The results obtained with active compounds were processed by probit analysis in order to get an ED_{50} with confidence interval.

Acetic Acid Induced Writhing Syndrome. At different times after administration of the test compounds, 10 mL/kg of a 2% acetic acid solution was injected intraperitoneally. Five minutes later, the number of animals protected from more than one writhing syndrome (extension movement of body and hind legs) were counted during 5 min. The ED_{50} is defined as the dose that protects 50% of the animals from more than one writhing syndrome.

Hot Plate Test. One hour after administration of the test compounds, the animals were placed on a hot plate that was maintained at 60 ± 1 °C. Untreated and unprotected animals began to lick their paws within 10 s. The latest time of removal from the plate was 12 s. The ED_{50} is defined as the dose at which 50% of the animals start to lick their paws later than 10 s.

Acknowledgment. The biological data given herein were kindly supplied by Drs. Lislott Aeppli, Willy P. Burkard, Rudolf Cumin, Hans H. Keller, and Rudolf Schaffner; we express our thanks for their assistance in preparing this manuscript. The authors are also indepted to the members of our Central Research Units for obtaining the spectral and microanalytical data. A generous gift of both enantiomers of butaclamol from the Ayerst Research Laboratories, Montreal, is kindly acknowledged.

Registry No. (+)-**3**·HCl, 55528-07-9; (−)-**3**·HCl, 55528-08-0; (±)-**5a**, 87922-63-2; (±)-**5a**·HCl, 75384-69-9; (±)-**5b**, 87937-30-2; (±)-**5b**·HCl, 75384-70-2; (±)-**5c**, 87922-64-3; (±)-**5c**·HCl, 87922-65-4; (±)-**5d**, 87922-66-5; (±)-**5d**·HCl, 75422-23-0; (±)-**5e**, 87922-67-6; (+)-**5e**, 87982-61-4; (−)-**5e**, 87982-62-5; (±)-**5e**·HCl, 75384-71-3; (+)-**5e**·HCl, 87982-63-6; (−)-**5e**·HCl, 87982-64-7; (−)-**5e**·(−)-BNPPA, 87982-65-8; (+)-**5e**·(+)-BNPPA, 87982-66-9; (±)-**5f**, 87922-68-7; (±)-**5f**·HCl, 87937-11-9; (±)-**5g**, 75384-68-8; (+)-**5g**, 87982-67-0; (−)-**5g**, 87982-68-1; (±)-**5g**·HCl, 87922-69-8; (+)-**5g**·HCl, 87982-69-2; (−)-**5g**·HCl, 87984-06-3; (±)-**5h**, 87922-70-1; (±)-**5h**·HCl, 75384-72-4; (±)-**6a**, 87922-71-2; (±)-**6b**, 87922-72-3; (±)-**6c**, 87922-73-4; (±)-**6d**, 87922-74-5; (±)-**6e**, 87922-75-6; (±)-**6f**, 87922-76-7; (±)-**6g**, 87922-77-8; (±)-**6h**, 87922-78-9; (±)-**7a**, 87922-79-0; (±)-**7b**, 87922-80-3; (±)-**7c**, 87922-81-4; (±)-**7d**, 87922-82-5; (±)-**7e**, 87922-83-6; (±)-**7f**, 87922-84-7; (±)-**7g**, 87922-85-8; (±)-**7h**, 87922-86-9; **8**, 37734-05-7; (±)-**9a**, 75385-04-5; (±)-**9b**, 75385-06-7; (±)-**9c**, 87922-87-0; (±)-**9d**, 75385-07-8; (±)-**9e**, 75385-10-3; (±)-**9f**, 87922-88-1; (±)-**9g**, 75385-05-6; (±)-**9h**, 75385-11-4; (±)-**10a**, 75384-96-2; (±)-**10b**, 75384-98-4; (±)-**10c**, 87922-89-2; (±)-**10d**, 75401-00-2; (±)-**10e**, 75385-01-2; (±)-**10f**, 87922-90-5; (±)-**10g**, 75384-97-3; (±)-**10h**, 75385-02-3; (±)-**11a**, 75384-88-2; (±)-**11b**, 75384-89-3; (±)-**11c**, 87922-91-6; (±)-**11d**, 75384-90-6; (±)-**11e**, 75384-93-9; (±)-**11f**, 87922-92-7; (±)-**11g**, 75400-98-5; (±)-**11h**, 75384-94-0; **12**, 87922-93-8; **12**·HCl, 75384-73-5; (±)-**13**, 87982-70-5; (±)-**13**·HCl, 75384-74-6; (±)-**14**, 87922-94-9; (±)-**14**·HCl, 75384-75-7; (±)-**15**, 87922-95-0; (±)-**16** (isomer 1), 87922-96-1; (±)-**16** (isomer 2), 87922-97-2; (±)-**17**, 75385-86-3; (±)-**17**·HCl, 75384-79-1; (±)-**18**, 87922-98-3; (±)-**18**·HCl, 75399-57-4; (±)-**19**, 75384-78-0; (+)-**19**, 75385-13-6; (−)-**19**, 75385-14-7; (±)-**20**, 87922-99-4; (±)-**22**, 87923-00-0; (±)-**22**·HCl, 75385-15-8; (±)-**23**, 87923-01-1; (+)-**23**, 87982-71-6; (−)-**23**, 87982-72-7; (±)-**23**·HCl, 87923-02-2; (−)-**23**·HCl, 87982-73-8; (+)-**23**·HCl, 87982-74-9; (±)-**23** tosylate, 87923-03-3; (±)-**24**, 87923-04-4; (±)-**24**·HCl, 75385-16-9; (±)-**25**, 87923-05-5; (±)-**25**·HCl, 87923-06-6; (±)-**26**, 75385-18-1; (−)-**26**, 75385-20-5; (+)-**26**, 75385-19-2; (+)-**26**·(+)-BNPPA, 88033-95-8; (+)-**26**·HCl, 88033-96-9; (−)-**26**·HCl, 88033-97-0; (±)-**26**·HCl, 87923-07-7; (±)-**27**, 87923-08-8; (±)-**27**·HCl, 87923-09-9; (±)-**28**, 87923-10-2; (±)-**28**·HCl, 75385-24-9; (±)-**29**, 87923-11-3; (±)-**29**·HCl, 75385-25-0; $PhCH_2CN$, 140-29-4; $4\text{-}ClC_6H_4CH_2CN$, 140-53-4; $3,4\text{-}Cl_2C_6H_3CH_2CN$, 3218-49-3; $4\text{-}CF_3C_6H_4CH_2CN$, 2338-75-2; $2,4\text{-}Cl_2C_6H_3CH_2CN$, 6306-60-1; $3\text{-}ClC_6H_4CH_2CN$, 1529-41-5; $2\text{-}ClC_6H_4CH_2CN$, 2856-63-5; $2,6\text{-}Cl_2C_6H_3CH_2CN$, 3215-64-3; methyl acrylate, 96-33-3.

