Articles

Guanylhydrazones with Potential Antileukemic Activity. 2. Synthesis and Structure-Activity Relationships of Analogues of 4,4'-Diacetyl-*N*,*N*'-diphenylurea Bis(guanylhydrazone)[1,2]

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Synthesis of the antileukemic agent 4,4'-diacetyl-*N*,*N*'-diphenylurea bis(guanylhydrazone) (DDUG) is described in detail. The compound was characterized in terms of its ^1H and ^{13}C NMR spectra. To determine the importance of structural features to its biological activity, a series of related compounds was synthesized. The aminoguanidine groups in DDUG were replaced with other groups, such as thiosemicarbazone or nitroaminoguanidine. In addition, one or both H atoms of the urea moiety of DDUG were replaced with a methyl group. One urea N was replaced with O, giving a urethane moiety. The two guanylhydrazone groups in DDUG were separated with one or two methylene groups, and the effects of such separation were investigated in relation to the biological activity of the compound. In contrast to the modifications that we carried out earlier in the phenyl ring, modifications at and around the urea moiety of DDUG caused decreases in the growth-inhibitory activity of the compound against mouse mammary adenocarcinoma (TA3) cells in culture. Preliminary results indicate that at least some antileukemic activity is retained on single N-methylation and on extension of the molecule with one methylene group. Most of the DDUG analogues inhibited DNA polymerase, and the degree of inhibition was less sensitive to structural modification in this system than in cellular test systems or in vivo.

4,4'-Diacetyl-*N*,*N*'-diphenylurea bis(guanylhydrazone) [DDUG, **3b** (Scheme I)] has been found to have a marked inhibitory activity against a wide spectrum of tumors and is the most potent bis(guanylhydrazone) that has been tested against mouse leukemia L1210.[3-5] We have shown earlier that replacing one or both of the phenyl groups in DDUG with pyridine moieties does not abolish the antileukemic and antiproliferative activity of the parent compound.[2]

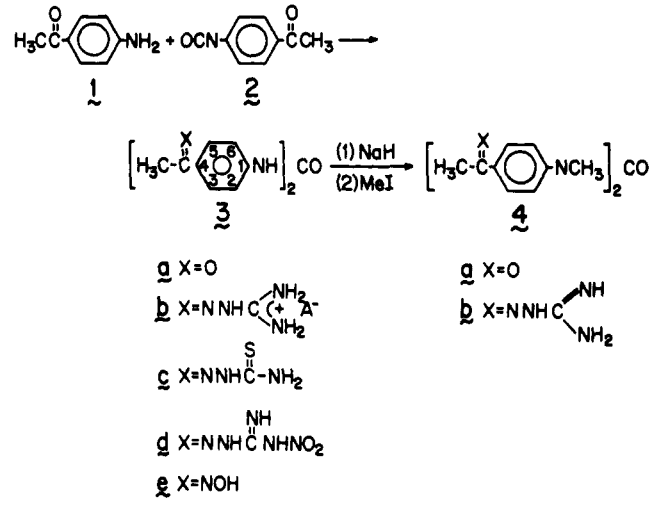
Development of other structurally related bis(guanylhydrazones) was motivated by our interest in finding substances with comparable antileukemic activity but decreased toxicity and in determining the structural features associated with the biological activity of this class of compounds. In contrast to methylglyoxal bis(guanylhydrazone) (CH_3-G), a clinically active bis(guanylhydrazone) which significantly lost biological activity on structural modification, the aromatic bis(guanylhydrazones) have been found to be considerably more permissive in this respect.[5]

In the present study, the aminoguanidine group in DDUG has been replaced with other groups, such as the thiosemicarbazone or nitroaminoguanidine group (Scheme I). In addition, one or each H atom of the urea moiety of DDUG has been replaced with a methyl group (**4b** and **6**). Similarly, one urea N has been replaced with O, giving a urethane moiety (**7b**, Scheme II). The effects of separating the two guanylhydrazone groups in DDUG were investigated by extending the molecule with one or two methylene groups, as shown in Scheme III.

The effects of these modifications have been studied in regard to inhibition of the growth of mouse mammary adenocarcinoma cells in culture, antileukemic activity in mice, and interference with DNA polymerase catalysis. This last type of activity has been previously correlated with the in vivo antileukemic activy of DDUG and its analogues.[6]

Chemistry. Scheme I depicts the synthesis of DDUG itself and its *N*,*N*'-dimethyl analogue. Although the

Scheme I



synthesis of this much-studied compound has been outlined in a preliminary communication,[7] the details of the synthesis have so far not been published, even though problems have been encounted in relation to its purity.[8] Condensation of 4-acetylphenyl isocyanate (**2**) with *p*-aminoacetophenone (**1**) gave the symmetrical urea **3a**, which was subsequently treated with aminoguanidine sulfate to give DDUG (**3b**). An analytically pure *p*-toluenesulfonate salt of DDUG has been obtained and has been characterized in terms of its ^1H and ^{13}C NMR spectra (see below). Likewise, condensation of **3a** with other carbonyl reagents gave the analogues **3c–e**.

Syntheses of the unsymmetrically N-methylated DDUG **6b** and the urethane **7b** are represented in Scheme II. *N*-Methylaminoacetophenone (**5**) was obtained by acetylating the amino group in **1**, followed by methylation with MeI and NaH and subsequent hydrolysis. The urethane analogue **7b**, in which one of the urea N atoms in DDUG has been replaced with O, was obtained by con-

