Syntheses and Structure−Activity Relationships of Nonnatural *β-C*-Nucleoside 5′-Triphosphates Bearing an Aromatic Nucleobase with Phenolic Hydroxy Groups: Inhibitory Activities against DNA Polymerases

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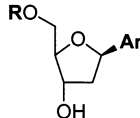
Five nonnatural *β-C*-nucleoside 5′-triphosphates bearing a 3,4-dihydroxyphenyl (**1TP**), a 2-hydroxyphenyl (**2TP**), a 3-hydroxyphenyl (**3TP**), a 4-hydroxyphenyl (**4TP**), or a phenyl (**5TP**) group were synthesized, and their structure−activity relationships were examined for a series of DNA polymerase reactions in vitro under typical polymerase chain reaction conditions. We found that the 5′-triphosphates (**1TP**−**5TP**) are not incorporated into DNA strands but inhibit the DNA polymerase reactions in the presence of natural nucleoside 5′-triphosphates (dNTPs). **1TP** having two phenolic hydroxy groups at the nucleobase moiety showed the most potent inhibitory effect against DNA synthesis by Ex Taq polymerase ($IC_{50} = 30 \ \mu M$). The competition assay indicated that **1TP** and dNTPs are most likely to affect DNA polymerase reactions competitively. This finding may raise the appealing possibility that artificial nucleoside 5′-triphosphates having phenolic hydroxy groups could exhibit potent inhibitory activity against DNA-directed enzymatic reactions.
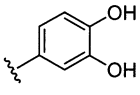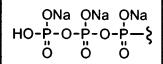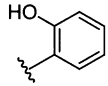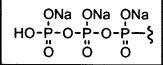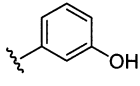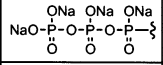
Introduction

DNA is a biopolymer that stores genetic information, and transcription and replication of the information involve hydrogen-bond-based recognition between two pairs of complementary nucleobases (A−T and G−C). To expand the genetic alphabet or to address a question on whether the four-letter alphabet is the only solution for the genetic code, unnatural base pairs that would be mediated by alternative hydrogen bonding[1] or by hydrophobic packing interactions[2,3] have been exploited so far. Natural nucleoside 5′-triphosphates act as substrates for DNA polymerases and then are incorporated into DNA strands with extremely high accuracy in a template-directed manner. In this context, nonnatural nucleoside 5′-triphosphates with some specific functional groups could possibly have an inhibitory effect on DNA and RNA syntheses, and therefore, they have long been recognized as promising candidat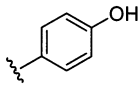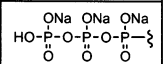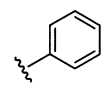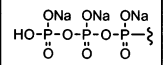es for antiviral and antineoplastic agents.[4−7] In addition, some unnatural base pairs based on altered hydrogen bonding[1b,c] and hydrophobic interactions[2,3] have been enzymatically incorporated into DNA strands leading to the chain elongation.

We have recently envisioned metal coordination as an alternative driving force for base pairing and reported a few examples of artificial *β-C*-nucleosides bearing a phenylenediamine,[8] a catechol,[9] a 2-aminophenol,[9a,10] and pyridine[11] as the nucleobase.[12−15] In this

Chart 1. Nucleosides (**1**−**5**) and Their 5′-Triphosphates (**1TP**−**5TP**) Used in This Study

study, the in vitro activity of the synthetic catechol-bearing nucleoside 5′-triphosphate **1TP** (Chart 1), which could potentially form either two-point hydrogen bonding or metal complexes, was examined for DNA poly-

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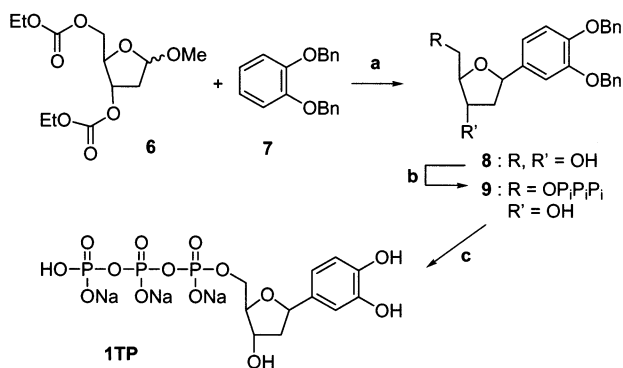† The University of Tokyo.
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Scheme 1[a]

[a] Reagents: (a) (1) SnCl₄, CH₂Cl₂, −20 °C; (2) K₂CO₃, MeOH; (b) (1) POCl₃ in trimethyl phosphate, proton sponge, then tributylammonium pyrophosphate, tributylamine, (2) NaClO₄; (c) H₂, Pd−C, H₂O.

Scheme 2[a]

[a] Reagents: (a) Cp₂HfCl₂−AgClO₄, CH₂Cl₂, −78 to −75 °C; (b) aqueous NH₃, MeOH; (c) (1) POCl₃ in trimethyl phosphate, proton sponge, then tributylammonium pyrophosphate, tributylamine; (2) NaClO₄.

Scheme 3[a]

[a] Reagents: (a) (1) THF, −78 °C, (2) Et₃SiH, BF₃·OEt₂, CH₂Cl₂, −78 °C; (b) TBAF, THF; (c) (1) POCl₃ in trimethyl phosphate, proton sponge, then tributylammonium pyrophosphate, tributylamine, (2) NaClO₄; (d) H₂, Pd−C, MeOH−H₂O.
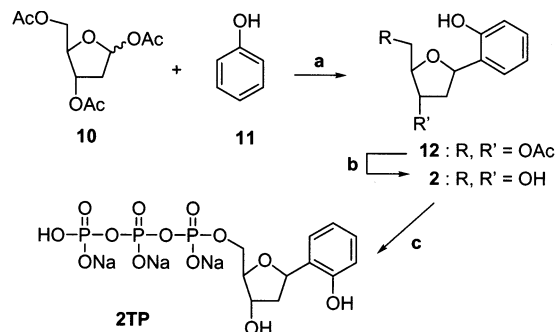
merase reactions under typical polymerase chain reaction (PCR) conditions. As a result, we found that **1TP** is not incorporated into DNA strands and that it inhibits the DNA polymerase reaction in the presence of natural nucleoside 5′-triphosphates (dNTPs, where N = A, T, G, or C). To investigate the structure−activity relationship, we further synthesized a series of β-C-nucleosides with one or two hydroxy groups at the 2-, 3-, or 4-position (**2**−**4**), and examined the effect of their 5′-triphosphates (**2TP**−**4TP**) on a series of DNA polymerase reactions.
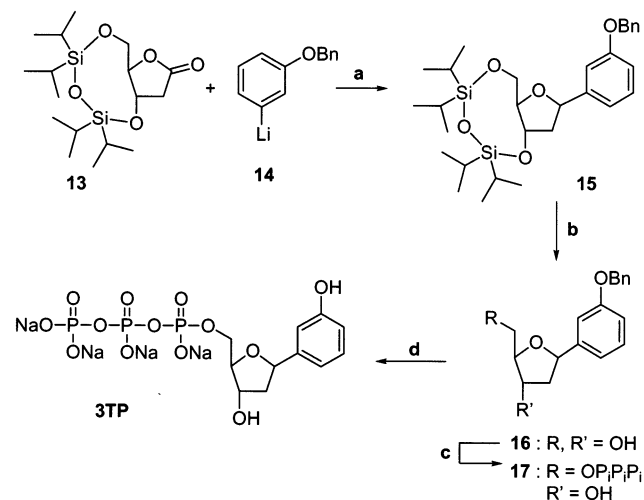
Results and Discussion

Syntheses of Nucleosides and Their 5′-Triphosphate Derivatives. Five β-C-nucleosides bearing a 3,4-dihydroxyphenyl (**1**), a 2-hydroxyphenyl (**2**), a 3-hydroxyphenyl (**3**), a 4-hydroxyphenyl (**4**), or a phenyl (**5**) group and their 5′-triphosphate derivatives (**1TP**−**5TP**, respectively) were prepared to examine their effect on DNA polymerase reactions (Chart 1).

The synthetic routes for the 5′-triphosphates are depicted in Schemes 1−5. A 3,4-dihydroxyphenyl derivative **1TP** was prepared from *O*-protected catecholbearing nucleoside **8** (Scheme 1).[9a] The Friedel−Crafts approach via electrophilic aromatic substitution was chosen to build up the carbon skeleton of the nucleoside **8**, where SnCl₄ was used as a Lewis acid. The coupling reaction of *O*-protected catechol **7** and 3,5-protected methylglycoside **6** afforded the base-coupled nucleosides as a mixture of α- and β-anomers (α/β = 1:2) in 32% yield. The removal of ethyl carbonate groups with K₂CO₃ in MeOH followed by purification by column chromatography afforded the desired β-C-nucleoside **8** in 14% yield. Its 5′-triphosphate derivative **9** was then prepared by treatment with POCl₃ in trimethyl phosphate followed by the addition of tributylammonium pyrophosphate and tributylamine in DMF.[16] After purification by reversed-phase HPLC, the triethylammonium counterions were exchanged with sodium ions using the modified ion exchange method.[17] The protective benzyl groups were removed by catalytic hydrogenation to afford the desired 5′-triphosphate **1TP** in 40% overall yield from **8**.

