Butylphenyl dGTP: a selective and potent inhibitor of mammalian DNA polymerase alpha

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ABSTRACT

BuPdGTP, the 2'-deoxyribonucleoside 5'-triphosphate of the DNA polymerase alpha (pol α)-specific inhibitor, N²-(p-n-butylphenyl)guanine, was examined with respect to its mechanism and its capacity to inhibit the mammalian DNA polymerases, pol α , pol β , and pol γ . BuPdGTP was specifically inhibitory for pol α , with no discernible activity on pol β and pol γ . The potency of BuPdGTP is unprecedented, with an apparent K_i less than 10 nanomolar. The unusual potency of the BuPdGTP is derived primarily from the 5' α and β phosphoryl moieties, whose binding to enzyme complements that of the base-linked butylphenyl substituent. BuPdGTP is competitive with dGTP and apparently not subject to polymerization. Experiments employing BuPdGTP in the presence of a non-complementary template suggest that the core polymerase or an associated coprotein contains dNTP binding sites which recognize specific nucleic acid bases. The partial sensitivity of selected, non-mammalian DNA polymerases suggests that modification of the N² substituent of dGTP will be a useful route to the design of novel, polymerase-specific affinity-probes.

INTRODUCTION

6-(Arylamino)uracils and N²-arylguanines of the prototype shown in Figure 1 are dGTP analogs, which, by appropriate design, can be targeted to inhibit specific DNA polymerases (1). The credentials of these inhibitors as dGTP analogs reside in their base substituents; as indicated in Figure 1, these substituents endow them with the capacity to form hydrogen bonds with template cytosine. The potency of these inhibitors, although dependent on their base pairing domains, is derived primarily from the aryl substituent, which binds strongly to the sensitive polymerase in the vicinity of its active site, sequestering the enzyme in a catalytically non-productive, protein-drug-template:primer complex (1).

The selectivity of the arylguanines and arylaminouracils for a specific DNA polymerase is determined by the nature of the aryl substituent and its substituents - particularly the substituents in the *para* and *meta* positions, depicted as X and Y in Figure 1. For example, the analogs 6-(p-n-butylanilino)uracil (BuAU) and N²-(p-n-butylphenyl)guanine (BuPG),

Figure 1. Structure of prototypic DNA polymerase inhibitors showing substituents involved in base pairing with template cytosine and substituents involved in determining enzyme selectivity and binding.

in which X is *n*-butyl and Y is H, are highly selective inhibitors of mammalian DNA polymerase alpha (pol α) with no discernible activity against any other eukaryotic or prokaryotic polymerases (2,3). Conversely, TMAU and TMPG, analogs in which positions X and Y are bridged by a trimethylene moiety ($-\text{CH}_2-\text{CH}_2-\text{CH}_2-$), do not inhibit pol α . Rather, they act specifically and with high potency on the replication-specific, type III DNA polymerase (pol III) of Gram-positive bacteria (3).

Exploiting the guanine nucleus as a structural basis, we have attempted to develop a more potent inhibitor of mammalian pol α and to find novel routes to the successful design of selective inhibitors of other important DNA polymerases. The first experiments in this effort have included the synthesis and investigation of the mechanism of action of 2'-deoxyribonucleosides and 2'-deoxyribonucleotides of BuPG. This paper summarizes the results of these experiments and reports in detail the preliminary characterization of the properties of BuPdGTP, a pol α -specific inhibitor with remarkable potency and excellent potential as a pol α -specific molecular probe.

MATERIALS AND METHODS

Substrates, Polymers, and Inhibitors.

Radioactive materials were obtained from New England Nuclear. dATP, dCTP, dGTP, and TTP, were purchased from P-L Laboratories. Poly(dA), poly(dC), and oligo (dT) [(d)T₁₂₋₁₈] and oligo(dG) [(d)G₁₂₋₁₈] were obtained from Collaborative Research, and calf thymus DNA was from Worthington. N²-(*p*-*n*-Butylphenyl)-9-(2-deoxy- β -D-ribofuranosyl)guanine

(BuPdG), its 5'-monophosphate (BuPdGMP), its 5'-diphosphate (BuPdGDP), and its 5'-triphosphate (BuPdGTP) were prepared and purified according to the methods of Wright and Dudycz (4).

Cells and Culture Conditions.

