Synthetic Nucleosides and Nucleotides. XXVII. Selective Inhibition of Deoxyribonucleic Acid Polymerase \( \alpha \) by 1-\( \beta \)-D-Arabinofuranosyl-5-styryluracil 5’-Triphosphates and Related Nucleotides: Influence of Hydrophobic and Steric Factors on the Inhibitory Action

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Eight kinds of 5-substituted 1-\( \beta \)-D-arabinofuranosyluracil 5’-triphosphates \((E)-(3-nitrostyryl) (6), (E)-(3-aminostyryl) (9), (E)-(4-nitrostyryl) (7), (E)-(4-aminostyryl) (10), (E)-styryl (8), phenethyl (11), (RS)-(3-azido-2-hydroxypropyl) (17), and (RS)-(3-amino-2-hydroxypropyl) (18) derivatives) were synthesized. Among these analogs, araUTPs bearing strongly hydrophobic styryl groups at the 5-position \((6 – 10)\) were shown to have selective and strong inhibitory action on deoxyribonucleic acid (DNA) polymerase \( \alpha \) purified from cherry salmon \((Oncorhynchus masou)\) testes. The 5-azidopropyl derivative (17) also inhibited this polymerase. The compounds with a nitro and an amino group on the 5-styryl substituent showed essentially the same activity, but the 5-phenethyl derivative (11) and the 5-aminopropyl derivative (18) showed greatly reduced inhibitory action. On the other hand, in the case of DNA polymerase \( \beta \), all the analogs showed similar inhibitory effects. The influence of hydrophobic and steric effects of substituents at the 5-position of araUTP on DNA polymerases \( \alpha \) and \( \beta \) are discussed.

Keywords—5-(E)-styryl araUTP; DNA polymerase \( \alpha \); DNA polymerase \( \beta \); hydrophobic effect; cherry salmon testes

At least three species of deoxyribonucleic acid (DNA)-dependent DNA polymerase, termed \( \alpha \), \( \beta \), and \( \gamma \), exist in eukaryotic tissues or cells. These polymerases, as well as viral DNA polymerases, have been purified from several sources, and their properties have been examined. To obtain information about the precise roles of these polymerases in vivo, species-specific inhibitors would be useful tools. For example, aphidicolin (which specifically inhibits DNA polymerase \( \alpha \)) and 2’,3’-dideoxythymidine 5’-triphosphate (which shows preferential inhibitory action on DNA polymerase \( \beta \) and \( \gamma \)) are available.\(^3\)–\(^5\) However, their specificity in vivo is not satisfactory. Thus, we have been searching for new classes of nucleotide analogs which act more efficiently and more selectively on various DNA polymerases. These compounds might also be potential antitumor or antiviral agents.

Arabinofuranosyl nucleotides (e.g., araTTP) inhibit DNA polymerase \( \alpha \) more than \( \beta \).\(^6\)–\(^7\) Recently we reported that 2’-azido-2’-deoxy araATP showed a more selective inhibitory effect than araATP, but its amino counterparts inhibited both polymerases to similar extent.\(^9\) We also previously found that a hydrophobic group at the 5-position of araUTP could increase the affinity of the analog for DNA polymerase \( \alpha \), based on experiments using several araUTPs modified at the 5-position with alkyl chains.\(^9\) Concerning hydrophobic effects in the purine series, 2-(p-n-butylanilino)-6-substituted purine nucleotides were found to have strong affinities for DNA polymerase \( \alpha \).\(^10\)

On the other hand, DeClercq et al. reported that 2’-deoxyUMP\(s\) modified with strongly hydrophobic styryl groups at the 5-position showed remarkable inhibitory effects on
eukaryotic thymidylate synthase. Thus, we have synthesized several 5-(substituted)styryl araUTPs and related compounds, and examined their inhibitory effects on DNA polymerases \( \alpha \) and \( \beta \) purified from developing cherry salmon (Oncorhynchus masou) testes.

**Synthesis**

Many procedures for the introduction of substituents at the 5-position of a uracil ring have been reported. The method with palladium as a catalyst is very convenient, and Bigge et al. used it to synthesize various 5-styryl-2'-deoxyuridines and 5'-monophosphate derivatives. We applied this method for the present synthetic study (Chart 1).