Supplementary Material Available: Tables I to VII and VIII to XIV list atom coordinates, bond lengths, bond angles, anisotropic temperature factors, hydrogen coordinates, some torsion angles, and two least-square planes for (−)-**5e**·(−)-BNPPA and (+)-**26**·(+)-BNPPA, respectively (9 pages). Ordering information is given on any current mast head page.

Synthesis and Characterization of N^2-(*p*-*n*-Butylphenyl)-2′-deoxyguanosine and Its 5′-Triphosphate and Their Inhibition of HeLa DNA Polymerase α

George E. Wright* and Lech W. Dudycz

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605.
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N^2-(*p*-*n*-Butylphenyl)-2′-deoxyguanosine (BuPdG) and its 5′-triphosphate (BuPdGTP), expected to be inhibitors of eukaryotic DNA polymerase α, have been synthesized. BuPdG was synthesized by two methods and characterized by ^1H NMR and by chemical relation to guanosine. Direct synthesis involving silylated N^2-(*p*-*n*-butylphenyl)guanine (BuPG) and 1-chloro-3,5-di-*p*-toluoyl-2-deoxyribofuranose in the presence of trimethylsilyl trifluoromethanesulfonate gave one α and two β isomers of deoxyribonucleoside as determined by ^1H NMR. However, NMR and UV spectra were equivocal in distinguishing between 7 and 9 isomers. The identity of the desired 9-β-BuPdG was ultimately proved by its independent synthesis from the corresponding ribonucleoside. ^1H NMR spectra of the O′-acetylated ribonucleosides of BuPG showed characteristic patterns of O′-acetylated guanosines, and their identity was proved by relating the products of the reaction of isomeric O′-acetylated 2-bromoinosines with *p*-*n*-butylaniline and with ammonia: the 2-bromoinosine which gave guanosine also gave the suspected 9-β-ribonucleoside, BuPGr, and that which gave N^7-β-ribofuranosylguanine also gave the 7-β isomer of BuPGr. BuPGr was transformed in a multistep procedure to give BuPdG, identical with the major β isomer obtained by direct deoxynucleoside synthesis. The 5′-monophosphate of BuPdG was obtained by treatment of the nucleoside with phosphoryl chloride in trimethyl phosphate; the monophosphate reacted as the phosphoimidazolyl derivative with pyrophosphate to yield the 5′-triphosphate, BuPdGTP. The BuPG deoxynucleosides inhibited DNA polymerase α from HeLa cells with potencies similar to that found for BuPG itself. BuPdGTP, however, was at least a 1000-fold more potent inhibitor of DNA polymerase α than BuPG.

Our interest in the preparation of the 2-amino-substituted 2′-deoxyguanosine, N^2-(*p*-*n*-butylphenyl)-2′-deoxyguanosine (BuPdG, **3**), and its 5′-triphosphate (BuPdGTP, **19**) is a direct result of our biological studies with 6-anilinouracils and N^2-phenylguanines. Certain of these analogues are potent, selective inhibitors of bacterial DNA

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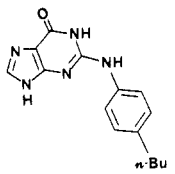

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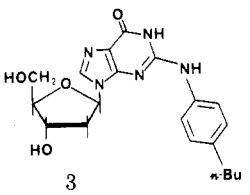

3

polymerase III[1,2] and mammalian DNA polymerase α.[3,4] Their activity is based partly on their ability to mimic the base-pairing capacity of 2′-deoxyguanosine 5′-triphosphate (dGTP), but selectivity is strongly dependent on substituents in the phenyl ring. A bulky, linear *p*-*n*-butyl group in these series [6-(*p*-*n*-butylanilino)uracil (BuAU) and N^2-(*p*-*n*-butylphenyl)guanine (BuPG, **2**)] causes selective inhibition of DNA polymerase α from HeLa[3,4a] and calf thymus[4b] cells. Both derivatives also inhibit the growth of a variety of normal and cancer cells in culture,[3,4] although cells are not uniformly sensitive to these compounds. For example, a human lung cancer cell line was considerably more sensitive to BuAU and BuPG than a human melanoma line.[4b] We felt that additional analogues in this series might be designed that would probe the basis for the different sensitivities of human cells and also aid in the development of anticancer agents.

In the present work we postulated that based on the known mechanism of action of inhibitors such as BuPG (**2**), a closer structural analogue of its competitor dGTP might cause significantly increased potency as an inhibitor of DNA polymerase α. This analogue, the 5′-triphosphate of BuPdG, might also serve as a substrate for the enzyme and cause termination of DNA synthesis. Further, if BuPdG (**3**) could be activated intracellularly by kinases to BuPdGTP, a very potent cell-growth inhibitor may result.

In order to pursue these hypotheses, we first needed an efficient method to convert BuPG (**2**) into its 9-β-2′-deoxyribonucleoside and unequivocal proof of the structure of the nucleoside. We have applied recent methods for nucleoside synthesis to solve this problem. One of these, which utilizes silylated heterocycles in the presence of Friedel–Crafts catalysts,[5] has been used with our improvement of the reaction conditions to achieve higher yields of isomeric deoxyribonucleosides. The second method employs a general procedure for the specific conversion of ribonucleosides to 2′-deoxyribonucleosides.[6,7]

Our original synthesis of BuPG employed the expensive substrate 2-chlorohypoxanthine.[3] This intermediate has been replaced by 2-bromohypoxanthine, which was readily obtained from purine ring closure of 5,6-diamino-2-thiouracil[8] and replacement of the mercapto group by bromine in an aqueous methanolic solution of concentrated hydrobromic acid.[9] 2-Bromohypoxanthine (**1**) reacted smoothly with *p*-*n*-butylaniline in boiling aqueous 2-methoxyethanol to give 85% of BuPG (**2**), identical with an authentic sample.[3]

A mixture of isomeric BuPG 2′-deoxyribonucleosides was prepared in one simple synthetic step in good yield (overall yield of acylated nucleosides ca. 70%). Silylation of **2** was conducted in dry 1,2-dichloroethane with an excess of bis(trimethylsilyl)acetamide (BSA)[10] until a clear solution was obtained. The silylated base reacted with 1-chloro-3,5-di-*p*-toluoyl-2-deoxyribofuranose in the presence of trimethylsilyl trifluoromethanesulfonate (TMSTF) as the Friedel–Crafts catalyst. The nucleoside mixture was deblocked with sodium methoxide and contained three components as found by thin-layer chromatography. As is typical for 2′-deoxyribonucleoside syntheses, especially with guanine derivatives, a mixture of 7-α and 7-β and 9-α and 9-β isomers was expected.

The assignment of the N-glycosidic linkage of guanine nucleosides is usually based on their characteristic UV spectra, but the presence of the phenyl ring in the N^2 position of BuPG nucleosides led to confusing results and made the assignment impossible. The UV spectra of the N^7 isomer of guanosine show a characteristic large difference in λ_{max} between pH 2 and 12 (λ_{max} 250 and 283 nm, respectively[11,12]). In contrast, guanosine exhibits a much smaller difference (9 nm) in λ_{max} between acid and alkaline solutions. BuPG deoxyribonucleosides, however, showed only small differences in λ_{max} between pH 2 and 12 (3–5 nm). Two of them (**4** and **5**) had λ_{max} in the region 263–266 nm and one (**3**) in the region 272–278 nm.

