Cyclic Orthoester Functions as New Protecting Groups in Nucleosides

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Abstract: Four kinds of cyclic orthoester functions have been studied as protecting groups of hydroxyl groups in nucleosides. Among them, the 1,3-benzodithiol-2-yl (BDT) group was found to have several promising properties. This group can be easily introduced into nucleoside hydroxyl groups in high yields by employing 1,3-benzodithiolium tetrafluoroborate in the presence of pyridine in methylene chloride. The preparation and properties of deoxyribonucleoside derivatives protected with the orthoester-type protecting groups involving the BDT group were described in detail. The synthesis of oligothymidylates is also achieved by utilizing the BDT group as the 5′-phosphate protecting group.

The protecting group of hydroxyl functions is one of the permanent subjects in organic synthesis. Although a number of protecting groups have been explored,[1-3] only a few have survived in the field of nucleoside and nucleotide chemistry,[4-6] since very limited conditions have been required for chemical transformations. For the projected reactions, a protecting group should fulfill the following requirements: (1) the reagent can be easily prepared, (2) introduction of the blocking group must be carried out under mild conditions in high yields, (3) no new chiral centers should be generated in its introduction, (4) the protecting group must be stable during the projected reactions, (5) removal of the protecting group should be highly selective and quantitative and must be performed without damage of the other functional groups. Especially for the synthesis of complicated molecules such as oligonucleotides, protecting groups that are stable under alkaline conditions and can be removed under mildly acidic conditions are quite useful. To date, the trityl group and its analogues have been designed for selective protection of the 5′-hydroxyl function.[7] Tetrahydropyranyl (THP)[8-10] and 4-methoxytetrahydropyranyl (mTHP)[11,12] groups have also been known as the widely applicable protecting groups belonging to the above category. The THP group, however, produces a pair of diastereomers that often cause difficult separation of the products in oligonucleotide synthesis. Introduction of the mTHP group into nucleoside hydroxyl groups requires 4-methoxydihydropyran, which has been prepared as crude material containing tetrahydropyran-4-one and 4,4-dimethoxytetrahydropyran by a series of sluggish reactions. Another drawback in both the pyranylations is that large quantities (10–20 equiv) of the reagent must be used for obtaining satisfactory yields of products. The starting material remains usually to some extent since the equilibrium between the unreacted material and the product lies in the pyranylation. Incomplete reactions cause sometimes difficult preparation of desired products on a large scale.

In this paper, we report a new and promising method for protection of hydroxyl groups in nucleosides utilizing orthoester structures as the skeletons of protecting groups.

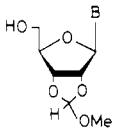
Table I. Stability of **1a–d** under Acidic Conditions

compd	0.01 M HCl–dioxo (1:1, v/v), 25 °C[a] $t_{1/2}$	0.01 M HCl–dioxo (1:1, v/v), 25 °C[a] $t_{complete}$	80% acetic acid, 25 °C[b] $t_{1/2}$	80% acetic acid, 25 °C[b] $t_{complete}$
1a	10 s	2 min	15 s	30 s
1b	2 h	20 h	30 s	20 min
1c	(1% after 4 days)		8 min	30 min (100 °C)
1d	24 h	3 days	20 min	3 h

[a] The concentration of the substrate was 8.9 μmol/mL (dioxo = 1,4-dioxane). [b] The concentration of the substrate was 12.4 μmol/mL.

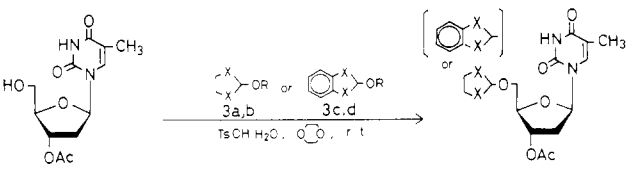
Results and Discussion

The methoxymethylidene group is well-known as the protecting group of ribonucleoside *cis*-2′,3′-diol.[13] From a different point of view, it could be also recognized that methanol is protected by the cyclic orthoester structure of the nucleoside backbone (I). This



I

principal idea led us to study a new methodology for the protection of nucleoside hydroxyl groups with cyclic orthoester structures. For protection of hydroxyl groups, symmetrical cyclic orthoester structures are suitable because the cyclic chiral centers are induced in protected molecules. From the above reasons, we first tried to synthesize 3′-*O*-acetyl-5′-*O*-(1,3-dioxolan-2-yl)thymidine (**1a**)



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a X = O, R = Me
b X = S, R = Me
c X = O, R = Me
d X = S, R = iAm

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Table II. Comparison of the BDT Group with Previously Known Acid-Labile Protecting Groups[a,b]

	DMTrT	mTHPT	BdtT	MMTrT	TBdt	THPT
$t_{1/2}$	3 min	23 min	38 min	48 min	2.5 h	3.5 h
$t_{complete}$	15 min	2.5 h	3 h	3 h	8 h	15 h

[a] These experiments were carried out in 80% acetic acid at 15 °C, and the concentration of the substrate was 10 μmol/mL. [b] DMTrT, mTHPT, BdtT, MMTrT, TBdt, and THPT refer to 5′-*O*-di-*p*-methoxytritylthymidine, 5′-*O*-(4-methoxytetrahydropyran-4-yl)thymidine,[15] 5′-*O*-(1,3-benzodithiol 2-yl)thymidine (7), 5′-*O*-mono-*p*-methoxytritylthymidine, 3′-*O*-(1,3-benzodithiol-2-yl)thymidine (8), and 5′-tetrahydropyranylthymidine,[16] respectively.

by an orthoester exchange reaction of 3′-*O*-acetylthymidine (**2**). When **2** was allowed to react with a large excess of 2-methoxy-1,3-dioxolane (**3a**) in the presence of *p*-toluenesulfonic acid in dioxane at room temperature for 1 day, **1a** was obtained as a crystalline material in 81% yield.

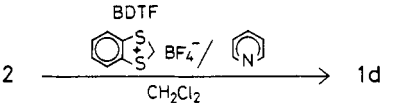
The reaction was very similar to that of tetrahydropyranylation or methoxytetrahydropyranylation. The orthoesterification proceeded increasingly with repeated addition of **3a** or the acid catalyst. An alternative method for the synthesis of **1a** was achieved by the reaction of **2** with **3a** in the presence of pyridinium *m*-nitrobenzenesulfonate (PNBS) in methylene chloride. The reaction was relatively slow, but the very clean reaction mixture was obtained. Thus, the usual workup after 2 days gave **1a** in 85% yield. For such reactions, the previously known catalyst, pyridinium *p*-toluenesulfonate,[14] which has proved to be a useful catalyst, was quite hygroscopic. The new catalyst PNBS might be useful for such ester exchange reactions because it consists of nonhygroscopic crystals and is highly soluble in methylene chloride. The stability of **1a** was examined under acidic conditions of 0.01 M hydrochloric acid–dioxane (1:1, v/v) and 80% acetic acid. These results are summarized in Table I. The table implies that the dioxolan-2-yl group was extremely acid labile. In fact, the storage of **1a** in the solid state at room temperature for 6 months caused complete decomposition to the starting material **2**.