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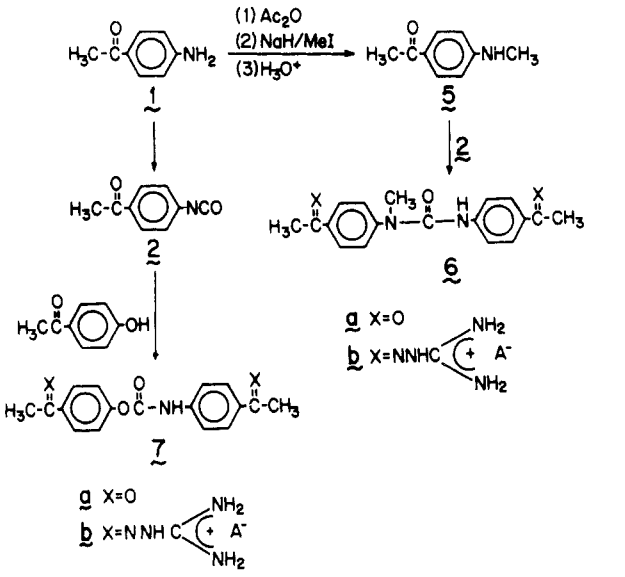
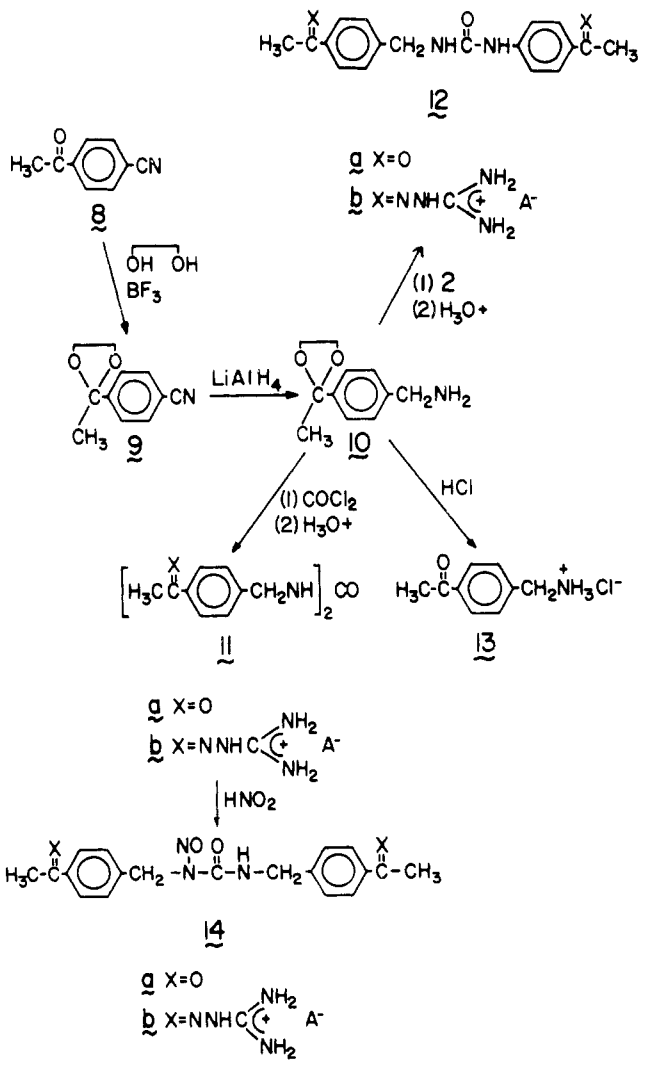
Scheme II



Scheme III



densing the isocyanate **2** with *p*-acetylphenol, giving **7a**, which was then converted to its bis(guanylhydrazone).

Scheme III shows synthesis of the two homologues of DDUG in which the molecule is extended with a methylene group inserted between the central urea moiety and one or each phenyl group. *p*-Cyanoacetophenone (**8**) was first ketolyzed with ethylene glycol, and the resulting ketal **9** was reduced to the methylamine derivative **10**, which was hydrolyzed to **13**. The reduced ketal was used for synthesizing the symmetrical (**11a**) and unsymmetrical (**12**) homologous ureas, which were converted to bis(guanylhydrazones). In order to introduce a potentially reactive center into the molecule, an *N*-nitroso derivative of **11** was obtained by treating **11a** with nitrous acid, giving **14a**, which again was converted to a bis(guanylhydrazone), **14b**.

The target guanylhydrazones were obtained either as free bases or as salts ($A^- = Br^-$, methanesulfonate, toluenesulfonate). Each compound represented a special problem with regard to the most suitable salt from the standpoint of physical properties and conditions for crystallization.

NMR Spectra. As has already been pointed out, considerable difficulty was experienced in obtaining analytically pure samples of DDUG and its analogues. A reliable method for structural characterization of these compounds is NMR spectroscopy. We have assigned both ^1H and ^{13}C NMR spectra to structural features of DDUG and its analogues.

^1H NMR Spectra. ^1H NMR spectra of DDUG and some of its analogues were determined in D_2O and Me_2SO-d_6 and are characterized by an AB quadruplet due to the 2, 3, 4, and 5 protons of phenyl rings. The assignment of these resonances follows from comparing the proton resonances of para-substituted acetophenone derivatives in which the *p*-amino group has been modified by electron-donating and -withdrawing groups.

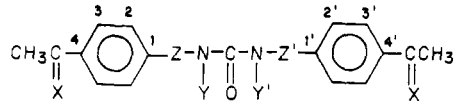
Predictably the protons ortho to the acetyl group in this series of compounds resonate at a lower field (7.8–8.1 ppm) than do the meta protons, the positions of which varied greatly on modifying the amino group. In the parent compound **1**, resonance was at 6.73 ppm, whereas in the isocyanate **2**, it was at 7.20 ppm, and in the *N*-acetyl derivative of **1**, it was at 7.75 ppm. Although the spectra of the preceding compounds were determined in $CDCl_3$ and those of the following in Me_2SO-d_6, the general trend is expected to be maintained. In the urea **3a**, the 2,6 protons were at 7.69, whereas the 3,5 protons were at 8.05 ppm. In the DDUG molecule, corresponding protons were at 7.55 and 7.92 ppm, respectively, with $J_{2,3} = J_{4,5} = 8.8$ Hz.

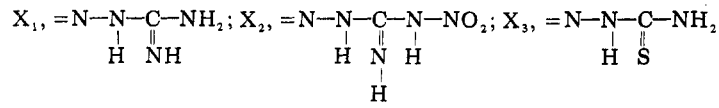
Since ^{13}C NMR resonances of the carbons bearing the aromatic protons could be readily assigned (see below), the preceding proton assignment has been confirmed by a selective decoupling technique.

^{13}C NMR Spectra. The availability of a series of structurally related analogues of DDUG made it possible to assign ^{13}C NMR spectra. All spectra were determined in Me_2SO-d_6, in which all compounds were soluble, and the shifts are given in parts per million from Me_4Si as an internal standard (Table I). Off-resonance decoupling established the number of protons on each of the carbon atoms. Initial assignments were derived from inspection of spectra for compounds **3a–f**. In this series, the oxygen in the terminal acetyl group is replaced, and the substitution causes the carbon resonances to change appreciably around this position only. The relatively invariant resonances are the urea carbonyl at 151.5–151.9 ppm, the phenyl C_1 at 129.3–130.6 ppm, and the phenyl C_2, C_6 at 117.2–117.5 ppm.