Products **4** and **5**, with virtually identical UV spectra, were identified as β and α anomers, respectively, and **3** was identified as a β anomer by ^{1}H NMR spectroscopy (Table I). In the ^{1}H NMR spectra, the signal for the anomeric (1′) proton of the β isomer of 2′-deoxyribonucleosides appears as a pseudotriplet (**3** and **4**) and for the α isomer as a pseudoquartet (**5**). The methylene protons, H-2′ and H-2″, next to the anomeric center of **5** display more chemical-shift nonequivalence than those in **4** [$\Delta\delta$ (H-2′, H-2″) = 0.43 and 0.11 ppm, respectively]. The determination of anomeric configuration, based on empirically determined rules,[13] was finally proved for **3** by chemical transformation of BuPG 9-β-ribonucleoside (BuPGr) to the corresponding deoxynucleoside (see below).

A general method for the specific conversion of ribonucleosides to 2′-deoxyribonucleosides[6,7] was used for two reasons: to identify the 9-β isomer of BuPG deoxyribonucleosides and to test this method as a more efficient procedure for the synthesis of the desired deoxyribonucleoside than the direct synthetic method. The β-ribonucleosides of BuPG were obtained in one synthetic step. The base was silylated with an excess of BSA in dry acetonitrile. Tetra-*O*-acetylribofuranose and TMSTF were added, and the reaction was conducted in boiling acetonitrile. Prolonged heating[14] of the reaction mixture gave

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(7) Pankiewicz, K.; Matsuda, A.; Watanabe, K. A. *J. Org. Chem.* **1982**, *47*, 485.
(8) Elion, G. B.; Lange, W. H.; Hitchings, G. H. *J. Am. Chem. Soc.* **1956**, *78*, 217.
(9) Beaman, A. G.; Gerster, A. F.; Robins, R. K. *J. Org. Chem.* **1962**, *27*, 986.
(10) In comparison with other one-pot nucleoside syntheses (see, for example: Vorgruggen, H.; Bennua, B. *Tetrahedron Lett.* **1978**, *15*, 1339; *Chem. Ber.* **1981**, *114*, 1279), the use of BSA does not introduce harmful basic or acidic byproducts into the reaction mixture and provides continuous regeneration of silylated base and catalyst (see, for example: Imazawa, M.; Eckstein, F. *J. Org. Chem.* **1979**, *44*, 2039).
(11) Gulland, M. J.; Story, L. F. *J. Chem. Soc.* **1938**, 692.
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(13) Srivastava, P. C.; Robins, R. K. *J. Heterocycl. Chem.* **1981**, *18*, 1659, and references cited therein.

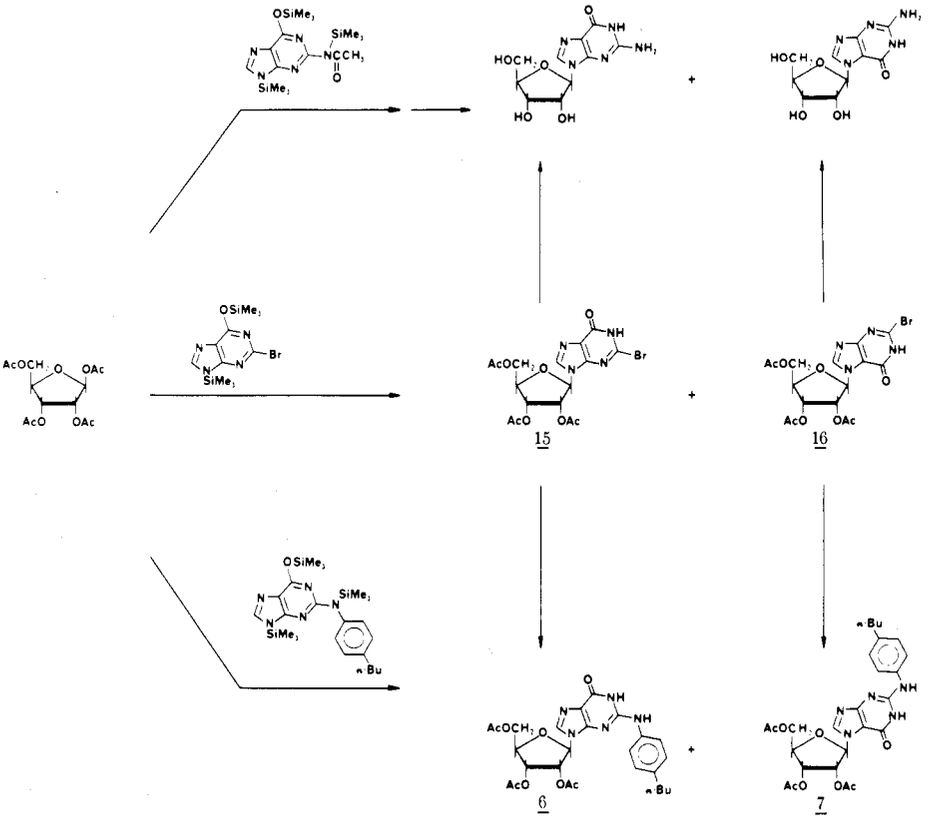
Table I. 250-MHz ^{1}H NMR Data for BuPG Nucleosides in Me_2SO-d_6

	chemical shifts,[a] δ							
nucleoside	H-1'	H-2'	H-2''	H-3'	H-4'	H-5'	H-5'	H-8
9-β-2'-deoxyribo- (3)	6.22 (t)	2.59	2.31	4.37	3.87	3.58	3.54	7.97
7-β-2'-deoxyribo- (4)	6.49 (t)	2.46	2.35	4.34	3.87	3.65	3.59	8.31
7-α-2'-deoxyribo- (5)	6.53 (dd)	2.68	2.25	4.30	4.18	3.48[b]		8.25
9-β-ribo- (8)	5.82	4.48		4.16	3.96	3.67	3.59	8.02
7-β-ribo- (9)	6.05	4.37		4.16	3.97	3.72	3.60	8.32

	coupling constants,[c] Hz								
nucleoside	$J_{1',2}$	$J_{1',2''}$	$J_{2',2''}$	$J_{2',3'}$	$J_{2'',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	$J_{5',5''}$
9-β-2'-deoxyribo- (3)	7.2	6.3	−13.3	6.1	3.4	3.1	4.2	4.2	−11.6
7-β-2'-deoxyribo- (4)	7.1	5.8	−13.3	6.4	3.3	3.2	4.0	4.3	−11.8
7-α-2'-deoxyribo- (5)	7.4	2.6	−14.3	6.7	2.4	2.6	d	d	
9-β-ribo- (8)	5.3			5.3		4.3	3.5	4.2	−11.9
7-β-ribo- (9)	5.3			5.2		3.9	3.2	3.5	−12.1

[a] Chemical shifts are relative to internal tetramethylsilane. Chemical shifts of 1-H, 2-NH and p-butylphenyl groups were nearly identical with those of BuPG and are not reported for each nucleoside. [b] Chemical shifts for H-5' and H-5'' were identical. [c] Assignment of coupling constants was made by selective spin decoupling and spectral simulation with a programmable calculator. [d] Because of the identical chemical shifts of H-5' and H-5'', J_{av} [$^1/_2(J_{4',5'} + J_{4',5''})$] = 3.7 Hz.

