Synthesis of 6-(Phenylhydrazino)uracils and Their Inhibition of a Replication-Specific Deoxyribonucleic Acid Polymerase

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Several 6-(para-substituted phenylhydrazino)uracils were synthesized, using two different methods. Derivatives with H, Me, Et, F, Cl, Br, NO_2, or SO_2Me as the para substituent were prepared by reaction of 6-aminouracil with the appropriate phenylhydrazine. *p*-Hydroxy derivatives were synthesized by condensation of 6-hydrazinouracil with a *p*-benzoquinone and reduction of the resulting azophenol with aqueous sodium dithionite. In one instance, reaction of 6-hydrazinouracil with tetrafluoro-*p*-benzoquinone yielded a pyrimido[4,5-*c*]cinnoline. Whereas *p*-hydroxy derivatives readily reverted to the azo form, the remaining compounds were quite stable as the hydrazines. Experiments examining the effects of the various derivatives on DNA polymerase III of *Bacillus subtilis* suggested that structural prerequisites for inhibitory activity include (1) a hydrazino moiety which, in conjunction with the uracil ring, can form specific hydrogen bonds with cytosine residues in template DNA, and (2) a phenyl ring, which apparently provides a major site of drug-enzyme interaction.

6-(*p*-Hydroxyphenylazo)uracil (HPUra) and 6-(*p*-hydroxyphenylazo)-2-amino-4-pyrimidone (HPiCyt) exemplify several synthetic 6-(arylazo)pyrimidines which selectively inhibit the growth and division of gram-positive bacteria.† HPUra and HPiCyt, when applied to sensitive organisms, exert their effects by inhibiting, selectively, replicative DNA synthesis.[1,2] The DNA-specific action of HPUra and HPiCyt derives from a unique capacity to inhibit selectively DNA polymerase III, a replication-specific enzyme.[3-7] Studies on the molecular basis of drug action indicate that the active, inhibitory species of HPUra and HPiCyt are the respective hydrazino (H_2) forms. Evidence from nmr analysis[6] and kinetic studies employing synthetic primer-templates[5] suggests that the inhibitory activity of H_2-HPUra and H_2-HPiCyt derives from a capacity to pair in a novel fashion (Figure 1) with specific pyrimidine moieties of the template strand of template-primer DNA and promote the formation of a catalytically inactive ternary complex composed of drug, enzyme, and DNA.

A detailed investigation of the mechanism of action of the *p*-hydroxy derivatives has been complicated by a tendency of the active hydrazino forms to oxidize during assay to the inactive azo compounds. In an attempt to remedy this problem and, further, to obtain more information regarding the relationship of drug structure and activity, we have synthesized and tested several hydrazino analogs containing various substituents on the phenyl ring. This paper describes the synthesis, characterization, and inhibitory properties of compounds in the uracil series.

Chemistry. Scheme I illustrates the general methods used for the synthesis of phenylhydrazinouracils. Compounds **2-9** and **21** could be prepared by direct substitution, whereas derivatives with a *p*-hydroxy group required the preparation and reduction of the intermediate azophenols **11-15**.

6-(Phenylhydrazino)uracils. The reaction of 6-aminouracil (**1**) with the appropriate phenylhydrazines in refluxing water gave the desired phenylhydrazinouracils directly and in good yields. These compounds, their yields, melting points, elemental analyses, and mass spectral molecular ions are presented in Table I. The crude reaction products varied in color, depending upon the extent of oxidation to the azo form. Compounds containing electron-withdrawing groups (*e.g.*, **8**, **9**) appeared to oxidize most readily. Solutions of phenylhydrazinouracils in hot water developed colors varying from light yellow to red. Consequently, during their crystallization from water, sodium dithionite was added to maintain the reduced forms.