The Chinese Hamster Ovary (CHO) cell line, SC-1, used as the major source of CHO pol α , was obtained from Dr. Larry Thompson. SC-1 cells were grown at 37° in room air atmosphere in spinner culture in Tricine (Sigma)-buffered McCoy's medium (Gibco) containing 10 percent fetal calf serum (HyClone) and 50 mM Tricine-Na (pH 7.4).

Enzymes.

HeLa cell DNA polymerases α , β , and γ , purified from synchronously dividing cells (5), were kindly provided by Dr. Earl Baril. CHO pol α was extracted from cells in the exponential phase of growth and purified through step VI of the method of Khan and Brown (6). *B.subtilis* pol III was purified by the immunoabsorbent method of Barnes and Brown (7). *E.coli* pol I and its large Klenow fragment were purchased from P-L Laboratories.

DNA Polymerase Assay.

The assay conditions for HeLa and CHO DNA polymerases were those described by Novak and Baril (8). Unless specified otherwise, assays employing natural template primer used [³H]TTP as the labeled precursor and DNAse I-activated calf thymus DNA. The apparent inhibitor constant (K_i' ; equal to the concentration of inhibitor required to reduce polymerase activity by 50 percent) of each inhibitor was determined by employing either the truncated, dGTP-deficient, direct assay method described by Wright and Brown (9) or conventional kinetic analysis. Conditions of truncated assay were devised to ensure that incorporation of [³H]TMP was linear throughout the assay period. Poly(dA):oligo(dT) and poly(dC):oligo(dG) were prepared by annealing polymer to oligomer in a molar base ratio of 10:1 (i.e. 10 moles of adenine: 1 mole thymine or 10 moles of cytosine: 1 mole of guanine). Annealing was carried out in 10 mM Tris-HCL (pH 7.5) at 50° for 10 min at a polymer concentration of 3.25 mM (as nucleotide).

RESULTS

Effects of BuPG and its 2'-Deoxyribonucleoside Derivatives on CHO DNA pol α .

Figure 2 summarizes the results of experiments examining the effect of BuPG, BuPdG, BuPdGMP, BuPdGDP, and BuPdGTP on the incorporation of [³H]TMP into activated calf thymus DNA in conditions of truncated, dGTP-deficient assay. The addition of the 2-deoxyribosyl group to BuPG had little effect on

Figure 2. Effect of BuPG, its 2'-deoxyribonucleoside, and its 2'-deoxyribonucleoside 5'-phosphates on CHO pol α . The enzyme was assayed with activated calf thymus DNA as described in the methods section, in the presence of several concentrations of each inhibitor and in the presence or absence of dGTP (truncated assay). 100 percent activity in the presence and absence of dGTP was represented by the incorporation of, respectively, 750 pmol and 1800 pmol of $[^3\text{H}]\text{TMP}$.

potency, decreasing K_i' only slightly from 12 to 10 μM . The addition of the 5'-phosphate to the 9- β -D deoxyribonucleoside to form BuPdGMP improved potency considerably, decreasing K_i' nearly 10 fold to a value of approximately 1 μM . The addition of the β 5'-phosphate to form BuPdGDP had a very dramatic effect, reducing K_i' another 50 fold to a value of approximately 15 nM. The effect of adding the γ phosphate, reflected by the curve for BuPdGTP, increased potency slightly, depressing K_i' to a value of less than 10 nM.

The Inhibitory Effects of the 2'-Deoxyribonucleoside Derivatives of BuPG are Antagonized by dGTP.

A series of experiments, the complete results of which are not shown, was executed to determine whether the inhibitory effect of each of the deoxyribonucleoside derivatives of BuPG, like that of the parent base, is subject to specific antagonism by dGTP. The experiments examined the capacity of each of the four triphosphates to alter inhibitor action; they employed activated calf thymus DNA, an appropriately devised truncated assay mixture containing a base-labeled deoxyribonucleoside triphosphate, inhibitor at a concentration equal to twice its K_i' , and unlabelled competitor

Figure 3. Competition between BuPdGTP and dGTP. CHO pol α was assayed with activated calf thymus DNA as described in the methods section, in the presence of dGTP and BuPdGTP at the indicated concentrations. The labeled substrate was [^3H]dGTP; unlabeled TTP, dCTP and dATP were present at a saturating concentration of 100 μM . V_{max} was equivalent to the incorporation of 750 pmol of [^3H]dGMP.

deoxyribonucleotide at 800 μM . The results indicated that dGTP specifically and completely antagonized the action of each inhibitor derivative; dATP, dCTP, and TTP at 800 μM had no antagonistic effects.