Scheme I



an overall yield of ca. 80% of two BuPG per-O-acetylated β-ribonucleosides and, as expected from Baker's rule,[15] no α anomers. The mixture was separated on a silica gel column to give pure **6** and **7** in yields of 69 and 11%, respectively.

The ^{1}H NMR spectra of **6** and **7** showed characteristic patterns of guanine ribonucleosides[16] (Table II). Based on these spectra, **6** is assigned as the 9-β-ribonucleoside and **7** as the 7-β isomer. Spectra of the N^9 isomers exhibit very small or no difference in the chemical shift of H-1' and H-2' resonances in comparison with N^7 isomers. The latter, because of the proximity of the 6-oxo group to H-1' and H-2', exhibit large nonequivalence in the chemical shifts of H-1' and H-2' (Table II).

Unequivocal structure proof of BuPG ribonucleosides was done by the procedure depicted in Scheme I, permitting comparison of the well-characterized N^9 and N^7 isomers of β-ribofuranosylguanine with **6** and **7**. Peracetylated 2-bromohypoxanthine β-ribonucleosides (2-bromoinosines, **15** and **16**) reacted with p-n-butylaniline to give tri-O-acetylated BuPG ribonucleosides identical with **6** and **7** obtained from direct synthesis, respectively.

(14) After 0.5 h, no sugar remained in the reaction mixture, and two nucleosides, whose initial relative proportion was 4:1, changed during the reaction time to ca. 1:4. This isomerization was also found during the synthesis of other purine nucleosides (Dudycz, L.; Wright, G. E., preliminary results presented at Fifth International Round Table on Nucleosides, Nucleotides and Their Biological Applications, Research Triangle Park, NC, Oct 1982).

(15) Baker, B. R. *Ciba Found. Symp. Chem. Biol. Purines* **1957**, 120.

(16) Azuma, T.; Isono, K. *Chem. Pharm. Bull.* **1977**, *25*, 3347.

Table II. Comparison between ^{1}H NMR Spectra of Acetylated BuPG Ribosides and Guanosines

compound	chemical shifts,[a] δ						
	H-8	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″
6	7.66	5.96 (m)		5.39 (m)	4.31	4.12	4.06
per-*O*′-acetylguanosine[b]	7.74	5.92 (m)		5.65 (m)		4.42 (m)	
7	8.02	6.16 (d)	5.82 (t)	5.42 (m)		4.35 (m)	
per-*O*′-acetyl-*N*^{7}-β-D-ribofuranosylguanine[b]	8.07	6.32 (d)	5.75 (t)	5.46 (t)		4.42 (m)	

[a] Spectra were determined in Me_2SO-d_6 with internal tetramethylsilane at 250 MHz. [b] Spectra reported at 100 MHz. (from ref 16).

Ammonolysis of **15** and **16** gave guanosine and its N^7 isomer, respectively, as determined by their characteristic UV spectra.[12] The results of these transformations prove that **6** is a 9-β-nucleoside and that **7** is a 7-β-nucleoside.

Deacetylation of **6** and **7** with ammonia in aqueous ethanol gave free BuPG ribonucleosides, **8** and **9**, respectively. The ^{1}H NMR spectra of **8** and **9** (Table I) showed virtually identical chemical shifts of H-3′, H-4′, H-5′, and H-5″ but a somewhat larger value, Δδ (H-1′, H-2′), for the N^7 isomer (**9**). In comparison with **8**, H-1′ of **9** is shifted downfield, and H-2′ is shifted upfield. Similar patterns were observed for the β isomers of the 2′-deoxyribonucleosides **3** and **4** (see above). The greater chemical-shift difference between H-1′ and H-2′ of **4** suggested that it was the 7-β isomer and, therefore, that **3** was the desired BuPG 9-β-2′-deoxyribonucleoside.

For conclusive proof of the structural assignment of **3**, we adapted a procedure for the specific transformation of BuPGr (**8**) to the corresponding 2′-deoxyribonucleoside. The 3′- and 5′-hydroxyl groups of **8** were protected by treatment with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine to give the 3′,5′-*O*-(1,1,3,3-tetraisopropyldisilox-1,3-diyl) intermediate (**10**). It was converted to the 2′-*O*-(imidazol-1-yl)thiocarbonyl derivative (**11**) by treatment with 1,1′-(thiocarbonyl)diimidazole in dimethylformamide. Reduction of this intermediate by tri-*n*-butyltin hydride in the presence of 2,2′-azobis[2-methylpropionitrile] gave the 2′-deoxy derivative **12**, isolated in crystalline form. After the deblocking of **12** with tetrabutylammonium fluoride in tetrahydrofuran, the product was purified by chromatography on silica gel with chloroform–methanol containing 0.4% boric acid.[17] The pure product of this transformation was identical with a sample of **3** obtained by direct synthesis.

The overall yield of **3** from **8** was about 50%, but somewhat less (35%) when calculated from BuPG itself. This procedure gave about twice the yield of **3** compared to direct deoxyribonucleoside synthesis, but the time and reagents involved in the six steps make it less efficient than direct synthesis. It was valuable, however, in providing conclusive proof for the structure of **3**.

The 5′-monophosphate of BuPdG (BuPdGMP, **18**) was prepared in 60% yield by the reaction of **3** with phosphoryl chloride in trimethyl phosphate.[18] The product was purified by column chromatography on DEAE-cellulose, bicarbonate form, in a linear gradient of ammonium bicarbonate, and finally on a silica gel column equilibrated and developed with a solvent mixture consisting of 2-propanol/ammonia/water. The corresponding 5′-triphosphate (BuPdGTP, **19**) was prepared by the reaction of **18** with 1,1′-carbonyldiimidazole and pyrophosphate in hexamethylphosphoramide as solvent.[19] Chromatography on DEAE-cellulose, bicarbonate form, in a linear gradient of triethylammonium bicarbonate yielded the pure triphosphate **19** (47%). The major side-product from this reaction was the corresponding 5′-diphosphate (BuPdGDP, **20**), isolated in 4% yield. All nucleotides were identified by phosphate analyses (see Experimental Section).

Table III. Inhibition of HeLa DNA Polymerase α by BuPG, Its Deoxyribonucleosides and BuPdGTP

compd	K_i,[a] μM
BuPG (**2**)	7.5
BuPdG (**3**)	2.3
7-β-BuPdG (**4**)	22
7-α-BuPdG (**5**)	37
BuPdGTP (**19**)	0.001

[a] HeLa DNA polymerase α was assayed as described previously.[20] K_i values were obtained by assaying the enzyme with activated DNA in the absence of dGTP.