1-\(\beta\)-D-Arabinofuranosyluracil (1) was lead to the 5-chloromercuri derivative (2) by treatment with mercuric acetate and sodium chloride, and then 3-nitrostyrene, 4-nitrostyrene, or styrene itself was reacted in the presence of palladium as a catalyst in methanol. 1-\(\beta\)-D-Arabinofuranosyl-5-(E)-(3-nitrostyryl)uracil (3), 1-\(\beta\)-D-arabinofuranosyl-5-(E)-(4-nitrostyryl)uracil (4), and 1-\(\beta\)-d-arabinofuranosyl-5-(E)-styryluracil (5) were obtained in yields of 22%, 19%, and 49%, respectively. The proton nuclear magnetic resonance (\(^1\)H-NMR) spectra of these nucleosides showed that the coupling constants (\(J\)) of the vinyl moieties of all styryl groups at the 5-position were in the range of 16.1—16.6 Hz, indicating that the all styryl substituents were “trans,” as reported by Bigge et al. All the synthesized nucleosides were converted to the corresponding 5'-monophosphates by phosphorylation with phosphoryl chloride in triethyl phosphate. Further phosphorylation to the 5'-triphosphate derivatives was performed by the phosphoroimidazolidate method with some modifications. In all cases, total yields of phosphorylation from nucleoside were about 50%. The nitro groups of 5-(E)-(3-nitrostyryl) araUTP (6) and 5-(E)-(4-nitrostyryl) araUTP (7) were reduced with hydrazine hydrate in the presence of a catalytic amount of Raney Ni (W-2), giving 5-(E)-(3-aminostyryl) araUTP (9) and 5-(E)-(4-aminostyryl) araUTP (10) in 78% and 83% yields, respectively. The vinyl group of 5-(E)-styryl araUTP (8) was reduced under 1 atm pressure of hydrogen in the presence of palladium–carbon, and 5-phenethyl araUTP (11) was obtained in a yield of 50%.

![Chart 1](image-url)
On the other hand, for comparison of the effect of hydrophobicity of substituents at the 5-position of araUTP, we also synthesized 5-alkyl derivatives (Chart 2). 5-Allyl-1-β-D-arabinofuranosyluracil (12) was synthesized from 2 with allyl chloride in the presence of palladium as a catalyst. The hydroxy groups at the 2', 3', and 5'-positions of 12 were acetylated, and the allyl group was oxidized with m-chloroperbenzoic acid followed by cleavage of the resulting epoxy ring with lithium azide. 1H-NMR spectra showed that compound 14 or 15 was a mixture of (R)- and (S)-products in the ratio of about 1:1. However, we used these compounds as such, since the isomers were difficult to separate. Compound 15 was treated with methanolic ammonia to give the free nucleoside (16). Then 16 was converted to its 5'-triphosphate in the same manner as described above. 5-(RS)-(3-Azido-2-hydroxypropyl) araUTP (17) was converted to the 3-amino counterpart (18) by reduction with hydrogen sulfide in 50% aqueous pyridine.

All chemically synthesized nucleotide analogs were purified by paper chromatography and paper electrophoresis.

**Biological Evaluation**

Inhibitory Effects of Various AraUTP Analogons on DNA Polymerases α and β—The inhibitory effects of eight newly synthesized araUTP analogons on DNA-dependent DNA polymerases α and β purified from developing cherry salmon (Oncorhynchus masou) testes were examined with activated salmon sperm DNA as a template-primer. The activity without analogs was taken as 100%, and the remaining activity (%) at various concentrations of analogs was measured. The resulting inhibition curves are shown in Fig. 1-A (DNA...
polymerase $\alpha$) and Fig. 1-B (DNA polymerase $\beta$). As can be seen in Fig. 1-A, all araUTP analogs (6–10) having styryl groups at the 5-position inhibited DNA polymerase $\alpha$ very strongly. A strong inhibitory effect was also found in the case of the 5-azidopropyl derivative
(17), but the corresponding amino derivative (18) showed a greatly reduced inhibitory effect on this polymerase. In contrast, there was no marked difference of inhibitory effects between the compounds with a nitro group and an amino group on the 5-styryl moiety. However, the inhibitory effect of 5-phenethyl araUTP (11) was less than that of 5-styryl araUTP (8).

On the other hand, in the case of DNA polymerase β (Fig. 1-B), all the analogs showed similar inhibitory effects, and the kind of substituent at the 5-position had no influence on the activity.

### Analyses of Inhibition Mode and Determination of Kinetic Constants (Ki Values)

We next examined the mode of inhibition of DNA polymerases α and β by these araUTP analogs using Lineweaver–Burk plots. In this analysis, activated DNA was used as a template-primer. As a typical example, plots of data for the inhibition of DNA polymerases α and β by 5-(E)-(3-nitrostyryl) araUTP (6) and 5-(E)-(3-aminostyryl) araUTP (9) are shown in Fig. 2. All the araUTP analogs were essentially competitive with natural substrate dTTP for both DNA polymerases α and β.

Based on these analyses, the kinetic constants (Ki values) were determined by replots of apparent Km value versus the concentration of the analog, and the results are summarized in Table I. For DNA polymerase α, the Ki values of all analogs except for 5-phenethyl araUTP (11) and 5-(RS)-(3-amino-2-hydroxypropyl) araUTP (18) were much smaller than the Km value of the natural substrate dTTP. For DNA polymerase β, the Ki values of all analogs were very similar to the Km value of dTTP.