Replacement of hydrogens with methyl groups in the urea moiety shifts the carbonyl to lower fields. Monomethylation makes the molecule nonsymmetrical, thus "doubling" most of the resonances. The effect is pro-

Table I. ^{13}C NMR Spectra of 4,4'-Diacetyl-*N,N'*-diphenylurea and Related Compounds





Compd	C[a]	4- and 4'-CH_3	Z, Z' -CH_2-	Y, Y' -CH_3	Ph C_1	Ph C_2, C_6	Ph C_3, C_5	Ph C_4	Urea C=O[d]	C at X[d]	X	C in X[d]
3a		26.2			130.6	117.2	129.3	143.7	151.6	193.9	O	
3b ($CH_3SO_2O^-$)		13.7			129.9	117.3	127.2	141.1	151.5*	151.9*	X_1	155.3
3b (free base)		13.2			133.7	117.4	125.6	138.7	152.0	147.1	X_1	159.0
3c		13.6			129.3	117.4	127.1	140.5	151.9	147.5	X_2	195.9
3d		14.0			130.3	117.4	127.4	140.9	151.9	152.9	X_3	158.0
3e		11.2			130.2	117.5	125.8	139.7	151.9	152.1		
4b		13.1		38.1	135.7	122.9	125.3	143.9	159.7*	146.5	X_1	159.2*
6a	u	26.2		37.0	130.5	118.3	128.9	144.3	154.9	196.0	O	
	p	26.5			133.1	124.6	129.0	148.0		196.5		
6b	u	14.4		37.3	129.9	118.7	126.8	141.4	154.1	151.2	X_1	155.6
	p	14.7			133.3	125.0	127.3	145.0		151.5		
11a		26.5	42.6		135.1	126.7	127.9	146.2	157.7	197.1	O	
11b		14.2	42.5		134.8	126.4	126.5	142.6	157.8	151.5	X_1	155.5
12a	u	26.1	42.5		129.7	116.5	128.1	144.8	154.5	195.8	O	
	p	26.5			135.3	126.9	129.3	145.4		197.1		
12b	u	14.0	42.3		129.0	116.6	126.6	141.8[b]	154.8	151.6	X_1	155.4
	p	14.3			135.0	126.5	127.2					
Aminoguanidine sulfate[c]												159.9

[a] Carbon atom(s): u, unprimed; p, primed. [b] u + p. [c] D_2O, Me_2SO-d_6, δ 3.1; pD 5.3–10.8. [d] An asterisk means "could be interchanged".

gressively attenuated toward the ends of the molecule, although it is quite noticeable even at the two acetyl termini, as is shown by the differences in the methyl ^{13}C NMR resonances.

From electronic considerations, the differences in C_2, C_5 carbons would also be expected to be relatively small, which was found to be the case. As a rule, no difficulty was encountered in assigning the carbon resonances to the two moieties by comparing the resonances of **6a** with those of **3a** and the resonances of **6b** with those of **3b**.

It is obvious from the spectra that DDUG and its analogues each exist in one geometrical isomer, which is most probably anti.

Biological Activity. The test results in three systems are summarized in Table II. Initially, analogues of DDUG were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA-3) cells in culture. Subsequently, they were tested for their ability to prolong the average survival time of syngeneic DBA/2J female mice inoculated intraperitoneally with 1×10^6 L1210 cells on day 0 and treated ip with compounds at 30 mg/kg once a day on days 1–3.

The antileukemic activity of DDUG has been found to correlate with the ability of DDUG to inhibit DNA biosynthesis catalyzed by DNA polymerase;[9a] this inhibition is presumably caused by binding to the DNA template. Indeed, these bis(guanylhydrazones), like DDUG, but in contrast to aliphatic bis(guanylhydrazones) and the mono(guanylhydrazone) of DDUG,[6] showed a characteristic biphasic spectral shift in the presence of varying concentrations of DNA. Accordingly, compounds were also tested for their ability to inhibit DNA biosynthesis catalyzed by cytoplasmic DNA polymerase α obtained from mouse leukemia L1210 cells[9b] in a cell-free system of the type described by Bollum.[10]

The results in Table II show that lengthening the DDUG molecule with one methylene group (**12b**) decreased the growth-inhibitory effect to about one-third, whereas the antileukemic activity was maintained. Further lengthening of the molecule with a second methylene group (**11b**) further decreased the growth-inhibitory activity, and the antileukemic activity appeared to be marginal. Extensive dose–response evaluation of antileukemic activity was not feasible, however, because of the limited availability of the compound, and, hence, the data are of a preliminary nature. The growth-inhibitory activity of the *N*-nitroso derivative **14b** was considerably lower, and the compound was also found to be too toxic in mice to be of any further interest.

The growth-inhibitory activity of DDUG was affected to a lesser degree by extension of the molecule with methylene groups than by progressive N-methylation of the urea moiety. Thus, on replacing one hydrogen with a methyl group (**6b**), although the growth-inhibitory activity dropped about two orders of magnitude, the compound retained appreciable antileukemic activity. The inhibition of DNA polymerase by the analogue was also similar to that of the parent compound.

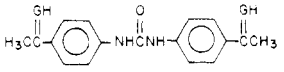
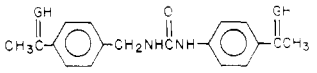
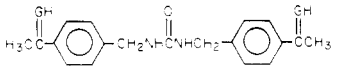
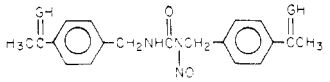
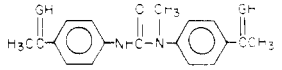
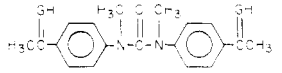
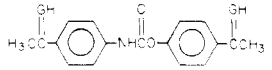
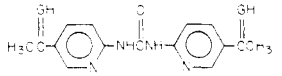
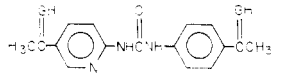
These data should be compared with those for compounds in which a carbon atom in the phenyl ring had been replaced with nitrogen (Table II, last two compounds). Such aza analogues of DDUG showed no difference in growth-inhibitory activity but apparently a decrease in antileukemic activity.

Thiosemicarbazones of various aldehydes and ketones are known to possess antiviral and antitumor activity, and the nitroguanylhydrazone of 2-biphenylcarboxaldehyde exhibits antineoplastic activity.[11] Unfortunately, it was impossible to determine the biological effects of replacing the aminoguanidine moiety in DDUG with other moieties, such as thiosemicarbazone or nitroguanidine (**3c–e**), because of the relative insolubility of such compounds in aqueous solvents.

Discussion

Consideration of the possible modes of interaction of DDUG and its analogues with DNA by building molecular

Table II. Biological Activity of DDUG and Its Analogues

	Compd	Inhibn of TA-3 cells in culture, ID_{50}, μM	Inhibn of DNA polymerase, μM	L1210 leukemia in vivo,[a] av survival ± SD (days)[c]
None				8.2 ± 1.1 (5)
3b		6	60	25 (1), > 30 (2)
12b		20	110	23.0 ± 4.0 (3), $p < 0.05$*[b]
11b		90	Insufficiently soluble	10.7 ± 3.8 (3)
14b		> 100 (growth-promoting at 10 μM)	230	10.5 ± 1.5 (2), 7 (1)
6b		180	80	14.3 ± 2 (3), $p < 0.05$*
4b		100	310	1.7 ± 0.6 (3) (toxic)
7b		180	170	Not tested
		7	160	11.0 ± 2 (3), $p < 0.05$*
		7	Insufficiently soluble	11.3 ± 2.1 (3), $p < 0.05$*

[a] Since the compounds were available in limited quantity, these tests were carried out on a limited schedule on three mice per group. The number of mice per test is given in parentheses. [b] An asterisk indicates that the p value < 0.05 in comparison with control. [c] See ref 6.

