L- and D-Enantiomers of 2',3'-Dideoxycytidine 5'-Triphosphate Analogs as Substrates for Human DNA Polymerases

IMPLICATIONS FOR THE MECHANISM OF TOXICITY*

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5'-Triphosphates of β-D and β-L-enantiomers of 2',3'-dideoxycytidine (ddC), 2',3'-dideoxy-5-fluorocytidine (FddC), 1,3-dioxolane-cytidine (OddC), and 1,3-dioxolane-5-fluorocytidine (FOddC) were evaluated as inhibitors and substrates for human DNA polymerases α, β, γ, δ, and ε. L-ddCTP was not a substrate or inhibitor for any DNA polymerase studied; L-FddCTP was not an inhibitor or substrate for replicative DNA polymerases and was a less potent inhibitor of DNA polymerases γ and β than its ω-enantiomer by 2 orders of magnitude. In contrast, all L-dioxolane analogs were potent inhibitors and chain terminators for all cellular DNA polymerases studied. The Ki values of their 5'-triphosphates for DNA polymerase γ were found to be in the following order: d-ddC < d-FddC < l-FddC < l-OddC < l-FOddC < l-FddC. The Ki values of l-OddCTP for the reactions catalyzed by DNA polymerases α, δ, ε, β, and γ were 6.0, 1.9, 0.4, 3.0, and 0.014 μM, respectively, and those of l-FOddCTP were 6.5, 1.9, 0.7, 19, and 0.06 μM, respectively. The Km values for incorporation of l-OddCTP into the standing points of primer extension were also evaluated and determined to be 1.3, 3.5, 1.5, 2.8, and 0.7 μM for DNA polymerases α, δ, ε, β, and γ, respectively. The incorporation of dioxolane analogs into DNA by replicative DNA polymerases could explain their potent cellular toxicity.

Several 2',3'-dideoxynucleoside analogs have been approved for the treatment of patients with AIDS (1, 2). Among them, ddC has been shown to be one of the most potent inhibitors of HIV replication. Recently, Sddc was found to have potent activity against HIV (3, 4) and hepatitis B virus (5) in cell culture. It was subsequently shown that the stereoisomer L-SddC (3TC) was responsible for the antiviral effect, while cytotoxicity was associated mainly with the ω-enantiomer (6–9). L-ddC and L-FddC were also found to be active against hepatitis B virus and HIV in culture without much toxicity (10–12). In contrast to L-SddC or L-FddC, L-OddC and L-FOddC were markedly more toxic than their ω-enantiomers against human leukaemic CEM cells (13). The spectrum of L-OddC toxicity against human tumor cells differed from that of cytosine arabinoside. L-OddC was also shown to have activity against solid human tumors in nude mice (14). Currently, L-OddC is being evaluated further as an anticancer agent. The reason why L-OddC but not L-ddC or L-SddC has potent antitumor activity is not clear, but it is probably associated with its interference in cellular DNA synthesis. L-SddC and L-OddC were shown to be phosphorylated by cellular kinases to the corresponding 5'-triphosphate metabolites inside cells. The antiviral effects of nucleoside analog are due to preferential interference with viral replication caused by incorporation into DNA by DNA polymerase. The interaction of 5'-triphosphate metabolites of L-OddC or L-FddC with cellular DNA polymerases may be one of the key factors in determining L-OddC cytotoxicity.

In the present paper, the interaction of 5'-triphosphates of β- and δ- enantiomers of 2',3'-dideoxycytidine and 1,3-dioxolane-cytidine as well as their 5-fluoro-derivatives with human pol α, pol β, pol ε, pol δ, and pol γ is reported.