### Discussion

Recently we have reported that the inhibitory effect of 5-ethyl araUTP on eukaryotic DNA polymerase α was less than that of araUTP or araTTP owing to the steric hindrance of the substituent at the 5-position of the uracil ring, but longer alkyl chain-modified derivatives, 5-n-propyl and 5-n-butyl araUTP, recovered inhibitory action as a result of their hydrophobic effect. In order to examine the hydrophobic effect of the substituent at the 5-position of araUTP in more detail, we have synthesized several araUTPs modified at the 5-position with strongly hydrophobic styryl groups, and examined their inhibitory effects on DNA polymerases α and β purified from cherry salmon (Oncorhynchus masou) testes.

As can be seen in Fig. 1, all 5-styryl derivatives of araUTP (6—10) showed very strong inhibitory effects on DNA polymerase α. The Ki values of these analogs were much smaller than the Km value of the natural substrate dTTP; 5-(E)-(3-nitrostyryl) araUTP (6) showed the smallest Ki value of 0.25 and its Ki/Km value was 0.10. Although the Ki values of 5-aminostyryl derivatives (9, 10) were somewhat higher than those of 5-nitrostyryl derivatives (6, 7), the

<table>
<thead>
<tr>
<th>Compound</th>
<th>DNA polymerase α</th>
<th>DNA polymerase β</th>
<th>Ki/Km for pol. α</th>
<th>Ki/Km for pol. β</th>
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<tbody>
<tr>
<td>dTTP</td>
<td>2.5 (Km)</td>
<td>20 (Km)</td>
<td>0.25</td>
<td>0.43</td>
</tr>
<tr>
<td>5-(E)-(3-Nitrostyryl) araUTP (6)</td>
<td>0.25 0.10 8.5 0.43</td>
<td>0.24</td>
<td></td>
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<tr>
<td>5-(E)-(3-Aminostyryl) araUTP (9)</td>
<td>0.80 0.32 33 1.65</td>
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<tr>
<td>5-(E)-(4-Nitrostyryl) araUTP (7)</td>
<td>0.45 0.18 8.5 0.43</td>
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<tr>
<td>5-(E)-(4-Aminostyryl) araUTP (10)</td>
<td>0.60 0.24 30 1.50</td>
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<tr>
<td>5-(E)-Styryl araUTP (8)</td>
<td>0.50 0.20 10 0.50</td>
<td></td>
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<tr>
<td>5-Phenethyl araUTP (11)</td>
<td>4.9 1.96 16 0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-(RS)-(3-Azido-2-hydroxypropyl) araUTP (17)</td>
<td>2.0 0.80 22 1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-(RS)-(3-Amino-2-hydroxypropyl) araUTP (18)</td>
<td>61 24.4 23 1.15</td>
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</table>
K_i/K_m values were about 0.3 suggesting that these analogs still have strong affinity for DNA polymerase α. In contrast, among the 5-alkyl derivatives of araUTP, the K_i value of the 5-aminopropyl derivative (18) was remarkably larger than that of the 5-azidopropyl derivative (17). This indicates that the hydrophobic effect of the substituent at the 5-position of araUTP has a greater effect on the affinity for DNA polymerase α than does the kind of substituent. However, 5-phenethyl araUTP (11) which is thought to have similar hydrophobicity at the 5-position to the 5-styryl derivative showed a K_i/K_m value of 1.96 and was found to show lower affinity for DNA polymerase α. This finding indicates that not only hydrophobicity but also steric hindrance of the 5-substituent on the uracil ring affects the affinity of the analog for DNA polymerase α. Therefore, as reported in the case of 5-(E)-(2-bromovinyl)-2'-deoxyuridine, intramolecular hydrogen bonding between the vinyl group at the 5-position of 5-(E)-(substituted)styryl araUTP and O^4 of the uracil ring could be important for the affinity to this polymerase.

In the case of DNA polymerase β, all analogs showed weak inhibitory effects, and K_i values of all analogs including 5-alkyl derivatives were essentially similar. Thus, neither hydrophobicity nor steric hindrance at the 5-position of araUTP affects the affinity of the analogs for DNA polymerase β. Knowledge of these differences between DNA polymerases α and β will be useful for the design of species-specific inhibitors.

A smaller value of the ratio of (K_i/K_m value for DNA polymerase α)/(K_i/K_m value for DNA polymerase β), indicates selectivity for DNA polymerase α, although the K_i values of the 5-aminostyryl derivatives (9, 10) for DNA polymerase α were larger than those of the 5-nitrostyryl derivatives (6, 7), these two 5-aminostyryl derivatives of araUTP were found to be more selective inhibitors for DNA polymerase α than the styryl derivatives.

We are attempting to prepare a new affinity adsorbent for specific purification of DNA polymerase α, by using a strong and selective inhibitor for this enzyme as a ligand. 5-(E)-(3-Aminostyryl) araUTP (9) and 5-(E)-(4-aminostyryl) araUTP (10) are potential candidates for this affinity ligand. Affinity chromatography on resin carrying these compounds as ligands is being examined.