The nature of the dGTP-mediated antagonism of BuPdGTP, the inhibitor examined in the remainder of this study, is shown by the Lineweaver-Burk plot depicted in Figure 3. The plot displays surmountable, competitive antagonism, identical to that demonstrated for the effect of dGTP on the action of the pol α - and pol III-specific base prototypes (2,3). Analysis of the data of Figure 3 yielded a K_i' of 9 nM, a value in close agreement with the K_i' value derived from truncated assay in the experiment of Figure 2.

Of the Mammalian DNA Polymerases, Pol α is the Specific Target of BuPdGTP.

Table I summarizes the results of experiments examining the effects of BuPdGTP on HeLa cell DNA polymerases α , β , and γ , the three enzymes exploited in the development of BuAU (2) and BuPG (3). (Purified HeLa pol α also has been examined for its sensitivity to BuPdGTP in preliminary studies by Wright and Dudycz (4)). The enzyme specificity of the nucleotide was identical to that established for BuPG (3). Like CHO pol α (Fig. 2), HeLa pol α was sensitive to BuPdGTP in the nanomolar range (K_i' , approximately 1 nM), whereas pol β and pol γ were completely resistant to the drug at a

TABLE I ENZYME SPECIFICITY OF BuPdGTP

ENZYME	BuPdGTP CONCENTRATION	PERCENT OF CONTROL ACTIVITY
HeLa pol α	1 μ M	< 1
HeLa pol β	400 μ M	100
HeLa pol γ	400 μ M	100

Each enzyme was assayed with activated DNA using the dGTP-deficient, truncated assay. The values for [3 H]TMP incorporation for the control in the α , β and γ assays were, respectively, 25, 8.3, and 2.5 pmoles.

concentration as high as 400 micromolar. The results of experiments by other investigators to whom we have supplied BuPdGTP corroborate our observation of pol α specificity among the mammalian enzymes. For example, KB cell pol α (Dr. Theresa Wang, personal communication) and pol α of regenerating rat liver (Dr. Gary Ostroff, this laboratory) display nanomolar-level sensitivity to BuPdGTP. Further, pol α of calf thymus is typically sensitive to BuPdGTP while calf thymus pol β is entirely resistant (Dr. Sam Wilson, personal communication).

BuPdGTP-Induced Inhibition, Although Antagonized by dGTP, Does Not Require Cytosine in Primer:Template.

Figure 4 summarizes the effects of BuPdGTP on pol α -catalysed synthesis on the homopolymeric template:primers poly(dC):oligo(dG) and poly(dA):oligo(dT). The action of BuPdGTP on poly(dC)-directed synthesis (part A) was consistent with the results of the experiments done with activated calf thymus DNA as primer:template (cf Fig. 2). The K_i' of the inhibitor was approximately 20nM, and its action was competitively antagonized by dGTP.

BuPdGTP unexpectedly inhibited poly(dA)-directed synthesis of oligo (dT) (Fig. 4, part B), and, even more unexpectedly, the inhibition was not antagonized by TTP, but by dGTP. The resistance to TTP is displayed by the curves of the reciprocal plot of Fig. 4B in which V_{max} was inversely related to BuPdGTP concentration and independent of TTP concentration. The nature of the BuPdGTP - dGTP antagonism is depicted in Fig. 5. dGTP at concentrations considerably below values determined for its K_m reversed inhibitor action almost completely, in a concentration-dependent manner, and it acted specifically; neither dATP, dCTP nor ribo-GTP at high concentration affected inhibitor potency on the poly(dA):oligo(dT)-based reaction.