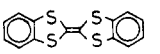
For searching appropriately stable protecting groups, we synthesized three kinds of orthoester derivatives (**1b–d**) of **2** by similar orthoester exchange reactions (see Experimental Section). These orthoesters were chosen since they have no chiral centers and simple structures. The 1,3-dithiolan-2-yl group is a little more stable than the dioxolan-2-yl group but still less than the DMTr group. On the other hand, the 1,3-benzodioxol-2-yl group was quite stable and even more stable than the trityl group. This group could, however, be removed by the action of 80% acetic acid at 100 °C for 30 min. Therefore, the 1,3-benzodioxol-2-yl group can be also used in carbohydrate chemistry where a protecting group more stable than the trityl group is required. In addition, spots containing this group turned reddish gray on TLC when heated on a hot plate.

Compared with the oxygen derivative, the 1,3-benzodithiol-2-yl (BDT) group was found to possess a proper stability as the protecting group. The BDT group was very smoothly cleaved from **1d** by treatment with 80% acetic acid at room temperature for 3 h. The stability of the BDT group was compared with those of previously known acid-labile protecting groups in 80% acetic acid. These results are summarized in Table II. The BDT group was situated between the mTHP and MMTr groups or nearer the latter group.

Furthermore, the BDT group was found to be stable under the conditions of iodine, silver acetate, or silver nitrate (30 equiv each) in pyridine–water (2:1, v/v) at room temperature for 1 day where the phenylthio group of phosphodiester type was activated or removed.

Since the BDT group has especially interesting properties in its stability among the above-mentioned orthoester-type protecting groups, we further tried to improve the method for introduction of this group into nucleoside hydroxyl groups. Nakayama[17] reported that 1,3-benzodithiolium tetrafluoroborate (BDTF)[18,19] underwent facile addition reactions with a large excess of alcohols to give 2-alkoxy-1,3-benzodithioles. Therefore, we examined this type of orthoesterification of **2** by using BDTF under varied conditions. Consequently, we found that **2** was successfully orthoesterified by treatment with 1.2 equiv of BDTF in the presence of pyridine in an excellent yield of 95%. In this reaction, the use of solvents such as pyridine or acetonitrile resulted in slow orthoesterification (~5 h), while the reaction in methylene chloride proceeded much faster and was completed within 30 min. Pyridine was found to be suitable as the base since a strong base like triethylamine led to the dimerization of BDTF, giving rise to dibenzotetrathiafulvalene (**4**).[17,20]





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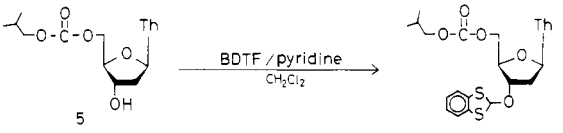
Generally, tetrahydropyranylation or 4-methoxytetrahydropyranylation requires more than 10 equiv of the reagent and accompanies more or less polymeric materials that appear higher than the desired product on TLC. When the pyranylated product is highly lipophilic, it is sometimes difficult to obtain the pure product by column chromatography owing to contamination with the polymers. Contrary to these drawbacks in the usual pyranylations, the orthoesterification using BDTF is of great value because nearly stoichiometric amounts of the reagent can be used and no polymeric materials are formed. Moreover, it is also noteworthy that the BDT group can serve as a marker since the product containing this group was detected very easily as the dark gray spot on TLC when heated on a hot plate as in the case of the 1,3-benzodioxol-2-yl group.

A secondary hydroxyl group of the thymidine derivative (**6**)[21] could be masked with the BDT group in high yield. In this case, 1.5 equiv of BDTF was required for satisfactory yield. The reaction was relatively slower than that with primary hydroxyls. Thus, 3′-*O*-(1,3-benzodithiol-2-yl)-5′-*O*-[(isobutyloxy)-carbonyl]thymidine (**6**) was obtained in 83% yield after 9 h.



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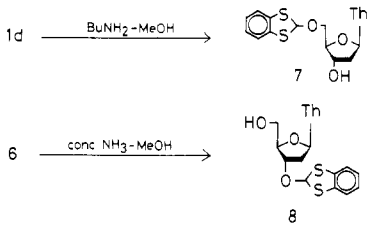
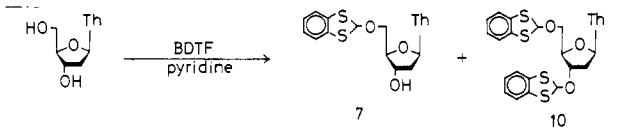
Selective deacylation of **1d** and **6** was achieved by 0.2 M NaOH–dioxane (1:1, v/v), butylamine–methanol, or concentrated ammonia–methanol to afford 5′-*O*-(1,3-benzodithiol-2-yl)thymidine (**7**) and 3′-*O*-(1,3-benzodithiol-2-yl)thymidine (**8**) in ex-



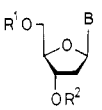
cellent yields (see Experimental Section). The 5′-regioisomer **7** was distinguishable from the 3′-isomer **8** on TLC.

Next, several experiments were conducted with thymidine (**9**)



to see if the 5′-oxygen of a nucleoside with free 3′- and 5′-hydroxyl groups could be preferentially orthoesterified. The reaction of **9** with 1.2 equiv of BDTF in the presence of pyridine in methylene chloride resulted in the predominant formation of 3′,5′-bis-*O*-(1,3-benzodithiol-2-yl)thymidine (**10**) in 41% yield (based on **9**). In this reaction, **7** was obtained in only 12% yield. This seems to be apparently due to the insolubility of **9** in methylene chloride while the initially formed **7** could be soluble in the solvent. Therefore, we examined the orthoesterification in pyridine, where **9** was dissolved, by using 1∼2 equiv of BDTF. Consequently, **7** was obtained as the main product in these reactions. The yield of **7** was optimized (83%) when 1.3 equiv of BDTF was employed, and the reaction was continued at room temperature for 1 day.

In order to ascertain the generality of our new technique for the orthoesterification of nucleoside hydroxyl groups, we examined the reactions of BDTF with a deoxycytidine derivative (**11**) and

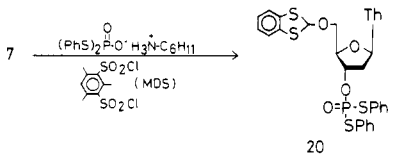


11: $R^1=R^2=H$, $B=Cy^{An}$ 16: $R^1=BDT$, $R^2=H$, $B=Gu^{iBu}$
12: $R^1=R^2=H$, $B=Ad^{Bz}$ 17: $R^1=R^2=BDT$, $B=Cy^{An}$
13: $R^1=R^2=H$, $B=Gu^{iBu}$ 18: $R^1=R^2=BDT$, $B=Ad^{Bz}$
14: $R^1=BDT$, $R^2=H$, $B=Cy^{An}$ 19: $R^1=R^2=BDT$, $B=Gu^{iBu}$
15: $R^1=BDT$, $R^2=H$, $B=Ad^{Bz}$

purine deoxynucleoside derivatives (**12**) and (**13**) under similar conditions. Consequently, we found that BDTF underwent exclusive alkylation of the alcoholic functions without the C–C bond formation at the purine bases expected from the Friedel–Crafts type of reactions. Thus, compounds **14**, **15**, and **16** were obtained in 73%, 87%, and 85% yields, respectively. Any alkylations of the base residues with BDTF were not observed in these reactions. In all cases, the only byproducts were the 3′,5′-dialkylated compounds (**17**, **18**, and **19**), which were also isolated in 9–15% yields.