**Experimental**

**General Methods**—Melting points were determined with Yanaco MP-3 apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-240 recording spectrophotometer. ^1^H-NMR spectra were obtained on a JEOL JNM-FX 200 NMR spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet), and m (multiplet). Mass spectra (MS) were measured on a JEOL JMS-D-300 mass spectrometer. Infrared (IR) spectra were obtained on a JASCO IRA-2 spectrometer. Thin layer chromatography was performed with pre-coated Silica gel 60 F254 plates (Merck) and silica gel Column chromatography was carried out on Wako-gel C-200. Radioactivity was measured with a Packard TRI-CARB C-480 liquid scintillation counter with toluene scintillator.

1-β-D-Arabinofuranosyl-5-chloromercuriuracil (2)—1-β-D-Arabinofuranosyluracil (1) (3.0 g, 12.4 mmol) was dissolved in 17 ml of water, then 4.1 g (12.9 mmol) of mercuric acetate in 24 ml of water was added, and the mixture was stirred for 24 h at 50 °C. The mixture was cooled to room temperature, and the white precipitate was collected by filtration, and washed with 100 ml of 0.16 M aqueous sodium chloride and 5 ml of ethanol. The white solid was ground and dried. 5.8 g (97%), mp 173-174 °C (dec.). Anal. Calcd for C_{9}H_{11}N_{2}O_{6}HgCl·H_{2}O: C, 21.70; H, 2.63; N, 5.62. Found: C, 21.69; H, 2.63; N, 5.47.

1-β-D-Arabinofuranosyl-5-(E)-(3-nitrostyryl)uracil (3)—A mixture of a suspension of 2.0 g (4.19 mmol) of 2 in 10 ml of methanol, 1.89 g (12.6 mmol) of 3-nitrostyrene and 35 ml (3.5 mmol) of methanolic solution of 0.1 M lithium tetrachloropalladate was refluxed for 24 h. The solution was filtered, and hydrogen sulfide gas was bubbled through the filtrate for 90 s. The precipitate of mercuric sulfide was filtered off through celite, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel (100 g) column chromatography. Elution was performed with 8—12% methanol in chloroform. The fractions containing 3 were combined and evaporated to dryness. The residue was crystallized from methanol to give 3 as yellow crystals. 360 mg (22%), mp 249.5—250.5 °C. UV λ_{max}^0 nm (e): 298 (19800), λ_{min} nm: 232, λ_{max}^0 nm: 298, λ_{min}^0 nm: 232, λ_{max} nm: 306, λ_{min} nm: 242.
1-β-D-Arabino furanosyl-5-(E)-(4-nitrostyryl)uracil (4) — A mixture of a suspension of 1.26 g (2.6 mmol) of 2 in 10 ml of methanol, 1.0 g (6.77 mmol) of 4-nitrostyrene and 26 ml (26 mmol) of methanolic solution of 0.1 M lithium tetrachloropalladate was refluxed for 24 h. The mixture was filtered, and hydrogen sulfide gas was bubbled through the filtrate for 90 s, followed by filtration through celite. The filtrate was evaporated, and the residue was purified by silica gel (130 g) column chromatography. Elution was performed with 10% methanol in chloroform. The fractions containing 4 were combined and evaporated to dryness. The residue was crystallized from methanol to afford 4 as orange-yellow crystals. 196 mg (19%). mp 243-244 °C. UV λmax nm (ε): 366 (15900), λmin nm: 284, λmax nm: 366, λ0.1NHCImax nm: 284, λ0.1NHCImin nm: 366. 1H-NMR (DMSO-d6 + D2O): 8.28 (s, 1H, H-6), 8.1-7.6 (m, 4H, aromatic, J= 8.8 Hz), 7.62 (d, 2H, aromatic, J= 8.8 Hz), 7.57 (d, 1H, vinylic, J= 16.1 Hz), 7.34 (d, 1H, vinylic, J= 16.1 Hz). MS m/z: 259 (heterocyclic base), 188, 142, 115. Anal. Caled for C17H17N3O8: C, 52.18; H, 4.38; N, 10.74. Found: C, 52.08; H, 4.36; N, 10.57.

1-β-D-Arabinofuranosyl-5-(E)-(4-styryl)uracil (5) — A mixture of a suspension of 1.5 g (3.1 mmol) of 2 in 15 ml of methanol, 1.0 g (9.6 mmol) of styrene and 30 ml (3.0 mmol) of methanolic solution of 0.1 M lithium tetrachloropalladate was refluxed for 24 h. The mixture was filtered, and hydrogen sulfide gas was bubbled through the filtrate for 90 s, followed by filtration through celite. The filtrate was evaporated and the residue was purified by silica gel (100 g) column chromatography. Elution was performed with 8—10% methanol in chloroform. The fractions containing 5 were combined and evaporated to dryness. The residue was crystallized from methanol—water to give 5 as colorless crystals. 530 mg (49%). mp 237-238 °C. UV λmax nm (ε): 308 (18000), λmin nm: 238, λmax nm: 308, λmax nm: 238, λ0.1NHCImax nm: 244. 1H-NMR (DMSO-d6 + D2O): 8.06 (s, 1H, H-6), 7.8-7.3 (m, 5H, aromatic), 7.28 (d, 1H, vinylic, J= 16.6 Hz), 6.90 (d, 1H, vinylic, J= 16.1 Hz), 6.05 (d, 1H, H-1', J= 4.9 Hz), 4.10 (m, 1H, H-2'), 3.98 (m, 1H, H-3'). MS m/z: 346 (molecular ion), 214 (heterocyclic base), 143, 115. Anal. Caled for C17H18N2O10: C, 50.71; H, 5.20; N, 6.57. Found: C, 50.84; H, 5.73; N, 6.05.