EXPERIMENTAL PROCEDURES

Materials and Compounds—L- and d-stereoisomers of ddC and OddC and their 5-fluoro-derivatives were synthesized as described previously (11, 13). The triphosphate forms of these compounds were synthesized in our laboratory (15). Unlabeled dNTP and ddNTP were purchased from Bio-Rad Mannheim. Poly(rA)-oligo(dT)12-18 and poly(IA)-oligo(dT)12-18 were obtained from Pharmacia Biotech Inc. ssDNA cellulose was obtained from Sigma. DEAE cellulose (DE-52), phosphocellulose (P11), and S-Sepharose fast flow were purchased from Pharmacia Biotech Inc. and their 5-fluoro-derivatives were synthesized as described previously (16). Oligonucleotides were synthesized on an Applied Biosystems 380A DNA synthesizer at the Yale Oligonucleotide Synthesis Facility. The primer oligonucleotides were labeled at the 5′ position with T4 polynucleotide kinase using γ-32P]ATP, annealed to M13 mp10 and M13 mp18 phage ssDNA were isolated as described previously (16). Oligonucleotides were synthesized on an Applied Biosystems 380A DNA synthesizer at the Yale Oligonucleotide Synthesis Facility. The primer oligonucleotides were labeled at the 5′ position with T4 polynucleotide kinase using γ-32P]ATP, annealed to M13 mp18 phage ssDNA as described in Ref. 16, purified on a Sephadex G-25 column, and used as substrates for elongation reactions. BuAdATP was a kind gift from Professor George Wright (University of Massachusetts).

Enzymes—Human pol α, pol δ, and pol γ from K562 chronic myelogenous leukemia cells and pol β from KB cells were partially purified using P11, DE-52, S-Sepharose, and ssDNA cellulose chromatography (6). All DNA polymerases were distinguished from each other using several biochemical criteria, including chromatographic behavior, template preference, sensitivity to specific inhibitors and associated exonuclease activities. The ability to use poly(rA)-oligo(dT)18 as template and primer in the presence of 80 mM KCl at pH 7.5–8.0 and high sensitivity to ddNTP indicated that the DNA polymerase activity was that of pol γ (17–19). pol δ and pol α were eluted from a ssDNA-cellulose column at 0.32 M and 0.4 M KCl, respectively. Additional purifications were performed on DE-52 and S-Sepharose columns. pol δ and pol α activities
were monitored on poly(dA)·oligo(dT)₁₅₋₁₈ in the presence of proliferating cell nuclear antigen and activated DNA, respectively, pol α was very sensitive to the inhibitor BuAtDATP, displaying 25% residual activity in the presence of 5 μM of this analog. In contrast, pol δ was much less sensitive, showing 80–90% residual activity with the same concentration of drug. pol δ displayed significant 3′→5′ exonuclease activity in contrast with the pol α preparation, which showed only traces of the 3′→5′ exonuclease activity, which were probably due to cross-contamination. As expected, the activity of pol δ was stimulated by proliferating cell nuclear antigen using poly(dA)·oligo(dT)₁₂₋₁₈ as template–primer, but proliferating cell nuclear antigen had no effect on pol α (20–22). pol β that was separated from other polymerases using ssDNA cellulose chromatography was resistant to 3 mM N-ethylmaleimide (23). pol ε was purified to near homogeneity from human placenta (24).

pol α and pol δ activities were routinely assayed in 20 μl of 20 mM Tris-HCl buffer (pH 7.4) containing 6 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM EDTA, 200 μg/ml heat-inactivated bovine serum albumin, 150 μg/ml activated calf thymus DNA, 20 μM each of dATP, dTTP, and dGTP, and 1 μCi of [³²P]dCTP or (³²P)ddCTP at concentrations no less than the Kᵅ values for the appropriate enzymes. The same assay conditions were used for other polymerases with the following modifications: pol β, pH 8.5; pol γ, pH 8.0 and 80 mM KCl; and pol ε, 8 mM MgCl₂. Assays for DNA polymerases contained 2 μl of enzyme (1 unit of pol α, pol ε, or pol γ, 2 units of pol δ, 0.75 unit of pol β). One unit of enzyme activity is defined as the amount of enzyme needed to incorporate 1 nmol of [³²P]dTMP/h into the acid-insoluble fraction at 37°C. The reactions were allowed to proceed at 37°C for 10–60 min, after which 15-μl aliquots were removed and spotted onto Whatman DE-81 discs. The filters were washed with 0.3 M KCl containing 0.5 mM EDTA and then fixed with ethanol. The incorporation of the radiolabeled substrate into the DNA chain was measured by liquid scintillation counting.