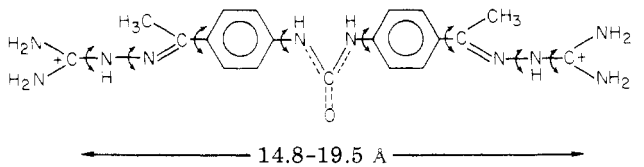


Figure 1.

models could help explain the extent of inhibition of DNA polymerase. It was established earlier that the two guanylhydrazone groups in DDUG are essential for its binding to DNA and are a prerequisite for its antileukemic activity.[9a] Because of the considerable basicity of the guanylhydrazone group, it is resonable to assume that both guanylhydrazone groups bind to the phosphate groups of the DNA molecule; this has been indicated from studies of the effects of binding on UV-spectral shifts.[9a] The central urea moiety of DDUG provides a certain amount of rigidity to the molecule, because of the double bond character of its C–N bonds and the bulk of its substituted phenyl moieties (Figure 1). The configuration around the C=N bonds of the terminal guanylhydrazone moieties is also fixed, and it is most likely anti. We did not detect any mixed syn–anti configuration by NMR spectroscopy.

On the basis of these considerations (Figure 1), a CPK space-filling model of DDUG has been built. The distances between the two guanylhydrazone carbons in the most extended and the least extended conformations were 19.5 and 14.8 Å, respectively. It could be demonstrated on a CPK model of a DNA fragment in the B conformation that this range between the guanylhydrazone groups in DDUG is adequate to bridge the phosphate residues of the DNA either along the same strand, involving every third nucleotide, or across strands. In the latter case, part of the phenyl rings becomes intercalated between the bases. This would account for the shifts of UV absorption observed when DDUG is bound to DNA.[9a]

Insertion of methylene groups as in **11b** and **12b** increases somewhat the lengths of the most extended conformation (to 20.5 and 19.8 Å, respectively), but the most striking effect is the increase in the flexibility of the molecule. This flexibility should increase the possibilities of ionic interactions with DNA, but we find in fact a marginal decrease in inhibition of DNA polymerase by the monomethylene analogue **12b**.

N-Methylation of the urea moiety as in **4b** and **6b** prevents it from achieving coplanarity because of the C–N double bond character and at the same time restricts rotation around the single bond between the phenyl and the C–N. The overall effect is an increase in the flexibility of the molecule, particularly for the dimethyl analogue **4b**. The DNA inhibition data indicate again that progressive methylation induces a marginal but progressive decrease in inhibition of DNA polymerase. The same is true of the urethane analogue **7b**, in which flexibility is increased by replacing N with O.

The most striking aspect of the effects of modification of the urea moiety is the relatively narrow range of the inhibitory effects on DNA polymerase (Table II). This is probably due to dominance of the contribution of the two guanylhydrazone groups to ionic binding to DNA, which outweighs any other structural features of the rest of the molecule. Assuming that these analogues, like DDUG, exert their cytotoxic activity by their ability to inhibit DNA polymerase reaction, the observed differences in their growth-inhibitory activity on TA-3 cells may suggest differences in the transport of these compounds. Differences in the transport of DDUG have indeed been

reported to account for observed differences in the sensitivity of various cell lines toward this drug.[12]

Experimental Section

General Chemical Methods. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. For TLC, glass plates coated with silica gel were used. IR spectra were determined with a Perkin-Elmer 457 spectrophotometer and NMR spectra with a Varian A-60A or XL100 instrument, using 8–15% solutions in $CDCl_3$, Me_2SO-d_6, or D_2O; positions of peaks are expressed in parts per million with Me_4Si, or dioxane (δ 3.70), as an internal standard. Evaporations were carried out in vacuo. Petroleum ether used had bp 36.6–55.5 °C.

Methods for Biological Evaluation. The mouse mammary adenocarcinoma (TA-3) cells were grown in stationary tube cultures in RPMI medium 1640 containing 10% horse serum. An inoculum of 50 000 cells in 1 mL of medium was supplemented with 1 mL of medium containing the compound to be tested. The tubes were incubated in an upright position for 3 days, and growth was estimated by protein assay. The growth in controls varied from six- to tenfold. Every concentration was tested in five tubes each. For compounds found to be inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50% inhibitory concentration.

For testing antileukemic activity, female DBA/2J mice weighing 18–20 g were used. The L1210 leukemia has been maintained in our laboratory by weekly intraperitoneal passages in these syngeneic mice for several years. The compounds were administered intraperitoneally at 30 mg/kg, since this dosage was found to be optimal for the parent compound DDUG.[3,5]

DNA polymerase α was obtained from L1210 cells as a crude extract, as described earler.[9b] The compounds in different concentrations were added to the assay mixture prior to the addition of the enzyme, and the initial velocity of the reaction was determined. From these data the 50% inhibitory concentration was then estimated.

4-Acetylphenyl Isocyanate (2). Compound **2** was prepared by a general method, using *p*-aminoacetophenone (**1**, 5.05 g, 37.4 mmol) and phosgene.[13] After filtration, the crystals obtained were washed with petroleum ether: yield 4.66 g (77.6%). They were then recrystallized from hot dry CCl_4: mp 74–79 °C. The extreme reactivity of **2** made it impossible to prevent self-condensation to the symmetrical urea **3a**, even on storage in a vacuum desiccator. Just before use, **2** was separated from its condensate by stirring the orange-yellow solid with hot dry CCl_4, filtering, and evaporating to dryness. The IR spectrum of the white material in KBr gives a very intense peak at 2265 cm^{-1}: NMR (CCl_4, dry) δ 2.53 (s, 3, CH_3), 7.20 (d, 2, 6 H, J = 8.9 Hz), 7.98 (d, 3, 5 H, J = 8.9 Hz).

4,4'-Diacetyl-*N*,*N'*-diphenylurea [*N*,*N'*-Bis(4-acetylphenyl)urea][14] (3a). To a stirred and ice-cooled solution of **1** (0.101 g, 0.746 mmol) in EtOAc (2 mL) and Et_3N (1 mL), a solution of **2** (0.12 g, 0.746 mmol) in dry EtOAc (2 mL) was added dropwise over a 5- to 10-min period, moisture being carefully excluded. The funnel was washed with dry EtOAc (2 mL). After stirring overnight, the contents of the flask was filtered. The precipitate was washed first with water and then with ether: yield 113 mg (51.2%). The product was recrystallized twice from hot MeOH: mp 263–265.5 °C (with evolution of gas); IR ν_{max}^{KBr} 3340 (NH), 1709, 1656 cm^{-1} (C=O); NMR (Me_2SO-d_6) δ 2.55 (s, 6, CH_3), 7.69 (d, J = 9 Hz, 3, 5 H), 8.05 (d, J = 9 Hz, 2, 6 H), 9.33 (s, 2, NH). Anal. ($C_{17}H_{16}H_2O_3$) C, H, N.