A 2-hydroxyphenyl derivative **2TP** was prepared as shown in Scheme 2. A hafnium-based Lewis acid in combination with AgClO₄ was used for the *C*-glycosi-

dation from triacetylated deoxyribose **10** to **12**.[18] The coupling reaction preferentially took place at the α-position of the hydroxy group of phenol **11**, and consequently the β-anomer was isolated as the main product in 34% yield.[19] Subsequent removal of acetyl groups afforded **2**, which was then converted to its 5′-triphosphate **2TP** in 11% yield by the same method as that for **1TP**.

The 3-hydroxyphenyl derivative **3TP** was prepared via nucleoside **16** (Scheme 3). The coupling reaction between lithiated **14** and lactone **13** furnished a hydroxyketone, which was subsequently reduced by Et₃SiH−BF₃·Et₂O to provide β-C-glycoside **15** selectively in 35% yield. The following desilylation afforded β-C-nucleoside **16** and was further converted to its 5′-triphosphate **3TP** in 11% yield.

A key step in the synthesis of **4TP** was the coupling reaction of organozinc derivative **19** with *O*-protected deoxyribose **18** followed by epimerization at the C1′ position (Scheme 4). Although this coupling reaction selectively produced α-anomer **20** (α/β = 7:1), epimerization with benzenesulfonic acid in the presence of a small amount of water[20] led to partial conversion from **20** to the desired β-anomer **21** in 39% yield (α/β = 1:2.2). The resulting β-anomer **21** was converted to **4TP** by deprotection and phosphorylation.

Scheme 4[a]

[a] Reagents: (a) THF, reflux; (b) $PhSO_3H$, H_2O, H_2SO_4, toluene, reflux; (c) NaOMe, MeOH; (d) (1) $POCl_3$ in trimethyl phosphate, proton sponge, then tributylammonium pyrophosphate, tributylamine, (2) $NaClO_4$; (e) H_2, Pd−C, MeOH−H_2O.
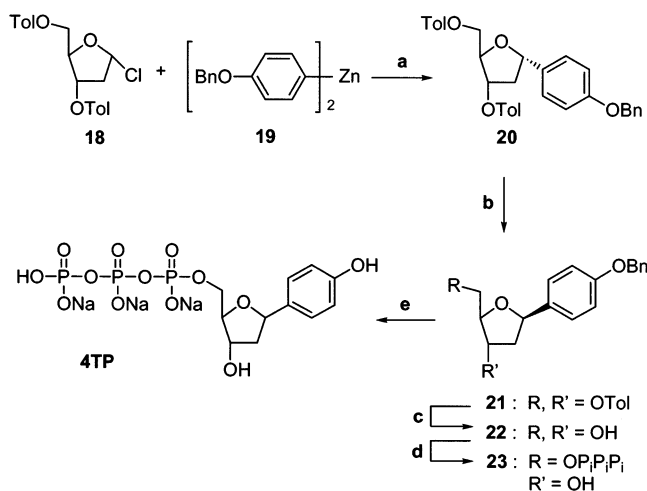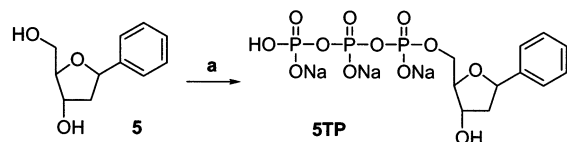
Scheme 5[a]

[a] Reagents: (a) (1) $POCl_3$ in trimethyl phosphate, proton sponge, then tributylammonium pyrophosphate, tributylamine, (2) $NaClO_4$.

The phenyldeoxynucleoside 5′-triphosphate **5TP** was derived from nucleoside **5**, which was prepared according to a previous report by Kool et al. (Scheme 5).[21]

The anomeric configuration of the C1′ position of each free nucleoside was determined to be β by X-ray analyses (see Supporting Information). The β-anomeric structure was also confirmed by comparison of the ^{1}H NMR splitting patterns for H1′ of each free nucleoside with previously reported data.[20,22] In all the cases, the 5′-triphosphates **1TP−5TP** were identified by ^{1}H and ^{31}P NMR, mass spectrometry, and elemental analyses (see Experimental Section).

Effect of the β-C-Nucleoside 5′-Triphosphates on DNA Polymerase Reactions. The effect of five β-C-nucleoside 5′-triphosphates (**1TP−5TP**) on DNA syntheses was evaluated using a series of DNA polymerases (Ex Taq, LA-Taq, Z-Taq, Pfu, and Pyrobest). The phenyl-bearing nucleoside 5′-triphosphate (**5TP**) without aromatic hydroxy groups was used as the reference compound for those (**1TP−4TP**) with one or two hydroxy groups. To increase the detection level, PCR was employed throughout this study. The PCR was performed using plasmid DNA (0.5 ng) as template and a pair of primers (30-nucleotide-long lacUV501 and 28-nucleotide-long lacUV502). Under these PCR conditions, a DNA product of 180 bases in length is produced (for instance, see Figure 1, lane 1). The amount of each PCR product was determined by 8% acrylamide gel electrophoresis. When one of the β-C-nucleoside 5′-triphosphates **1TP−5TP** was used as a substrate in place of natural nucleoside 5′-triphosphates, virtually no DNA synthesis was detected, and this result indicates that these artificial 5′-triphosphates are not incorporated into
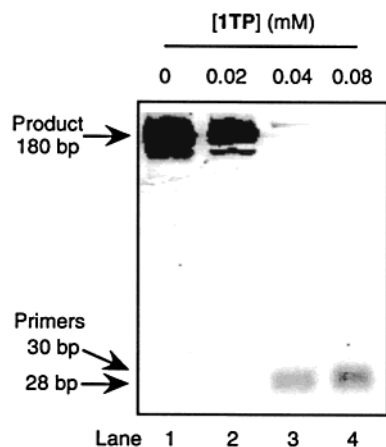
Figure 1. DNA amplification by Ex Taq in the presence of **1TP**: (lane 1) dNTPs, 0.2 mM each; (lane 2) dNTPs, 0.2 mM each + 0.02 mM **1TP**; (lane 3) dNTPs, 0.2 mM each + 0.04 mM **1TP**; (lane 4) dNTPs, 0.2 mM each + 0.08 mM **1TP**.
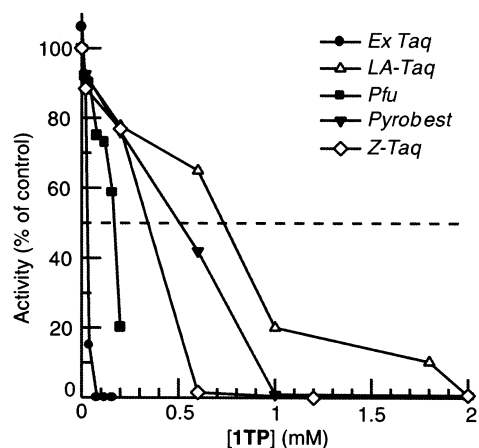
Figure 2. Effect of **1TP** on DNA polymerase reactions.

DNA strands. We then examined the influence of these β-C-nucleoside 5′-triphosphates on DNA syntheses in the presence of four regular substrates.

The artificial β-C-nucleoside 5′-triphophates **1TP−4TP** with one or two hydroxy groups showed inhibitory activities against DNA polymerases when added to PCR mixtures (Figures 2−5). In all the cases with inhibitory activities, the formation of a full-length product was reduced by the addition of β-C-nucleoside 5′-triphosphates **1TP−4TP** in a dose-dependent manner, whereas the quantity of unused primers was increased (for instance, see Figure 1, lanes 2−4). No products shorter than 180 bases were obtained, indicating that there is no specific attenuation position of DNA chain elongation along the template.