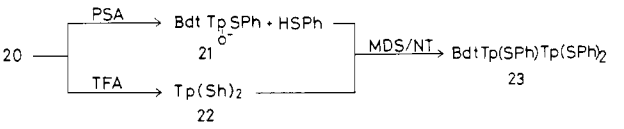
It is noteworthy that the base residues of both the pyrimidine and purine nucleosides were not modified by BDTF and the highly selective alkylations toward the hydroxyl groups were achieved. This characteristic might increase a potential utility of the BDT group as the hydroxyl protecting group for the four common deoxyribonucleosides.

Recently, we have shown new approaches for the synthesis of oligodeoxyribonucleotides[22] and oligoribonucleotides[10] where phenylthio groups were utilized as the internal and 3′-phosphate protecting groups. In order to see if the BDT group can be used as the hydroxyl protecting group in the phosphotriester approach, we synthesized *S*,*S*-diphenyl 5′-*O*-(1,3-benzodithiol-2-yl)thymidine 3′-phosphorodithioate (**20**) in 87% yield by the phosphorylation of **7** with cyclohexylammonium *S*,*S*-diphenyl phosphorodi-



thioate[23-25] in the presence of mesitylene 1,3-disulfonyl chloride (MDS).[22]

Previously, we have also reported that one of two phenylthio groups can be removed selectively from *S*,*S*-diphenyl *O*-nucleoside phosphotriesters by pyridinium phosphinate (PSA) in pyridine under very mild conditions.[22,25] Compound **20** underwent also selective and smooth dephenylthiolation with PSA without damage of the BDT group to give *S*-phenyl 5′-*O*-(1,3-benzodithiol-2-yl)thymidine 3′-phosphorothioate (**21**) as triethylammonium salt.



On the other hand, the BDT group could be readily cleaved from **20** by the action of 2% trifluoroacetic acid in chloroform at 0 °C for 20 min to afford *S*,*S*-diphenyl thymidine 3′-phosphorodithioate (**22**) in 97% yield. The usual condensation of **21** with **22** by use of MDS and 3-nitro-1*H*-1,2,4-triazole as the condensing agent gave the dimer (**23**) in 87% yield.

For an alternative preparation of **21** from **20**, basic conditions could also be employed. When **20** was treated with triethylamine–water–pyridine (2:1:2, v/v/v) at room temperature for 15 min, **21** was obtained simply by evaporation of the mixture. The removal of the phenylthio group under these conditions is similar to that of the β-cyanoethyl group[28,29] from triester units having the latter and *o*-chlorophenyl groups as the 3′-phosphate protecting groups. On the other hand, we have noticed in our long experience that thiophenol does not undergo phosphorylation with dialkyl phosphates in the presence of any known condensing agents.[30] There was, indeed, no reaction between thiophenol and **21** when they were mixed in the presence of MDS in dry pyridine. These findings led us to try a more convenient coupling procedure for the synthesis of **13**. The reaction mixture obtained by treatment of **20** with triethylamine in aqueous pyridine was evaporated and dried by repeated coevaporation with dry pyridine to afford a mixture of **21** and thiophenol. Then, the mixture was allowed to react with **22** in the presence of MDS and 3-nitro-1,2,4-triazole (NT). According to our expectation, the above successive reaction gave the dimer **23** in 89% yield. During the coupling reaction, dealkylation based on the nucleophilicity of thiophenol present in the mixture was not observed.[31] During the workup and the coupling thiophenol may be air-oxidized to diphenyl disulfide and partially react with MDS to be converted to a nonreactive sulfonate derivative. For the selective removal of the 3′-terminal phenylthio group, 5 M pyridinium phosphinate in pyridine was conveniently used. Thus, a fully protected tetramer **24** was obtained in 80% yield from the synthetic intermediates **25** and **26**.

Final deprotection of all the protecting groups from the tetramer was carried out as follows. When the protected tetramer was treated with 0.2 N NaOH–dioxane followed by subsequent treatment with iodine in aqueous pyridine and 80% acetic acid, $(Tp)_4$ was obtained in 86% yield. The enzyme analysis of the

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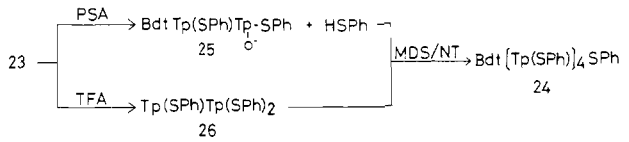

tetramer using spleen phosphodiesterase gave the sole degradation product of Tp.

These results obtained above suggest that the BDT group can be applied as the 2′-hydroxyl protecting group to the synthesis of oligoribonucleotides in which PSA will be available for the selective dephenylthiolation.

Conclusion

The new protecting groups of the orthoester type described in this paper could be widely used in carbohydrate chemistry as well as nucleoside and nucleotide chemistry. Especially, the conditions for introduction of the BDT group into hydroxyl groups are near neutral rather than acidic, while the previously known THP and mTHP groups were introduced under acidic conditions. Since the BDT group can be smoothly and nearly stoichiometrically introduced into hydroxyl groups under very mild conditions, acid-labile protecting groups can exist in target molecules in its introduction. Finally, we add that our preliminary study suggests that the BDT group can be used as the promising protecting group of 2′-hydroxyls in oligoribonucleotide synthesis. These results will shortly be reported elsewhere.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 100 MHz on a JEOL JNM PS-100 spectrometer using tetramethylsilane (Me_4Si) as an internal standard. UV spectra were obtained on a Hitachi 124 spectrophotometer. Paper chromatography was performed by descending technique with Whatman 3 MM papers using solvent I (2-propanol–concentrated ammonia–water, 7:1:2, v/v/v) and solvent II (1-propanol–concentrated ammonia–water, 55:10:35, v/v/v). Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd., and a minipump for a goldfish basin was conveniently used to gain a medium pressure for rapid chromatographic separation. Thin-layer chromatography was performed on precoated TLC plates Silica Gel 60 F-254 (Merck). The R_f values of the protected nucleoside derivatives were measured after development with CH_2Cl_2–MeOH (9:1, v/v) unless otherwise noted. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves (3A). CH_2Cl_2 was dried over P_4O_{10} overnight, decanted, distilled from K_2CO_3, and stored over molecular sieves (3A). Spleen phosphodiesterase was purchased from Boehringer Co. Ltd. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta.