5-Allyl-1-β-D-arabinofuranosyluracil (12) — A mixture of a suspension of 3.0 g (6.3 mmol) of 2 in 50 ml of methanol, 5.2 ml (63.7 mmol) of allyl chloride and 16 ml (1.6 mmol) of 0.1 M methanolic solution of lithium tetrachloropalladate was refluxed for 24 h at room temperature. The solution was filtered, and hydrogen sulfide gas was bubbled through the filtrate for 40 s, followed by filtration through celite. The filtrate was neutralized with Dowex 1×2 (bicarbonate form), and the resin was removed by filtration and washed with methanol. The combined filtrate and washing were evaporated, and the residue was purified by silica gel (50 g) column chromatography. Elution was performed with 8—10% methanol in chloroform. The fractions containing 12 were combined and evaporated to dryness. The residue was crystallized from acetonitrile to give 12 as colorless crystals. 1.15 g (64%). mp 161.5—163 °C. UV λmax nm (ε): 267 (9000), λmin nm: 267, λmax nm: 267, λmax nm: 267, λ0.1NHCImax nm: 243. 1H-NMR (DMSO-d6 + D2O): 7.51 (s, 1H, H-6), 6.00 (d, 1H, H-1', J= 4.9 Hz), 5.9—5.7 (m, 1H, 5-CH2CH =CH2), 5.1—5.0 (m, 2H, 5-CH2CH= CH2), 4.03 (m, 1H, H-2'), 3.91 (m, 1H, H-3'), 3.7—3.5 (m, 3H, H-4' and H-5'), 2.95 (d, 2H, 5-CH2CH= CH2, J= 6.1 Hz). MS m/z: 284 (molecular ion), 181, 152. Anal. Caled for C12H16N2O12: C, 50.70; H, 5.67; N, 9.86. Found: C, 50.84; H, 5.73; N, 10.05.

5-Allyl-1-β-D-(2,3,5-tri-O-acetylarabinofuranosyl)uracil (13) — A mixture of a solution of 1.70 g (5.98 mmol) of 12 in 30 ml of pyridine and 2.5 ml (26.5 mmol) of acetic anhydride was stirred for 24 h at room temperature. The solution was cooled in an ice-water bath, then 3 ml of methanol was added and the whole was stirred for 20 min. The solvent was evaporated off, and removed completely by co-evaporation with ethanol. Crystallization of the residue from ethanol gave 13 as colorless crystals. 2.3 g (94%). mp 138.5—139.5 °C. MS m/z: 419 (molecular ion), 259, 139, 97, 43. Anal. Caled for C12H16N2O12: C, 50.68; H, 5.40; N, 6.83. Found: C, 52.55; H, 5.50; N, 6.96.

5-(RS)-(2,3,5-Epoxypropyl)-1-β-D-(2,3,5-tri-O-acetylarabinofuranosyl)uracil (14) — Compound 13 (2.0 g, 4.87 mmol) was dissolved in 50 ml of methylene chloride, and 2.6 g (15.1 mmol) of m-chloroperoxybenzoic acid was added. The mixture was stirred for 24 h at room temperature. After being washed with saturated NaHCO3 aqueous solution and water, the organic layer was filtered through Whatman 1 PS filter paper, and the solvent was evaporated off. The residue was purified by silica gel (100 g) column chromatography. Elution was performed with 3—4% methanol in chloroform. The fractions containing 14 were combined and evaporated to dryness. The residue was crystallized from ethanol to give 14 as colorless crystals. 196 mg (19%). mp 136.5—137 °C. UV λmax nm (ε): 267 (9000), λmin nm: 235, λmax nm: 235, λmax nm: 235, λ0.1NHCImax nm: 267, λ0.1NHCImin nm: 235. 1H-NMR (DMSO-d6 + D2O): 7.48 and 7.45 (s, 1H, total H-6), 6.3 (m, 1H, H-1'), 5.4 (m, 1H, H-2'), 5.1 (m, 1H, H-3'), 4.4—4.2 (m, 3H, H-4' and H-5'), 3.0 (m, 1H, 5-CH2CH(CO2CH3)2, 2.8 (m, 2H, 5-CH2CH(CO2CH3)2, 2.5 (m, 2H, 5-CH2CH(CO2CH3). MS m/z: 426 (molecular ion), 307, 259, 139, 97, 43. Anal. Caled for C12H16N2O12: C, 50.71; H, 5.20; N, 6.57. Found: C, 50.46; H, 5.23; N, 6.53.