The Km values for each compound for all DNA polymerases studied were determined using a competitive inhibition equation as described in Ref. 25.

Chain Termination Assays—Triphosphates of ddCTP analogs were analyzed for their chain terminating activity using M13 phage DNA hybridized with a 5′-[³²P]-primer. Incorporation of one template-complementexonuclease residuum into the 3′-terminus of the primer was carried out in 8 μl of a mixture containing the appropriate buffer solution as described above, enzyme, 0.01 μl primer-template complex, and the analog under investigation. After incubation, the reaction was stopped by adding 4.5 μl of formamide containing dyes and EDTA, and the reaction products were separated by denaturing 15% polyacrylamide gel electrophoresis. Chain termination assays in the presence of four natural dNTPs were based on previously published methods (26) and described in Fig. 6.

Quantitation of the Primer Extension Assay—The bands on the x-ray film, representing the incorporation of dNTP analogs into the standing position of 3′-ends of 5′-[³²P]-primers annealed with template, were quantitated with the aid of a densitometer (Molecular Dynamics) as described previously (27). The bands were scanned, and the efficiency of the primer extension and its dependence on substrate concentration was measured as a Lineweaver-Burk plot (28). Experimental measurements were carried out within the linear range of the counts versus integration curve. The incubation time for all experiments was chosen to have a linear dependence between yield of product and time. The measurements were carried out within the linear range of the counts versus integration curve. The incubation time for all experiments was chosen to have a linear dependence between yield of product and time.

Inhibition of Human DNA Polymerases by L- and D-Enantiomers of ddCTP Analogs—The structures of ddCTP analogs evaluated for their ability to interact with human DNA polymerases are shown in Fig. 1. The Km values of dCTP for pol α, pol δ, pol ε, pol β, and pol γ were 0.6 μM, 0.4 μM, 2.0 μM, 1.9 μM, and 0.16 μM, respectively (6, 20). Table I lists the Km values for L- and D-ddCTP analogs for these enzymes. As shown in Table I, L-OddCTP and L-FoddCTP were potent inhibitors of replicative DNA polymerases. The Km values of L-FoddCTP were about the same as those of its D-enantiomer for pol δ, pol δ, and pol γ, but were less for pol α and pol ε. The 5′-triphosphates of L-FddC and L-ddC at concentrations up to 20 μM had no inhibitory effect on pol α, pol β, pol δ, and pol ε. The results obtained for D-ddCTP and D-FddCTP were similar to those published previously (29, 30). pol γ was much more sensitive than any other polymerase to all compounds tested with the exception of L-ddCTP. The triphosphate of L-OddC, which was shown to have antitumor activity (14), was a potent inhibitor of all DNA polymerase studied.

Table I lists the Km values of the appropriate enzymes. The same assay conditions were used for other polymerases with the following modifications: pol β, pH 8.5; pol γ, pH 8.0 and 80 mM KCl; and pol ε, 8 mM MgCl₂. Assays for DNA polymerases contained 2 μl of enzyme (1 unit of pol α, pol ε, or pol γ, 2 units) of pol δ, 0.75 unit of pol β). One unit of enzyme activity is defined as the amount of enzyme needed to incorporate 1 nmol of [³²P]dTMP/h into the acid-insoluble fraction at 37°C. The reactions were allowed to proceed at 37°C for 10–60 min, after which 15-μl aliquots were removed and spotted onto Whatman DE-81 discs. The filters were washed with 0.3 M KCl containing 0.5 mM EDTA and then fixed with ethanol. The incorporation of the radiolabeled substrate into the DNA chain was measured by liquid scintillation counting.