3′-*O*-Acetyl-5′-*O*-(1,3-dioxolan-2-yl)thymidine (1a). Method A. To a solution of 142 mg (0.5 mmol) of **2**[32] in 5 mL of dry dioxane was added 1 g of molecular sieves (3A) and 95 mg (0.5 mmol) of *p*-toluenesulfonic acid monohydrate. After the mixture was stirred for 15 min, 1.85 mL (20 mmol) of 2-methoxy-1,3-dioxolane (**3a**)[33] was added. The mixture was continuously stirred for 24 h and then quenched by addition of 2 mL of pyridine. The mixture was transferred into a separatory funnel with 10 mL of CH_2Cl_2 and 10 mL of water. The organic phase was collected, and the aqueous layer was extracted further with three 10-mL portions of CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on a column of 5 g of silica gel with CH_2Cl_2 to give 144 mg (81%) of **1a**, which was recrystallized from ether to give an analytically pure sample: R_f 0.40; mp 151–153 °C; ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3, CH_3), 2.06–2.54 (m, 2, 2′-H), 2.11 (s, 1, $COCH_3$), 3.82 (s, 1, 5′-H_a), 3.85 (s, 1, 5′-H), 3.88–4.26 (m, 6, 3′-H, 4′-H, and O–CH_2CH_2–O), 5.89 (s, 1, O–CH–O), 6.42 (dd, J = 5.7 and 8.3 Hz, 1, 1′-H), 7.66 (s, 1, =CH), 9.34 (br s, 1, NH).

Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.56; H, 5.66; N, 7.86. Found: C, 50.43; H, 5.76; N, 7.83.

Method B. To a solution of 142 mg (0.5 mmol) of **2**[32] in 5 mL of dry CH_2Cl_2 was added 141 mg (0.5 mmol) of pyridinium *m*-nitrobenzenesulfonate (PNBS) and 1 g of molecular sieves (3A). The mixture was stirred for 15 min, 0.52 mL (10 mmol) of **3a**[33] was added, and stirring was continued for 2 days. The molecular sieves were filtered and washed with 5 mL of CH_2Cl_2. The CH_2Cl_2 solution was transferred into a separatory funnel and shaken with 20 mL of water. The organic phase was collected and the aqueous layer was extracted further with two 10-mL portions of CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on a column of 5 g of silica gel to give 151 mg (85%) of **1a**.

Pyridinium *m*-Nitrobenzenesulfonate (PNBS). ^{1}H NMR spectrum of commerically available *m*-nitrobenzenesulfonic acid–*x*water purchased from Tokyo Kasei Co. Ltd. was measured in order to estimate the *x* value. The *x* value was calclated to be 0.45 by integration of the spectrum. To a suspension of 10 g (47.4 mmol) of the above acid in 200 mL of ether was added 4.5 g (56.8 mmol) of pyridine. After being stirred vigorously at room temperature overnight, the suspension was filtered. The precipitate was washed with 100 mL of ether and dried over sodium hydroxide pellets in vacuo for 1 h to give 9.3 g of the crude pyridinium salt. The filtrate and washings were combined, and the mixture was concentrated to a small volume. The residual solid was triturated with 100 mL of ether. The insoluble material was filtered, washed with 100 mL of ether, and dried over sodium hydroxide pellets in vacuo to give 3.8 g of additional crops of the salt. The total yield was 13.1 g (98%). The crude salt was recrystallized from acetone to give 10.4 g (78%) of the pure salt as slightly yellow crystals: mp 120–123 °C (soften at 115 °C); ^{1}H NMR ($CDCl_3$–Me_2SO-d_6, 5:1, v/v) δ 7.58 (t, J = 8 Hz, 1, ArH), 7.92–8.33 (m, 4, ArH), 8.42–8.78 (m, 2, ArH), 8.97 (m, 1, ArH), 12.47 (s, 1, SO_3H).

Anal. Calcd for $C_{11}H_{10}N_2O_5S$: C, 46.81; H, 3.57; N, 9.92. Found: C, 46.89; H, 3.56; N, 9.93.

3′-*O*-Acetyl-5′-*O*-(1,3-dithiolan-2-yl)thymidine (1b). To a solution of 142 mg (0.5 mmol) of **2**[32] in 5 mL of dry dioxane was added 1 g of molecular sieves (3A), 95 mg (0.5 mmol) of *p*-toluenesulfonic acid monohydrate, and 1.36 g (10 mmol) of **3b**.[34] The mixture was refluxed for 4 h. After the mixture was cooled to room temperature, the usual workup as described before gave 28.2 mg (15%) of **1b** as a foam: ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 1, CH_3), 2.00–2.52 (m, 2, 2′-H), 2.10 (s, 3, $COCH_3$), 3.32 (m, 4, S–CH_2CH_2–S), 3.83 (s, 1, 5′-H_a), 3.85 (s, 1, 5′-H_b), 4.19 (m, 1, 4′-H), 5.22 (d, J = 5.8 Hz, 1, 3′-H), 6.38 (dd, J = 6.4 and 8.4 Hz, 1, 1′-H), 6.39 (s, 1, S–CH–S), 7.56 (s, 1, =CH), 9.21 (br s, 1, NH).

Anal. Calcd for $C_{15}H_{20}N_2O_6S_2$: C, 46.38; H, 5.19; N, 7.21. Found: C, 46.49; H, 5.24; N, 7.44.

3′-*O*-Acetyl-5′-*O*-(1,3-benzodioxol-2-yl)thymidine (1c). To a mixture of 142 mg (0.5 mmol) of **2**[32] and 190 mg (1 mmol) of *p*-toluenesulfonic acid monohydrate in 5 mL of dry dioxane was added 0.5 g of molecular sieves (3A). The mixture was stirred for 10 min, and then 1.52 g (10 mmol) of **3c**[35] was added. After the solution was stirred for 29 h, the usual workup described previously gave 128 mg (63%) of **1c**, which was recrystallized from ether as white needles: R_f 0.41; mp 169–171 °C; ^{1}H NMR ($CDCl_3$) δ 1.87 (s, 3, CH_3), 2.11 (s, 3, $COCH_3$), 2.18 (m, 1, 2′-H_a), 2.42 (td, J = 2, 7, and 15 Hz, 1, 2′-H_b), 3.98 (m, 2, 5′-H), 4.19 (m, 1, 4′-H), 5.22 (d, J = 6 Hz, 1, 3′-H), 6.41 (dd, J = 5.6 and 9 Hz, 1, 1′-H), 6.91 (s, 4, ArH), 6.93 (s, 1, O–CH–O), 7.54 (s, 1, =CH), 9.37 (br s, 1, NH).

Anal. Calcd for $C_{19}H_{20}N_2O_8$: C, 56.43; H, 4.99; N, 6.93. Found: C, 55.88; H, 4.94; N, 6.96.