We also examined the effects of the nucleoside diphosphate, BuPdGDP, in the presence of poly (dA): oligo (dT) in experiments identical to those of

Figure 4. Effect of BuPdGTP on homopolymer-directed synthesis catalysed by CHO pol α . Primers were annealed to templates as described in the methods section. (A) poly(dC):oligo(dG) was present at a concentration of 0.6mM (as dCMP). The concentration of [^3H]dGTP, the sole monomeric substrate (sp.act. 10 cpm/pmol) and inhibitor were varied as indicated. V_{max} was equivalent to the incorporation of 636 pmol of [^3H]dGMP. (B) Poly(dA):oligo(dT) was present at a concentration of 0.6mM (as dAMP). The concentrations of [^3H]TTP, the sole monomeric substrate (sp. act. 15 cpm/pmol), and inhibitor were varied as indicated. V_{max} was equivalent to the incorporation of 833 pmol of [^3H]TMP.

Figure 5. Effect of dGTP and other non-complementary deoxyribonucleoside triphosphates on BuPdGTP - induced inhibition of poly (dA)-directed synthesis of oligo(dT). The conditions of assay, except for the inclusion of dATP, dCTP, or dGTP, were identical to those described for the experiment of part B of Figure 4.

Figures 4B and 5. The results are essentially identical to those of the BuPdGTP experiments. BuPdGDP inhibited dTMP incorporation (K_i approx. 50nM) and its action, like that of BuPdGTP, was specifically antagonized by dGTP (dGDP, used at 800 μ M, did not reverse the effect of 50 nM BuPdGTP).

BuPdGTP Apparently Does Not Undergo Polymerization. BuPG is clearly not a substrate for polymerization by pol α , whereas BuPdGTP is structurally eligible for reaction with and incorporation into a primer terminus. Until we prepare the appropriate radioactive derivative of BuPdGTP, we cannot determine unequivocally whether it is an effective pol α substrate. At present, we believe that polymerization of BuPdGTP, if it occurs at all, does not play a significant role in its inhibitory mechanism. Our belief is supported by two observations. The first is the observation that the behavior and potency of the diphosphate, BuPdGDP, an unlikely substrate for polymerization by a DNA polymerase, is essentially equivalent to that of BuPdGTP (see Fig. 2). The second observation is derived from the result of an unpublished experiment designed to determine whether DNA synthesized in the presence of BuPdGTP has poorly extensible or blocked termini. In this experiment, DNase I-activated calf thymus DNA was incubated for an extended period of time with pol α under conditions in which dGTP was replaced with BuPdGTP at the same concentration (67 μ M). The DNA, after purification and separation from inhibitor and enzyme, was not impaired in its capacity to serve as a template-primer when it was employed subsequently in a second pol α -catalysed reaction.

The Potency of BuPdGTP Varies Across a Spectrum of Non-Mammalian DNA Polymerases.

Several investigators consented to examine the effects of BuPdGTP on a variety of procaryotic and eucaryotic polymerases and in vitro DNA polymerization systems. Table II summarizes the results communicated from these laboratories.

The response of the systems varied from pol α -like sensitivity to complete resistance. Intact or fragmented E.coli pol I and B.subtilis pol III, like mammalian pol β and γ , were completely resistant to BuPdGTP. The alpha-like enzymes of Drosophila and Xenopus, not surprisingly, were very sensitive to the inhibitor. Saccharomyces pol I also was clearly alpha-like in its drug sensitivity. The wheat embryo polymerases displayed a variety of responses, ranging from total resistance (pol A) to pol α -like sensitivity. Three viral polymerases, the Vaccinia-, HSV-, and EBV-specific enzymes, were moderately sensitive to BuPdGTP. The multiprotein, in vitro