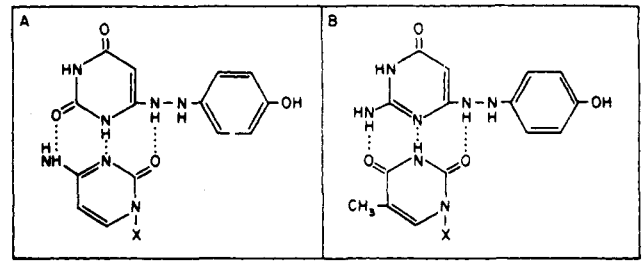

Figure 1. Proposed hydrogen bonding of (A) H_2-HPUra–cytosine and (B) H_2-HPiCyt–thymine in template DNA.

p-Hydroxyphenylhydrazinouracils obtained by dithionite reduction of the azo compounds (see below) reoxidized quite readily in solution or in the solid state. Therefore, the hydrazines were prepared freshly for nmr analysis and were generated *in situ* for enzyme inhibition studies.

Nuclear magnetic resonance data for the hydrazines are presented in Table II. The nmr spectra were useful for distinguishing the azo and hydrazino forms; H-5 of the uracil ring appeared consistently at δ 4.60-4.68 in the hydrazines but at δ 6.1-6.28 in the azo compounds. Furthermore, two broadened peaks in the region δ 7.2-9.0 were derived from the hydrazino NH protons. Assignment of the separate hydrazino resonances was based on the absence of the upfield NH peak of **2** (δ 7.90) in compound **21** in which the 1′-NH is substituted by Me. This assignment was corroborated by nmr studies[6] of hydrogen bond formation between 6-(*p*-hydroxyphenylhydrazino)uracil and cytosine or deoxycytidine; only the 6-NH, which is specifically involved in the hydrogen-bonded base pairing, was shifted downfield in the spectrum. The greater chemical shift of 1′-NH resonances in response to para substitution also served to justify the assignments in this series of compounds. Indeed, in the cases of the *p*-NO_2 (**8**) and the *p*-SO_2Me (**9**) derivatives, the 1′-NH resonance was *downfield* from the 6-NH resonance. With the exception of the *p*-hydroxy derivatives **16-20**, all compounds gave identifiable mass spectra, and in each case the molecular ion was present.

6-(Phenylazo)uracils. The 6-(*p*-hydroxyphenylazo)uracils were obtained in high yield by mixing a warm solution

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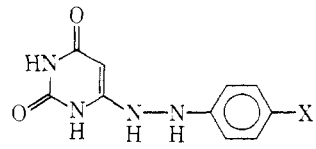
† B. Langley, personal communication. iCyt, isocytosine.

Table I. 6-(Phenylhydrazino)uracils



Compd	X	Yield, %	Mp, °C, dec	Analyses	Formula	M⁺
2	H	87	>330	C, H, N	$C_{10}H_{10}N_4O_2$	218
3	Me	82	>330	C, H, N	$C_{11}H_{12}N_4O_2 \cdot H_2O$	232
4	Et	61	>330	C, H, N	$C_{12}H_{14}N_4O_2$	246
5	F	76	>330	C, H, N	$C_{10}H_9N_4O_2F \cdot 0.5H_2O$	236
6	Cl	65	>330	C, H, N	$C_{10}H_9N_4O_2Cl \cdot 1.5H_2O$	252, 254
7	Br	43	>330	C, H, N	$C_{10}H_9N_4O_2Br \cdot 1.5H_2O$	296, 298
8	NO_2	26	276–279	C, H, N[a]	$C_{10}H_9N_5O_4 \cdot H_2O$	263
9	SO_2Me	40	177–182	C, H, N	$C_{11}H_{12}N_4O_4S \cdot H_2O$	296
21	H, 1′-NCH_3[b]	51	>330	C, H, N	$C_{11}H_{12}N_4O_2$	232

[a] H: calcd, 3.94; found, 3.41. [b] See Table II for numbering system.

of quinone in ethanol with a suspension of 6-hydrazinouracil (**10**) in 10% hydrochloric acid. Table III summarizes the physical data on these compounds. The product of the reaction between 6-hydrazinouracil and 2,6-dimethyl-p-benzoquinone was exclusively **15**; apparently steric influence of *o*-methyl groups prevented condensation at the 1 position. The proximity in chemical shift of the phenyl ring protons of **15** to the corresponding protons of **11**, both of which are adjacent to the deshielding azo linkage, further identified the product as the 3′,5′-dimethylazophenol. Compounds **12–14** were apparently the only isomers formed, since the nmr spectrum of each product indicated only one proton pattern consistent with the proposed structure. Mass spectra of these compounds appeared nonspecific, although small molecular ions were observed.