3′-*O*-Acetyl-5′-*O*-(1,3-benzodithiol-2-yl)thymidine (1d). To a solution of 284 mg (1 mmol) of **2**[32] in 5 mL of dry dioxane were added 2.27 g (10 mmol) of **3d**[36] and 0.2 g of molecular sieves (3A). To the mixture was added 190 mg (1 mmol) of *p*-toluenesulfonic acid monohydrate. After 2 min TLC showed that **1d** was formed in ca. 70%. After the mixture was stirred for 30 min, an additional amount of 190 mg (1 mmol) of *p*-toluenesulfonic acid monohydrate was added. After the mixture was stirred for 30 min, the reaction was stopped by addition of pyridine and the mixture was transferred into a separatory funnel with 20 mL of CH_2Cl_2 and 20 mL of water. The organic phase was collected and combined with further extracts with two 10-mL portions of CH_2Cl_2. After the CH_2Cl_2 solution was dried over Na_2SO_4, it was evaporated in vacuo and coevaporated with three 5-mL portions of toluene. The residue was chromatographed on a column of 25 g of silica gel with CH_2Cl_2 to give 231 mg (53%) of **1d**. Recrystallization from hexane–ether gave an analytically pure sample: R_f 0.56; mp 155–156 °C; ^{1}H NMR ($CDCl_3$) δ 1.87 (s, 1, CH_3), 2.00–2.46 (m, 2, 2′-H), 2.07 (s, 3, $COCH_3$), 3.71 (s, 1, 5′-H_a), 3.73 (s, 1, 5′-H_b), 4.13 (m, 1, 4′-H), 5.18 (d, J = 5.4 Hz, 1, 3′-H), 6.32 (dd, J = 5.7 and 9.2 Hz, 1, 1′-H), 6.78 (s, 1, S–CH–S), 7.00–7.48 (m, 4, Ar H), 7.42 (s, 1, =CH), 9.44 (br s, 1, NH).

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Anal. Calcd for $C_{19}H_{20}N_2O_6S_2$: C, 52.28; H, 4.64; N, 6.42; S, 14.69. Found: C, 52.22; H, 4.50; N, 6.39; S, 14.75.

Synthesis of 1d Using 1,3-Benzodithiolium Tetrafluoroborate (BDTF). To a suspension of 1.42 g (5 mmol) of **2**[32] and 1.44 g (6 mmol) of BDTF[17] in 20 mL of CH_2Cl_2 was added 567 μL (7 mmol) of pyridine to the suspension. Immediately after the addition of pyridine, a homogeneous solution was obtained and gradually white crystals precipitated. After the mixture was stirred for 30 min, 846 μL (6 mmol) of triethylamine was added to convert the excess salt to dibenzotetrathiafulvalene **4**.[17,19] After being stirred for 30 min, the mixture was transferred into a separatory funnel with 20 mL of CH_2Cl_2 and 20 mL of 0.2 M phosphate buffer (pH 7.2). The CH_2Cl_2 layers were collected, and the aqueous solution was extracted further with two 20-mL portions of CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was coevaporated with three 5-mL portions of toluene to remove the last traces of pyridine and chromatographed on a column of 60 g of silica gel with CH_2Cl_2 to give 2.1 g (96%) of **1d**.

3′-*O*-(1,3-Benzodithiol-2-yl)-5′-*O*-isobutyloxycarbonylthymidine (6). To a suspension of 171 mg (0.5 mmol) of **5**[21] and 180 mg (0.75 mmol) of BDTF in 2 mL of dry CH_2Cl_2 was added with stirring 97 μL (1.2 mmol) of pyridine. The resulting solution was stirred for 9 h, when a white precipitate appeared. The mixture was diluted with 10 mL of hexane–CH_2Cl_2 (2:1, v/v) and applied to a column of 15 g of silica gel. Elution with hexane–CH_2Cl_2 gave 206 mg (83%) of **6** as a foam: R_f 0.69; ^{1}H NMR ($CDCl_3$) δ 0.95 (d, J = 7 Hz, 6, $C(CH_3)_2$), 1.83 (s, 3, CH_3), 1.90–2.64 (m, 2, 2′-H), 2.10 (m, 1, CH), 3.92 (d, J = 7 Hz, 2, OCH_2), 4.08–4.22 (m, 2, 3′-H and 4′-H), 4.25 (m, 2, ′-H), 6.21 (t, J = 7 Hz, 1, 1′-H), 6.73 (s, 1, S–CH–S), 6.98–7.44 (m, 4, Ar H), 7.13 (s, 1, =CH), 9.35 (br s, 1, NH).

Anal. Calcd for $C_{22}H_{26}N_2O_7S_2$: C, 53.43; H, 5.30; N, 5.66; S, 12.97. Found: C, 53.35; H, 5.34; N, 5.58; S, 12.82.

When the reactions was carried out by using 2 equiv of BDTF, it was completed in 5 h to give 201 mg (81%) of **6**.

5′-*O*-(1,3-Benzodithiol-2-yl)thymidine (7). To a suspension of 87.3 mg (0.2 mmol) of **1d** in 2 mL of methanol was added 2 mL of butylamine. The mixture became immediately homogeneous and was stirred at room temperature for 3 h. The excess amine and the solvent were evaporated in vacuo, and the residue was chromatographed on a column of 4 g of silica gel with CH_2Cl_2–MeOH (100:0–99:1, v/v) to give 75.2 mg (96%) of **7**. Recrystallization from ether–hexane gave an analytically pure sample: R_f 0.43; mp 141 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.86 (s, 3, CH_3), 1.90–2.46 (m, 2, 2′-H), 3.31 (br s, 1, OH), 3.65 (m, 2, 5′-H), 4.06 (m, 1, 4′-H), 4.36 (m, 1, 3′-H), 6.27 (dd, J = 5.6 and 7.4 Hz, 1, 1′-H), 6.78 (s, 1, SCHS), 7.00–7.52 (m, 4, ArH), 7.39 (s, 1, =CH), 9.40 (br s, 1, NH).

Anal. Calcd for $C_{17}H_{18}N_2O_5S_2$: C, 51.76; H, 4.60; N, 7.10; S, 16.26. Found: C, 51.32; H, 4.47; N, 7.08; S, 16.13.

3′-*O*-(1,3-Benzodithiol-2-yl)thymidine (8). To a solution of 206 mg (0.42 mmol) of **6** in 10 mL of methanol was added 10 mL of concentrated ammonia. After 8 h the mixture was evaporated in vacuo and the residue was applied to a column of 10 g of silica gel. Elution with CH_2Cl_2–MeOH (100:0–99:1, v/v) gave 160 mg (95%) of **8** as a form, which gradually solidifed: mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.81 (s, 3, CH_3), 2.05–2.50 (m, 2, 2′-H), 2.74 (br s, 1, OH), 3.64 (m, 1, 5′-H_a), 3.83 (m, 1, 5′-H_b), 4.04 (m, 1, 4′-H), 4.30 (m, 1, 3′-H), 6.05 (t, J = 7.2 Hz, 1, 1′-H), 6.71 (s, 1, SCHS), 6.95–7.44 (m, 4, ArH), 7.15 (s, 1, =CH), 9.14 (br s, 1, NH).

Anal. Calcd for $C_{17}H_{18}N_2O_5S_2 \cdot 0.5H_2O$: C, 50.61; H, 4.75; N, 6.94. Found: C, 50.21; H, 4.71; N, 6.93.