First, we measured the inhibitory activity of each β-C-nucleoside 5′-triphophate on DNA syntheses catalyzed by the five different species of DNA polymerases (Ex Taq, LA-Taq, Pfu, Pyrobest, and Z-Taq). With all the DNA polymerases examined, DNA syntheses were inhibited by **1TP** with two hydroxy groups (Figure 2). The level of inhibition was, however, independent of each DNA polymerase. For determination of the inhibitory activities, the assay was repeated at least twice for each enzyme, and the results were evaluated quantitatively as summarized in Table 1. The concentrations of **1TP** required to exhibit 50% inhibition (IC_{50}) ranged from 0.03 (Ex Taq) to 0.73 mM (LA-Taq). **1TP** acted as

Table 1. Comparative Inhibitory Activities of Synthetic 5′-Triphosphates (**1TP**−**5TP**) on DNA Polymerases

compd	IC$_{50}$^{a} (mM) DNA polymerases				
	Ex Taq	Pfu	LA-Taq	Z-Taq	Pyrobest
1TP	0.03	0.2	0.7	0.3	0.5
2TP	1.7	0.8	2.4	3.1	1.4
3TP	1.9	0.9	3.1	6.2	2.7
4TP	1.6	0.9	3.7	3.7	1.5
5TP	>16	>16	>16	>16	>16

^{a} IC$_{50}$: concentrations of substrates to exhibit 50% inhibition (mM).
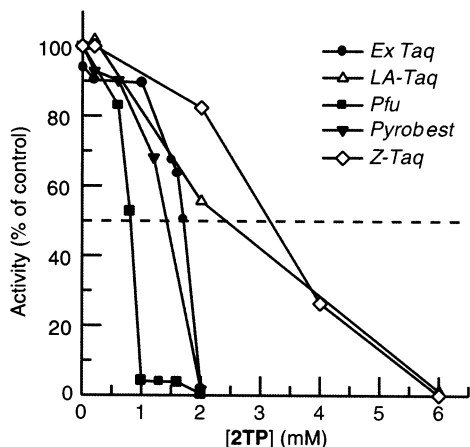
Figure 3. Effect of **2TP** on DNA polymerase reactions.

Figure 4. Effect of **3TP** on DNA polymerase reactions.

Figure 5. Effect of **4TP** on DNA polymerase reactions.

the most efficient inhibitor against Ex Taq DNA polymerase. The second strongest inhibitory effect was observed with Pfu, followed by Z-Taq, Pyrobest, and LA-Taq in this order. The IC$_{50}$ for LA-Taq with the lowest inhibitory effect was 30 times larger than that for Ex Taq with the highest inhibitory effect. The DNA amplification by Ex Taq was reduced to undetectable level when 80 μM **1TP** was added, while the other four polymerases retained more than 70% activity at the same concentrations.

To clarify the role of hydroxy groups of each substrate in the inhibitory activity, the effect of **2TP**, which has one hydroxy group at the 2-position, on PCR was first examined (Figure 3). PCR was carried out using the five DNA polymerases in the presence of various concentrations of **2TP** and a fixed concentration (0.2 mM) of regular nucleoside 5′-triphosphates (Figure 3). For all five polymerases used, the inhibition by **2TP** was less than that by **1TP**. The IC$_{50}$ of **2TP** ranged from 0.8 (Pfu) to 3.1 mM (Z-Taq) as shown in Table 1. **2TP** inhibited DNA synthesis by Pfu most efficiently. The second strongest inhibitory effect was observed with Pyrobest followed by Ex Taq, LA-Taq, and Z-Taq in this order. The IC$_{50}$ for Z-Taq with the lowest inhibitory effect was 4 times larger than that for Ex Taq with the highest inhibitory effect. The amplification activity by Pfu was reduced to less than 5% when 1 mM **2TP** was added, while the other four polymerases retained more than 80% activity.

The effect of **3TP**, which has one hydroxy group at the 3-position, on PCR using the five DNA polymerases was then examined in the presence of various concentrations of **3TP** (0−10.0 mM; Figure 4). The IC$_{50}$ of **3TP** ranged from 0.9 (Pfu) to 6.2 mM (Z-Taq) as shown in Table 1. As in the case of **2TP**, the second strongest
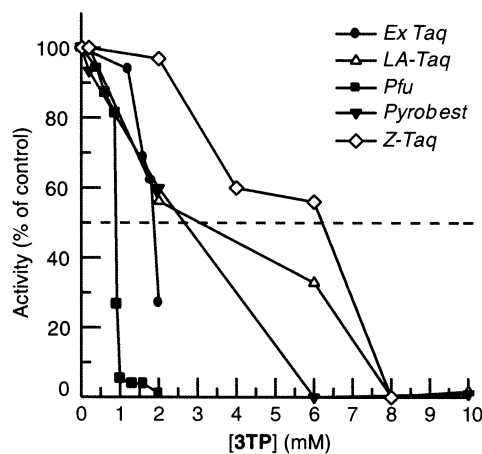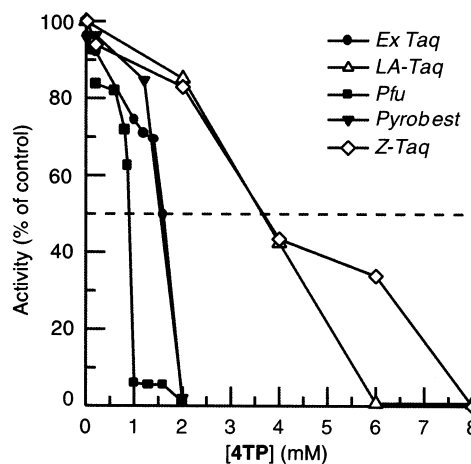
inhibitory effect was observed with Ex Taq followed by Pyrobest, LA-Taq, and Z-Taq in this order. The IC$_{50}$ for Z-Taq with the lowest inhibitory effect was 7 times larger than that for Ex Taq with the highest inhibitory effect. The amplification activity by Pfu was reduced to approximately 5% when 1 mM **3TP** was added, while the other four polymerases retained more than 80% activity.

DNA syntheses by the five DNA polymerases were also examined in the presence of various concentrations of **4TP** (0−8.0 mM) with one hydroxy group at the 4-position (Figure 5). The IC$_{50}$ of **4TP** ranged from 0.9 (Pfu) to 3.7 mM (Z-Taq) as shown in Table 1. Again, **4TP** acted as the most efficient inhibitor against DNA synthesis by Pfu. The second strongest inhibitory effect was observed with Pyrobest and Ex Taq followed by LA-Taq and Z-Taq. The IC$_{50}$ for LA-Taq and Z-Taq with the lowest inhibitory effect was 4 times larger than that for Pfu with the highest inhibitory effect. The DNA amplification by Pfu was reduced to approximately 5% when 1 mM **4TP** was added, while the other four polymerases retained more than 80% activity. On the basis of these results with **1TP** and three derivatives (**2TP**−**4TP**, with one hydroxy group at different positions (Figures 3−5)), we concluded that overall the inhibitory activity was strongest with **1TP** among the β-C-nucleoside 5′-triphosphates tested in this study. The three β-C-nucleoside 5′-triphosphates with one hydroxy group are weaker inhibitors compared with **1TP** which has two hydroxy groups at the 3- and 4-positions.
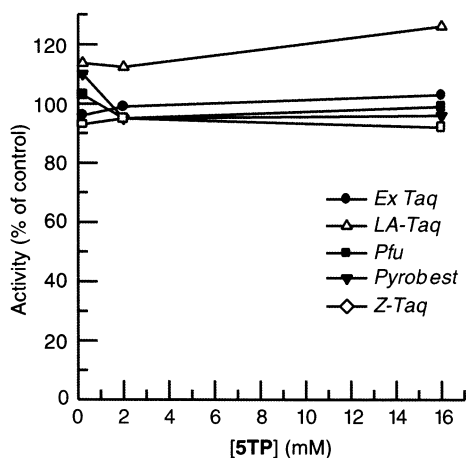
Figure 6. Effect of **5TP** on DNA polymerase reactions.

Figure 7. Comparative amplification activities for Z-Taq polymerase in the presence of **1TP** and/or excess concentration of natural nucleoside 5′-triphosphates: **1TP**, dNTPs 0.2 mM each + **1TP** 1 mM; **1TP** + T, dNTPs 0.2 mM each + **1TP** 1mM + dTTP 1 mM; **1TP** + G, dNTPs 0.2 mM each + **1TP** 1 mM + dGTP 1 mM; **1TP** + C, dNTPs 0.2 mM each + **1TP** 1 mM + dCTP 1 mM; **1TP** + A, dNTPs 0.2 mM each + **1TP** 1 mM + dATP 1 mM; T, dNTPs 0.2 mM each + dTTP 1 mM; G, dNTPs 0.2 mM each + dGTP 1 mM; C, dNTPs 0.2 mM each + dCTP 1 mM; A, dNTPs 0.2 mM each + dATP 1 mM.

As a control, we also examined the effect of **5TP** without hydroxy groups on DNA syntheses using the five DNA polymerases. As shown in Figure 6, however, the inhibition was not observed with **5TP** at least up to 16 mM. Owing to the solubility problem, we failed to test the effect of **5TP** at concentrations higher than 16 mM.

These results suggest that the hydroxy groups are essential for the inhibitory activities. The two hydroxy groups at the 3,4-positions of **1TP** are most likely to play a significant role in the inhibitory effect.[23] The two hydroxy groups may form two-point hydrogen bonding with some functional groups around the catalytic cavity of each DNA polymerase. In the cases of three 5′-triphosphates with one hydroxy group (**2TP**−**4TP**), however, the extent of inhibition was not correlated with the position of hydroxy group.