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Complexes of M13 phage DNA and primer used in this study were as follows.

M13mp18 phage DNA

3′-CATTTTGTCCGGCCTACGGG-5′

5′-GTAACACGGCGCGATT-3′

←17-mer primer→

M13mp10 phage DNA

3′-GTTAAGGTGCGCAATATTGCTCCGCGG-5′

5′-CAGTTCAAGCAGCAGGTTGAAACGGA-3′

←14-mer primer→

RESULTS

Inhibition of Human DNA Polymerases by L- and D-Enantiomers of ddCTP Analogs—The structures of ddCTP analogs evaluated for their ability to interact with human DNA polymerases are shown in Fig. 1. The Km values of dCTP for pol α, pol δ, pol ε, pol β, and pol γ were 0.6 μM, 0.4 μM, 2.0 μM, 1.9 μM, and 0.16 μM, respectively (6, 20).

![Fig. 1. Structure of L- and D-enantiomers of 2',3'-dideoxyctydine 5'-triphosphate analogs.](image-url)
no longer linear. Similar methods were applied for pol γ, pol ε, and pol δ. The $K_m$ values for dCTP and L-OddCTP are presented in Table II. In the evaluation of Table II, it should be emphasized that the kinetic values for the incorporation of natural nucleotides or analogs could be dependent on the nucleotide sequence of template-primer used (31). However, the ratio of the $K_m$ value of any nucleotide analog to $K_m$ value of the natural nucleotide should give a relative measure of the ability of that particular analog to be a substrate of DNA polymerase in comparison with the natural nucleotide.

As previously shown (6, 20), L-SddCTP was a weak inhibitor of pol α and pol δ and was not a substrate for either enzyme. We compared the ability of L-SddCTP and L-OddCTP to be incorporated at standing and running points of a DNA chain by pol α under similar conditions. As one can see in Fig. 5, L-SddCTP is incorporated neither into the first nor into the eighth position of an elongated primer. Conversely, L-OddCTP is a good substrate for pol α, and the DNA fragments terminated by L-OddCMP are accumulated in both cases.

Chain Termination of DNA Synthesis by L-OddCTP in the Presence of All Four dNTPs—L-OddCTP was also assayed as a terminating substrate for pol α in the presence of all four dNTPs following Sanger's method (26). The results are shown in Fig. 6. The comparison of the DNA sequence by Klenow fragment with d-ddCTP as a terminator is also presented. Synthesis was performed in the presence of M13 mp10 phage DNA annealed with 5'-32P-15-mer primer. DNA reaction products were analyzed using 12% polyacrylamide gel electrophoresis. The primer extension products of L-OddCTP were similar to the products observed for the reaction with d-ddCTP and Klenow fragment, as shown in lane 1 and lanes 2-5. These results indicate that L-OddCTP was incorporated into DNA at cytosine residue sites. Subsequent chase containing 100 μM of four dNTPs (lane 4), which failed to extend the 3'-L-OddCMP terminated products, showed the ability of pol α to incorporate L-OddCTP into the growing DNA chain in the presence of ddCTP. An accumulation of DNA fragments was observed at a length expected for the chain terminator with cytidine as a base.

**DISCUSSION**

Recently, nucleoside derivatives with the unnatural L-configuration were evaluated against a broad spectrum of viruses, and some of these analogs proved to be very potent against...
hepatitis B virus and HIV (6–12). At the same time, differences in their cellular toxicities were noticed. In contrast to L-SddC or L-ddC, both L- and D-enantiomers of FOddC and OddC were shown to be very cytotoxic (13, 14). The interaction of their 5'-triphosphate metabolites with cellular DNA polymerases may be one of the key factors in their varying toxicities. This possibility prompted us to evaluate the 5'-triphosphates of L- and D-enantiomers of dioxolane-cytidine as potential inhibitors or substrates of human DNA polymerases. For comparison, nucleoside 5'-triphosphates with the natural D-configuration were included in the study. The results presented here show that L-OddCTP and its 5-fluoro-derivative are potent inhibitors and chain terminators of cellular DNA polymerases including pol α, pol ε, and pol δ. The inhibition of polymerase activity is due to the incorporation of L-OddCTP into the DNA chain at cytidine residue sites but not to the inhibition of the rate of incorporation of other dNTPs. If the inhibition of synthesis was due to a dual mechanism including both incorporation and

