γ-Phosphate-substituted 2'-Deoxynucleoside 5'-Triphosphates as Substrates for DNA Polymerases*

Andrey A. Arzumanov, Dmitry G. Semizarov, Lyubov S. Victorova, Natalia B. Dyatkina, and Alexander A. Krayevsky‡

From the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov Street, Moscow 117984, Russia

Several 2'-deoxynucleoside 5'-triphosphate and 3'-azido-2',3'-dideoxynucleoside 5'-triphosphate analogs containing a hydrophobic phosphonate group instead of the γ-phosphate were synthesized and evaluated as substrates for human immunodeficiency virus (HIV) and avian myeloblastosis virus reverse transcriptases, human placental DNA polymerases α and β, and calf thymus terminal deoxynucleotidyl transferase. They were efficiently incorporated into the DNA chain by the retroviral enzymes but were not utilized by the mammalian ones. Also, some γ-ester and γ-amide derivatives of dTTP and 3'-azido-2',3'-dideoxynucleoside 5'-triphosphate (AZTTP) were synthesized and studied. They proved to be substrates for both the retroviral and mammalian enzymes under study. The Kₘ values for incorporation of the dTTP derivatives into the DNA chain were close to those for dTTP and AZTTP. The Kₘ for the AZTTP derivatives were one order of magnitude greater than those for dTTP and AZTTP. The results obtained indicate that HIV and avian myeloblastosis virus reverse transcriptases have no steric obstacles for binding the triphosphate fragment bearing a bulky substituent at the γ-position. Modification of the γ-phosphate in AZTTP increased the selectivity of HIV reverse transcriptase inhibition versus DNA polymerase α. γ-Methylphosphonate and γ-phenylphosphonate were dephosphorylated in human serum much less rapidly than AZTTP. Besides, they were shown to be markedly more hydrophobic than AZTTP. Thus, replacement of the γ-phosphate in AZTTP with γ-phosphonate markedly alters its substrate properties toward some cellular DNA polymerases and blood dephosphorylating enzymes but does not change its substrate activity with respect to HIV reverse transcriptase.

The γ-phosphate seems to play an important role in binding of dNTP to the enzyme + template-primer complex. Indeed, 2'-deoxynucleoside 5'-diphosphates display a hundredfold lower affinity to Escherichia coli DNA polymerase (1) and human placental DNA polymerase α (2, 3) as compared with dNTP. X-ray analysis of rat liver DNA polymerase β complexed with a template, terminated primer, and 2',3'-dideoxycytidine 5'-triphosphate revealed that the γ-phosphate appears to interact with residues Asp-190, Asp-192, Arg-149, and Gly-189 of the enzyme (4). In the molecule of HIV reverse transcriptase, Asp-110, Asp-185, and Asp-186 are likely to interact with the γ-phosphate of dNTP (5). Residue Asp-882 of E. coli DNA polymerase I is a catalytically important residue (6). According to the sequence, it is equivalent to Asp-190 of DNA polymerase β (5).

The process of substrate binding to E. coli DNA polymerase I has been earlier studied (7). It has been assumed that the preformed Mg²⁺-dATP complex binds to the enzyme as a β,γ-dicarboxylate. A chemical mechanism has been proposed for substrate binding to DNA polymerases (8). At the first step, dNTP binds to the enzyme and interacts with the enzyme-bound divalent cation only via its γ-phosphate. This complex has been detected by NMR spectroscopy (9). At the second verification step, only if a proper Watson-Crick base pair can form between the substrate and the corresponding residue of the template, the β-phosphate of dNTP also coordinates to the enzyme-bound metal cation and thus ensures the distance between the primer terminus and α-phosphate of dNTP appropriate for nucleophilic substitution at the phosphorus atom. It is noteworthy that the regions surrounding the polymerase active site correspond to motifs that are conserved in DNA polymerases (10, 11). Therefore, it seems likely that different DNA polymerases bind dNTPs by similar mechanisms.