The present results indicate that the β-C-nucleoside 5′-triphophates **1TP**−**5TP** are not incorporated into DNA chains but are possibly recognized by the DNA polymerases, resulting in the inhibition of polymerization of the regular nucleoside 5′-triphosphate substrates. If this is the case, the inhibition could be overcome by the addition of a large excess of regular substrates. To test this possibility, PCR was carried out in the presence of a higher concentration (1.2 mM) of dATP, dTTP, dGTP, or dCTP, where the concentrations of the other three dNTPs were fixed at 0.2 mM. The DNA amplification by Z-Taq in the presence of 1 mM **1TP** significantly increased by the addition of 1 mM dTTP, dGTP, or dCTP (Figure 7). The most remarkable recovery was found with dTTP (48%) followed by dGTP (33%) and dCTP (14%), whereas the addition of dATP failed to recover the DNA synthesis activity by Z-Taq but rather reduced the amplification activity. The addition of 1 mM dATP, dTTP, dGTP, or dCTP in the absence of **1TP** showed essentially the same activity as that under the standard PCR condition. Since the level of recovery differed between the nucleotides, the competition activity of the β-C-nucleoside 5′-triphophate **1TP** appears to depend on the base species. Their competitive activity may arise from the difference in the degree of structural analogy of the nucleobase moieties.

In addition, it should be noted that nonnatural 5′-triphosphates **1TP**−**4TP** interact with the DNA polymerase complexes, resulting in the shutting-off of further elongation of the DNA strand by DNA poly-
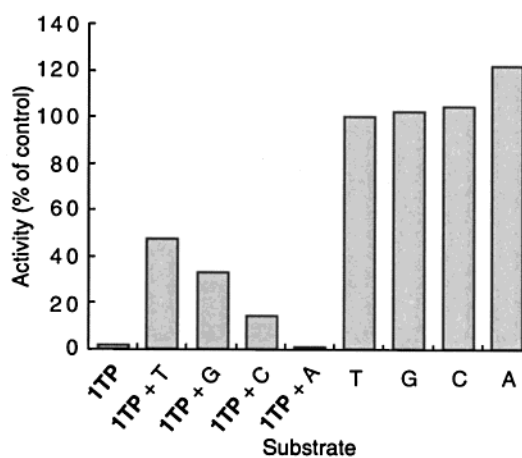
merases. The competitive inhibition of DNA synthesis indicates the possibility that each DNA polymerase forms a complex with nonnatural 5′-triphosphates (**1TP**−**4TP**), resulting in a reduction of the enzyme activity. The primary sequence of Taq polymerase used in this study is 38% identical to that of *Eschericia coli* DNA polymerase I, an enzyme that has served as a model for the study of DNA replication using enzymes of the DNA Pol I family. This sequence homology implies that **1TP**−**4TP** inhibit DNA polymerases other than those used in this study. It is also noteworthy that the nonnatural 5′-triphosphate **1TP** shows the inhibitory effect on RNA synthesis by *E. coli* RNA polymerase core enzyme (M. Shionoya et al., unpublished results).

Conclusion

The effect of five β-C-nucleoside 5′-triphosphates bearing a 3,4-dihydroxyphenyl (**1TP**), a 2-hydroxyphenyl (**2TP**), a 3-hydroxyphenyl (**3TP**), a 4-hydroxyphenyl (**4TP**), or a phenyl (**5TP**) group on DNA polymerase reactions was investigated. It was found that **1TP** with two hydroxy groups is a potent inhibitor against DNA syntheses by the five different DNA polymerases. A DNA amplification study using excess dNTPs indicated that **1TP** and dNTPs are recognized by these DNA polymerases but the level of inhibition differed among the five polymerases, presumably reflecting minor differences in their catalytic centers. Although blood samples are known as PCR inhibitors,[24] to our best knowledge, this is the first example of a chelate-type β-C-nucleoside 5′-triphosphate that shows potent inhibitory activity against DNA polymerases.[25] This finding may raise the appealing possibility that artificial nucleoside derivatives having phenolic hydroxy groups could exhibit potent inhibitory activity against DNA-directed enzymatic reactions. We will report elsewhere the template effect of the chelate-type β-C-nucleoside incorporated inside the DNA strands on DNA polymerase reactions.

Experimental Section

All reactions except for hydrogenation were carried out in oven-dried glassware under argon atmosphere. Trimethyl phosphate and tributylamine were distilled from BaO and CaH$_2$, respectively, under reduced pressure. Phosphorus oxychloride was distilled under atmospheric pressure. All other reagents were commercially available and used without further purification. All reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60, F-254. Column chromatography was conducted using Wakogel C-300 (silica gel, Wako). Purification via reversed-phase HPLC was performed with a TOSO TSK gel ODS-80Ts column (21.5 mm i.d. × 30 cm).

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX500 spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C), and ^{31}P NMR spectra were recorded on a JEOL AL270B (109 MHz). Chemical shifts (δ) are reported in ppm, and coupling constants (J) are reported in hertz. Mass spectra were recorded on an ESI-TOF mass spectrometer (LCT, Micromass) or a FAB mass spectrometer (JMS 700P, JEOL).

1,2-Dibenzyloxy-4-(2′-deoxy-β-D-ribofuranosyl)benzene (8). To a stirred solution of ribose **6** (595 mg, 2.0 mmol) in CH$_2$Cl$_2$ (1 mL) was added **7** (591 mg, 2.0 mmol). The mixture was cooled to −20 °C, and then SnCl$_4$ (600 µL, 2.0 mmol) was added dropwise to the reaction mixture over 10 min. The mixture was further stirred for 15 min before the reaction was quenched with water (4 mL). The reaction mixture was extracted with CH$_2$Cl$_2$ and washed with 1.0 M HCl aqueous solution and brine, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatograph with n-hexane−AcOEt (6:1). The title compound was obtained as a mixture of α- and β-anomers (357 mg).

Without separation, K$_2$CO$_3$ (50 mg) was added to the mixture (357 mg) in MeOH (5 mL), and the reaction mixture was then stirred for 3 h at room temperature. The mixture was extracted with CH$_2$Cl$_2$, and the organic phase was washed with water, dried over anhydrous MgSO$_4$, and evaporated. The crude product was chromatographed on silica gel with n-hexane−AcOEt (1:1). The title compound was obtained in 14% yield as a colorless solid. Its configuration was confirmed by X-ray analysis of a single crystal obtained by recrystallization from CHCl$_3$−AcOEt (for detailed data, see Supporting Information). Mp 105.0−106.0 °C. ^{1}H NMR (CDCl$_3$): δ 6.73−7.25 (13H, m, aromatic), 5.08 (4H, s, 2 × CH_2C$_6$H$_5$), 4.99 (1H, dd, J = 5.6, 9.8 Hz, H-1′), 4.28−4.34 (1H, m, H-3′), 3.88 (1H, ddd, J = 4.4, 4.4, 7.3 Hz, H-4′), 3.69 (1H, dd, J = 4.1, 10.1 Hz, H-5′), 3.62 (1H, dd, J = 6.6, 11.6 Hz, H-5′), 2.08 (1H, ddd, J = 1.9, 5.5, 13.3 Hz, H-2′), 1.90 (1H, ddd, J = 6.1, 10.0, 13.2 Hz, H-2′), 1.82 (1H, d, J = 6.3 Hz, OH), 1.75 (1H, d, J = 6.1 Hz, OH). Anal. Calcd for C$_{25}$H$_{26}$O$_5$: C, 73.87; H, 6.45. Found: C, 73.70; H, 6.36.

1-Hydroxy-2-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)benzene (12). To a stirred mixture of Cp$_2$HfCl$_2$ (2.4 g, 6.3 mmol), AgClO$_4$ (2.6 g, 13 mmol), phenol **11** (400 mg, 4.2 mmol), and powdered 4 Å molecular sieves (1.4 g) in CH$_2$Cl$_2$ (20 mL) was added 1,3,5-tri-O-acetyl-2-deoxyribofuranose **10** (546 mg, 2.1 mmol) in CH$_2$Cl$_2$ (30 mL) at −78 °C. The temperature was gradually raised to −40 °C over 2 h, and after the completion of initial O-glycosidation, the temperature was further raised to −5 °C over 2 h to convert to C-glycoside **12**. The reaction was quenched with saturated NaHCO$_3$ aqueous solution, and then the mixture was extracted with CH$_2$Cl$_2$. The organic phase was washed with saturated NaHCO$_3$ aqueous solution and brine, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatograph with n-hexane−AcOEt (30:1). The title compound **12** was obtained in 34% yield as a pale-yellow oil. ^{1}H NMR (CDCl$_3$): δ 7.60 (1H, s, C$_6$H$_4$OH), 7.19 (1H, t, J = 8.0 Hz, aromatic), 7.05 (1H, d, J = 7.6 Hz, aromatic), 6.87 (1H, d, J = 8.1 Hz, aromatic), 6.85 (1H, t, J = 7.4 Hz, aromatic), 5.19−5.23 (2H, m, H-1′ and H-3′), 4.42 (1H, dd, J = 3.3, 12.0 Hz, H-5′), 4.34 (1H, dd, J = 3.2, 12.0 Hz, H-5′), 4.24 (1H, ddd, J = 3.2, 3.2, 5.7 Hz, H-4′), 2.30−2.34 (2H, m, H-2′), 2.18 (3H, s, COCH_3), 2.15 (3H, s, COCH_3). ^{13}C NMR (CDCl$_3$): δ 170.65, 170.60, 155.2, 129.4, 127.4, 123.3, 120.0, 117.1, 83.4, 81.6, 76.0, 63.7, 39.4, 20.9, 20.6. ESI-TOF mass, m/z: found, 317.0985; calcd, [M + Na]$^+$ 317.1001.

