J_{5',5''}$ = 10.6, $J_{5'',4'}$ = 3.0), 3.78, 3.79 (6H, 2s), 3.99 (1H, m), 4.30 (2H, m), 4.56 (1H, m, 3′H), 6.32 (1H, m), 6.83−7.56 (13H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −2.2, 11.8, 17.8, 25.7, 29.9, 40.4, 54.9, 61.1, 63.4, 71.5, 72.5, 84.5, 85.9, 86.2, 110.6, 113.0, 124.9, 127.1, 127.8, 128.1, 128.3, 128.6, 129.4, 129.7, 130.8, 134.5, 135.4, 144.6, 150.3, 158.2, 163.9. Anal. Calcd for $C_{42}H_{56}N_2O_{10}SSi\cdot0.5H_2O$: C, 61.66; H, 7.02; N, 3.42; S, 3.92. Found: C, 61.62; H, 6.92; N, 3.49; S, 3.81.

5′-O-[3-(2-Hydroxyethyl)sulfonylmethyl-4′,4″-dimethoxytrityl]thymidine (11a). To a solution of **10a** (5.29 g, 6.77 mmol) in tetrahydrofuran (60 mL) was added tetrabutylammonium fluoride (2.13 g, 8.12 mmol), and the resulting slution was stirred at room temperature for 1 h. The solution was diluted with water (300 mL), and the organic materials were extracted three times with chloroform (300 mL). The extracts were combined, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (2:8, v/v) containing 0.5% pyridine to give **11a** (4.22 g, 94%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.55 (3H, d, $J_{5-Me,6}$ = 1.0 Hz), 2.27−2.47 (2H, m), 3.06 (2H, m), 3.33−3.45 (2H, m), 3.78, 3.79 (6H, 2s), 4.04 (3H, m), 4.60 (1H, m), 4.31 (2H, m), 6.31 (1H, m), 6.81−7.62 (13H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 11.9, 40.6, 53.4, 55.2, 56.1, 61.0, 63.5, 71.9, 84.9, 86.1, 86.5, 110.8, 113.2, 127.5, 128.3, 129.0, 129.6, 130.1, 134.4, 135.4, 135.9, 145.0, 150.6, 158.5, 164.2. Anal. Calcd for $C_{40}H_{52}N_2O_{10}SSi\cdot H_2O$: C, 59.64; H, 5.89; N, 4.09; S, 4.68. Found: C, 59.61; H, 6.07; N, 3.95; S, 4.51.

5′-O-[4-(2-Hydroxyethyl)sulfonylmethyl-4′,4″-dimethoxytrityl]thymidine (11b). Compound **11b** (5.7 g, 94%) was obtained from **10b** (7.1 g, 9.1 mmol) according to a procedure similar to that described in the case of **11a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.49 (3H, s), 2.17−2.43 (2H, m), 3.10 (2H, m), 3.25−3.50 (2H, m), 3.76 (6H, s), 4.03−4.05 (3H, m), 4.31 (2H, s), 4.51 (1H, m), 6.33 (1H, m), 6.80−6.83, 7.23−7.55 (13H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 11.6, 40.6, 53.4, 55.0, 55.7, 60.2, 63.4, 71.5, 84.7, 86.0, 86.3, 110.7, 113.0, 126.2, 128.1, 128.6, 129.6, 129.8, 130.4, 134.4, 134.9, 135.7, 145.0, 150.4, 158.3, 164.2. Anal. Calcd for $C_{40}H_{52}N_2O_{10}SSi\cdot0.3H_2O$: C, 60.76; H, 5.79; N, 4.17; S, 4.77. Found: C, 60.42; H, 5.55; N, 4.10; S, 4.48.