4,4'-Diacetyl-*N*,*N'*-diphenylurea Bis(guanylhydrazone) Bis(*p*-toluenesulfonate) [2,2'-Carbonylbis[(imino-4,1-phenyleneethylidyne)hydrazinecarboximidamide] Bis(4-methylbenzenesulfonate)] (3b). To **3a** (0.200 g, 0.676 mmol), aminoguanidine sulfate (0.199 g, 1.622 mmol), and *p*-toluenesulfonic acid (0.280 g, 1.47 mmol) was added MeOH (2 mL). After heating on a steam bath for 5–10 min, the mixture was stirred at room temperature for 1 h. The resulting precipitate was filtered, washed with water and ether, and dried: yield 0.375 g (71.8% on the basis of the monohydrate). The compound was reprecipitated twice from several milliliters of MeOH containing several milligrams of *p*-toluenesulfonic acid and enough DMF to give, while being heated on a steam bath, a somewhat cloudy but filterable solution. After filtration, the flask was stoppered, allowed to cool slowly for some time, and chilled in a refrigerator. The precipitate was filtered, washed with water, MeOH, and ether, and dried. The material softens and shrinks ~210 °C: mp 214–216 °C dec (yellow foam); IR ν_{max}^{KBr} 3300, 1683 cm^{-1}. Anal. ($C_{33}H_{40}N_{10}O_7O_2 \cdot H_2O$) C, H, N.

4,4'-Diacetyl-*N*,*N'*-diphenylurea Bis(thiosemicarbazone) [2,2'-Carbonylbis[(imino-4,1-phenyleneethylidyne)hydrazinecarbothioamide]] (3c). To a solution of thiosemicarbazide (3.22 g, 35.4 mmol) in glacial HOAc (15 mL) were added **3a** (4.18 g, 14.2 mmol) and MeOH (40 mL). After refluxing for 30 min, the mixture was cooled to room temperature. Filtration of the resulting precipitate was followed by washing with MeOH, water, and ether: yield 4.8 g (77.5%). The product was recrystallized from a mixture of DMF and ether, filtered, and washed with MeOH and ether: mp 239–243 °C, evolving gas and turning red; IR ν_{max}^{KBr} 1638 cm^{-1} (C=O). Anal. ($C_{19}H_{22}N_8OS \cdot 1.5H_2O$) C, H, N.

4,4'-Diacetyl-*N*,*N'*-diphenylurea Bis(nitroguanylhydrazone) [2,2'-Carbonylbis[(imino-4,1-phenyleneethylidyne)hydrazine-*N*-nitrocarboximidamide]] (3d). A mixture of **3a** (1.0 g, 3.38 mmol), DMF (2 mL), and 16% HBr (2 mL) with nitroaminoguanidine (0.886 g, 7.44 mmol) was heated just enough to give a clear solution. Filtration of the yellow precipitate that formed was followed by washing with water, EtOH, and ether: yield (after drying) 1.14 g (66.3%). The compound was dissolved in warm DMF (steam bath), and the solution was filtered to remove any polymeric material that might have formed. The DMF was evaporated off, and the residue was redissolved in a minimum volume of warm DMF. MeOH was then added to the solution until a slight cloudiness appeared. After a short while, there formed a yellow precipitate, which was filtered off, next washed with MeOH and then ether, and finally dried to a bright yellow powder. The compound slowly darkens ~200 °C but does not melt even at 400 °C: IR ν_{max}^{KBr} 3345 (NH), 1718 cm^{-1}. Anal. ($C_{19}H_{22}N_{12}O_5 \cdot 0.5H_2O$) C, H, N.

4,4'-Diacetyl-*N*,*N'*-diphenylurea Dioxime [2,2'-Carbonylbis[(imino-4,1-phenylene)-1-ethanone] 1,1'-Dioxime] (3e). To a solution of **3a** (0.100 g, 0.338 mmol) in a mixture of dry pyridine (0.5 mL) and MeOH (3 mL) was added $NH_2OH \cdot HCl$ (0.059 g, 0.845 mmol). The mixture was heated until a clear solution was obtained. After being allowed to cool slowly, the solution was chilled. The crystals that formed were filtered, washed with ether, and dried: yield 0.082 g (74.5%). The product was recrystallized from a minimum volume of a hot mixture of DMF and MeOH, but ether and petroleum ether had to be added in order to induce recrystallization. After filtration, the crystals were washed with ether and petroleum ether: mp 235 °C dec; IR ν_{max}^{KBr} 3288 (NOH and -NH), a very weak absorption at 3515 (free OH of =NOH), 1634 cm^{-1} (-C=O); NMR (Me_2SO-d_6) δ 2.13 (s, 6, CH_3), 7.53 (s, 8, aryl), 8.79 (br s, 2, -NH), 10.98 (s, 2, =NOH); NMR (CF_3COOH) δ 2.87 (s, 6, CH_3), 7.18 (s, 8, aryl). Anal. ($C_{17}H_{18}N_4O_3$) C, H, N.

***N*,*N'*-Dimethyl-4,4'-diacetyl-*N*,*N'*-diphenylurea [*N*,*N'*-Bis(4-acetylphenyl)-*N*,*N'*-dimethylurea] (4a).** To an ice-chilled, stirring mixture of DMF (10 mL, freshly distilled from CaH_2) and NaH (0.25 g, 10.4 mmol) was added, over a period of 10 min, **3a** (0.48 g, 1.62 mmol) dissolved in dry DMF (10 mL). After being stirred at room temperature for 45 min, the mixture was chilled in ice. MeI (0.4 mL, 0.913 g, 6.44 mmol) was added and the resulting mixture was stirred at room temperature until reaction was complete (ca. 1 h) on the basis of TLC. The flask was chilled in ice, and HOAc was cautiously added to destroy unreacted NaH. The contents of the flask were taken up in $CHCl_3$, and the mixture was washed three times with water and dried (Na_2SO_4). The compound gave a weak phenylhydrazine test. The mixture was chromatographed on Bio-Sil A (100–200 mesh), eluting with EtOAc. Crystals were obtained from a solution of the product in a 3:1 mixture of dry benzene and petroleum ether on chilling in a freezer; these crystals melted at room temperature. The product was used as an oil for preparing the guanylhydrazone: NMR ($CDCl_3$) δ 2.52 (s, 6, $-COCH_3$), 3.30 (s, 6, $-NCH_3$), 7.07 (d, J = 8.9 Hz, 2, 6 H), 7.80 (d, J = 8.9 Hz, 3, 5 H).