γ-Phosphate-substituted dNTPs differ in the substrate specificity toward DNA polymerases. In particular, AZT 5'-α-phosphate-β-phosphonoylaceta and 3'-α-phosphate-β-phosphonoylaceta, a compound in which the γ-phosphate is replaced by a carboxyl group, was a terminating substrate for HIV and AMV reverse transcriptases (12, 13). 2'-Deoxycytidine 5'-α-phosphoryl-β-phosphonoylaceta was shown to be a substrate for HIV and AMV reverse transcriptases, rat liver DNA polymerase β, and calf thymus terminal deoxynucleotidyl transferase, but it was not utilized by human placental DNA polymerase α and E. coli DNA polymerase I (13). γ-Methylamides of dTTP and dATP were not substrates for human placental DNA polymerase α (2). It has also been shown that γ-[4-(N-2-chloroethyl-N-methylamino)benzylamido]-dNTP covalently modifies E. coli DNA polymerase I (14) and reverse transcriptase (15).

Here we report the synthesis, substrate properties, and stability in human blood serum of γ-methyl and γ-phenylphosphonate diphosphates of 2'-deoxycytidine Ia and Ib and 3'-azido-2',3'-dideoxycytidine IIa and IIb and compare their properties with those of γ-phosphate-substituted nucleotides Ic, Id, Ile, and IIId (Structure 1).

* This work was supported by Grant 95-03-08142a from the Russian Foundation for Basic Research, International Science Foundation Grant N23000, and the National Institutes of Health (Career Development Award grant). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed. Tel.: 7-095-135-22-33; Fax: 7-095-135-14-05; E-mail: AAK@imb.imb.ac.ru.

1 The abbreviations used are: AZT, azidothymidine; HIV, human immunodeficiency virus; AMV, avian myeloblastosis virus; AZTTP, 3'-azido-2',3'-dideoxycytidine 5'-triphosphate; HPLC, high pressure liquid chromatography.
Materials and Methods

Methylphosphonic and phenylphosphonic dichlorides and phenyl phosphonic dichloride were from Aldrich. 2-Deoxynucleoside 5'-diphosphates were synthesized according to Ref. 16.

For DNA polymerase assays, the samples of Ia and IIa were purified by HPLC on a Daltosil C-18 column (150 × 4 mm, 4 μm); elution was with a linear gradient of 0–25% methanol in 0.05 M KH2PO4 (pH 6.0) with UV detection at 270 nm. The flow rate was 0.6 ml/min.

The yield of compounds Ia, Ib, Ia, and Ib was 0.11 mmol (20%) and 0.13 mmol (26%), respectively. The data of 31P NMR are presented in Table I.

Enzymes and DNA

HIV reverse transcriptase was isolated according to Ref. 17. DNA polymerases α and β were isolated from human placenta as described in Refs. 18 and 19. AMV reverse transcriptase and terminal deoxynucleotidyl transferase were from Omutninsk Chemicals (Omutninsk, Russia) and Amersham Corp., respectively.

 Primer Extension Assays

For the template-dependent DNA polymerases, the assay mixture (volume 6 μl) contained 0.01 μM template-primer (Scheme 1), compound under study or dTPP, enzyme (2 activity units of reverse transcriptases or 1 unit of DNA polymerases α and β), and the corresponding buffer. The reaction was carried out for 20 min at 37°C and terminated by adding 3 μl of deionized formamide containing 0.5 mM EDTA and 2% bromophenol blue and xylene cyanol. The reaction products were separated by electrophoresis in 20% polyacrylamide gel, and the gels obtained were radioautographed.

Kinetic measurements (22) were performed within the linear region of the product formation versus time curve. The reaction time was 2 min.