5′-O-[3-(2-Hydroxy-2-methylpropyl)sulfonylmethyl-4′,4″-dimethoxytrityl]thymidine (11c). To a solution of **10c** (11.0 g, 13.6 mmol) in tetrahydrofuran (100 mL) was added tetrabutylammonium fluoride (7.1 g, 27.2 mmol), and the resulting solution was stirred at room temperature for 10 h. The solution was diluted with water (500 mL), and the organic materials were extracted three times with chloroform (300 mL). The extracts were combined, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (2:8, v/v) containing 0.5% pyridine to give **11c** (9.2 g, 97%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.41 (6H, s), 1.56 (3H, s), 2.26−2.45 (2H, m), 3.02 (2H, s), 3.35 (1H, m), 3.44 (1H, m), 3.78, 3.80 (6H, 2s), 4.03 (1H, m), 4.28 (2H, m), 4.60 (1H, m), 6.31 (1H, dd, $J_{1',2'}$ = 6.6 Hz, $J_{1',2''}$ = 6.6 Hz), 6.81−6.87, 7.16−7.58 (13H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 12.0, 29.8, 40.7, 55.2, 61.0, 62.0, 63.5, 69.6, 71.7, 84.8, 86.1, 86.5, 110.8, 113.2, 127.5, 128.4, 128.9, 129.6, 130.1, 130.2, 134.4, 135.5, 135.7, 145.0, 150.5, 158.5, 158.6, 164.0. Anal. Calcd for $C_{42}H_{56}N_2O_{10}S\cdot0.5H_2O$: C, 61.44; H, 6.16; N, 3.84; S, 4.56. Found: C, 61.42; H, 6.23; N, 3.98; S, 4.20.

General Procedure for 1a, 1b, and 1c. Compound **11a, 11b** (162 mg, 0.243 mmol), or **11c** (170 mg, 0.243 mmol) was dissolved in dichloromethane (24.3 mL) and dried for 6 h over molecular sieves 4 Å (1.2 g). To this solution was added *N*-ethyldiiropropylamine (101 μl, 0.583 mmol) and dichloro(*N*,*N*-diisopropylamino)phosphine (49.1 mg, 0.243 mmol) at refluxing temperature. After the reaction was completed (2 h for **11a**, 6 h for **11b** and**11c**), the mixture was diluted with dichloromethane (50 mL). The solution was washed three times with saturated sodium bicarbonate (50 mL), and the organic layer was dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column with hexane−ethyl acetate containing 1% triethylamine to give **1a** (87.0 mg, 45%), **1b** (70 mg, 41%), or **1c** (98 mg, 49%).

Phosphoramidite Unit 1a: ^{31}P NMR (109 MHz, $CDCl_3$) δ 149.8, 143.0; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.08−1.13 (12H, m), 2.32 (0.5H, m), 2.54 (1.5H, m), 2.75 (0.5H, m), 2.98 (0.5H, m), 3.14−3.34 (2H, m), 3.48 (0.5H, m), 3.57−4.21 (12.5H, m), 4.36 (1H, m), 4.50 (0.5H, m), 4.68 (0.5H, m), 5.00−5.07 (1H, m), 6.13 (0.5H, m), 6.36 (0.5H, J = 6.6 Hz), 6.79−7.93 (13H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 11.7, 11.9, 24.4, 24.5, 39.7, 40.5, 43.5, 43.7, 50.1, 50.3, 51.0, 51.1, 55.2, 56.6, 56.9, 57.3, 57.6, 60.4, 60.7, 61.0, 63.7, 70.9, 71.1, 73.9, 83.4, 83.6, 83.9, 84.1, 84.7, 86.2, 86.3, 110.5, 111.0, 113.2, 113.3, 113.5, 127.7, 127.8, 128.6, 128.7, 128.9, 129.4, 129.6, 129.7, 130.7, 131.4, 132.5, 132.9, 134.8, 135.2, 137.1, 138.9, 143.7, 144.7, 150.1, 150.3, 158.3, 158.4, 158.8, 159.0, 163.8, 163.9; MS (ESI) 796 (M + H^+). Anal. Calcd for $C_{40}H_{50}N_3O_{10}PS\cdot H_2O$: C, 59.03; H, 6.44; N, 5.16; S, 3.94. Found: C, 58.74; H, 6.35; N, 5.10; S, 3.68.