***N*,*N'*-Dimethyl-4,4'-diacetyl-*N*,*N'*-diphenylurea Bis(guanylhydrazone) [2,2'-Carbonylbis[[[(methylnitrilo)-4,1-phenylene]ethylidene]hydrazinecarboximidamide]] (4b).**

Compound **4a** (0.526 g, 1.62 mmol), aminoguanidine sulfate (0.558 g, 4.54 mmol), DMF (1 mL), water (0.5 mL), and 16% HBr (0.5 mL) were heated together, giving a clear solution. The solvents were removed, and water (0.5 mL) was added, followed by 2 N NaOH until precipitation was complete. The product was filtered, washed with water, and dried. The material was dissolved in a mixture of MeOH and 16% HBr, and 2 N NaOH was added until the solution was basic to litmus. Then the reaction mixture was warmed to 60 °C, when crystals formed. These were filtered, washed with water, and dried: mp 210–215 °C, with the evolution of gas. Anal. ($C_{21}H_{28}N_{10}O\cdot0.5H_2O$) C, H, N.

4-(Acetylamino)acetophenone [1-[4-(Acetylamino)-phenyl]ethanone]. *p*-Aminoacetophenone (1, 4.5 g, 33.2 mmol) and dry pyridine (10 mL) were cooled in an ice bath for a few minutes. Then Ac_2O (10 mL) was added, the reaction mixture being protected from moisture by a drying tube ($CaCl_2$). After 1 h, the solvent was evaporated, and the solute was taken up in $CHCl_3$. Subsequent to washing with 5% $NaHCO_3$ and once with water, the solution was dried ($CaSO_4$). Evaporation of the solvent was followed by recrystallization of the residue from a mixture of MeOH and petroleum ether: mp 170.5–173.5 °C (lit.[15] mp 166–167 °C); yield 5.39 g (91.4%); IR ν_{max}^{KBr} 1675 cm^{-1}, center of broad absorption, C=O's; NMR ($CDCl_3$) δ 2.24 (s, 3, CH_3), 2.61 (s, 3, CH_3), 7.75 (d, J = 9.0 Hz, 2, 6 H), 8.04 (d, J = 9.0 Hz, 3, 5 H), 8.43 (br s, 1, NH). Anal. ($C_{10}H_{11}NO_2$) C, H, N.

4-(Methylamino)acetophenone [1-[4-(Methylamino)-phenyl]ethanone] (5). To NaH (1.38 g, 30.2 mmol) in ice-chilled DMF (70 mL, freshly distilled from CaH_2) was added, over a 20-min period, 4-(acetylamino)acetophenone (3.8 g, 21.3 mmol) in a minimum volume of dry DMF. The reaction mixture was stirred at room temperature for 15 min. The flask was again chilled, and MeI (1.57 mL, 25.2 mmol) was added. The mixture was stirred at room temperature until the reaction was complete on the basis of TLC. Then the flask was chilled in an ice bath, and concentrated HOAc was added slowly, to destroy excess NaH. The contents of the flask were evaporated to dryness. Then the residue was taken up in $CHCl_3$, and the solution was washed with water and dried ($CaSO_4$). The solvent was evaporated, and the residue was refluxed with 3 N HCl for 45 min. The solution was made basic with NaOH, and the solute was extracted into $CHCl_3$. The solvent was evaporated in vacuo, and the residue was crystallized from water. The product was recrystallized after treatment with charcoal: yield 0.62 g (24.5%); mp 95–100 °C; IR ν_{max}^{KBr} 3322 (–NH), 1654 cm^{-1} (–C=O); NMR ($CDCl_3$) δ 2.51 (s, 3, $-COCH_3$), 2.89 (s, 3, $-NCH_3$), 4.64 (br s, 1, –NH), 6.61 (d, J = 8.8 Hz, 2, 6 H), 7.92 (d, J = 8.8 Hz, 3, 5 H). Anal. ($C_9H_{11}NO$) C, H.

4,4′-Diacetyl-*N*,*N*′-diphenyl-*N*-methylurea [*N*,*N*′-Bis(4-acetylphenyl)-*N*-methylurea] (6a). To a stirring, ice-chilled suspension of **5** (0.120 g, 0.806 mmol) in dry EtOAc (2 mL) and dry Et_3N (1 mL), **2** (0.130 g, 0.806 mmol) in dry EtOAc (2 mL) was added dropwise over a 10-min period. EtOAc (2 mL) was used for washing the funnel. The reaction mixture was stirred overnight at room temperature and filtered. The precipitate obtained was washed with ether and dried: yield 0.132 g (52.8%). The product was recrystalized from hot MeOH. After filtration, the crystals were washed with ether. Two types of crystals were formed, the bulk of which melted at 162–166.5 °C; all had melted by 238 °C; IR ν_{max}^{KBr} 3325 (–NH), 1670 cm^{-1} (–C=O); NMR (Me_2SO-d_6) δ 2.45 (s, 3, $-CH_3$), 2.52 (s, 3, CH_3), 3.33 (s, 3, $-NCH_3$), ~7.75 (m, 8, aryl), 8.97 (s, 1, –NH). Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

4,4′-Diacetyl-*N*,*N*′-diphenyl-*N*-methylurea Bis(guanylhydrazone) [2-[1-[[[[1-[2-(Aminoiminomethyl)hydrazino]-ethyl]-4,1-phenylene]amino]carbonyl-*N*-methylamino-4,1-phenylene]ethylidene]hydrazinecarboximidamide] (6b) Hydrobromide. The unsymmetrical *N*-methylurea **6a** (0.0255 g, 0.0823 mmol), DMF (0.5 mL), and 16% HBr (0.2 mL) were heated on a steam bath with aminoguanidine sulfate (0.028 g, 0.231 mmol), giving a clear solution. After cooling to room temperature, water was added until the solution was slightly cloudy, and the mixture was put in a freezer. The resulting precipitate was filtered, washed with water and ether, and dried: yield 0.014 g (27.5%). The compound was then taken up in a minimum volume of hot DMF, the mixture was filtered, and 16% HBr (several drops) was added. Chilling resulted in the formation of crystals. After being filtered off, they were washed with water and ether. The compound is yellow on drying, but immediately turns white on exposure to air: mp 253–256 °C dec; IR ν_{max}^{KBr} 3280 (broad, guanylhydrazone salt), 1667 cm^{-1}. Anal. ($C_{20}H_{28}N_{10}OBr_2\cdot2H_2O$) C, H, N.