TABLE II EFFECTS OF BuPdGTP ON SELECTED DNA POLYMERASES AND DNA REPLICATION SYSTEMS FROM NON-MAMMALIAN SOURCES

ENZYME OR SYSTEM	LABORATORY OR SOURCE	SUSCEPTIBILITY*	ESTIMATED K_i '
<u>E.coli</u> pol I	This laboratory	Resistant	not possible to estimate
<u>E.coli</u> pol I, large Klenow fragment	Dr. Samuel Wilson and this laboratory	Resistant	not possible to estimate
<u>B.subtilis</u> pol III	This laboratory	Resistant	not possible to estimate
<u>Wheat embryo</u> polymerases:	Dr. Simon Litvak	Sensitive	< 5 μ M
pol A	"	Resistant	not possible to estimate
pol B	"	Sensitive	< 1 μ M
pol C I	"	Sensitive	> 100 μ M
pol C II	"	Sensitive	
<u>Saccharomyces</u> pol I	Dr. Akio Sugino	Sensitive	50nM
<u>Drosophila</u> pol α	Dr. I.R. Lehman (Dr. Ron Conaway)	Sensitive	<1 μ M
<u>Xenopus</u> pol α	Dr. Simon Litvak	Sensitive	<<1 μ M
<u>Epstein Barr Virus (EBV)</u> -Specific polymerase	Dr. Wendy Clough	Sensitive	<10 μ M
<u>Herpes simplex virus (HSV-1 and HSV-2)</u> -specific polymerases	Dr. Yung-Chi Cheng	Sensitive	3 μ M
<u>Adenovirus</u> -specific replication system (requires Adeno-specific polymerase)	Dr. Thomas Kelly (Dr. Jeff Ostrove)	Sensitive	50-100 μ M
<u>Vaccinia virus</u> specific polymerase	Dr. Samuel Wilson	Sensitive	<10 μ M
<u>Bacteriophage T7</u> -Specific polymerase	Dr. Charles Richardson (Dr. Robert Lechner)	Sensitive	<10 μ M
<u>Permeabilized Chinese Hamster Embryo Fibroblast system (Ribonucleoside 5'diphosphate dependent)</u>	Dr. Arthur Pardee (Dr. Prem Reddy)	Sensitive	< 1 μ M

*The term resistant is applied to systems or enzymes which were not inhibited by 500 μ M BuPdGTP in the presence of dGTP at a concentration of 100 μ M or less.

Adenovirus DNA-dependent replication system was the least sensitive of the viral polymerase activities assayed. Bacteriophage T7-specific DNA polymerase also was moderately sensitive to BuPdGTP. The ribonucleotide (CDP)-dependent system derived from permeabilized synchronized Chinese Hamster fibroblasts, like CHO pol α , was very sensitive to BuPdGTP.

DISCUSSION

Comparison of the mechanisms of BuPG and its 2'-deoxyribonucleoside 5'-polyphosphates

Similarities. We have studied in detail how N²-(alkylphenyl)guanines inhibit specific DNA polymerases (3). The results of our studies consistently have indicated that these inhibitors, although simple bases, behave specifically as analogs of dGTP, binding to the active site region of the target enzyme and to template cytosine. Considering the likelihood that these inhibitors react with the active site region of their polymerase targets, we hypothesized that their conversion to a 2'-deoxyribonucleoside 5'-triphosphate form would enhance their potency - possibly by facilitating their irreversible polymerization into primer termini. We chose BuPG, the pol α -specific inhibitor, as the model analog with which to examine this hypothesis. The results of our examination, presented in the previous section, indicate that the conversion of BuPG to the 2'-deoxyribonucleoside 5'-di- and triphosphates creates pol α -specific inhibitors of unprecedented potency. The characteristics of BuPdGTP, including its intraspecies enzyme specificity (Table I), its sensitivity to antagonism by dGTP (Figs. 3,4A and 5), and the apparent absence of its incorporation into DNA suggest that deoxyribonucleotidylation of BuPG does not change the basic bivalent mechanism by which it reacts with the active site region and H bonds to template cytosine.

Differences. The experiments with poly (dA):oligo (dT), displayed in Figures 4B and 5 indicate a major, qualitative difference of the mechanism of BuPG and that of its 2'-deoxyribonucleoside 5'-polyphosphates. The latter nucleotide form can inhibit pol α in a manner which does not involve their H bonding to template cytosine and the concomitant sequestration of enzyme into a protein:inhibitor:template-primer complex. We do not yet understand the basis for this template-independent, "secondary" action of the nucleoside polyphosphates. Considering the capacity of dGTP to selectively antagonize the secondary action (cf Fig. 5), we conclude that it depends on the reaction of the inhibitor nucleotide with a site or site(s) in pol α which can recognize a deoxyribonucleoside phosphate (in this case, dGTP) by its base.

There is considerable precedent for base-specific recognition of nucleoside 5'-di- and -triphosphates by proteins (10). The p21 protein product of the ras oncogene (11) specifically binds GDP and GTP or dGTP. The case of the regulatory subunit of ribonucleoside diphosphate reductase is perhaps an even more relevant example; it has multiple dNTP binding sites with an assortment of base specificities (12). Finally, there even exists evidence, based on kinetic studies of E.coli DNA polymerase I (13), that the active site of a DNA polymerase may display base-specific substrate binding domains.