Inhibition of DNA Polymerase α. The inhibitory effects of BuPdG, its 7-β and 7-α isomers, and BuPdGTP on DNA polymerase α from HeLa cells are summarized in Table III. The enzyme assay[20] used activated DNA and, when done in the absence of the expected competitive substrate dGTP, allows direct determination of K_i, the inhibitor concentration causing half-maximal inhibition of enzyme activity. The 9-β isomer of deoxynucleoside **3** was somewhat more active than the base BuPG itself (**2**) as an inhibitor of this enzyme, whereas the 7-β and 7-α isomers were less so. The target compound BuPdGTP (**19**) showed an extraordinary increase of potency compared with **2** and **3**, its K_i (ca. 0.001 μM) being at least 1000-fold lower than those of **2** and **3**. Preliminary observations (results not shown) indicate that BuPdGTP inhibits DNA polymerase α by the same mechanism as that established for BuPG;[4] i.e., its effect is reversible by dGTP (apparent K_i in the presence of 800 μM dGTP was 0.2 μM), and it does not inhibit HeLa DNA polymerase β or γ or *Bacillus subtilis* DNA polymerase III at 1 mM (results not shown). The selectivity and extreme potency of BuPdGTP toward DNA polymerase α strongly suggest that it and the family of inhibitors from which it was conceptually derived bind to the enzyme at a region overlapping the substrate binding site.

It is unlikely that the nucleotides of BuPdG will show significant cytotoxic activity against human cells. We are, however, examining the activity of BuPdG itself as a cytotoxic agent—even a small amount of metabolism intracellularly could generate enough BuPdGTP to profoundly inhibit replicative DNA synthesis in cells. Alternatively, active transport of the nucleoside and cleavage to the base

(17) This reaction is not always complete due to a side reaction (Rasmussen, J. R.; Slinger, C. J.; Kordish, R. J.; Newman-Evans, D. D. *J. Org. Chem.* **1981**, *46*, 4843), but the presence of boric acid in the eluting solvent permits very good separation of ribonucleosides from 2′-deoxyribonucleosides as a result of the formation of strong, charged complexes between boric acid and ribosides.

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could lead to a high concentration of BuPG within the cell, causing enhanced cytotoxic activity.

In the development of cytotoxic agents, BuPdGTP could be employed to probe differences among DNA polymerase α's derived from human cells. Any difference thus exposed in inhibitor binding-site structure, i.e., the enzyme region that binds the p-n-butylphenyl group, would prompt the synthesis of other substituted phenyl analogues of BuPdG to take advantage of this difference.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Ultraviolet spectra were determined with a Beckman Model 25 spectrophotometer. Nuclear magnetic resonance spectra were obtained on a Perkin-Elmer R-12B (60 MHz) or a Bruker WM-250 (250 MHz) spectrometer, both operating in the FT mode. Solvents were dried by standard methods; anhydrous acetonitrile from MCB was used without further purification. Elemental analyses (C, H, and N) were done by Schwarzkopf Microanalytical Laboratories, Woodside, NY. Nucleotides were analyzed for total phosphorus after ashing by the method of Ames and Dubin.[21] Thin-layer chromatography was performed with Merck Kieselgel 60 F-254 analytical plates. Column chromatography was done with Merck Kieselgel 60 (40–60 μm).

N^2-(p-n-Butylphenyl)guanine (BuPG, 2). A stirred solution of 2-bromohypoxanthine (1; 10.8 g, 50 mmol) and p-n-butylaniline (23.5 mL, 150 mmol) in a mixture of 2-methoxyethanol (300 mL) and water (100 mL) was heated at reflux. After 2.5 h, the mixture was chilled in an ice bath, and the fine precipitate was filtered and washed with concentrated aqueous ammonia (80 mL) and methanol (3 × 25 mL). The product was purified by dissolving the slightly yellow precipitate in hot 1 N sodium hydroxide (300 mL) and treating the mixture with activated charcoal. The hot mixture was filtered, acidified with glacial acetic acid, and chilled. The product was isolated by filtration, washed with methanol, and dried over phosphorus pentoxide to yield 12.0 g (85%) of fine colorless crystals, identical with an authentic sample: NMR (250 MHz; Me_2SO-d_6) δ 10.45 (s, 1-H), 8.60 (s, 2-NH), 8.00 (s, 8-H), 7.50 (d, J = 8.5 Hz, 2',6'-H), 7.14 (d, J = 8.5 Hz, 3',5'-H), 2.55 (t, CH_2), 1.56 (quintet, CH_2), 1.33 (sextet, CH_2), 0.90 (t, CH_3).

N^2-(p-n-Butylphenyl)-9-(2-deoxy-β-D-ribofuranosyl)guanine (N^2-(p-n-Butylphenyl)-2'-deoxyguanosine, BuPdG, 3) and Its 7-β and 7-α Isomers (4 and 5). A solution of BuPG (1.70 g, 6 mmol) in 1,2-dichloroethane (25 mL) was treated with BSA (10.5 mL, 42 mmol) and allowed to stand at room temperature for 1 h. A solution of 1-chloro-3,5-di-p-toluoyl-2-deoxyribofuranose (1.94 g, 5 mmol) in 1,2-dichloroethane (20 mL) and a solution of TMSTF (1.56 g, 7 mmol) in benzene (5 mL) were added to the silylated BuPG solution. After standing for 2 h at room temperature, the solution was heated to boiling and brought to room temperature. The solution was poured into a mixture of saturated aqueous sodium bicarbonate (150 mL) and chloroform (150 mL). The cloudy biphasic mixture was filtered, and the precipitate was washed with chloroform. The filtrate and washings were combined, and the organic phase was separated. The organic phase was washed with water (3 × 100 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The remaining syrup was triturated with cold ethanol (50 mL), and the solid was collected by filtration to give 2.35 g (67%) of nucleoside mixture. The solid was treated with sodium methoxide (230 mg of Na in 120 mL of methanol) at 50–60 °C for 1 h, and the solution was neutralized with glacial acetic acid. Silica gel (20 g) was impregnated with this mixture and, after evaporation of methanol, was placed on top of a silica gel column (24 × 4.5 cm). The column was washed with chloroform (1.2 L) to remove nonpolar components, and nucleosides were eluted in 15-mL fractions by a step gradient of methanol in chloroform containing 0.5% acetic acid/10% methanol (3.6 L), 20% methanol (2.5 L) and 25% methanol (2.0 L).

Fractions 13–44 were combined, and the solvents were evaporated. The resulting solid was dissolved in a hot mixture of ethanol (2 mL) and aqueous ammonia (5 mL). After standing at room temperature for 3 days, the colorless crystals were collected by filtration to give 312 mg (15.6%) of the 7-β isomer 4: mp 184–185 °C; UV λ_{max} (H_2O) 263 nm (ϵ 11 300), λ_{max} (pH 2) 263 nm (ϵ 13 100), λ_{max} (pH 12) 266 nm (ϵ 14 700). Anal. ($C_{20}H_{25}N_5O_4 \cdot 0.25H_2O$) C, H, N.