4-Acetylphenyl (4-Acetylphenyl)carbamate [1-[4-[[[(4-Acetylphenyl)amino]carbonyl]oxy]phenyl]ethanone] (7a). To a rapidly stirring, ice-chilled solution of *p*-hydroxyacetophenone (0.150 g, 1.10 mmol) in a mixture of dry EtOAc (2 mL) and Et_3N (0.5 mL) was added **2** (0.178 g, 1.10 mmol) dissolved in dry EtOAc (2 mL). The reaction mixture was allowed to stand at room temperature for 48 h, and the resulting precipitate was filtered, washed with water and petroleum ether, and dried: yield 0.189 g (57.6%). The substance was recrystallized twice from hot EtOAc and was washed with petroleum ether: mp 184–187 °C; Ir ν_{max}^{KBr} 3318 (–NH), 1748 (C=O), 1669 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.54 (s, 3, CH_3), 2.57 (s, 3, CH_3), 7.45 (m, 5, phenyl + NH), ~8.05 (m, 4, phenyl). Anal. ($C_{17}H_{15}NO_4$) C, H, N.

4-Acetylphenyl (4-Acetylphenyl)carbamate Bis(guanylhydrazone) [2-[1-[4-[[[[4-[1-[(Iminoaminomethyl)-hydrazono]ethyl]phenyl]amino]carbonyl]oxy]phenyl]-ethylidene]hydrazinecarboximidamide] (7b) Hydrobromide. To the urethane **7a** (0.200 g, 0.674 mmol) and aminoguanidine sulfate (0.232 g, 1.89 mmol) were added MeOH (1.0 mL), water (0.5 mL), 16% HBr (0.5 mL), and DMF (0.5 mL). The flask was heated to dissolve the solids and then was allowed to cool at room temperature. After crystals formed, the mixture was chilled in a freezer. The crystals were filtered, washed with water, and redissolved in a mixture of 16% HBr, MeOH, and a few drops of DMF with heating. Then the solution was cooled. The product was filtered, washed with cold water and ether, and dried: yield 0.290 g (75.5%); mp 195–205 °C dec; R_f in a 95/5 mixture of MeOH and HCOOH 0.8; the Sakaguchi test was positive; IR ν_{max}^{KBr} 3280 (broad guanylhydrazone salt), 1746 (C=O), 1673 cm^{-1}. Anal. ($C_{19}H_{25}N_9O_2Br_2$) C, H, N.

4-[1-(Ethylenedioxy)ethyl]benzonitrile [4-[1-[1,2-Ethanediylbis(oxy)]ethyl]benzonitrile] (9). *p*-Cyanoacetophenone (8, 1.0 g, 6.89 mmol) was dissolved in dry benzene (15 mL, dried over $CaSO_4$), and ethylene glycol (0.6 mL, 0.67 g, 10.8 mmol) was added. Then ether–BF_3 complex (0.1 mL, purified) was added, and water was removed by means of a Dean-Stark apparatus. Heating was stopped after 4.5 h, and the benzene layer was cooled, washed once with cold 5% $NaHCO_3$, and dried (Na_2SO_4): yield 1.29 g (98.5%). The product was recrystallized from a mixture of ether and petroleum ether: mp 67–71 °C; IR ν_{max}^{KBr} 2237 cm^{-1} (CN), no carbonyl; NMR ($CDCl_3$) δ 1.65 (s, 3, CH_3), 3.97 (m, 4, $-CH_2CH_2-$), 7.75 (s, 4, phenyl). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

4-[1-(Ethylenedioxy)ethyl]benzylamide [[4-[1-[1,2-Ethanediylbis(oxy)]ethyl]phenyl]methanamine] (10). The cyano ketal **9** (1.0 g, 5.29 mmol), dissolved in dry THF (10 mL), was added, over a 10-min period with ice-bath cooling under N_2, to a suspension of $LiAlH_4$ (0.402 g, 10.58 mmol) in THF (10 mL). After being stirred at room temperature for 1.5 h, the mixture was cooled in an ice bath again. EtOAc was added slowly, and then water was added very slowly, to decompose excess $LiAlH_4$. THF was removed in vacuo, and the residue was taken up in $CHCl_3$. The solution was washed with water and dried (Na_2SO_4). The yield of the oily product was 1.0 g (98%): IR (NaCl plates, neat), loss of CN peak; NMR ($CDCl_3$) δ 1.58 (s, 3, CH_3), 3.82 (s, 2, $-CH_2NH_2$) (conceals part of the two complex multiplets of the ketal protons), 7.38 (s, 4, phenyl).

4,4-Diacetyl-*N*,*N*′-dibenzylurea [*N*,*N*′-Bis[(4-acetylphenyl)methyl]urea] (11a). A suspension of **10** (2.0 g, 10.36 mmol) in 1 N NaOH (10.36 mL, 10.36 mmol) was cooled in an ice bath with rapid stirring. An ice-cold 12.5% solution of $COCl_2$ in benzene (9.85 mL, 12.43 mmol) was added dropwise with vigorous stirring. After about 0.5 h at room temperature, more 1 N NaOH was added to basify the solution and destroy any unreacted $COCl_2$. To ensure that the ketal had been cleaved, concentrated HCl was added to acidify the mixture, which was then stirred at room temperature for 1 h. The resulting precipitate was collected on a filter, washed with water and then petroleum ether, and dried: yield 1.16 g (69%). The product was recrystallized from hot MeOH and washed with ether: mp 200–203 °C; IR ν_{max}^{KBr} 3320 (NH), 2882, 1675 cm^{-1} (–C=O); NMR (Me_2SO-d_6)

δ 2.52 (s, 6, $-CH_3$), 4.31 (d, 4, $-CH_2$), 6.63 (m, 2, NH), 7.68 (s, 8, phenyl). Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

4,4'-Diacetyl-*N*,*N*'-dibenzylurea Bis(guanylhydrazone) [2,2'-Carbonylbis[(iminomethylene-4,1-phenyleneethylidyne)hydrazinecarboximidamide]] (11b). To the symmetrical methyleneurea **11a** (0.057 g, 0.176 mmol) and aminoguanidine sulfate (0.061 g, 0.493 mmol) were added DMF (0.75 mL), water (0.3 mL), and 16% HBr (0.3 mL). After warming to dissolve the solids, the mixture was chilled. The resulting precipitate was filtered, washed with acetone and ether, and recrystallized twice from a hot mixture of DMF and 16% HBr, acetone being added each time to force out the precipitate. The product was filtered, washed with acetone and ether, and dried: yield 0.061 g (58.2%); mp 260–270 °C dec; IR ν_{max}^{KBr} 3300 (broad guanylhydrazone salt), shoulder 2879, 1675 cm^{-1} (C=O). Anal. ($C_{21}H_{30}N_{10}OBr_2$) C, H, N.