1-Hydroxy-2-(2′-deoxy-β-D-ribofuranosyl)benzene (2). To a stirred solution of **12** (110 mg, 0.38 mmol) in MeOH (4 mL) was added dropwise 28% NH$_3$ aqueous solution (1 mL). The reaction mixture was stirred for 5 h at room temperature. The solvent was removed in vacuo, and the residue was chromatographed on silica gel with n-hexane−AcOEt−MeOH (25:5:1). Compound **2** was obtained in 76% yield as a colorless solid. Its configuration was confirmed by X-ray analysis of a single crystal obtained by recrystallization from CHCl$_3$−AcOEt (for detailed data, see Supporting Information). Mp 135.5−136.5 °C. ^{1}H NMR (D$_2$O−CD$_3$OD = 1:1): δ 7.26 (1H, d, J = 7.6 Hz, aromatic), 7.08 (1H, t, J = 7.6 Hz, aromatic), 6.82 (1H, t, J = 7.6 Hz, aromatic), 6.77 (d, 1H, J = 7.9 Hz, aromatic), 5.30 (1H, dd, J = 5.5, 10.7 Hz, H-1′), 4.30−4.38 (1H, m, H-3′), 3.92 (1H, ddd, J = 2.4, 4.6, 4.6 Hz, H-4′), 3.75−3.83 (2H, m, H-5′), 2.18 (1H, ddd, J = 1.5, 5.5, 13.4 Hz, H-2′), 1.95 (1H, ddd, J = 5.5, 10.7, 13.4 Hz, H-2′). ^{13}C NMR (D$_2$O−CD$_3$OD = 1:1): δ 158.6, 132.6, 131.5, 129.7, 123.4, 119.7, 91.5, 83.2, 77.2, 66.3, 45.5. Anal. Calcd for C$_{11}$H$_{14}$O$_4$: C, 62.85; H, 6.71. Found: C, 62.79; H, 6.71.

1-Benzyloxy-3-[3′,5′-O-((1,1,3,3)-tetraisopropyl)disiloxandiyl)-2′-deoxy-β-D-ribofuranosyl]benzene (15). To a solution of 3-benzyloxybromobenzene (262 mg, 1.0 mmol) in anhydrous THF (2.5 mL) under argon atmosphere was added n-BuLi (0.6 mL, 1.6 M solution in n-hexane, 0.96 mmol) at −78 °C, and the reaction mixture was further stirred for 30 min at −78 °C. A solution of **13** (220 mg, 0.59 mmol) in anhydrous THF (2.5 mL) was added to the mixture at −78 °C. After 1 h, the reaction mixture was quenched with saturated NH$_4$Cl aqueous solution and extracted with CH$_2$Cl$_2$. The organic phase was washed with saturated NH$_4$-Cl aqueous solution, water, and brine, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The resulting yellowish oil was used for the next reaction without further purification.

A solution of the crude oil in CH$_2$Cl$_2$ (2.5 mL) was treated with Et$_3$SiH (3 equiv) and BF$_3$·OEt$_2$ (3 equiv) at −78 °C under an argon atmosphere. The resulting solution was stirred for 6 h at −78 °C, and the reaction was quenched with saturated NaHCO$_3$ aqueous solution. The resulting mixture was extracted with CH$_2$Cl$_2$, and the organic phase was then washed with saturated NaHCO$_3$ aqueous solution, water, and brine, dried over anhydrous MgSO$_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatography with n-hexane−AcOEt (20:1), and the desired product **15** was obtained as a colorless oil in 35% yield. ^{1}H NMR (CDCl$_3$): δ 7.43 (2H, d, J = 7.0 Hz, CH$_2$C$_6$H$_5$), 7.38 (2H, t, J = 7.0 Hz, CH$_2$C$_6$H$_5$), 7.32 (1H, d, J = 7.0 Hz, C$_6$H$_4$), 7.24 (1H, t, J = 7.9 Hz, CH$_2$C$_6$H$_5$), 6.98 (1H, s, C$_6$H$_4$), 6.93 (1H, d, J = 7.6 Hz, C$_6$H$_4$), 6.86 (1H, dd, J = 2.4, 8.2 Hz, C$_6$H$_4$), 5.01−5.04 (1H, m, H-1′), 5.05 (2H, s, CH_2C$_6$H$_5$), 4.52 (1H, dt, J = 4.5, 7.6 Hz, H-3′), 4.14 (1H, dd, J = 3.0, 11.0 Hz, H-5′), 3.84−3.96 (2H, m, H-4′ and H-5′), 2.35 (1H, ddd, J = 4.5, 6.7, 12.8 Hz, H-2′), 2.07 (1H, dt, J = 7.9, 12.8 Hz, H-2′), 0.92−1.15 (28H, m, 4 × CH(CH_3)$_2$). ^{13}C NMR (CDCl$_3$): δ 158.9, 143.8, 137.0, 129.5, 128.6, 127.9, 127.6, 118.5, 113.7, 112.4, 86.5, 78.9, 73.5, 69.9, 63.8, 43.2, 17.6, 17.5, 17.44, 17.3, 17.16, 17.12, 17.02, 13.54, 13.44, 13.06, 12.59. HRMS (ESI-TOF) calcd for C$_{30}$H$_{46}$O$_5$Si$_2$Na 565.2781, found 565.2772.

1-Benzyloxy-3-(2′-deoxy-β-D-ribofuranosyl)benzene (16). To a stirred mixture of **15** (227 mg, 0.42 mmol) in anhydrous THF (8.4 mL) was added dropwise n-Bu$_4$NF (1.3 mL, 1.0 M solution in anhydrous THF, 1.3 mmol). The resulting mixture was stirred for 2 h at room temperature, and saturated NaHCO$_3$ and 10% NaOH aqueous solutions were added to the reaction mixture for quenching. The mixture was extracted with Et$_2$O, and the organic extract was washed with saturated NaHCO$_3$ and 10% NaOH aqueous solutions, water, and brine. The combined organic layer was dried over anhydrous MgSO$_4$ and concentrated in vacuo. The crude product was purified by silica gel column chromatography with CH$_2$Cl$_2$−MeOH (19:

1). The desired product **16** was obtained as a colorless solid in 83% yield. Its configuration was confirmed by X-ray analysis of a single crystal obtained by recrystallization from $CHCl_3$–AcOEt (for detailed data, see Supporting Information). Mp 97.5–98.5 °C. ^1H NMR ($CDCl_3$–CD_3OD = 1:1): δ 7.41 (2H, d, J = 7.0 Hz, $CH_2C_6H_5$), 7.37 (2H, t, J = 7.0 Hz, $CH_2C_6H_5$), 7.32 (1H, d, J = 7.1 Hz, C_6H_4), 7.23 (1H, t, J = 7.9 Hz, $CH_2C_6H_5$), 6.98 (1H, s, C_6H_4), 6.90 (1H, d, J = 7.8 Hz, C_6H_4), 6.88 (1H, dd, J = 2.7, 8.2 Hz, C_6H_4), 5.12 (1H, dd, J = 5.7, 10.1 Hz, H-1′), 5.04 (2H, s, C$H_2C_6H_5$), 4.33 (1H, dt, J = 2.2, 6.3 Hz, H-3′), 3.97 (1H, ddd, J = 4.9, 4.9, 3.0 Hz, H-4′), 3.68 (2H, d, J = 5.0 Hz, H-5′), 2.21 (1H, ddd, J = 1.9, 5.7, 13.3 Hz, H-2′), 1.95 (1H, ddd, J = 6.3, 10.2, 13.3 Hz, H-2′). ^{13}C NMR ($CDCl_3$): δ 158.9, 142.9, 136.9, 129.6, 128.6, 127.9, 127.5, 118.6, 113.8, 112.7, 87.3, 79.9, 73.4, 69.9, 63.3, 50.6, 43.5. Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.70; H, 6.65.

1-Benzyloxy-4-(3′,5′-di-*O*-toluoyl-2′-deoxy-α-D-ribofuranosyl)benzene (20). To a solution of finely dried magnesium turnings (500 mg, 21 mmol) and a few crystals of iodine in anhydrous THF (1 mL) was added dropwise 4-benzyloxylbromobenzene (5.0 g, 19 mmol) in THF (19 mL) under an argon atmosphere. The reaction mixture was required to be heated at about 40 °C for initiation. The concentration of the Grignard reagent was determined to be 0.45 M by the acid–base titration.