Phosphoramidite Unit 1b: ^{31}P NMR (109 MHz, $CDCl_3$) δ 147.3, 153.5; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.21−1.28 (12H, m), 1.38, 1.51 (3H, 2s), 2.15−2.26 (1H, m), 2.34−2.44 (1, m), 2.83−3.86 (16H, m), 4.27−4.44 (3H, m), 6.20 (0.5H, m), 6.36 (0.5H, m), 6.78−6.92, 7.21−7.79 (13H, m, ArH); ^{13}C NMR (68 MHz, $CDCl_3$) δ 12.56, 24.36, 24.41, 24.44, 24.51, 24.57, 24.70, 40.03, 40.06, 40.48, 43.45, 43.60, 43.64, 43.79, 53.58, 53.68, 54.33, 54.44, 55.30, 55.31, 56.93, 57.30, 57.38, 57.67, 61.13, 61.90, 63.62, 70.42, 70.79, 74.71, 74.80, 84.37, 84.45, 84.64, 84.86, 85.43, 86.56, 87.01, 110.96, 111.20, 113.31, 113.37, 113.64, 113.70, 127.81, 127.90, 128.32, 128.46, 128.89, 129.35, 132.12, 133.35, 134.36, 135.20, 135.52, 136.91, 137.61, 142.20, 142.34, 149.78, 149.90, 158.33, 158.44, 158.52, 158.56, 163.28, 163.31; MS (ESI) 796 (M + H^+). Anal. Calcd for $C_{40}H_{50}N_3O_{10}PS\cdot0.5H_2O$: C, 59.67; H, 6.39; N, 5.22; S, 3.98. Found: C, 59.57; H, 6.06; N, 5.39; S, 3.69.

Phosphoramidite Unit 1c: ^{31}P NMR (109 MHz, $CDCl_3$) δ 136.5, 140.9; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.11−1.26 (12H, m), 1.46−1.71 (9H, m), 2.32−2.69 (2.5H, m), 3.14−3.28 (2H, m), 3.45−3.82 (9.5H, m), 4.06−4.18 (2H, m), 4.34−4.39 (0.5H, m), 4.56−4.65 (0.5H, m), 4.80−4.89 (1H, m), 6.11−6.18 (1H, m), 6.75−6.91, 7.23−7.37, 7.54, 7.58, 7.75 (13H), 8.20−8.50 (1H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ 12.06, 12.22, 23.89, 23.96, 24.45, 24.48, 24.57, 24.61, 28.19, 28.31, 28.39, 28.62, 30.12, 30.20, 30.32, 39.39, 40.65, 40.70, 43.48, 43.66, 44.18, 44.37, 55.16, 55.23, 59.41, 59.65, 59.79, 61.15, 61.55, 62.59, 62.76, 69.88, 70.09, 70.88, 70.99, 75.04, 75.22, 75.26, 84.31, 84.41, 84.56, 84.63, 85.94, 86.19, 86.23, 110.53, 110.68, 113.21, 113.29, 113.32, 113.37, 127.89, 128.04, 128.44, 128.72, 128.84, 129.33, 129.44, 129.49, 129.63, 129.87, 129.99, 130.64, 131.01, 133.06, 133.75, 135.00, 136.08, 136.63, 138.52, 143.67, 146.22, 149.99, 150.16, 158.28, 158.39, 158.67, 158.94, 163.95, 164.06; MS (ESI) 824 (M + H^+). Anal. Calcd for $C_{42}H_{54}N_3O_{10}PS\cdot0.2H_2O$: C, 60.56; H, 6.66; N, 5.04; S, 3.84. Found: C, 60.39; H, 6.86; N, 4.94; S, 3.67.

Typical Procedure for Dimer Synthesis and Determination of Diastereomer Ratio Using Phosphoramidite 1a or 1b. Compound **1a** (39.8 mg, 50 μmol) and 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (16.2 mg, 45.5 μmol) were dissolved in acetonitrile (0.5 mL). To this solution was added 1*H*-tetrazole (7.0 mg, 100 μmol), and the resulting solution was stirred at room temperature. After 5 min, *N*,*N*,*N*′,*N*′-tetraethylthiuram disulfide (44.5 mg, 150 μmol) was added, and the solution was stirred for another 30 min. To this solution was added CD_3CN (50 μL), and the diastereomer ratio was deter-

mined by ^{31}P NMR spectroscopy. The sample was recovered, diluted with ethyl acetate (5 mL), and washed with saturated sodium bicarbonate (5 mL). The organic layer was collected, dried over sodium sulfate, filtered, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with hexane−ethyl acetate (45:55, v/v) to give **13a** (36.5 mg, 80%).