5-(RS)-(3-Azido-2-hydroxypropyl)-1-β-D-(2,3,5-tri-O-acetylarabinofuranosyl)uracil (15) — A solution of 700 mg (1.64 mmol) of 14 in 20 ml of dimethylformamide (DMF) was treated with 240 mg (4.90 mmol) of lithium azide. The mixture was stirred for 24 h at 65 °C, then the solvent was evaporated off. The residue was dissolved in
30 ml of chloroform, and washed with water three times. The organic layer was filtered through Whatman 1PS filter paper, and the filtrate was evaporated. The residue was purified by silica gel (70 g) column chromatography. Elution was performed with 4—5% methanol in chloroform. The fractions containing 15 were combined and evaporated to dryness, giving a foam. 380 mg (49%). UV λ max MeOH nm (ε): 267 (9000), λ max H 2O nm: 253, λ max HCl nm: 253, λ max NaOH nm: 253, λ max D 2O nm: 243. 1H-NMR (CDCl 3 + D 2O): 7.54 and 7.49 (s, 1H, total H-6), 6.3 (m, 1H, H-1'), 5.4 (m, 1H, H-2'), 5.1 (m, 1H, H-3'), 4.6—4.5 (m, 3H, H-4' and H-5'), 4.3 (m, 1H, 5-CH 2CH(OH)CH 2N 3), 3.3 (m, 2H, 5-CH 2CH(OH)CH 2N 3), 2.6 (m, 2H, 5-CH 2CH(OH)CH 2N 3). IR (Nujol): 2080 cm -1 (—N 3).

1-β-D-Arabinofuranosyl-5-(RS)-(3-azido-2-hydroxypropyl)uracil (16)—Compound 15 (310 mg, 0.66 mmol) was dissolved in 0.5 ml of methanol, and 3.8 ml (0.99 mmol) of 0.26 M methanolic sodium methoxide was added. The mixture was stirred for 5 h at room temperature, neutralized with Dowex 50 × 8 (proton form) and filtered. The resin was washed with methanol, and the combined filtrate and washings were evaporated to dryness to give a foam. 200 mg (90%). This compound was used for the next reaction immediately.

**General Method for Phosphorylation**—Phosphoryl chloride (1.5 mmol) was added to 0.5 mmol of synthetic nucleoside (3, 4, 5, or 16) in 5 ml of triethyl phosphate under cooling below 0 °C. The mixture was stirred for 24 h at room temperature. The solution was poured into 30 ml of saturated NaHCO 3 aqueous solution and extracted with chloroform (30 ml) three times. The combined organic layers were re-extracted with water (15 ml). The aqueous layers were combined and diluted with water to give a final volume of 300 ml, and applied to a column of diethylaminoethyl (DEAE)-cellulose (2.9 × 25 cm, bicarbonate form). The column was washed with 500 ml of water, and eluted with a linear gradient from water (1 l) to 0.2 M triethylammonium bicarbonate (1 l). Fractions containing desired nucleotide were combined and evaporated to dryness, and co-evaporation with 50% aqueous ethanol was carried out to remove residual triethylamine. The obtained 5'-monophosphate derivative was converted to the 5'-triphosphate by the following method. The 5'-monophosphate derivative (0.2 mmol) was dissolved in 1 ml of DME and evaporated to dryness. This process was repeated twice, then 1 mmol of N,N'-carbonyldiimidazole was added to the residue in 2 ml of DMF, and the mixture was stirred for 3.5 h at room temperature. After confirmation of the complete conversion to the 5'-phosphorimidazolidinate derivative by paper electrophoresis (in 50 mm triethylammonium bicarbonate, 700 V, 30 min), 0.8 ml of methanol was added to decompose excess reagent, and the mixture was stirred for 30 min at room temperature. Then 2 ml (2 mmol) of 1 M tributylammonium pyrophosphate in DMF was added, and the mixture was stirred for 24 h at room temperature. Water (10 ml) was added and the nucleotide was adsorbed on 1 g of charcoal. The mixture was filtered through celite. After washing with 100 ml of water, elution was carried out with 200 ml of 50% aqueous ethanol—3% NH 4OH. The eluate was evaporated, and the residue was dissolved in 100 ml of water and applied to a column of DEAE-cellulose (2.9 × 25 cm, bicarbonate form). The column was washed with 500 ml of water, and then eluted with a linear gradient from water (1 l) to 0.5 M triethylammonium bicarbonate (1 l). The combined fractions containing 9 were evaporated and co-evaporated with 50% aqueous ethanol to dryness. Through the above procedure, 5-(E)-(3-nitrostyryl) araUTP (6), 5-(E)-(4-nitrostyryl) araUTP (7), 5-(E)-styryl araUTP (8), and 5-(RS)-(2-hydroxy-3-azidopropyl) araUTP (17) were synthesized from the corresponding nucleosides in yields of about 50%.