![FIG. 4. Concentration-dependent incorporation of L-OddCTP into DNA by pol α (lanes 1–7) and pol δ (lanes 8–14) into the 3'-end of 5'-32P-15-mer primer annealed with M13 mp10 phage DNA. DNA primer extension was carried out as described under “Experimental Procedures” with the exception that incubation time was 10 min, and 0.2 unit of pol α and 0.25 unit of pol δ were used to assure that less than 30% of the primer was consumed during experiments. A, incorporation of 2.5 μM dCTP (lanes 1 and 8); 0.2, 0.5, 1, 2, 5, and 10 μM L-OddCTP (lanes 2–7 and lanes 9–14, respectively. B, the intensity of each track from A was quantitated by computer densitometry and plotted as 1/V versus 1/[S] for the reactions catalyzed by DNA pol α (– –) and pol δ (–△–). 1/V is presented as relative value of density.

| Polymerase | Km(dCTP) | Km(L-OddCTP) | Km(L-OddCTP)/Km(dCTP) |
|------------|----------|--------------|------------------------|
| pol α      | 0.6 μM   | 1.3 ± 0.5 μM | 2.2                    |
| pol δ      | 0.4 μM   | 3.5 ± 0.8 μM | 8.8                    |
| pol ε      | 2.0 μM   | 1.5 ± 0.5 μM | 0.8                    |
| pol β      | 1.9 μM   | 2.8 ± 1.2 μM | 1.5                    |
| pol γ      | 0.16 μM  | 0.7 ± 0.3 μM | 4.4                    |

![FIG. 5. Incorporation of L-OddCTP (A, lane 2, and B, lanes 1 and 2) and L-SddCTP (A, lanes 3 and 4, and B, lanes 3–5) in standing (A) and running (B) points of 15-mer primer (A) or 14-mer primer (B) annealed to M13 mp10 phage DNA by pol α. Panel A, lane 1, shows the incorporation of dCTP. The reactions were performed as described under “Experimental Procedures.”

![FIG. 6. Autoradiograph of chain terminating sequencing reaction with L-OddCTP using pol α (lanes 2–6) and M13 mp10 phage DNA annealed with 5'-32P-15 mer primer. The letters on the left side of the figure indicate the sequence of the growing DNA chain after the primer. Lane 1 (control) shows the DNA sequence by Klenow fragment with 20 μM ddCTP. Lanes 2–5, DNA sequence by pol α with L-OddCTP at concentrations 10, 20, 20, and 40 μM, respectively. Lane 4, after the reaction, 100 μM mixture containing all four dNTP was added, and the reaction continued 20 min more. Track 6, reaction without L-OddCTP. The mixture for pol α contained 2.5 μM dCTP and 20 μM each of three other dNTPs.}
interference with the incorporation of other nucleosides, we would expect to see DNA fragments of a size inconsistent with DNA products terminated at cytidine residues (pauses) (Fig. 6). The interaction of L-OddCTP and L-FOddCTP with human DNA polymerases is the first example of the lack of enantioselectivity described for human replicative DNA polymerases. This phenomenon was described for HIV reverse transcriptase, pol γ, and pol β with respect to the L- and D-enantiomers of SddCTP (6, 20, 32) and to carbobir triphosphate (33). It should be mentioned that replicative DNA polymerases are believed to be more sensitive to changes in the conformation of substrates. Indeed, this is true for SddCTP whose L-isomer is not a substrate for replicative DNA polymerases. However, substitution of sulfur for oxygen at the 3′-position of the ribose residue leads to the opposite effect. L-OddCTP is a good terminating substrate for replicative DNA polymerases, inhibiting them with Kₐ values in the pharmacologically relevant concentration range.