To finely dried $ZnCl_2$ (497 mg, 3.7 mmol) was added the Grignard reagent (13 mL, 0.45 M solution in anhydrous THF, 6.0 mmol) under an argon atmosphere. After heating for 3 h at 80 °C, the reaction mixture was cooled to room temperature. A solution of 1′-α-chrolo-3′,5′-di-*O*-toluoyl-2′-deoxyribose **18** (970 mg, 2.5 mmol) in anhydrous THF (15 mL) was introduced into the reaction mixture at room temperature, which was further heated at reflux for 5 h. The reaction was quenched with distilled water, and then the solvent was removed in vacuo. The residue was taken up into AcOEt, and the organic layer was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatograph with *n*-hexane–AcOEt (20:1). The major α-anomer **20** and the minor β-anomer **21** were obtained as a pale-yellow oil in 36% and 5% isolated yields, respectively.

20. ^1H NMR ($CDCl_3$): δ 7.96 (2H, d, J = 8.2 Hz, $C_6H_4CH_3$), 7.76 (2H, d, J = 8.2 Hz, $C_6H_4CH_3$), 7.42 (2H, d, J = 7.5 Hz, $CH_2C_6H_5$), 7.34–7.38 (4H, m, aromatic), 7.30 (1H, t, J = 7.3 Hz, $CH_2C_6H_5$), 7.21 (2H, d, J = 7.9 Hz, aromatic), 7.17 (2H, d, J = 8.0 Hz, aromatic), 6.96 (2H, d, J = 8.7 Hz, C_6H_4), 5.58 (1H, ddd, J = 3.5, 3.9, 7.1 Hz, H-3′), 5.29 (1H, dd, J = 6.8, 6.8 Hz, H-1′), 5.04 (2H, s, C$H_2C_6H_5$), 4.66 (1H, ddd, J = 4.6, 4.6, 6.9 Hz, H-4′), 4.58 (1H, dd, J = 5.1, 11.7 Hz, H-5′), 4.54 (1H, dd, J = 4.5, 11.5 Hz, H-5′), 2.89 (1H, ddd, J = 6.8, 7.1, 13.6 Hz, H-2′), 2.38 (3H, s, $C_6H_4CH_3$), 2.37 (3H, s, $C_6H_4CH_3$), 2.27 (1H, ddd, J = 4.4, 6.4, 13.6 Hz, H-2′).

21. ^1H NMR ($CDCl_3$): δ 7.98 (2H, d, J = 8.1 Hz, $C_6H_4CH_3$), 7.94 (2H, d, J = 8.1 Hz, $C_6H_4CH_3$), 7.21–7.42 (11H, m, aromatic), 6.93 (2H, d, J = 8.7 Hz, C_6H_4), 5.58–5.63 (1H, m, H-3′), 5.20 (1H, dd, J = 4.9, 10.2 Hz, H-1′), 5.05 (2H, s, C$H_2C_6H_5$), 4.62–4.68 (2H, m, H-5′), 4.50–4.55 (1H, m, H-4′), 2.4–2.5 (1H, m, H-2′), 2.43 (3H, s, $C_6H_4CH_3$), 2.40 (3H, s, $C_6H_4CH_3$), 2.23 (1H, ddd, J = 6.1, 11.0, 13.9 Hz, H-2′). HRMS (ESI-TOF) calcd for $C_{34}H_{32}O_6Na$ 559.2099, found 559.2099.

1-Benzyloxy-4-(3′,5′-di-*O*-toluoyl-2′-deoxy-β-D-ribofuranosyl)benzene (21). To a stirred solution of **20** (965 mg, 1.8 mmol) in toluene (40 mL) were added a catalytic amount of benzenesulfonic acid (10%), two drops of concentrated H_2SO_4, and seven drops of H_2O. The reaction mixture was heated at reflux for 2 h. The mixture was then poured into 5% $NaHCO_3$ aqueous solution (100 mL) and extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product was purified by silica gel chromatography eluting with *n*-hexane–AcOEt (20:1). The desired product **21** was obtained as a pale-yellow oil in 39% yield.

1-Benzyloxy-4-(2′-deoxy-β-D-ribofuranosyl)benzene (22). Compound **21** (19 mg, 0.035 mmol) was dissolved in a mixture

of anhydrous MeOH (1 mL) and $CHCl_3$ (0.5 mL) and treated with 28% MeOH solution of NaOMe (80 μL). The reaction mixture was stirred for 1.5 h at room temperature under an argon atmosphere, and then the reaction was quenched with solid NH_4Cl. After filtration, the solvent was removed in vacuo. The residue was extracted with AcOEt, and then the organic phase was washed with saturated $NaHCO_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel column chromatograph with Et_2O to Et_2O–EtOH (75:1). The product **22** was obtained as a colorless solid in 76% yield. Its configuration was confirmed by X-ray analysis of a single crystal obtained by recrystallization from $CHCl_3$–AcOEt (for detailed data, see Supporting Information). Mp 113.0–114.0 °C. ^1H NMR ($CDCl_3$–CD_3OD = 1:1): δ 7.42 (2H, d, J = 7.1 Hz, $CH_2C_6H_5$), 7.38 (2H, t, J = 7.7 Hz, $CH_2C_6H_5$), 7.32 (1H, d, J = 7.2 Hz, $CH_2C_6H_5$), 7.27 (2H, d, J = 8.7 Hz, C_6H_4), 6.96 (2H, d, J = 8.7 Hz, C_6H_4), 5.12 (1H, dd, J = 5.6, 10.2 Hz, H-1′), 5.06 (2H, s, C$H_2C_6H_5$), 4.39–4.43 (1H, m, H-3′), 3.98 (1H, ddd, J = 4.4, 4.4, 6.6 Hz, H-4′), 3.81 (1H, dd, J = 3.8, 11.5 Hz, H-5′), 3.72 (1H, dd, J = 4.3, 11.3 Hz, H-5′), 2.20 (1H, ddd, J = 1.9, 5.6, 13.3 Hz, H-2′), 2.04 (1H, ddd, J = 6.3, 10.2, 13.2 Hz, H-2′). ^{13}C NMR ($CDCl_3$–CD_3OD = 1:1): δ 158.4, 136.9, 133.1, 128.6, 127.9, 127.47, 127.41, 114.9, 87.1, 79.8, 73.8, 70.0, 63.3, 43.8. Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.66; H, 6.73.

1,2-Dibenzyloxy-4-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (9). Compound **8** (85 mg, 0.21 mmol) and 1,8-bis(dimethylamino)naphthalene (proton sponge, 73 mg, 0.34 mmol) were dissolved in trimethyl phosphate (1.7 mL), and the mixture was cooled to 0 °C. After addition of phosphorus oxychloride (23 μL, 0.25 mmol) dropwise, the solution was stirred for 3 h at 0 °C. A solution of tributylamine (330 μL, 1.4 mmol) and tributylammonium pyrophosphate (132 mg, 0.39 mmol) in dry DMF (2.3 mL) was added to the reaction mixture, and the solution was stirred for 1 min and then quenched with 1.0 M triethylammonium bicarbonate (TEAB, 33 mL, pH 8.5). After standing for 2.5 h at room temperature, the reaction mixture was concentrated by lyophilization to approximately 2 mL. Purification by RP-HPLC (30–46% CH_3-CN gradient over 50 min at a flow rate of 4 mL/min in 0.10 M TEAB) yielded **9** as triethylammonium salts (285 mg) with a retention time of 40 min. ESI-TOF mass, *m/z*: found, 644.90; calcd, $[M + 3H]^-$ 645.07. The triethylammonium salts were dissolved in water (1 mL), and then $NaClO_4$ (255 mg, 2.1 mmol) was added. After the mixture was stirred overnight, acetone (15 mL) was added to the reaction mixture. The resulting precipitate was collected by centrifugation, and the sodium salts **9** were obtained as a colorless solid in 40% yield. ^1H NMR (D_2O): δ 7.25–7.34 (10H, m, aromatic), 6.88–7.00 (3H, m, aromatic), 5.09 (2H, s, C$H_2C_6H_5$), 5.05 (1H, dd, J = 5.4, 10.6 Hz, H-1′), 4.38–4.42 (1H, m, H-3′), 4.03–4.08 (1H, m, H-4′), 3.88–4.02 (2H, m, H-5′), 2.04 (1H, ddd, J = 5.5, 5.9, 13.5 Hz, H-2′), 1.93 (1H, ddd, J = 5.8, 10.7, 13.6 Hz, H-2′). ^{31}P NMR (D_2O): δ −10.3 (1P, d, J = 18.3 Hz), −11.1 (1P, d, J = 18.3 Hz), −22.9 (1P, t, J = 18.3 Hz) (external reference, 20 mM H_3PO_4 in D_2O). Anal. Calcd for $C_{25}H_{26}Na_3O_{14}P_3$·$3H_2O$: C, 39.18; H, 4.21. Found: C, 39.41; H, 4.19.