5-(E)-(3-Aminostyryl) araUTP (9)—A mixture of 6 (2000 OD 298 units, 0.10 mmol) in 5 ml of water, 100 μl of hydrazine hydrate and a catalytic amount of Raney Ni (W-2) was stirred for 4 h at room temperature. The solution was filtered through celite, and the filter was washed with 200 ml of 50% aqueous ethanol—3% NH 4OH. The combined filtrate and washings were evaporated to dryness. The residue was dissolved in 250 ml of water and applied to a column of DEAE-cellulose (2.9 × 25 cm, bicarbonate form). The column was washed with 500 ml of water, and eluted with a linear gradient from water (1 l) to 0.5 M triethylammonium bicarbonate (1 l). The combined fractions containing 9 were evaporated and co-evaporated with 50% aqueous ethanol to dryness. 1370 OD 300 units (78%). UV λ max MeOH nm (ε): 300 (15000), λ max H 2O nm: 253, λ max HCl nm: 239, λ min HCl nm: 253, λ max NaOH nm: 308, λ max D 2O nm: 262. Ninyhydrin positive (violet).

5-(E)-(4-Aminostyryl) araUTP (10)—A mixture of a solution of 1240 OD 298 units (80 μmol) of 9 in 5 ml of water, 100 μl of hydrazine hydrate and a catalytic amount of Raney Ni (W-2) was stirred for 4 h at room temperature. The mixture was filtered through celite, and the filter was washed with 200 ml of 50% aqueous ethanol—3% NH 4OH. The combined filtrate and washings were evaporated to dryness. The residue was dissolved in 250 ml of water, and applied to a column of DEAE-cellulose (2.9 × 25 cm, bicarbonate form). After washing of the column with 500 ml of water, elution was performed with a linear gradient from water (1 l) to 0.5 M triethylammonium bicarbonate (1 l). The combined fraction containing 10 were evaporated and co-evaporated with 50% aqueous ethanol to dryness. 970 OD 298 units (83%). UV λ max MeOH nm (ε): 325 (15000), λ max H 2O nm: 247, λ max HCl nm: 310, λ min HCl nm: 240, λ max NaOH nm: 325, λ max D 2O nm: 310. Ninyhydrin positive (violet).

5-Phenethyl araUTP (11)—A mixture of 1800 OD 300 units (0.10 mmol) of 8 in 1 ml of water and 0.1 g of palladium—carbon was stirred under 1 atm pressure of hydrogen for 24 h at room temperature. The solution was filtered through celite, and the filter was washed with 200 ml of 50% aqueous ethanol—3% NH 4OH. The combined filtrate and washings were evaporated to dryness. The residue was dissolved in 200 ml of water, and applied to a column of DEAE-cellulose (2.9 × 25 cm, bicarbonate form). After washing of the column with 500 ml of water, elution was performed with a linear gradient from water (1 l) to 0.5 M triethylammonium bicarbonate (1 l). The
combined fractions containing 11 were evaporated and co-evaporated with 50% aqueous ethanol to dryness. 1800 OD$_{267}$ units (50%). UV $\lambda_{\text{max}}$ nm ($\varepsilon$): 267 (9000), $\lambda_{\text{min}}$ nm: 236, $\varepsilon$ 0.1N$\text{HC}$_{1}$nm$: 267, \lambda_{\text{min}}$ 0.1N$_{\text{HC}1}$nm$: 235, \lambda_{\text{min}}$ 0.1N$_{\text{NaOH}3}$nm$: 267, \lambda_{\text{min}}$ 0.1N$_{\text{NaOH}3}$nm$: 245.

5-(RS)-(3-Amino-2-hydroxypropyl) araUTP (18)—Hydrogen sulfide gas was gently bubbled into a solution of 1000 OD$_{267}$ units (0.11 mmol) of 17 in 1 ml of 50% aqueous pyridine for 6 h at room temperature. The solvent was evaporated off, and the residue was dissolved in 500 ml of water, elution was performed with a linear gradient from water (1 l) to 0.5 M triethylammonium bicarbonate (1 l). The combined fractions containing 18 were evaporated and co-evaporated with 50% aqueous ethanol to dryness. 640 OD$_{267}$ units (64%). UV $\lambda_{\text{max}}$ nm ($\varepsilon$): 267 (9000), $\lambda_{\text{min}}$ nm: 235, $\varepsilon$ 0.1N$\text{HC}$_{1}$nm$: 267, \lambda_{\text{min}}$ 0.1N$_{\text{HC}1}$nm$: 235, \lambda_{\text{min}}$ 0.1N$_{\text{NaOH}3}$nm$: 267, \lambda_{\text{min}}$ 0.1N$_{\text{NaOH}3}$nm$: 243. Ninhydrin positive (violet).