Fractions 73–100 contained a solid, which was dissolved in methanol and, after filtration, diluted with water and slowly evaporated to give a cloudy solution. Slow cooling produced colorless needles of the 7-α isomer 5 (110 mg, 5.5%): mp 193–195 °C; UV λ_{max} (H_2O) 264 nm (ϵ 14 600), λ_{max} (pH 2) 265 nm (ϵ 18 000), λ_{max} (pH 12) 267 nm (ϵ 20 300), 239 (13 900). Anal. ($C_{20}H_{25}N_5O_4 \cdot 0.67H_2O$) C, H, N.

Fractions 113–144 contained a solid, which was crystallized from ethanol–aqueous ammonia to give the 9-β isomer 3 as colorless crystals (368 mg, 18.4%): mp 196–197 °C; UV λ_{max} (H_2O) 273 nm (ϵ 19 800), λ_{max} (pH 2) 272 nm (ϵ 20 300), λ_{max} (pH 12) 278 nm (ϵ 23 500), 231 (16 700). Anal. ($C_{20}H_{25}N_5O_4 \cdot 0.25H_2O$) C, H, N.

N^2-(p-n-Butylphenyl)-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)guanine (6) and Its 7-β Isomer (7). A solution of BuPG (8.15 g, 28.8 mmol) in anhydrous acetonitrile (100 mL) was treated with BSA (36 mL, 144 mmol). After 1 h at room temperature, a solution of tetra-O-acetylribofuranose (7.63 g, 24 mmol) in acetonitrile (100 mL) was added, followed by TMSTF (6.32 mL, 34.8 mmol). After the solution was heated at reflux for 2 h, an additional portion of BSA (7 mL, 28 mmol) was added, and the reaction mixture was heated at reflux for 7 h. The reaction mixture was concentrated unver vacuum, the residue was dissolved in chloroform (300 mL), and this solution was poured into water (300 mL). The biphasic mixture was filtered to remove unreacted BuPG, and the organic phase was separated, washed with water (3 × 150 mL), dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was crystallized from ethanol (100 mL) to give 9.01 g (69%) of the 9-β isomer 6 as colorless crystals, mp 241–243 °C. Anal. ($C_{26}H_{31}N_5O_8$) C, H, N.

The filtrate from the crystallization of 6 was evaporated to dryness and chromatographed on a silica gel column (30 × 2.5 cm). Elution with 0.1% methanol in chloroform (1.5 L) gave the 7-β isomer 7 as a foam (1.4 g, 11%), mp 143–146 °C. Anal. ($C_{26}H_{31}N_5O_8$) C, H, N.

N^2-(p-n-Butylphenyl)-9-β-D-ribofuranosylguanine (BuPGr, 8) and Its 7-β Isomer (9). The O-acetyl derivatives 6 and 7 were deblocked by treatment with a mixture of equal volumes of concentrated aqueous ammonia and ethanol for 3 days. The resulting gels were heated, and, after cooling, the solutions deposited fine colorless crystals, which were collected by filtration and dried over phosphorus pentoxide in vacuo. Compound 6 (9.01 g) gave 6.76 g (98%) of BuPGr (8), mp 213–223 °C dec. Anal. ($C_{20}H_{25}N_5O_5 \cdot 0.5H_2O$) C, H, N. Compound 7 (1.19 g) gave 900 mg (99%) of the 7-β isomer (9), dec pt 225 °C. Anal. ($C_{20}H_{25}N_5O_5$) C, H, N.

N^2-(p-n-Butylphenyl)-9-[2-deoxy-3,5-O-(1,1,3,3-tetraisopropyldisilox-1,3-diyl)-β-D-ribofuranosyl]guanine (12). BuPGr (8; 6.23 g, 15 mmol) was dried by coevaporation with pyridine (2 × 30 mL). The resulting syrup was dissolved in pyridine (65 mL) and treated with 1,1,3,3-tetraisopropyl-1,3-dichlorodisiloxane (5.25 g, 16.5 mmol), and the mixture stood overnight at room temperature. An additional 0.7 mL of reagent was added, and the reaction stood for 4 h. The solvent was removed in vacuo, the residual syrup was dissolved in chloroform (250 mL), and the solution was extracted with water (4 × 200 mL). The organic phase was separated by filtration through a phase separator and then dried over anhydrous sodium sulfate, and the solvent was evaporated. The resulting foam was chromatographed on a silica gel column (50 × 4.5 cm) in a stepwise gradient of methanol in chloroform: 1% (2 L), 2% (4 L), and 4% (5 L). Fractions containing the product were collected and evaporated to dryness. The colorless foam of 10 (8 g, 81%) was dried over phosphorus pentoxide in vacuo and used without purification in the next step: NMR (60 MHz; Me_2SO-d_6) δ 7.87 (s, 8-H), 5.77 (s, H-1'), 1.04 [s, $SiCH(CH_3)_2$].

Compound 10 (1.98 g, 3 mmol) dissolved in dimethylformamide (50 mL) was treated with 1,1'-(thiocarbonyl)diimidazole (1.33 g, 7.5 mmol). After 4.5 h at 35 °C, the reaction mixture was brought to room temperature, and the solvent was removed in vacuo. The residue was dissolved in chloroform (200 mL) and washed with water (6 × 50 mL). The chloroform layer was filtered through

(21) Ames, B. N.; Dubin, D. T. *J. Biol. Chem.* **1960**, *235*, 769.

a phase separator, dried over anhydrous sodium sulfate, and evaporated. The residue was crystallized from ethanol to give 1.91 g (83%) of **11**: mp 237–240 °C dec; NMR (60 MHz; Me_2SO-d_6) δ 8.58, 7.89, and 7.10 (imidazole H), 7.89 (s, 8-H), 6.35–6.47 (m, H-1′ and H-2′), 1.04 [s, $SiCH(CH_3)_2$].

To a solution of compound **11** (1.9 g, 2.48 mmol) in boiling dry toluene (20 mL) was added dropwise a mixture of tri-*n*-butyltin hydride (1.74 g, 9.88 mmol) and 2,2′-azobis[2-methylpropionitrile] (270 mg) in dry toluene (20 mL) over 1 h. After the solution was refluxed for an additional 2 h, the solvent was evaporated in vacuo, and the residue was dissolved in chloroform and chromatographed on a silica gel column (40 × 4.5 cm). The column was washed with chloroform (1 L) and 1% methanol in chloroform (2 L), and the product was eluted with 4% methanol in chloroform (2 L). The product was crystallized from methanol to give 1.46 g (92%) of **12**: mp 224–226 °C; NMR (60 MHz, Me_2SO-d_6) δ 7.92 (s, H-8), 6.19 (dd, H-1′), 1.04 [s, $SiCH(CH_3)_2$]. Anal. ($C_{32}H_{51}N_5O_5Si_2 \cdot CH_3OH$) C, H, N.