For terminal deoxynucleotidyl transferase, the assay mixture (volume, 5 μl) contained 0.1 μM 5'-32P-labeled tetradeoxynucleotide primer (as in Scheme 1), compound under study, 2 units of the enzyme, 100 mM sodium cacodylate (pH 7.2), 10 mM MgCl2, 1 mM CaCl2, and 1 mM dithiothreitol.

DNA synthesis inhibition assays were carried out as described in Ref. 23. The assay mixture (volume, 6 μl) contained 0.02 μM template-primer, 20 μM dGTP and dCTP, 10 μM dATP (1 μCi of [α-32P]dATP), 3 μM dTTP, II, or AZTTP in different concentrations, enzyme, and the appropriate buffer.
The assay mixture containing 2.5 μl of 10 mM solution of IIa-d or AZTTP and 47.5 μl of 1% fetal blood serum was incubated at 37 °C, mixed with 50 μl of water and 230 μl of methanol, and cooled for 30 min at −20 °C. The samples were centrifuged for 10 min at 12,000 rpm, and the supernatants were concentrated to 100 μl and analyzed by HPLC.

The extent of hydrolysis was assessed by measuring the amount of the starting compound.

RESULTS

Compounds Ia, Ib, IIa, and IIb were synthesized at high yields using the standard method of phosphate activation by 1,2,4-triazole (24). It includes interaction of methyl- or phenylphosphonic bis-(1,2,4-triazolides) with 5'-diphosphates of 2'-deoxythymidine and 3'-azido-2',3'-dideoxythymidine. Compounds Ic and IIc were prepared as described in Ref. 25. Compounds IIa and IIc were prepared by one-pot synthesis using the procedure described in Ref. 16. The corresponding derivatives of ATP, dATP, UTP, and GTP have earlier been synthesized by reacting aniline with trimetaphosphate prepared in situ from nucleoside triphosphates (26). The 31P NMR data are presented in Table I.

Compounds I and II were evaluated as substrates for HIV and AMV reverse transcriptases, human placental DNA polymerases α and β, and calf thymus terminal deoxynucleotidyl transferase. The results of HIV reverse transcriptase assays are shown in Figs. 1 and 2.

Compounds Ib-d were proved to be substrates for HIV reverse transcriptase at 2 μM; they were incorporated into the growing DNA chains one (Fig. 1, lanes 3, 7, and 11), two (lanes 4 and 8, 9, and 12 and 13), and up to one hundred (lanes 6, 10, and 14) times. Similar results were obtained for Ia (data not shown). The presence of octadecanucleotide bands on lanes 4, 8, and 12 is due to the error prone properties of the enzyme.

All modified nucleoside 5'-triphosphates under study were utilized by AMV reverse transcriptase (data not shown). Table II lists the kinetic parameters for incorporation of I and II into the DNA chain by AMV reverse transcriptase. Clearly, $K_m$ for I are close to the values for dTTP and AZTTP, whereas $K_m$ (II) are 1 order of magnitude greater than $K_m$ for dTTP and AZTTP. The $V_{max}$ values are close for compounds studied.

It was shown that IIa (Fig. 2A, lanes 4 and 5), IIb (Fig. 2A, lanes 9 and 10), IIc (Fig. 2B, lanes 4 and 5), and IId (Fig. 2B, lanes 6 and 7) are incorporated into the DNA chain by HIV reverse transcriptase, the efficiency of incorporation being close for all compounds.

It can be seen in Fig. 3 that phenylphosphonate Ib was a very poor substrate for DNA polymerase α (Fig. 3, lanes 3 and 4), whereas Ic (Fig. 3, lanes 5 and 6) and Id (Fig. 3, lanes 7 and 8) were efficiently utilized by this enzyme. Methylphosphonate Ia (Fig. 3, lanes 9 and 10) was a slightly better substrate than its phenyl counterpart Ib. DNA polymerase β did not recognize Ib as a substrate even at 80 μM (Fig. 4, lanes 6–9) and utilized Ic only to a small extent (Fig. 4, lanes 13–19). In the control assays (Fig. 4, lanes 2–5), only natural dNTPs were used. At the same time, the enzyme incorporated IId and, less efficiently, Ia into the DNA chain (Fig. 5).