Selective Introduction of the BDT Group into Thymidine at the 5′-Hydroxyl Group. Method A. To a suspension of 242 mg (1 mmol) of thymidine in 4 mL of dry CH_2Cl_2 was added 396 mg (1.2 mmol) of BDTA and then 190 μL (2.4 mmol) of dry pyridine. The resulting heterogeneous solution was stirred with vigorous stirring for 4 h and then 168 μL (1.2 mmol) of triethylamine was added. After 30 min, the mixture was transferred into a separatory funnel with 20 mL of CH_2Cl_2 and 20 mL of water. The organic layer was collected, and the aqueous layer was extracted further with two 10-mL portions of CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was coevaporated with two 5-mL portions of toluene and chromatographed on a column of 15 g of silica gel with hexane–CH_2Cl_2 to give 47 mg (12%) of **7** and 269 mg (41%) of **10** as foam. For **10**: R_f 0.62 ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3, CH_3), 1.88 (m, 1, 2′-H_a), 2.38 (m, 1, 2′-H_b), 3.48 (dd, J = 2 and 10.4 Hz, 1, 5′-H_a), 3.69 (dd, J = 2 and 10.4 Hz, 1, 5′-H_b), 6.24 (t, J = 6.8 Hz, 1, 1′-H), 6.65 (s, 1, S–CH–S), 6.79 (s, 1, S–CH–S), 7.08–7.58 (m, 9 Ar H and =CH), 9.15 (br s, 1, NH).

Anal. Calcd for $C_{24}H_{22}N_2O_5S_4$: C, 52.73; H, 4.06; N, 5.12. Found: C, 52.85; H, 4.22; N, 5.08.

Method B. To a solution of 242 mg (1 mmol) of thymidine in 5 mL of dry pyridine was added 312 mg (1.3 mmol) of BDTA. The mixture was stirred for 24 h. In order to convert the excess reagent into **4** 0.18 mL (1.3 mmol) of triethylamine was added. After the mixture was stirred for 15 min, it was transferred into a separatory funnel with 10 mL of CH_2Cl_2 and shaken with 10 mL of 0.2 M phosphate buffer (pH 7.2). The organic phase and further CH_2Cl_2 extracts (3 × 10 mL) were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on a column of 15 g of silica gel with CH_2Cl_2–MeOH (100:0–99.5:0.5, v/v) to give 327 mg (83%) of **7**.

5′-*O*-(1,3-Benzodithiol-2-yl)-*N*⁴-anisoyldeoxycytidine (14). To a solution of 361 mg (1 mmol) of N^4-anisoyldeoxycytidine (**11**),[37] dried by repeated coevaporations with dry pyridine, in 5 mL of dry pyridine was added 288 mg (1.2 mmol) of BDTF. The mixture was stirred for 21 h. After the usual workup, chromatography on a column of 20 g of silica gel with CH_2Cl_2–MeOH (100:0–99:2, v/v) gave 377 mg (73%) of **14** and 100 mg (15%) of **17**. For **14**: R_f 0.47; mp 78–80 °C (C_6H_6); ^{1}H NMR (Me_2SO_4-d_6–$CDCl_3$, 2:1, v/v) δ 2.05 (m, 1, 1′-H), 2.38 (m, 1, 1′-H_b), 3.66 (dd, J = 2.8 Hz, J = 10.1 Hz, 1, 5′-H_a), 3.84 (dd, J = 2.0 Hz, J = 10.1 Hz, 1, 5′-H_b), 4.14 (m, 1, 4′-H), 4.30 (m, 1, 3′-H), 5.23 (d, J = 4.0 Hz, 1, OH), 6.27 (t, J = 6.0 Hz, 1, 1′-H), 6.83 (s, 1, SCHS), 7.00–7.60 (m, 8, Ar H), 8.05 (d, J = 10.8 Hz, 5-H), 8.13 (d, J = 10.8 Hz, 6-H), 10.91 (br s, 1, NH).

Anal. Calcd for $C_{24}H_{23}N_3O_6S_2$: C, 56.13; H, 4.51; N, 8.18. Found: C, 56.72; H, 4.61; N, 7.75.

For **17**: R_f 0.82; mp >200 °C (Me_2SO_4–$CHCl_3$, 3:1, v/v); ^{1}H NMR (Me_2SO_4-d_6–$CDCl_3$, 4:1, v/v) δ 2.55 (m, 2, 2′-H), 3.63 (m, 1, 4′-H), 4.21 (m, 1, 3′-H), 6.02 (t, J = 6.0 Hz, 1, 1′-H), 6.87 and 6.90 (s, 2, SCHS), 6.95–7.60 (m, 13, ArH), 7.88 (d, J = 7.0 Hz, 1, 5-H), 8.02 (d, J = 7.0 Hz, 1, 6-H), 10.52 (br s, 1, NH).

Anal. Calcd for $C_{31}H_{27}N_3O_6S_4$: C, 55.92; H, 4.09; N, 6.31. Found: C, 55.66; H, 4.01; N, 6.33.

When 1.3 equiv of BDTF was used in this reaction, the yields of **14** and **17** were 352 (69%) and 121 mg (18%), respectively.

5′-*O*-(1,3-Benzodithiol-2-yl)-*N*⁶-benzoyldeoxyadenosine (15). To a solution of 355 mg (1 mmol) of N^6-benzoyldeoxyadenosine (**12**),[37] dried by repeated coevaporations with dry pyridine, in 5 mL of dry pyridine was added 312 mg (1.3 mmol) of BDTF. The mixture was stirred for 24 h. The same workup as described before gave 442 mg (87%) of **15** and 62 mg (9%) of **18**. For **15**: R_f 0.45; ^{1}H NMR ($CDCl_3$) δ 2.62 (m, 2, 2′-H), 3.69 (m, 2, 5′-H), 4.10 (m, 1, 4′-H), 4.65 (m, 1, 3′-H), 6.53 (d, J = 6.3 Hz, 1, 1′-H), 6.84 (s, 1, SCHS), 7.00–7.65 (m, 7, Ar H), 8.06 (m, 2, ArH), 8.33 and 8.71 (s, 2, 2-H and 8-H), 9.40 (br s, 1, NH).

Anal. Calcd for $C_{24}H_{21}N_5O_4S_2$: C, 56.79; H, 4.17; N, 13.80. Found: C, 56.22; H, 4.36; N, 13.73.

For **18**: R_f 0.79; ^{1}H NMR ($CDCl_3$) δ 2.63 (m, 2, 2′-H), 3.53 (m, 2, 5′-H), 4.10–4.50 (m, 2′,3′,4′-H), 6.29 (t, J = 6.4 Hz, 1, 1′-H), 6.64 and 6.69 (s, 2, SCHS), 6.9207.60 (m, 7, Ar H), 7.95 (m, 2, ArH), 7.97 and 8.50 (s, 2, 2-H and 8-H), 9.50 (br s, 1, NH).