N^2-(*p*-*n*-Butylphenyl)-2′-deoxyguanosine (BuPdG, 3). A 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (2 mL) was added dropwise to a solution of **12** (2.08 g, 3.24 mmol) in tetrahydrofuran (20 mL), and the reaction was followed by TLC. After 2 h, the mixture was evaporated, and the residue was dissolved in ethanol and passed through a column filled with Dowex 50Wx4, pyridinium form. Silica gel (30 g) was coated with the mixture and placed on top of a column of silica gel (4.5 × 20 cm). The column was washed with chloroform (1 L), and the product was eluted with mixtures of 2% methanolic boric acid and chloroform (1:4 and 3:7, v/v). Fractions containing the product were evaporated, and the residue coevaporated with methanol to remove boric acid. Crystallization by slow evaporation from ethanol/aqueous ammonia gave 1.13 g (87.4%) of **3**, mp 196–197 °C, identical with that obtained from direct synthesis.

2-Bromo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)hypoxanthine (15) and Its 7-β Isomer (16). A solution of 2-bromohypoxanthine (2.59 g, 12 mmol) in acetonitrile (50 mL) was treated with BSA (9 mL, ca. 36 mmol). After 1 h at room temperature, a solution of tetra-*O*-acetylribofuranose (3.18 g, 10 mmol) in acetonitrile (50 mL) was added, followed by TMSTF (2.80 mL, ca. 15 mmol). After 0.5 h, the solution was heated at reflux for 15 min, and the reaction was quenched by quick cooling in an ice bath. The solvent was evaporated, and the residual syrup was dissolved in chloroform (300 mL). The solution was washed with water until the pH of the aqueous phase was neutral (4 × 200 mL) and then dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The residue was purified by flash chromatography on a silica gel column with 1% methanol in chloroform. The 7 isomer (**16**, 770 mg) was eluted first, followed by a mixture of **15** and **16** (ca. 1 g), and finally pure **9** isomer (**15**, 540 mg). Compound **16**: mp 87–92 °C; UV λ_{max} (ethanol, pH 1) 256 nm, λ_{max} (ethanol) 257 nm, λ_{max} (ethanol, pH 13) 266 nm. Anal. ($C_{16}H_{17}O_8N_4Br$) C, H, N.

The UV spectra of **15** at pH 2 and 12 were identical with those reported for the 9 isomer,[22] although the melting point of **15** was 160–161 °C, lower than that (178–180 °C) in the literature.[18]

Ammonolysis of Compounds 15 and 16. The 2-bromohypoxanthine tri-*O*′-acetylribonucleosides **15** and **16** (90 mg, 0.19 mmol) were dissolved in concentrated aqueous ammonia (3 mL) and placed in sealed ampules for 3 h at 150 °C. The reaction mixture from **15** was cooled, and the resulting colorless precipitate was purified by preparative TLC on cellulose plates with isopropyl alcohol/concentrated aqueous ammonia/water (7:2:1). The pure product (24 mg, 48%) had UV spectra identical with those of guanosine.[12]

The reaction mixture from **16** was evaporated to dryness, and the residue was chromatographed on an anion-exchange column (Dowex 1x4, [HCO_3^-], 2.5 × 20 cm). Elution was done in 10-mL fractions with a gradient of 0.05–0.3 M ammonium bicarbonate (4 h, flow 2.5 mL/min; fractions 1–40 contained a trace of **16**) and then with 0.3 M ammonium bicarbonate. Fractions 41–55 containing the product were concentrated and coevaporated with triethylamine and ethanol to yield 16 mg (32%) of **17** as fine crystals. The UV spectra of **17** were identical with those reported for N^7-β-D-ribofuranosylguanine.[12]

Reaction of Compounds 15 and 16 with *p*-*n*-Butylaniline. The 2-bromohypoxanthine tri-*O*′-acetylribonucleosides were heated at reflux with *p*-*n*-butylaniline (3 equiv) in 2-methoxyethanol (5 mL) for 1.5 h. Compound **15** (140 mg, 0.3 mmol) gave, after evaporation of the reaction mixture and crystallization from methanol, 90 mg (56%) of **6**: mp 240–243 °C; mmp with the compound prepared from BuPG and tetraacetylribofuranose, 240–243 °C.

Compound **16** (310 mg, 0.65 mmol) gave, after evaporation of the reaction mixture and purification by preparative TLC (silica gel; 5% methanol in chloroform), 200 mg (57%) of **7** as a colorless foam. This product was identical (UV, NMR) with that obtained from BuPG and tetraacetylribofuranose.

N^2-(*p*-*n*-Butylphenyl)-2′-deoxyguanosine 5′-Phosphate (BuPdGMP, 18). Phosphoryl chloride (150 μL, 1.64 mmol) was added to a cold solution of **3** (399 mg, 1 mmol) in trimethyl phosphate (6 mL). The mixture was kept at 0–3 °C for 3 h and at −20 °C overnight. An additional portion of phosphoryl chloride (100 μL, 1.1 mmol) was added, and, after 5 h at 0–5 °C, cold water (15 mL) was poured into the reaction mixture. The thick slurry was neutralized with triethylamine, and the slightly basic solution was diluted with water to a total volume of 250 mL and applied on a DEAE-cellulose column (20 × 2.5 cm). Elution was carried out in a linear gradient of 0.05–0.50 M ammonium bicarbonate during 16 h at a flow rate of 2 mL/min. Fractions 56–116 (13 mL each) containing desired product were combined and evaporated to dryness. The residue was dissolved in several milliliters of water and evaporated several times with triethylamine and finally with ethanol to dryness. The solid triethylammonium salt was dissolved in water and passed through 15 mL of Dowex 50Wx8, ammonium form. The eluate was lyophilized to give the ammonium salt of **18**, 340 mg (66%). The crude product was purified by chromatography on a silica gel column (25 × 2.5 cm) with the solvent 2-propanol/concentrated ammonia/water (7:2:1). Fractions 6–35 (12 mL each) containing pure **18** were combined and evaporated. The product was converted into its ammonium salt as above and lyophilized. The recovery of **18** after purification was 94%: UV λ_{max} (H_2O) 276 nm (ε 17 300), λ_{max} (pH 1) 276 nm (ε 16 500), λ_{max} (pH 13) 282 nm (ε 19 700). Anal. Calcd for $C_{20}H_{32}N_7O_7P$: P, 6.04. Found: P, 6.09.