Fully Protected Dimer 13a: ^{31}P NMR (109 MHz, CD_3CN) δ 67.0, 65.3; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.07−0.09 (6H, m), 0.87−0.88 (9H, m), 1.50, 1.53 (3H, 2s), 1.86, 1.88 (3H, 2s), 2.14−2.44 (4H, m), 3.01−3.28 (4H, m), 3.74−3.80 (6H, m), 4.03−4.57 (9H, m), 5.38 (1H, m), 6.02−6.28 (2H,m), 6.75−6.93 (4H, m), 7.14−7.47 (8H, m), 7.71−7.93 (2H, m), 9.62−9.97 (2H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ −4.69, −4.50, 12.42, 12,61, 17.96, 25.74, 29.74, 36.76, 40.42, 52.57, 52.65, 55.29, 55.31, 60.67, 60.77, 61.64, 67.37, 71.38, 75.80, 75.85, 82.87, 83.01, 84.57, 84.64, 84.71, 85.71, 86.78, 111.00, 111.12, 111.24, 111.33, 113.34, 113.44, 113.68, 113.78, 124.90, 125.35, 126.51, 127.26, 127.51, 128.18, 128.23, 128.37, 128.85, 129.39, 130.46, 131.40, 132.80, 133.20, 133.61, 134.05, 134.05, 134.85, 135.87, 136.79, 137.75, 142.22, 150.07, 150.16, 158.26, 158.45, 158.50, 158.54, 163.71, 163.86**.** HRMS (ESI) *m/z* (M + H) Calcd for $C_{50}H_{64}N_4O_{15}PS_2Si$: 1082.3317. Found: 1082.3394.

Fully Protected Dimer 13b: ^{31}P NMR (109 MHz, CD_3CN) δ 68.6, 65.4; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06−0.09 (6H, m), 0.87−0.89 (9H, m), 1.65−1.89 (6H, m), 2.11−2.50 (4H, m), 3.09−4.38 (19.2H, m), 4.93−5.00 (0.8H, m), 6.09−6.22 (2H,m), 6.75−6.92 (4H, m), 7.17−7.73 (10H, m), 9.40−9.60 (2H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ −4.67, −4.48, 12.08, 12.17, 12.56, 17.99, 25.74, 38.92, 40.44, 49.54, 49.68, 55.33, 61.10, 61.25, 62.64, 68.01, 71.07, 71.39, 76.22, 82.30, 82.46, 83.84, 84.76, 84.86, 85.79, 85.89, 86.46, 86.71, 110.28, 110.98, 111.33, 113.18, 113.47, 113.55, 128.06, 128.14, 128.40, 128.63, 128.72, 128.85, 129.97, 129.62, 129.79, 130.29, 130.55, 130.97, 132.41, 132.50, 134.82, 135.46, 136.33, 136.84, 137.25, 140.20, 144.22, 144.77, 150.19, 150.27, 158.59, 158.99, 159.08, 164.13, 164.40**.** HRMS (ESI) *m/z* (M + H) calcd for $C_{50}H_{64}N_4O_{15}PS_2Si$: 1082.3317. Found: 1082.3290.

Typical Procedure for Dimer Synthesis and Determination of Diastereomer Ratio Using Phosphoramidite 1c. Compound **1c** (50 μmol) was coupled with 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (16.2 mg, 45.5 μmol) according to the procedure described above except that the coupling time was extended from 5 to 15 min. The sulfurization and NMR measurement procedure were identical to those described above. **Fully protected dimer 13c**: ^{31}P NMR (109 MHz, CD_3CN) δ 60.1, 56.2; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.07−0.10 (6H, m), 0.88−0.90 (9H, m), 1.62−1.91 (12H, m), 2.05−2.60 (4H, m), 2.94−2.99 (0.7H, m), 3.23−3.54 (2.3H, m), 3.76−4.56 (15H, m), 4.83−4.89 (0.7H, m), 5.42 (0.3H, m), 6.15−6.37 (2H, m), 6.74−6.92 (4H, m), 7.28−7.87 (10H, m), 9.00−9.24 (2H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 4.68, −4.63, −4.54, −4.62, 12.29, 12.34, 12.60, 12.71, 18.00, 25.74, 25.77, 28.09, 28.75, 229.00, 29.12, 39.26, 39.29, 39.49, 39.60, 40.27, 40.68, 55.28, 55.30, 56.38, 61.89, 62.01, 62.73, 71.41, 71.56, 79.07, 79.14, 82.31, 82.41, 84.27, 84.35, 84.49, 84.67, 84.81, 85.01, 85.40, 85.98, 86.57, 86.92, 111.06, 111.12, 111.31, 113.15, 113.31, 113.40, 113.45, 113.60, 127.54, 127.71, 128.38, 128.66, 128.80, 129.63, 129.85, 129.90, 130.07, 130.43, 130.81, 132.63, 133.17, 133.27, 134.60, 135.38, 135.92, 136.93, 138.53, 142.67, 143.95, 150.09, 150.17, 158.17, 158.54, 158.90, 163.44, 163.53, 163.56, 163.62**.** HRMS (ESI) *m/z* (M + H) Calcd for $C_{52}H_{68}N_4O_{15}PS_2Si$: 1111.3629. Found: 1111.3646.