The amount of L-OddCMP present at the DNA terminus depends not only on the efficiency of incorporation of L-OddCTP by polymerases but also on the rate of excision by 3′ → 5′-exonucleases (34). The ability of 3′ → 5′-exonucleases associated with pol δ and pol ε are currently evaluated. The anticancer activity of L-OddC may be a result of termination of DNA synthesis after L-OddCTP incorporation into proliferating cells coupled with inefficient excision of incorporated L-OddCMP from DNA.

In the present study, we also made an attempt to address the impact of the substitution of fluorine for hydrogen at the 5′-position of cytidine 5′-triphosphate analogs on their ability to serve as substrates for cellular DNA polymerases. As shown in Table 1, both L- and D-enantiomers of ddCTP and FddCTP were not substrates for pol α, pol ε, and pol δ and inhibited pol β and pol γ at the same range of concentrations. Similar results were obtained for the L-enantiomers of OddCTP and FOddCTP. No significant differences were seen between L-OddCTP and its 5-fluoro-derivatives in terms of their interaction with DNA polymerases. Both L-enantiomers were equally potent inhibitors for these DNA polymerases. With respect to pol β, L-OddCTP was 6 times more potent than its 5-fluoro-analog. At present, we cannot explain the differences in the interaction of L-OddCTP and its 5-fluoro-derivative with pol β.

In summary, L-OddC is the first L-nucleoside shown to have potent antitumor activity. This activity could be related to the ability of L-OddCTP to be utilized as a substrate by human replicative DNA polymerases. Surprisingly, this property is not shared with L-SddC and L-ddC, which are relatively noncytotoxic. The discovery that the L-enantiomers of chain-terminating nucleotides can be incorporated by replicative DNA polymerases could lead to the development of a new class of anticancer compounds.