***N*-(4-Acetylbenzyl)-*N*'-(4-acetylphenyl)urea [*N*-(4-Acetylphenyl)-*N*'-[(4-acetylphenyl)methyl]urea] (12a).** Compound **10** (0.5 g, 2.59 mmol) was suspended in dry EtOAc (2 mL), with stirring, in an ice bath. A solution of **2** (0.417 g, 2.59 mmol) in dry EtOAc (2 mL) was added dropwise over a 10-min period. After stirring overnight at room temperature, the reaction mixture was filtered. The precipitate obtained was discarded, and the filtrate was evaporated to dryness. The residue was dissolved in MeOH (4 mL), and concentrated HCl (0.25 mL) was added (final concentration ~0.7 N). After being stirred at room temperature for 1 h, the solution was evaporated to dryness. The residue was dissolved in hot MeOH and allowed to crystallize. After filtration, the crystals were washed with petroleum ether. With a second crop, the total combined yield was 0.436 g (54.3%). The compound was again recrystallized from hot MeOH: mp 188–191 °C; IR ν_{max}^{KBr} 3330 and 3295 (–NH), 1667 cm^{-1} (C=O); NMR (Me_2SO-d_6) δ 2.52 (s, 3, CH_3), 2.58 (s, 3, CH_3), 4.47 (d, 2, $-CH_2$), 6.97 (t, 1, NH), ~7.58 (m, 4, phenyl, ~8.01 (m, 4, phenyl), 9.18 (s, 1, NH). Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

***N*-(4-Acetylbenzyl)-*N*'-(4-acetylphenyl)urea Bis(guanylhydrazone) [2-[1-[[[[1-[2-(Aminoiminomethyl)hydrazono]ethyl]-4,1-phenylene]imino]carbonyliminomethylene-4,1-phenylene]ethylidene]hydrazinecarboximidamide] Hydrobromide (12b).** To **12a** (0.052 g, 0.168 mmol) and aminoguanidine sulfate (0.058 g, 0.470 mmol) were added DMF (0.5 mL) and 16% HBr (0.3 mL). After being heated to dissolve the solids, the mixture was chilled. Water was added dropwise to precipitate the product, and the mixture was chilled again in a freezer. The product was filtered, washed with acetone and ether, and dried: yield 0.086 g (85.3%). It was recrystallized twice from a mixture of MeOH, DMF, and 16% HBr. The crystals were washed with acetone and ether: mp 213–230 °C dec; IR ν_{max}^{KBr} 3280 (broad guanylhydrazone salt), 1668 cm^{-1}. Anal. ($C_{20}H_{28}N_{10}OBr_2 \cdot H_2O$) C, H, N.

(4-Acetylbenzyl)amine [(4-Acetylphenyl)methanamine] Hydrochloride (13). The protected ketone **10** was dissolved in a mixture of equal volumes of MeOH and 1 N HCl, and the solution was stirred at room temperature for 1 h. The cleavage could be followed by TLC if spotted from a neutral aqueous solution to prevent self-condensation. The solvents were evaporated, and the residue was taken up in MeOH. The compound was filtered, and ether was added for crystallization. The product was recrystallized once from a hot mixture of MeOH and ether and once from a hot mixture of absolute EtOH and acetone. After filtration, the crystals were washed with anhydrous ether: mp 222–228 °C (turns red). The compound gives a positive ninhydrin test, but a negative phenylhydrazine test: IR ν_{max}^{KBr} 1687 cm^{-1} (–C=O); NMR (Me_2SO-d_6) δ 2.62 (s, 3, CH_3), 4.17 (s, 2, $-CH_2$), 7.73 (d, J = 8.8 Hz, 2, 6 H), 8.07 (d, J = 8.8 Hz, 3, 5 H). Anal. ($C_9H_{12}NOCl$) C, H, N.

***N*-Nitroso-4,4'-diacetyl-*N*,*N*'-dibenzylurea [*N*,*N*'-Bis[(4-acetylphenyl)methyl]-*N*-nitrosourea] (14a).** A suspension of **11a** (0.206 g, 0.636 mmol) in HCOOH (7 mL) was chilled in an ice bath for 10 min. Then $NaNO_2$ (0.110 g, 1.59 mmol) was added in small portions, with vigorous stirring. After 15 min, TLC (ethyl acetate as eluent) showed essentially one spot with an R_f of 0.95 as compared with 0.6 for the starting material. After 80 min, the solution was evaporated to dryness. The residue was taken up in $CHCl_3$, and the solution was washed twice with water and dried (Na_2SO_4). Crystals were obtained from a mixture of dry $CHCl_3$ and petroleum ether. The product was recrystallized from this mixture: yield 0.198 g (84%); mp 136–139 °C, with vigorous evolution of gas; IR ν_{max}^{KBr} 1480 (–NN=O), 1673 (C=O, unsubstituted side), 1726 cm^{-1} (C=O, substituted side); NMR ($CDCl_3$) δ 2.55 (s, 3, CH_3), 2.58 (s, 3, CH_3), 4.80 (d, 2, J = 6 Hz, $-CH_2NH$), 5.1 (s, 2, $-CH_2NN$=O), ~7.47 (m, 5, phenyl and NH), ~8.00 (m, 4, phenyl). Anal. ($C_{19}H_{19}N_3O_4 \cdot H_2O$) C, H, N.

***N*-Nitroso-4,4'-diacetyl-*N*,*N*'-dibenzylurea Bis(guanylhydrazone) [2-[1-[[[[1-[2-(Aminoiminomethyl)hydrazono]ethyl]-4,1-phenylene]methyl]nitrosoiminocarbonyliminomethylene-4,1-phenylene]ethylidene]hydrazinecarboximidamide] Hydrobromide (14b).** DMF (1 mL) and 16% HBr (0.5 mL) were chilled in an ice bath for 5–10 min. Then the *N*-nitrosourea derivative **14a** (0.027 g, 0.073 mmol) was added, followed by aminoguanidine bicarbonate (0.030 g, 0.221 mmol). The suspension was stirred at room temperature for 5 h, by the end of which there resulted a clear solution. The solvents were evaporated to dryness without heating, and a few drops of water was added, giving a precipitate. After filtration, the precipitate was washed with water and then acetone: yield 0.024 g (51.2%); mp 255 °C dec. The compound was stored in a refrigerator: IR ν_{max}^{KBr} 3280 (center of very broad guanylhydrazone salt peak), 1723, 1670 cm^{-1}. Anal. ($C_{21}H_{29}N_{11}O_2Br_2 \cdot H_2O$) C, H, N.

Acknowledgment. This study was supported in part by research grants (CA-08793, CA-13038, and CA-16056) from the National Institutes of Health. We thank Dr. E. Mihich for his encouragement of this program. We are indebted to Dr. M. T. Hakala and Miss A. I. Mulhern of our department for the tests on the mouse mammary adenocarcinoma cells and to Ms. Onda Simmons for the NMR spectra.

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(14) Chemical names in brackets are set up in accordance with rules given in "Naming and Indexing of Chemical Substances for *Chemical Abstracts* During the Ninth Collective Period (1972–1976)" where those rules are significantly different from more traditional rules. The names not in brackets were chosen to indicate, as clearly as possible, the structural relationships of the compounds to the reference compound, which is generally called "diacetyldiphenylurea guanylhydrazone" ("DDUG") in the pharmacological literature.
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