As a basis from which to dissect further the mechanism of the template independent action of the BuPG deoxyribonucleotides we propose alternative working models - models which we term, respectively, "core" and "coprotein". Both models consider the holoenzyme type of complexity characteristic of mammalian pol alphas (5).

The coprotein model envisions a site which is located on an alpha-specific accessory protein, tightly bound to the catalytically active subunit but distinct and resolvable from it. For example, the protein could be a dNTP-reactive protein like the component of calf thymus pol α suggested by Steinberg et al (14) to be a non-catalytic, polymerase regulatory protein.

The core model envisions the dGTP/BuPdGTP binding site to be a specific subsite or domain of the substrate binding region of the catalytically active core of pol α . This model embraces the aforementioned concept of Travaglini et al (13), that the active substrate binding site of a DNA polymerase may not be simply a template-directed, generic site (15) with equivalent affinity for all dNTP's, but a site which contains (or, under appropriate catalytic conditions, develops) base-specific dNTP binding domains.

Presently, we find the core model the more attractive of the alternatives, primarily because it invokes a single dGTP binding site to explain the template-dependent and template-independent actions of all of the inhibitor forms - be they a base, a nucleoside, or a nucleotide.

Attractiveness notwithstanding, the models can be resolved experimentally through the use of appropriate purification techniques to isolate core pol α from its coproteins and subsequently, by the application of appropriately designed assay and binding studies exploiting labeled derivatives of BuPdGDP and BuPdGTP. Studies exploiting the latter approaches are in progress.

Comment on the utility of cross reactivity of N²-substituted purine nucleotides among DNA polymerases

Prior to developing BuPdGDP and BuPdGTP, we have sought to exploit the simple substituted purine nucleus to determine whether the manipulation of the

N²-substituent - an approach which succeeded in the production of pol α - and pol III-specific reagents - also would be a productive direction to take with other DNA polymerases and other guanine nucleotide binding proteins. Unfortunately, no base, including BuPG has sufficient solubility or appropriately strong credentials as dGTP to produce a signal upon which to base effective structure-activity studies. As the results of Table II indicate, the use of nucleotide forms of the purine avoids the deficiencies inherent in the simple bases and, thus, permits a feasible strategy to develop potent and selective probes of a spectrum of DNA and RNA polymerases and nucleotide binding proteins.

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REFERENCES

1. Brown, N.C. and Wright, G.E. (1977) *Pharmac. Ther.* 1, 437-456.
2. Wright, G.E., Baril E.F., and Brown, N.C. (1980) *Nucleic Acids Res.* 8, 99-119.
3. Wright, G.E., Baril, E.F., Brown, V.M., and Brown, N.C. (1982) *Ibid.* 10, 4431-4440.
4. Wright, G.E. and Dudycz, L.W. (1984) *J. Med. Chem.*, 27, 175-181.
5. Lamothe, P., Baril, B., Chi, A., Lee, L., and Baril, E. (1981) *Proc. Nat. Acad. Sci. U.S.A.* 78, 4723-4727.
6. Khan, N.N. and Brown, N.C. manuscript in preparation.
7. Barnes, M.H. and Brown, N.C. (1979) *Nucleic Acids Res.* 6, 1213-1219.
8. Novak, B. and Baril, E.F. (1978) *Biochim. Biophys. Acta* 432, 37-48.
9. Wright, G.E. and Brown, N.C. (1976) *Biochim. Biophys. Acta* 432, 37-48.
10. Hughes, S.M., (1983) *Febs. Lett.* 164, 1-8.
11. Ellis, R.W., Lowy, D., and Scolnick, E.M. (1982) *Adv. in Viral Oncol.* 1, 107-126.
12. Eriksson, S., Thelander, L., and Akerman, M. (1979) *Biochemistry* 18, 2948-2952.
13. Travaglini, E.C., Mildvan, A.S., and Loeb, L.A. (1975) *J. Biol. Chem.* 250, 8647-8656.
14. Steinberg, J.A., Otten, M., and Grindey, G.B. (1979) *Cancer Res.* 39, 4330-4335.
15. Englund, P.T., Huberman, J.A., Jovin, T.M., and Kornberg, A. (1969) *J. Biol. Chem.* 244, 3038-3044.