Typical Procedure for Dimer Synthesis and Determination of Diastereomer Ratio Using 5-(9-Phenanthryl)-1*H*-tetrazole as an Activator. Phosphoramidite **1a**, **1b**, or **1c** (50 μmol) was coupled with 3′-*O*-(*tert*-butyldimethylsilyl)-thymidine (16.2 mg, 45.5 μmol) in the presence of 5-(9-phenanthryl)-1*H*-tetrazole (100 mmol) according to the procedure descried above except that the coupling time was extended from 5 to 60 min. The sulfurization and NMR measurement procedure were identical to those described above.

Thymidyl(5′-3′)Thymidine Phosphorothioate (14). Compound **13a** (10 mg, 9.23 μmol), which was synthesized by using 1*H*-tetrazole as an activator, was dissolved in 80% acetic acid (1 mL), and the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure by repeated coevaporation three times with water. To this residue was added concentrated ammonia−ethanol (2:1, v/v, 1 mL), and the resulting solution was stirred for 1 h. The solvent was removed under reduced pressure, and the residue was co-evaporated repeatedly with water until the pH became neutral. Finally, the residue was dissolved in 50% trifluoroacetic acid (1 mL). The solution was stirred for 1 h, and the solvent was removed under reduced pressure. The residue was dissolved in water (1 mL) and washed three times with ethyl acetate (1 mL). The aqueous layer was concentrated under reduced pressure, and the residue was chromatographed on a C-18 reversed phase HPLC column to give the title compound (116 A_{260}, 46%). R_p isomer: Retention time 19.7 min. S_p isomer: Retention time 21.1 min. The diastereomer ratio of R_p:S_p was determined to be 70:30 from the integration of the peak area detected at 254 nm. The structure was confirmed by the enzymatic digestion described below. A similar procedure using **13b** and **13c** gave the title compound in 50% and 58%, respectively.

Enzymatic Digestion by Nuclease P1. Compound R_p-**14** or S_p-**14** (0.5 A_{260}) was dissolved in 25 μL of 0.1 M acetic acid−sodium acetate buffer (pH 5.3, 0.2 mM $ZnCl_2$), and the total volume was adjusted to 50 μL by adding distilled water. To this solution was added nuclease P1 (4 unit), and the resulting mixture was incubated at 50 °C for 5 h. The reaction was quenched by heating at 100 °C for 30 s, and the reaction mixture was analyzed by reversed phase HPLC.

Enzymatic Digestion by Snake Venom Phosphodiesterase and Alkaline Phosphatase. Compound R_p-**14** or S_p-**14** (0.5 A_{260}) was dissolved in 5 μL of 0.5 M Tris-HCl buffer (pH 9.0, 10 mM $MgCl_2$), and the total volume was adjusted to 50 μL by adding distilled water. To this solution were added snake venom phosphodiesterase (0.2 unit) and alkaline phosphatase (calf intestine) 5 μL, and the resulting mixture was incubated at 37 °C for 6 h. The reaction was quenched by heating at 100 °C for 30 s, and the reaction mixture was analyzed by reversed phase HPLC.

Coupling of Phosphoramidite with Various Alcohols. The phosphoroamidite **1a** (19.9 mg, 25 μmol) in CH_3CN (250 μL) was preactivated with 4,5-dicyanoimidazole (5.9 mg, 50 μmol) for 1 min. To this solution was added 100 μL (42−220 equiv excess to **1a**) of an appropriate alcohol, and resulting mixture was stirred for an additional 1 min. The reaction was quenched with triethylamine, and the diastereomer ratios were determined by ^{31}P NMR spectroscopy.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**13**; ^{31}P NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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