It is evident from Fig. 6 that Ic (lanes 5 and 6) and Id (lanes 7 and 8) are incorporated into the primer by terminal deoxynucleotidyl transferase to yield long oligonucleotide products; Ib (lanes 3 and 4) and Ia (lanes 9 and 10) also serve as substrates for the enzyme.

It was found that 3'-azido-2',3'-dideoxythymidine derivatives II are not substrates for DNA polymerases α and β and terminal deoxynucleotidyl transferase (data not shown). This finding is of no surprise, because AZTTP is a very poor substrate for these enzymes. However, both AZTTP and Ia-d at rather high concentrations inhibit DNA synthesis catalyzed by DNA polymerase α and terminal deoxynucleotidyl transferase (Table III). Compounds IIIa-d were as efficient as AZTTP in

### Table I

| Compound | $\delta$ ($ppm$) | $J$ ($Hz$) |
|----------|-----------------|-----------|
|          | $\alpha$-$p$    | $\beta$-$p$ | $\gamma$-$p$ | $\alpha$-$\beta$ | $\beta$-$\gamma$ |
| Ia       | −11.47d         | −22.60dd   | 18.18d       | 19.3           | 22.5           |
| Ib       | −10.66d         | −22.20m    | 6.91d        | 19.0           | 25.4           |
| Ic       | −11.20d         | −22.43m    | −9.71d       | 17.1           | 17.7           |
| Id       | −10.24d         | −23.22m    | −10.57d      | 19.4           | 19.8           |
| IIa      | −10.74d         | −21.83m    | 19.77d       | 19.0           | 20.3           |
| IIb      | −10.82d         | −22.26m    | 6.80d        | 18.3           | 25.2           |
| IIc      | −14.08d         | −23.26m    | −11.62d      | 20.2           | 20.8           |
| IId      | −11.53d         | −20.29m    | −10.02d      | 17.7           | 18.2           |

**Hydrolysis of Compounds IIa, IIb, IIc, IId, and AZTTP in Human Blood Serum**

The assay mixture containing 2.5 μl of 10 mM solution of IIa-d or AZTTP and 47.5 μl of 1% fetal blood serum was incubated at 37 °C, mixed with 50 μl of water and 230 μl of methanol, and cooled for 30 min at −20 °C. The samples were centrifuged for 10 min at 12,000 rpm, and the supernatants were concentrated to 100 μl and analyzed by HPLC.

**Fig. 1. Primer extension by HIV reverse transcriptase in the presence of Ib, Ic, and IId.** Lane 1, template-primer + enzyme; lane 2, as in lane 1 + dTTP; lane 3, as in lane 1 + Ib; lane 4, as in lane 1 + Ib + dGTP; lane 5, as in lane 1 + Ib + dATP + dCTP; lane 6, as in lane 1 + Ib + dGTP + dATP + dCTP; lane 7, as in lane 1 + Ic; lane 8, as in lane 1 + Ic + dGTP; lane 9, as in lane 1 + Ic + dGTP + dATP; lane 10, as in lane 1 + Ic + dGTP + dATP + dCTP; lane 11, as in lane 1 + IId; lane 12, as in lane 1 + IId + dGTP; lane 13, as in lane 1 + IId + dGTP + dATP; lane 14, as in lane 1 + IId + dGTP + dATP + dCTP. The concentration of all compounds was 2 μM.