N^2-(*p*-*n*-Butylphenyl)-2′-deoxyguanosine 5′-Triphosphate (BuPdGTP, 19). BuPdGMP (**18**; 140 mg, 0.27 mmol) was converted to its tributylammonium salt by passing an aqueous solution through Dowex 50Wx8, pyridinium form, and, subsequently, by coevaporation of the eluate with tributylamine (130 μL, 0.54 mmol). The residue was dissolved in a small volume of water and lyophilized. A suspension of the dry substrate in hexamethylphosphoramide (1.5 mL) was treated with 1,1′-carbonyldiimidazole (203 mg, 1.25 mmol). After 5 min, a clear solution was obtained, and the reaction was quenched after 4.5 h by the addition of 175 μL of methanol. TLC showed complete conversion of **18** into its imidazolyl derivative. A solution of tetrakis(tributylammonium) pyrophosphate, prepared from tetrasodium pyrophosphate (588 mg, 1.25 mmol), in hexamethylphosphoramide (10 mL) was added dropwise with stirring. After 24 h at room temperature, the reaction mixture was poured onto 40 g of ice, and the solution was applied to a DEAE-cellulose column, bicarbonate form (25 × 4.4 cm). Elution was carried out in a linear gradient of triethylammonium bicarbonate, pH 7.8, 0.05–0.5 M, during 24 h at a flow rate of 4 mL/min. Fractions 102–117 (16 mL each) contained **18**, fractions 348–374 contained the 5′-diphosphate **20**, and fractions 384–436 contained the major product, **19**. The fractions containing **19** were combined and evaporated. The product was converted to its ammonium salt and lyophilized to give 90 mg (47%) of **19**: UV λ_{max} (H_2O) 276 nm (ε 17 700), λ_{max} (pH 1), 276 nm (ε 17 400), λ_{max} (pH 13) 282 nm (ε 20 500). Anal. Calcd for $C_{20}H_{40}N_9O_{13}P_3$: P, 13.15. Found: P, 13.40.

Fractions containing **20** were treated as above. The yield of the 5′-diphosphate (**20**) as the ammonium salt was 7 mg (4.3%): UV λ_{max} (H_2O) 276 nm (ε 18 600), λ_{max} (pH 1) 276 nm (ε 18 100), λ_{max} (pH 13) 282 nm (ε 20 700). Anal. Calcd for $C_{20}H_{36}N_8O_{10}P_2$: P, 10.16. Found: P, 10.37.

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Registry No. 1, 87781-93-9; 2, 83173-14-2; 3, 87781-96-2; 4, 87781-94-0; 5, 87781-95-1; 6, 87781-97-3; 7, 87781-98-4; 8, 87781-99-5; 9, 87782-00-1; 10, 87782-08-9; 11, 87782-09-0; 12, 87782-01-2; 15, 41623-91-0; 16, 87782-02-3; 17, 15373-27-0; 18, 87782-03-4; 18·2NH_3, 87782-04-5; 19, 87782-05-6; 19·4NH_3, 87782-06-7; 20·3NH_3, 87782-07-8; *p*-butylaniline, 104-13-2; 1-chloro-3,5-di-*p*-toluoyl-2-dioxyribofuranose, 3601-89-6; tetra-*O*-acetylribofuranose, 28708-32-9; DNA polymerase, 9012-90-2.

Quantitative Structure–Activity Relationships of 6-Anilinouracils as Inhibitors of *Bacillus subtilis* DNA Polymerase III

George E. Wright* and Joseph J. Gambino

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605.
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Quantitative structure–activity relationships (QSAR) of a series of 6-anilinouracil derivatives were developed for their inhibitory activity against the wild-type DNA polymerase III (pol III) and a mutant enzyme, pol III/*azp-12*, derived from *Bacillus subtilis*. Interaction between inhibitors and both enzymes appears to result solely from hydrophobic binding. Comparison of the substituent contributions indicates increased hydrophobic character and a minor change of shape of the inhibitor binding site of the mutant enzyme. Because the two enzymes have identical K_m values for substrates, the inhibitor binding site is thought to be distinct from the enzyme active site.

6-(Phenylhydrazino)-, 6-(benzylamino)-, and 6-anilinouracils have been shown to be potent inhibitors of *B. subtilis* DNA polymerase III (pol III).[1-3] All three series of analogues inhibit pol III by the immobilization of the enzyme because of an interaction between the phenyl group of the inhibitors and a hydrophobic binding site on the enzyme thought to be physically near the active site. The qualitative data show several trends for substituents. Compounds with hydrophobic groups in both the meta and para positions of the phenyl ring are most active, and those with only meta substituents are generally more active than those with the same substituent in the para position. Compounds with large alkyl groups in the para position or with polar substituents tend to be the least active inhibitors.

A preliminary attempt to correlate substituent hydrophobic (π) constants with activity showed a rough linear relationship for meta-substituted anilinouracils but no direct correlation for para-substituted compounds.[3] A more detailed derivation of quantitative structure–activity relationships for these inhibitors was sought for two purposes. The first was to facilitate the synthesis of more potent inhibitors as potential antibacterial agents by indicating the type and position of substituent needed for strong interaction with the enzyme binding site. The second purpose was to determine the nature of and difference between the inhibitor binding sites of the wild-type enzyme (WT) and of a mutant, "inhibitor-resistant" enzyme, pol III/*azp-12*.[3,4] This mutant enzyme was obtained from *B. subtilis* colonies selected for resistance to 6-[(*p*-hydroxyphenyl)hydrazino]uracil and 6-[(*p*-hydroxyphenyl)hydrazino]isocytosine. Originally, this mutation was thought to be the result of a structural change in the inhibitor binding site, which repulsed polar groups in the phenyl ring, such as *p*-hydroxyl and *p*-amino, based on the observation that inhibitors with H and Me groups were equiactive against both wild-type and mutant polymerases.[1] Further study with other substituted inhibitors showed that the pattern of sensitivity of this enzyme was more complex. For example, in the 6-anilinouracil series, compounds with meta substituents had decreased inhibitory activity toward the mutant enzyme compared with the WT enzyme, while para-substituted compounds usually had increased inhibitory activity against the mutant enzyme.[3]

For the determination of QSAR's for these enzymes, 6-anilinouracil analogues presented several advantages over the 6-(phenylhydrazino)uracil[1] series: they show a wider range of inhibitory activity, they do not need reduction in the assay mixture as do the oxidizable 6-(phenylhydrazino)uracils, and they are expected to be conformationally less mobile than both the hydrazino and benzylamino analogues.

K_i, the concentration of 6-anilinouracil required to achieve 50% inhibition of enzyme activity, was used in the calculation of the QSARs because it has been shown to be identical with the concentration needed for half-maximal complex formation between DNA, inhibitor, and enzyme.[4] It is thus thought to represent a true binding constant for the inhibitor–enzyme interaction.

QSAR of Inhibitors of DNA Polymerase III. Equation 1 was derived for the compounds listed in Table I by using the substituent constants tabulated by Hansch[5] and multiple regression analysis. The equation was developed by the sequential addition and deletion of parameters thought to be important in binding based on the qualitative assessment of the experimental data. The *F* test was used to determine the significance of each change, and Table II shows the final sequence used.

$$\log (1/K_i) = 1.00\ (\pm 0.13)\ \pi_m + 0.71\ (\pm 0.12)\ \pi_p + 3.87\ (\pm 0.48)\ MR_m - 2.30\ (\pm 0.27)\ (MR_m)^2 + 2.86\ (\pm 0.54)\ MR_p - 2.18\ (\pm 0.26)\ (MR_p)^2 + 2.50\ (\pm 0.30) \quad (1)$$

$$n = 37;\ r = 0.942;\ F_{(6,30)} = 40.01\ (0.001)$$

The hydrophobic parameter π_m contributed the most to binding among all single parameters, but it accounted for only 18% of the variance in the data (eq 1a). The

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