1,2-Dihydroxy-4-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (1TP). Compound **9** (31 mg, 0.042 mmol) was dissolved in distilled water (4 mL), and Pd–C (36 mg, 10% Pd) was added to the reaction mixture. The suspended mixture was stirred vigorously for 4 h under an H_2 atmosphere. After filtration and removal of the solvent, **1TP** was obtained quantitatively as a colorless solid. ^1H NMR (D_2O): δ 7.01–7.03 (1H, m, C_6H_4), 6.81–6.83 (3H, m, C_6H_4), 5.02 (1H, dd, J = 6.6, 8.9 Hz, H-1′), 4.47–4.52 (1H, m, H-3′), 4.02–4.12 (3H, m, H-4′ and H-5′), 2.07–2.14 (2H, m, H-2′). ^{31}P NMR (D_2O): δ −9.28 (1P, d, J = 21.3 Hz), −10.87 (1P, d, J = 18.3 Hz), −22.68 (1P, dd, J = 21.3, 18.3 Hz) (external reference, 20 mM H_3PO_4 in D_2O). HRMS (FAB) calcd for $C_{11}H_{15}O_{14}P_3Na$ 486.9572, found 486.9565.

1-Hydroxy-2-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (2TP). Compound **2** (88 mg, 0.42 mmol) and

proton sponge (288 mg, 1.3 mmol) were dissolved in trimethyl phosphate (1.5 mL), and the mixture was cooled to 0 °C. After addition of phosphorus oxychloride (77 μL, 0.84 mmol) dropwise in trimethyl phosphate (1.5 mL), the solution was stirred for 3 h at 0 °C. A solution of tributylamine (1.3 mL, 5.6 mmol) and tributylammonium pyrophosphate (530 mg, 0.98 mmol) in dry DMF (4.7 mL) was added to the reaction mixture, and the solution was stirred for 1 min and then quenched with 1.0 M TEAB (70 mL, pH 8.5). After standing for 1 h at room temperature, the reaction mixture was lyophilized to dryness. Purification by RP-HPLC (10−25% CH$_3$CN gradient over 1 h at a flow rate of 5 mL/min in 0.10 M TEAB) yielded **2TP** as triethylammonium salts with a retention time of 26 min. ESI-TOF mass, m/z 449.0 (M + 3H)$^-$. The obtained salts were dissolved in distilled water (1 mL), and NaClO$_4$ (403 mg, 3.3 mmol) was added to the solution. After the mixture was stirred for 3 h, acetone (20 mL) was added to the solution. The resulting precipitate was collected by centrifugation, washed with acetone, and dried in vacuo. The sodium salts **2TP** were obtained as a colorless solid in 11% yield. ^{1}H NMR (D$_2$O): δ 7.33 (1H, d, J = 7.7 Hz, C$_6$H$_4$), 7.10 (1H, t, J = 7.8 Hz, C$_6$H$_4$), 6.86 (1H, t, J = 7.5 Hz, C$_6$H$_4$), 6.76 (1H, d, J = 8.1 Hz, C$_6$H$_4$), 5.32 (1H, dd, J = 5.5, 10.4 Hz, H-1′), 4.41−4.45 (1H, m, H-3′), 4.03−4.06 (1H, m, H-4′), 3.93−4.02 (2H, m, H-5′), 2.14 (1H, ddd, J = 1.9, 5.5, 13.5 Hz, H-2′), 2.03 (1H, ddd, J = 6.0, 11.0, 14.0 Hz, H-2′). ^{31}P NMR (D$_2$O): δ −10.1 (1P, d, J = 15.2 Hz), −11.0 (1P, d, J = 18.3 Hz), −22.75 (1P, dd, J = 15.3, 18.3 Hz) (external reference, 20 mM H$_3$PO$_4$ in D$_2$O). Anal. Calcd for C$_{11}$H$_{14}$Na$_3$O$_{13}$P$_3$·3H$_2$O: C, 23.17; H, 3.54. Found: C, 23.28; H, 3.62. HRMS (FAB) calcd for C$_{11}$H$_{15}$O$_{13}$P$_3$Na 470.9623, found 470.9627.

1-Benzyloxy-3-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (17). Compound **16** (39 mg, 0.18 mmol) and proton sponge (60 mg, 0.28 mmol) were dissolved in trimethyl phosphate (1.4 mL) and cooled to 0 °C. After addition of phosphorus oxychloride (19 μL, 0.21 mmol) dropwise in trimethyl phosphate (0.5 mL), the solution was stirred for 3 h at 0 °C. A solution of tributylamine (278 μL, 1.2 mmol) and tributylammonium pyrophosphate (192 mg, 0.35 mmol) in dry DMF (2 mL) was added to the reaction mixture, and the solution was stirred for 1 min. Then the reaction was quenched by addition of 1.0 M TEAB (30 mL, pH 8.5). After standing for 30 min at room temperature, the reaction mixture was lyophilized to dryness. Purification by RP-HPLC (0−50% CH$_3$-CN gradient over 50 min at a flow rate of 5 mL/min in 0.10 M TEAB) yielded compound **17** with a retention time of 51 min. ESI-TOF mass, m/z: found, 538.8; calcd, [M + 3H]$^-$ 539.0.

The salts thus obtained were dissolved in distilled water (1 mL), and NaClO$_4$ (161 mg, 1.3 mmol) was added to the solution. After the mixture was stirred for 2 h, acetone (20 mL) was added to the reaction mixture. The resulting precipitate was collected by centrifugation, washed with acetone, and dried in vacuo. The sodium salts **17** were obtained as a colorless solid in 11% yield. ^{1}H NMR (D$_2$O): δ 7.38 (2H, d, J = 7.2 Hz, CH$_2$C$_6$H$_5$), 7.33 (2H, t, J = 7.2 Hz, CH$_2$C$_6$H$_5$), 7.29 (1H, d, J = 7.2 Hz, C$_6$H$_4$), 7.23 (1H, t, J = 5.5 Hz, CH$_2$C$_6$H$_5$), 6.96−7.02 (2H, m, C$_6$H$_4$), 6.86−6.90 (1H, m, C$_6$H$_4$), 5.07 (2H, s, CH$_2$C$_6$H$_5$), 5.03 (1H, dd, J = 5.4, 10.5 Hz, H-1′), 4.41−4.47 (1H, m, H-3′), 4.05−4.12 (1H, m, H-4′), 3.95−4.05 (2H, m, H-5′), 2.13 (1H, dd, J = 5.6, 13.6 Hz, H-2′), 1.96−2.04 (1H, m, H-2′). ^{31}P NMR (D$_2$O): δ −8.89 to −9.17 (1P, m), −10.8 (1P, d, J = 18.3 Hz), −22.06 to −22.38 (1P, m) (external reference, 20 mM H$_3$PO$_4$ in D$_2$O). Anal. Calcd for C$_{18}$H$_{19}$Na$_4$O$_{13}$P$_3$·4H$_2$O: C, 30.70; H, 4.09. Found: C, 30.70; H, 4.09.

1-Hydroxy-3-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (3TP). To a stirred solution of **17** (18 mg, 0.092 mmol) in a mixture of distilled water (1 mL) and MeOH (1 mL), Pd−C (30 mg, Pd 10%) was added. The suspended mixture was stirred overnight under an H$_2$ atmosphere. After filtration and removal of the solvent, **3TP** was produced quantitatively as a colorless solid. ^{1}H NMR (D$_2$O): δ 7.13−7.18 (1H, m, C$_6$H$_4$), 6.82−6.94 (2H, m, C$_6$H$_4$), 6.68−6.72 (1H, m, C$_6$H$_4$), 5.00 (1H, dd, J = 4.9, 10.1 Hz, H-1′), 4.41 (1H, m, H-3′), 4.02 (1H, m, H-4′), 4.00 (1H, m, H-5′), 3.88 (1H, m, H-5′),

2.09−2.15 (1H, m, H-2′), 2.00 (1H, ddd J = 1.2, 5.5, 10.7 Hz, H-2′). HRMS (FAB) calcd for C$_{11}$H$_{13}$O$_{13}$P$_3$Na$_3$ 514.9262, found 514.9252.

1-Benzyloxy-4-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (23). Compound **22** (58 mg, 0.19 mmol) and proton sponge (66 mg, 0.31 mmol) were dissolved in trimethyl phosphate (2.0 mL) and cooled to 0 °C. After addition of phosphorus oxychloride (21 μL, 0.23 mmol) in trimethyl phosphate (1 mL) dropwise, the solution was stirred for 3 h at 0 °C. A solution of tributylamine (310 μL, 1.3 mmol) and tributylammonium pyrophosphate (122 mg, 0.22 mmol) in dry DMF (2.0 mL) was added to the reaction mixture, and the solution was stirred for 1 min and then quenched by addition of 1.0 M TEAB (30 mL, pH 8.5). After standing for 1.5 h at room temperature, the reaction mixture was lyophilized to dryness. Purification by RP-HPLC (20−55% CH$_3$CN gradient over 60 min at a flow rate of 5 mL/min in 0.10 M TEAB) yielded **23** as triethylammonium salts with a retention time of 38 min. ESI-TOF mass, m/z 539.1 (M + 3H)$^-$, 561.1 (M + Na + 2H)$^-$.