**Fig. 2. Primer extension catalyzed by HIV reverse transcriptase.** A, lanes 1 and 6, template-primer + enzyme; lanes 2 and 7, as in lane 1 + 2 μM dTTP; lanes 3 and 8, as in lane 1 + 2 μM AZTTP; lane 4, as in lane 1 + 2 μM Ia; lane 5, as in lane 1 + 20 μM IIa; lane 9, as in lane 1 + 2 μM IId; lane 10, as in lane 1 + 20 μM IId. B, lane 1, template-primer + enzyme; lane 2, as in lane 1 + 2 μM dTTP; lane 3, as in lane 1 + 2 μM AZTTP; lane 4, as in lane 1 + 2 μM IIa; lane 5, as in lane 1 + 20 μM IIa; lane 6, as in lane 1 + 2 μM IId; lane 7, as in lane 1 + 20 μM IId.
inhibiting HIV reverse transcriptase, but their inhibitory effect on DNA polymerase α was 5–6-fold smaller than that of AZTTP.

We studied the stability of γ-phosphate-modified AZTTP derivatives in human blood serum. In all assays we did not observe formation of AZT 5'-diphosphate; only AZT 5'-monophosphate and AZT were formed (data not shown). It is evident from Table IV that for γ-methylphosphonate IIa and γ-phenylphosphonate IIb the half-lives are 8 and 11 times, respectively, greater than that for AZTTP. The dephosphorylation rates for IIc and IId were intermediate between the rates for IIa, IIb, and AZTTP.

It should be noted that compounds IIa, IIb, IIa, and IIb are surprisingly highly hydrophobic. The retention time for IIb and IIb on a reversed-phase HPLC column is even higher than that for AZT 5'-monophosphate (Table IV). TLC on Silica gel plates also revealed high hydrophobicity of IIa, IIb, IIa, and IIb.

**DISCUSSION**

The literature data outlined in the introduction indicate that replacement of the γ-phosphate residue with a carboxyl main-
tains the substrate properties of these dNTP analogs toward several DNA polymerases (12, 13). Consequently, for these enzymes one negative charge in the γ-phosphate is sufficient to ensure formation of a catalytically competent complex. Modification of the triphosphate residue in sugar-substituted dNTPs slightly reduces their affinity to reverse transcriptases but increases their selectivity toward these enzymes (23, 27).

The data obtained in this work may be summarized and analyzed as follows.

Replacement of the γ-Phosphate with Methylphosphonate or Phenylphosphonate—Replacement of the γ-phosphate with methylphosphonate or phenylphosphonate has a minor effect on the substrate properties of dTTP and AZTTP toward retroviral reverse transcriptases. Compounds II and AZTTP inhibited HIV reverse transcriptase-catalyzed DNA synthesis by 50% at close concentrations, but the $K_m$ values for the γ-phosphate-modified AZTTP derivatives differed 10–20-fold from that for AZTTP. This contradiction may be ascribed to the fact that the $K_m$ were measured in a standing start single-substrate incorporation assay, whereas the 50% inhibition values were determined in a system containing all four dNTPs. High substrate efficiency of γ-phosphate-modified analogs of dTTP and AZTTP suggests that the dNTP-binding site of HIV and AMV reverse transcriptases has no sterical obstacles for binding triphosphate fragments bearing bulky substituents at the γ-position. We have earlier studied the substrate properties of dTTP derivatives bearing methyl (23), phenyl, and decyl (28) residues at the $P^\gamma$ atom. It has been shown that modification of the α-phosphate markedly decreases the substrate efficiency of dTTP. The data obtained in this work indicate that modification of the γ-phosphate has a minor effect on the substrate properties of dTTP toward reverse transcriptases and some DNA polymerases. These results are easily explainable, because the α-phosphate is the reactive group of dNTP, whereas the γ-phosphate is not involved directly in the reaction, although it is assumed to play a role in dNTP binding to the enzyme.