Anal. Calcd for $C_{31}H_{25}N_5O_4S_4$: C, 56.43; H, 3.82; N, 10.71. Found: C, 56.23; H, 3.91; N, 10.48.

5′-*O*-(1,3-Benzodithiol-2-yl)-*N*²-isobutyryldeoxyguanosine (16). To a solution of 355 mg (1 mmol) of N^2-isobutyryldeoxyguanosine monohydrate,[38] dried by repeated coevaporations with dry pyridine, in 5 mL of dry pyridine was added 312 mg (1.3 mmol) of BDTF. The mixture was stirred for 24 h. The same workup as described before gave 417 mg (85%) of **16** and 73 mg (11%) of **19**. For **16**: R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 1.21 (d, J = 6.9 Hz, 6, $C(CH_3)_2$), 2.41 (m, 2, 2′-H), 2.82 (septet, J = 6.9 Hz, $CH(Me)_2$), 3.65 (m, 2, 5′-H), 4.09 (m, 1, 4′-H), 4.46 (m, 1, 3′-H), 5.42 (d, J = 5.8 Hz, 1, OH), 6.31 (t, J = 7.0 Hz, 1, 1′-H), 6.90 (s, 1, SCHS), 7.00–7.49 (m, 4, ArH), 7.97 (s, 1, 8-H), 10.80 and 11.07 (br s, 2, NH).

Anal. Calcd for $C_{21}H_{23}N_5O_5S_2$: C, 51.52; H, 4.73; N, 14.30. Found: C, 50.97; H, 4.86; N, 13.83.

For **19**: R_f 0.61; ^{1}H NMR (Me_2SO_4-d_6–$CDCl_3$, 4:1, v/v) δ 1.22 (d, J = 6.4 Hz, 6, $C(CH_3)_2$), 2.40 (m, 2, 2′-H), 2.73 (m, 1, $CH(Me)_2$), 3.48 (m, 2, 5′-H), 4.09–4.40 (m, 2, 2′,3′,4′-H), 6.10 (t, J = 7.0 Hz, 1, 1′-H), 6.65 and 6.70 (s, 2, SCHS), 6.90–7.50 (m, 8, ArH), 7.60 (s, 1, 8-H), 11.02 and 11.27 (br s, 2, NH).

Anal. Calcd for $C_{28}H_{27}N_5O_5S_4$: C, 52.40; H, 4.24; N, 10.91. Found: C, 52.16; H, 4.34; N, 10.65.

***S*,*S*-Diphenyl 5′-*O*-(1,3-benzodithiol-2-yl)thymidine 3′-Phosphorodithioate (20).** To a solution of 458 mg (1.2 mmol) of cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate[23-25] in 5 mL of dry pyridine was added 381 mg (1.2 mmol) of MDS. After the mixture was stirred for 5 min, 395 mg (1 mmol) of **7** was added, and stirring was continued for 5 h. The mixture was diluted with 20 mL of CH_2Cl_2, transferred to a separatory funnel, and shaken with 20 mL of water. The organic phase

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was collected, and the aqueous layer was extracted with three 10-mL portions of CH_2Cl_2. The combined CH_2Cl_2 extracts were dried, evaporated in vacuo, and chromatographed on a column of 20 g of silica gel with CH_2Cl_2 to give 570 mg (87%) of **20** as a foam, which was precipitated from its acetone solution into hexane to give an analytically pure sample including one-half molecule of hexane: R_f 0.62; ^{1}H NMR ($CDCl_3$) δ 1.86 (s, 3, CH_3), 1.95–2.36 (m, 2, 2′-H), 3.60 (s, 1, 5′-H_a), 3.62 (s, 1, 5′-H_b), 4.08 (m, 1, 4′-H), 5.10 (m, 1, 3′-H), 6.22 (dd, J = 5.4 and 8.5 Hz, 1, 1′-H), 6.71 (s, 1, SCHS), 7.00–7.60 (m, 14, Ar H), 7.42 (s, 1, =CH), 8.58 (br s, 1, NH).

Anal. Calcd for $C_{29}H_{27}N_2O_6PS_4 \cdot 0.5C_6H_{14}$: C, 54.76; H, 4.88; N, 3.99. Found: C, 55.37; H, 5.05; N, 4.03.

Synthesis of the Dimer 23. Removal of the BDT Group from 20. To a solution of 329 mg (0.5 mmol) of **20** in 20 mL of $CHCl_3$ at 0 °C was added 0.4 mL of trifluoroacetic acid. The solution was stirred for 20 min. The mixture was transferred into a separatory funnel with 15 mL of $CHCl_3$ and washed with 10 mL of 5% $NaHCO_3$ solution. The aqueous solution was further extracted with two 10-mL portions of 5% $NaHCO_3$ solution. The organic extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on a column of 6 g of silica gel with CH_2Cl_2–MeOH (100:0–99:1, v/v) to afford 245 mg (97%) of **22**[25] (R_f 0.45).

Method A: Commercially available phosphinic acid (Kanto Co. Ltd., 30%) (9.1 g, 41.3 mmol) was concentrated by a rotary evaporator and coevaporated with five 30-mL portions of dry pyridine. The residue was dissolved in a mixture of 8.25 mL of pyridine and 4.13 mL of triethylamine. To the solution was added 362 mg (0.55 mmol) of **20**, and the mixture was stirred at room temperature for 15 min. The mixture was diluted with 20 mL of CH_2Cl_2, transferred into a separatory funnel, and shaken with 20 mL of 1 M TEAB solution. The aqueous layer was collected, and the CH_2Cl_2 layer was washed with two 20-mL portions of 1 M TEAB solution. The aqueous layer and washings were combined and extracted with 20 mL of CH_2Cl_2. The latter CH_2Cl_2 extract was washed further with two 20-mL portions of 1 M TEAB solution and combined with the first CH_2Cl_2 extract. The CH_2Cl_2 solution was dried over Na_2SO_4, evaporated in vacuo, and rendered anhydrous by repeated coevaporation with dry pyridine. The residue was mixed with 253 mg (0.5 mmol) of **22** and 171 mg (1.5 mmol) of 3-nitro-1*H*-1,2,4-triazole. The mixture was rendered anhydrous by coevaporation with two 5-mL portions of dry pyridine and dissolved in 2 mL of dry pyridine. To the solution was added 476 mg (1.5 mmol) of MDS. The mixture was stirred at room temperature for 40 min. The usual workup gave 488 mg (87%) of **23**: R_f 0.49; 1.86 (m, 6, CH_3), 2.22 (m, 4, 2′-H), 3.57, 3.71 (m, 2, 5′-terminal 5′-H), 4.07 (m, 2, 4′-H), 4.28 (m, 2, 3′-terminal 5′-H), 5.12 (m, 2, 3′-H), 6.25 (m, 2, 1′-H), 6.74, 6.78 (s, 1, diastereomeric SCHS), 7.00–7.74 (m, 21, Ar H and 6-H), 9.40, 9.47, 9.56 (br s, 2, diastereomeric NH).

Anal. Calcd for $C_{45}H_{44}N_4O_{12}P_2S_5$: C, 51.23; H, 4.20; N, 5.31. Found: C, 51.22; H, 4.32; N, 5.22.