The salts thus obtained were dissolved in distilled water (1 mL), and NaClO$_4$ (150 mg, 1.2 mmol) was added to the solution. After the mixture was stirred for 2 h, acetone (20 mL) was added to the reaction mixture. The resulting precipitate was collected by centrifugation, washed with acetone, and dried in vacuo. The sodium salts **23** were obtained as a colorless solid in 15% yield. ^{1}H NMR (D$_2$O): δ 7.36 (2H, d, J = 7.5 Hz, CH$_2$C$_6$H$_5$), 7.24−7.32 (5H, m, aromatic), 6.93 (2H, d, J = 8.4 Hz, aromatic), 5.02 (2H, s, CH$_2$C$_6$H$_5$), 4.99 (1H, dd, J = 7.5, 8.7 Hz, H-1′), 4.42−4.45 (1H, m, H-3′), 4.00−4.04 (1H, m, H-4′), 3.92−3.98 (2H, m, H-5′), 2.03−2.06 (2H, m, H-2′). ^{31}P NMR (D$_2$O): δ −9.08 and −9.81 (1P, m), −11.00 (1P, d, J = 18.3 Hz), −22.28 and −23.03 (1P, m) (external reference, 20 mM H$_3$PO$_4$ in D$_2$O). Anal. Calcd for C$_{18}$H$_{20}$Na$_3$O$_{13}$P$_3$·4H$_2$O: C, 31.87; H, 4.16. Found: C, 31.35; H, 3.99.

1-Hydroxy-4-(2′-deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (4TP). Compound **23** (25 mg, 0.028 mmol) was dissolved in a mixture of distilled water (1 mL) and MeOH (1 mL), and then Pd−C (30 mg, 10% Pd) was added to the reaction mixture. The suspended mixture was stirred overnight under an H$_2$ atmosphere. After filtration and removal of the solvent, **4TP** was obtained quantitatively as a colorless solid. ^{1}H NMR (D$_2$O): δ 7.27 (2H, d, J = 8.5 Hz, C$_6$H$_4$), 6.81 (2H, d, J = 8.5 Hz, C$_6$H$_4$), 5.02 (1H, t, J = 8.2 Hz, H-1′), 4.45−4.49 (1H, m, H-3′), 4.04−4.08 (1H, m, H-4′), 3.94−4.03 (2H, m, H-5′), 2.07−2.11 (2H, m, H-2′). ^{31}P NMR (D$_2$O): δ −9.11 (1P, d, J = 18.3 Hz), −10.9 (1P, d, J = 18.3 Hz), −22.4 (1P, t, J = 18.3 Hz) (external reference, 20 mM H$_3$PO$_4$ in D$_2$O). FAB mass, m/z: found, 470.9612, 492.9494, 514.9336; calcd, [M + Na + 2H]$^-$ 470.9623, [M + 2Na + H]$^-$ 492.9443, [M + 3Na]$^-$ 514.9262.

(2′-Deoxy-β-D-ribofuranosyl)benzene 5′-Triphosphate (5TP). Compound **5** was prepared via the procedure reported by Kool et al.[21] The β-configuration of **5** was confirmed by X-ray analysis (see Supporting Information). Compound **5** (48 mg, 0.25 mmol) and proton sponge (84 mg, 0.39 mmol) were dissolved in trimethyl phosphate (2.0 mL), and the mixture was cooled to 0 °C. After addition of phosphorus oxychloride (27 μL, 0.30 mmol) dropwise in trimethyl phosphate (1 mL), the solution was stirred for 3 h at 0 °C. A solution of tributylamine (390 μL, 1.6 mmol) and tributylammonium pyrophosphate (268 mg, 0.49 mmol) in dry DMF (2.7 mL) was added to the reaction mixture, and the solution was stirred for 1 min. Then the reaction was quenched with the addition of 1.0 M TEAB (39 mL, pH 8.5). After standing for 1 h at room temperature, the reaction mixture was concentrated in vacuo and chromatographed on a DEAE−Sephadex A25 with a gradient of TEAB (0.10−1.0 M, pH 7.5). The appropriate fractions were collected and concentrated in vacuo. Purification by RP-HPLC (0−15% CH$_3$CN gradient over 30 min and fixed to 15% for an additional 15 min at a flow rate of 5 mL/min in 0.10 M TEAB) yielded **5TP** as triethylammonium salts with a retention time of 43 min. ESI-TOF mass, m/z: found, 433.0, 455.0; calcd, [M + 3H]$^-$ 433.0, [M + Na + 2H]$^-$ 455.0.

The obtained residue was dissolved in distilled water (1 mL), and $NaClO_4$ (170 mg, 1.4 mmol) was added to the solution. After the mixture was stirred for 2 h, addition of acetone (20 mL) resulted in precipitation. The precipitate was collected by centrifugation, washed with acetone, and dried in vacuo. The sodium salts **5TP** were obtained as a colorless solid in 25% yield. ^1H NMR (D_2O): δ 7.31−7.36 (2H, m, C_6H_5), 7.28 (2H, t, $J = 7.6$ Hz, C_6H_5), 7.22 (1H, t, $J = 7.3$ Hz, C_6H_5), 5.03 (1H, dd, $J = 5.8, 10.0$ Hz, H-1′), 4.43−4.47 (1H, m, H-3′), 4.20−4.34 (1H, m), 4.02−4.12 (2H, m, H-5′), 3.89−3.99 (1H, m), 2.01−2.13 (2H, m, H-2′). ^{31}P NMR (D_2O): δ −5.55 (1P, d, $J = 21.3$ Hz), −10.6 (1P, d, $J = 18.3$ Hz), −21.4 (1P, dd, $J = 18.3, 21.3$ Hz) (external reference, 20 mM H_3PO_4 in D_2O). Anal. Calcd for $C_{11}H_{14}Na_3O_{12}P_3 \cdot 4H_2O$: C, 23.09; H, 3.88. Found: C, 23.12; H, 3.99.

PCR Amplification Conditions. Five DNA polymerases were examined for PCR amplification: TaKaRa Ex Taq (TaKaRa Shuzo, Japan); native Pfu polymerase from *Pyrococcus furiosus* (Stratagene, CA); TaKaRa Z-Taq (TaKaRa Shuzo); TaKaRa LA-Taq (TaKaRa Shuzo); Pyrobest DNA polymerase (TaKaRa Shuzo) from *Pyrococcus sp.* PCR amplifications were carried out under the optimum conditions for each polymerase as suggested by the manufactures: in 1 × Ex Taq buffer containing 2 mM Mg^{2+} and a 0.2 mM concentration of each deoxynucleoside 5′-triphosphate (dNTP) with Ex Taq; in native Pfu buffer containig 2 mM Mg^{2+} and a 0.2 mM concentration of each dNTP with Pfu; in 1 × Z-Taq buffer containing 3 mM Mg^{2+} and a 0.2 mM concentration of each dNTP with Z-Taq; in 1 × LA-Taq buffer containing 2.5 mM Mg^{2+} and a 0.2 mM concentration of each dNTP with LA-Taq; in 1 × Pyrobest buffer containing 2 mM Mg^{2+} and a 0.2 mM concentration of each dNTP with Pyrobest. The standard reaction mixture contained 0.5 μL (0.4 ng/μL) of DNA template pGMI401 (4263 nt), 5 μL (5 pmol) of each primer, lacUV501 (d(5′-GAAGATCT-CAGCTGGCACGACAGGTTTC-3′), 28 nt), lacUV502 (d(5′-CGATGCATAGCTGTTTCCTGTGTGAAATTG-3′), 30 nt), various concentrations of synthetic 5′-triphosphates (**1TP**−**5TP**), 0.5 μL (5 U/μL) of DNA polymerase in a final volume of 50 μL. Reaction mixtures were incubated in a thermocycler (model Gene Amp PCR system 9700; PE Applied Biosystems) for 5 min at 95 °C, followed by 30 cycles at 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s and then by a final extension at 72 °C for 5 min in the case of Ex Taq, Pfu, and LA-Taq. Mixtures were also incubated at 95 °C for 5 min, followed by 25 cycles at 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s and then by a final extension at 72 °C for 5 min in the cases of Z-Taq and Pyrobest. The polymerase units were defined by the supplier, in all cases reflecting conversion of 10 nmol of dNTP into an acid-insoluble form in 30 min under defined reaction conditions.

Aliquots of PCR products (8 μL each) were analyzed by electrophoresis (8% acrylamide gel) and visualized by staining with SYBR gold. Band intensity measurement was employed with phosphorimager SI (Vista Fluorescence), and each data point is shown as an average of two independent experiments.

Supporting Information Available: The X-ray analytical data for mononucleosides **2**, **5**, **8**, **16**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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