Replacement of the γ-Phosphate with Phenylphosphonate—Replacement of the γ-phosphate with phenylphosphonate totally inactivates dTTP as substrate for DNA polymerases α and β and terminal deoxynucleotidyltransferase. However, the γ-ester and γ-amide derivatives (Ic and Id, respectively) are utilized by these enzymes. This is consistent with the data described in Refs. 2 and 3, which indicate that different 2′-deoxynucleoside γ-alkylamidotriphosphates are not substrates for DNA polymerase α.

It is unclear why the γ-aryl- and γ-alkylphosphonates differ in substrate activity from dTTP and its γ-amide and γ-ester derivatives. Conversion of 2′-deoxythymidine 5′-phosphate to 2′,5′-dideoxythymidine 5′-phosphate increases $pK_a$ from 6.5 to 7.0 (29). The angles O-P-O and C-P-O in 2′-aminoethylphosphate (30, 31) and 2′-aminoethylphosphonate (32) differ by 5°. The O-P bond in the first compound is longer than the C-P bond in the second one by 0.26 Å. The phosphoryl and phosphate groups differ in the distribution of electron density around the phosphorus atom. However, these data are not sufficient to explain the differences in the biochemical properties of γ-phosphates and γ-phosphonates.

Modification of the γ-Phosphate in AZTTP—Modification of the γ-phosphate in AZTTP increases the selectivity of HIV reverse transcriptase inhibition versus DNA polymerase α. Thus, modification of the triphosphate residue may be a useful step in designing selective inhibitors of HIV reverse transcriptase. Furthermore, γ-methylphosphonate IIa and γ-phenylphosphonate IIb are dephosphorylated in human serum much less rapidly than AZTTP. Besides, IIa and IIb are markedly more hydrophobic than AZTTP. Their TLC mobilities on silica gel plates in dioxane-NH$_2$OH-water (R$_f$ 0.40 and 0.45) are even higher than those for 2′-deoxythymidine 5′-monophosphate and AZT 5′-monophosphate (R$_f$ 0.38 and 0.42). For IIb and IIId the retention times in reversed-phase HPLC were 14 and 18 min, whereas for dTTP and AZTTP they were 6.8 and 7 min; for AZT 5′-monophosphate and AZT, they were 11 and 24 min. Thus, replacement of the γ-phosphate in AZTTP with γ-phosphonate markedly alters its substrate properties toward some cellular DNA polymerases and blood dephosphorylating enzymes but does not change its substrate activity with respect to HIV reverse transcriptase. It seems likely that modification of the triphosphate residue in nucleotide analogs will yield potent reverse transcriptase inhibitors stable in blood and hydrophobic enough to penetrate into the cell. Also, modified dNTPs of the proposed structure may be useful as tools for studying the cellular processes, in which nucleoside 5′-triphosphates serve as donors of γ-phosphates or nucleotide residues.