Method B: To a solution of 507 mg (0.77 mmol) of **20** in 15.4 mL of pyridine were added 7.7 mL of triethylamine and subsequently 7.7 mL of water. The mixture was kept at room temperature for 15 min. Then the solution was evaporated in vacuo to a gum, which was rendered anhydrous by repeated coevaporation with dry pyridine. The residue was mixed with 355 mg (0.7 mmol) of **22** and 240 mg (2.1 mmol) of 3-nitro-1*H*-1,2,4-triazole. The mixture was coevaporated with two 5-mL portions of pyridine and dissolved in 2 mL of dry pyridine. To the solution was added 666 mg (2.1 mmol) of MDS. After being stirred for 30 min, the mixture was diluted with 20 mL of CH_2Cl_2, transferred into a separatory funnel, and washed with three 20-mL portions of 5% $NaHCO_3$ solution. The washings were combined and again extracted with 20 mL of CH_2Cl_2. The latter CH_2Cl_2 extract was washed with two 20-mL portions of 5% $NaHCO_3$ and two 20-mL portions of water. The organic extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on a column of 6 g of silica gel with CH_2Cl_2–MeOH (99:1–98:2, v/v) to give 677.2 mg (92%) of **23**.

Synthesis of the Tetramer 24. Removal of the BDT Group from 23. To a solution of 422 mg (0.4 mmol) of **23** was added at 0 °C 0.32 mL of trifluoroacetic acid with stirring. After the mixture was stirred for 30 min, the usual workup gave 290 mg (80%) of **26**: R_f 0.45; ^{1}H NMR ($CDCl_3$) δ 1.87 (m, 6, CH_3), 2.34 (m, 4, 2′-H), 3.04 (br 1, OH), 3.76 (m, 2, 5′-terminal 5′-H), 4.10 (m, 2, 4′-H), 4.29 (m, 2, 3′-terminal 5′-H), 5.24 (m, 2, 3′-H), 6.21 (m, 2, 1′-H), 7.15–7.74 (m, 17, Ar H and 6-H), 9.74, 9.79, 9.94 (br s, 2, diestereomeric NH).

Anal. Calcd for $C_{38}H_{40}N_4O_{12}P_2S_3$: C, 50.55; H, 4.47; N, 6.21. Found: C, 50.93; H, 4.76; N, 5.85.

As described previously, 253 mg (0.24 mmol) of **23** was treated with a 5 M solution of PSA (18 mmol) in pyridine containing 1.8 mL of triethylamine. After 50 min, the reaction was complete. The mixture was transferred into a separatory funnel with 20 mL of $CHCl_3$. The $CHCl_3$ solution was washed with 30 mL of 0.2 M TEAB solution and two 20-mL portions of water. The washings were combined and extracted with 20 mL of $CHCl_3$–pyridine (3:1, v/v). The latter $CHCl_3$ extract was washed with three 20-mL portions of water. The organic extracts were combined, dried over Na_2SO_4, and evaporated in vacuo. The residue was mixed with 181 mg (0.2 mmol) of **26** and 68.5 mg (0.6 mmol) of 3-nitro-1*H*-1,2,4-triazole and coevaporated with dry pyridine several times. The mixture was dissolved in 4 mL of pyridine, and 190 mg (0.6 mmol) of MDS was added. The solution was stirred for 25 min. The usual workup gave 302 mg (80%) of **24**: R_f 0.47; ^{1}H NMR ($CDCl_3$) δ 1.94 (m, 12, CH_3), 2.33 (m, 8, 2′-H), 3.67 (m, 2, 5′-terminal 5′-H), 4.09 (m, 4, 4′-H), 4.32 (m, 6, internal 5′-H), 5.15 (m, 4, 3′-H), 6.21 (m, 4, 1′-H), 6.77, 6.81 (s, 1, diastereomeric SCHS), 7.00–7.74 (m, 28, Ar H and 6-H), 9.95 (m, 4, NH).

Anal. Calcd for $C_{77}H_{78}N_8O_{24}P_4S_7 \cdot 1.5H_2O$: C, 49.33; H, 4.35; N, 5.98. Found: C, 49.22; H, 4.36; N, 5.89.

Deprotection of the Tetramer 24. To a solution of 18.5 mg (10 μmmol) of **24** in 0.8 mL of dioxane was added 0.8 mL of 0.2 N sodium hydroxide at room temperature. After being stirred for 10 min, the mixture was neutralized with Dowex 50 W-X 2 (pyridinium form). At this time, TLC showed one spot with R_f values of 0.22 (solvent I) and 0.62 (solvent II). The resin was filtered and washed with 2 mL of pyridine–water (1:1, v/v). The filtrate was evaporated to a gum, which was dissolved in 1 mL of pyridine–water (2:1, v/v). To the solution was added 76 mg (0.3 mmol) of iodine. After being stirred for 15 min, the mixture was transferred into a separatory funnel with 10 mL of benzene and 10 mL of water. The aqueous layer was collected, and the benzene layer was further extracted with two 5-mL portions of water. The aqueous extracts were evaporated in vacuo. TLC of the residue showed three spots corresponding to TpTpTpTp (trace), BdtTpTpTpTp (main), and ammonium iodide with R_f values of 0.44, 0.57, and 0.67, respectively (solvent II). The residue was treated with 10 mL of 80% acetic acid at room temperature for 2.5 h. The solvent was removed in vacuo, and the residue was chromatographed on Whatman 3 MM papers with solvent II to give 287 OD (88%) of $(Tp)_4$: R_f 0.74 (solvent II), UV_{max} 267 nm, UV_{min} 234 nm. The tetramer (30 OD) was incubated with spleen phosphodiestrase (10 μg) in 0.45 mL of 0.05 M ammonium acetate at 37 °C overnight. The analysis using paper chromatography showed only one spot of Tp (32 OD).

Registry No. 1a, 84752-57-8; **1b**, 84752-58-9; **1c**, 84752-59-0; **1d**, 84752-60-3; **2**, 21090-30-2; **3a**, 19693-75-5; **3b**, 36069-31-5; **3c**, 6823-42-3; **3d**, 55315-56-5; **5**, 13084-59-8; **6**, 84752-62-5; **7**, 84752-63-6; **8**, 84752-64-7; **10**, 84752-65-8; **11**, 48212-99-3; **12**, 4546-72-9; **14**, 84752-66-9; **15**, 84752-67-0; **16**, 84752-68-1; **17**, 84752-69-2; **18**, 84774-89-0; **19**, 84752-70-5; **20**, 84774-90-3; **21**, 84752-72-7; **22**, 70900-95-7; **23**, 84752-73-8; **24**, 84752-74-9; **25**, 84774-91-4; **26**, 80585-49-5; BDTF, 5784-27-0; PNBS, 84752-61-4; BdtTpTpTpTp, 84752-56-7; $(Tp)_4$, 4719-57-7; thymidine, 50-89-5; N^2-isobutyryldeoxyguanosine, 68892-42-2.