**Analytical Results and Constants of Synthetic Nucleotide Analogs**—All chemically synthesized nucleotide analogs (6—11, 17, and 18) were purified by paper electrophoresis and paper chromatography. Phosphorus analysis data and $R_f$ values of products on paper electrophoresis (in 50 mm citrate buffer, pH 3.3) and on paper chromatography (in ethanol-0.5 M sodium acetate 1 : 1, v/v) are summarized in Table II.

**DNA Polymerases $\alpha$ and $\beta$**—DNA polymerases $\alpha$ (without primase activity) and $\beta$ were purified from cherry salmon (Oncorhynchus masou) testes as described previously.$^8$ All operations were carried out at 0—4 °C. Cherry salmon testes were homogenized in 50 mm Tris–HCl buffer (pH 7.5, containing 10 mm $\beta$-mercaptoethanol, 1 mm ethylenediaminetetraacetic acid (EDTA), 20% glycerol, 0.5 mm phenylmethylsulfonyl fluoride, and 0.5 mm benzamidine) with a Teflon pestle homogenizer (600 rev/min, 4 strokes). The homogenate was stirred for 40 min, and then centrifuged at 13000 × $g$ for 40 min. DNA polymerase $\alpha_1$ was extracted in the supernatant fraction. After the first phosphocellulose column chromatography, this enzyme was precipitated by the addition of 60% saturated ammonium sulfate and collected by centrifugation.

The extraction of DNA polymerase $\beta$ was performed by rehomogenization of the pellet obtained from the first step in 50 mm Tris–HCl buffer containing 0.5 m KCl using a Waring blender (1 min × 4). The homogenate was stirred for 40 min, and centrifuged at 13000 × $g$ for 40 min; the supernatant was collected. After the first phosphocellulose column chromatography, the enzyme was precipitated by the addition of 50—80% saturated ammonium sulfate and collected by centrifugation.

Each of the polymerases was further purified by means of sequential column chromatographies on DEAE-cellulose, phosphocellulose (second), hydroxyapatite, blue-agarose, and single-stranded DNA-cellulose. The final sample showed a specific activity of 100000 units/mg for DNA polymerase $\alpha_1$ with activated DNA as a template–primer, and 170000 units/mg for DNA polymerase $\beta$ with poly(rA)–oligo(dT)$_{12-18}$ as a template–primer.

**Assay Condition for DNA Polymerases $\alpha_1$ and $\beta$**—The standard assay mixture (25 µl) for DNA polymerase $\alpha_1$ was contained 100 µg/ml activated salmon sperm DNA, 50 mm Tris–HCl (pH 8.0), 4 mm MgCl$_2$, 15% glycerol, 1 mm dithiothreitol, 400 µg/ml bovine serum albumin, 100 µm dATP, 100 µm dGTP, 100 µm dCTP, 25 µm [H]$\text{dTPP}^5$ (5 cpm/pmol), 0—100 µm synthetic nucleotide analog, and 0.5 unit of enzyme. In the DNA polymerase $\beta$ assay system, the same mixture except for 50 mm glycine–KOH (pH 9.5) instead of Tris–HCl, and addition of 80 mm KCl, was used. The enzyme reaction was carried out for 30 min at 37 °C.

When kinetic analysis was performed, the concentrations of [H]$\text{dTPP}$ and inhibitors were varied.

### References and Notes


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**Table II. Analytical Results and Constants of Synthetic Nucleotides**

<table>
<thead>
<tr>
<th>Compound</th>
<th>UV, $\lambda_{\text{max}}$ nm ($\varepsilon$)</th>
<th>$\varepsilon$ (P) Found</th>
<th>Paper chromatography$^a$ ($R_f \times 100$)</th>
<th>Paper electrophoresis$^b$ ($R_f$ dTPP$^5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>298 (19800)</td>
<td>6600</td>
<td>28</td>
<td>0.9</td>
</tr>
<tr>
<td>9</td>
<td>300 (15000)</td>
<td>4900</td>
<td>23</td>
<td>0.8</td>
</tr>
<tr>
<td>7</td>
<td>366 (15900)</td>
<td>5100</td>
<td>22</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>325 (15000)</td>
<td>4800</td>
<td>15</td>
<td>0.8</td>
</tr>
<tr>
<td>8</td>
<td>308 (18000)</td>
<td>5900</td>
<td>37</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>267 (9000)</td>
<td>2800</td>
<td>38</td>
<td>1.0</td>
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<tr>
<td>17</td>
<td>267 (9000)</td>
<td>2900</td>
<td>42</td>
<td>1.0</td>
</tr>
<tr>
<td>18</td>
<td>267 (9000)</td>
<td>2900</td>
<td>36</td>
<td>0.9</td>
</tr>
</tbody>
</table>

$^a$) Solvent system, ethanol: 0.5 m sodium acetate (pH 7.5, 1:1, v/v).  
$^b$) In 0.5 m citrate buffer (pH 3.3, 700 V, 30 min).  
$c$) Relative value with respect to dTPP.