**REFERENCES**

1. Eglund, P. T., Huberman, J. A., Jovin, T. M., and Kernberg, A. (1969) J. Biol. Chem. 244, 3038–3044
2. Doronin, S. V., Lavrik, O. I., Nevinsky, G. A., and Podust, V. N. (1987) FEBS Lett. 216, 221–224
3. Knorre, D. G., Lavrik, O. I., and Nevinsky, G. A. (1988) Biochimie (Paris) 70, 655–661
4. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., and Kraut, J. (1994) Science 264, 1891–1903
5. Tantillo, C., Ding, J., Jacobo-Molina, A., Nanni, R. G., Boyer, P. L., Hughes, S. H., Powels, R., Andries, K., Janssen, P. A. J., and Arnold, E. (1994) Mol. Biol. 243, 369–387
6. Polesky, A. H., Dahlberg, M. E., Benkovic, S. J., Grindley, N. D. F., and Joyce, C. M. (1992) J. Biol. Chem. 267, 8417–8428
7. Burgers, P. M. J., and Eckstein, F. (1979) J. Biol. Chem. 254, 6889–6893
8. Ferrin, L. R., and Mildvan, A. S. (1986) Biochemistry 25, 5141–5145
9. Sloan, D. L., Loeb, L. A., Mildvan, A. S., and Feldmann, R. J. (1975) J. Biol. Chem. 250, 8913–8920
10. Polis, C., Sawagut, I., Delarue, M., and Tordo, N. (1999) EMBO J. 8, 3867–3874
11. Delarue, M., Polis, O., Tordo, N., Moras, D., and Argos, P. (1990) Protein Eng. 4, 461–467
12. Klesso, T. G., Tarusova, N. B., Atrashkevich, E. D., Kukanova, M. K., Shulezhen, S. V., Bokhov, A. F., Gareev, M. M., Galeyev, G. G., and Krayevsky, A. A. (1990) Biolog. Khim. 16, 530–536
13. Rosovskaya, T. A., Mishchenko, A. V., Tarusova, N. B., Kukanova, M. K., Krayevsky, A. A., and Beabalaashvili, R. Sh. (1993) Mol. Biol. (Moscow) 27, 1051–1060
14. Buneva, N. V., Demidova, T. V., Knorre, D. G., Kudriashova, N. V., Romanchedenko, A. G., and Starobrazova, M. G. (1980) Mol. Biol. Russian 14, 1080–1087
15. Buneva, N. V., Kudriashova, N. V., Nebrat, L. T., Romanchedeno, A. G., Chimistova, T. A., and Isakhsheva, L. F. (1985) Dokl. Akad. Nauk SSSR 286, 243–246
16. Ludwig, J. (1981) Acta Biochim. Biophys. Acad. Sci. Hung. 16, 131–133
17. Rosovskaya, T. A., Belogurov, A. A., Lukin, M. A., Chernov, D. N., and Kukanova, M. K. (1992) Mol. Biol. Russian 27, 618–630
18. Mozherin, D. J., Atrashkevich, A. M., and Kukanova, M. K. (1992) Mol. Biol. Russian 26, 999–1010
9. Kolocheva, T. A., and Nevinsky, G. A. (1993) Mol. Biol. Russian 27, 1368–1379
10. Kraev, A. A. (1988) Mol. Biol. Russian 22, 1164–1197
11. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 11.31–11.33, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Semizarov, D. G., Victorova, L. S., Krayevsky, A. A., and Kukhanova, M. K. (1993) FEBS Lett. 327, 45–48
13. Victorova, L. S., Dyatkina, N. B., Mozharev, D. J., Atrazhev, A. M., Krayevsky, A., and Kukhanova, M. K. (1992) Nucleic Acids Res. 20, 783–789
14. Arzumanov, A. A., and Dyatkina, N. B. (1994) Nucleosides Nucleotides 13, 1031–1038
15. Armstrong, V. W., and Eckstein, F. (1976) Eur. J. Biochem. 70, 33–38
16. Babkina, G. T., Zarytova, V. F., and Knorre, D. G. (1975) Bioorg. Chem. Russian 1, 611–615
17. Atrazhev, A. M., Dyatkina, N. B., Krayevsky, A. A., Kukhanova, M. K., Chidgeavaevadze, Z. G., and Beabealashvilli, R. S. (1987) Bioorg. Chem. Russian 13, 1045–1052
18. Dyatkina, N., Arzumanov, A., Victorova, L., Kukhanova, M., and Krayevsky, A. (1995) Nucleosides Nucleotides 14, 91–103
19. Chambers, R. D., O’Hagan, D., Lamont, R. B., and Jain, S. C. (1990) J. Chem. Soc. Chem. Commun. 1053–1057
20. Kraut, J. (1961) Acta Crystallogr. 14, 1146–1152
21. Perrier, W. G., Lindsey, A. R., and Young, D. W. (1962) Acta Crystallogr. 15, 616–618
22. Okaya, Y. (1966) Acta Crystallogr. 20, 712–715

γ-Phosphate-substituted 2'-Deoxynucleoside 5'-Triphosphates