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REFERENCES

1. Mitsuya, H., and Broder, S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1911–1915
2. Mitsuya, H., Weinhold, K. J., Furman, P. A., St. Clair, M. H., Lehrman, S. N., Galli, R. C., Bolognesi, D., Barry, D. W., and Broder, S. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7096–7100
3. Belleau, B., Dixit, D., Nguyen-Ba, N., and Kraus, J. L. (1989) Fifth International Conference on AIDS, Montreal, Canada, June 4–9, 1989, p. 515
4. Soudens, H., Yao, X. J., Gao, Q., Belleau, B., Kraus, J. L., Nguyen-Ba, N., Spiro, B., and Wainberg, M. A. (1991) Antimicrob. Agents Chemother. 35, 1390–1396
5. Doog, S. L., Tsai, C. H., Schinazi, R. F., Liotta, D. C., and Cheng, Y. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8495–8499
6. Chang, C. N., Skalski, V., Zhou, I. J., and Cheng, Y. Y. (1992) J. Biol. Chem. 267, 22144–221420
7. Coombs, A. V., Connemac, N. J., unkonikin, H. J., Mutton, I. M., Pearson, B. A., Storer, R., Cameron, J. M., and Penn, C. R. (1992) Antimicrob. Agents Chemother. 36, 202–205
8. Shibata, R. F., Chu, C. K., Peck, A., McMillan, A., Mathis, R., Cannon, D., Jeng, L. S., Beach, J. W., Choi, W. B., Yeha, S., and Liotta, D. C. (1992) Antimicrob. Agents Chemother. 36, 672–676
9. Beach, J. W., Jeng, L. S., Alves, J. J., Puhl, D., Kim, H. O., Chang, C. N., Doong, S. L., Schinazi, R. F., Cheng, Y. C., and Chu, C. K. (1992) J. Org. Chem. 57, 2217–2219
10. Lin, T. S., Luo, M. Z., Liu, M. C., Pai, B., Dutschman, G. E., and Cheng, Y. C. (1994) Biochem. Pharmacol. 47, 171–174
11. Lin, T. S., Luo, M. Z., Liu, M. C., Pai, B., Dutschman, G. E., and Cheng, Y. C. (1994) J. Med. Chem. 37, 798–803
12. Schinazi, R. F., Gosselin, G., Faraj, A., Korba, B. E., Liotta, D. C., Chu, C. K., Mathe, C., Imbach, J. L., and Sommadossi, J. P. (1994) Antimicrob. Agents Chemother. 38, 2172–2174
13. Kim, H. O., Shannuruganiathan, K., Alves, A. J., Jeng, L. S., Beach, J. W., Shinazi, R. F., Chang, C. N., Cheng, Y. C., and Chu, C. K. (1992) Tetrahedron Lett. 33, 6899–6902
14. Grove, K. L., Guo, X., Liu, S. H., Gao, Z., Chu, C. K., and Cheng, Y. C. (1995) Cancer Res. 55, 3008–3011
15. Chang, C. N., Doong, S. L., Zhou, J. H., Beach, J. W., Jeng, L. S., Chu, C. K., Tsai, C. H., and Cheng, Y. Y. (1992) J. Biol. Chem. 267, 13938–13942
16. Sansbrook, J., Fritch, E. F., and Manniatis, T. (1989) Molecular Cloning. A Laboratory Manual, 2nd Ed., pp. 1059–1061, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Knap, K. W., Yamado, M., and Websbach, A. (1976) Biochemistry 15, 4540–4548
18. Wernette, C. M., Conway, M. C., and Kagni, L. S. (1988) Biochemistry 27, 6046–6054
19. Insdorf, N. F., and Bogenhagen, D. F. (1989) J. Biol. Chem. 264, 21491–21497
20. Hart, G. J., Orr, D. C., Penn, C. R., Figuereido, H. T., Gray, N. M., Boehne, R. E., and Cameron, J. M. (1992) Antimicrob. Agents Chemother. 36, 1688–1694
21. Websbach, A. (1981) Enzyme 14, 67–86
22. Nickel, W., Austermann, S., Bialek, G., and Grosse, F. (1992) J. Biol. Chem. 267, 848–854
23. Cotopan, W. C., Chen, M. S., and Wang, T. S. F. (1992) J. Biol. Chem. 267, 21459–21464
24. Mozher, D. Y., Atrazheva, A. M., and Kukhanova, M. K. (1992) Mol. Biol. (Mosc.) 26, 665–671
25. Fersht, A. (1977) Enzyme Structure and Mechanism, pp. 84–100, W. H. Freeman and Company, Reading
26. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
27. Boosilis, M. S., Petruska, J., and Goodman, M. F. (1987) J. Biol. Chem. 262, 14689–14696
28. Lineweaver, H., and Burk, D. (1934) J. Am. Chem. Soc. 56, 658–666
29. Starnes, M. C., and Cheng, Y. Y. (1987) J. Biol. Chem. 262, 988–991
30. Wright, G. E., and Brown, N. C. (1990) Pharmacol. & Ther. 47, 447–497
31. Richetti, M., and Buc, H. (1990) EMBO J. 9, 1583–1593
32. Wilson, J. E., Martin, J. L., Borroto-Esoda, K., Hopkins, S., Painter, G., Liotta, D. S., and Furman, P. A. (1993) Antimicrob. Agents Chemother. 1720–1722
33. Miller, W. H., Daluge, S. M., Garvey, E. P., Hopkins, S., Reardon, J. E., Boyd, F. L., and Miller, R. L. (1992) J. Biol. Chem. 267, 21220–21224
34. Burgers, P. M. J. (1989) Prog. Nucleic Acid Res. Mol. Biol. 37, 235–380

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