When compound **10** was condensed with tetrafluoro-*p*-benzoquinone, the bright yellow crystalline product was not the desired tetrafluoroazophenol **22**, but a pyrimido[4,5-*c*]cinnoline (**23**). Scheme II depicts the proposed reaction mechanism and the spectroscopic data. Compound **23** shows only two broad, exchangeable resonances in the proton nmr (DMSO-d_6) at δ 11.8 and 12.7. These were undoubtedly derived from ring NH protons and were strong-

Scheme I

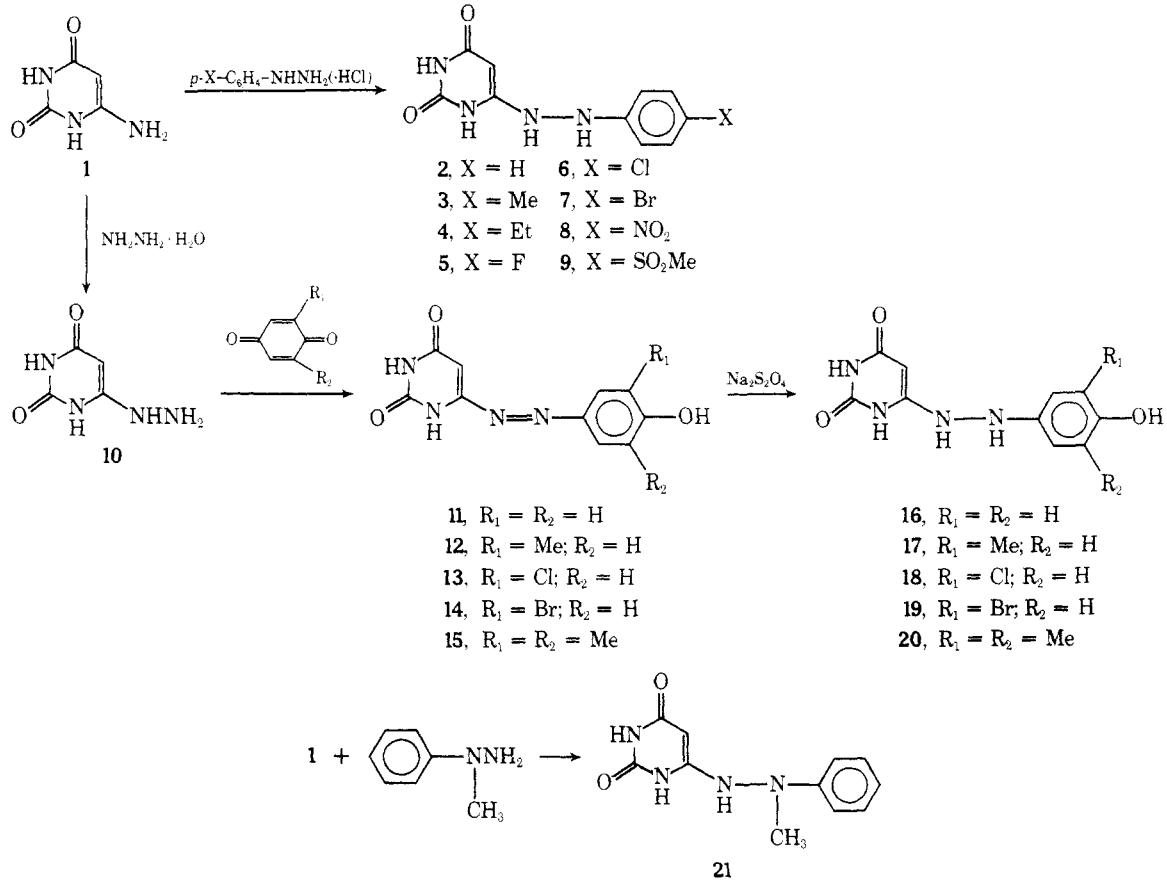
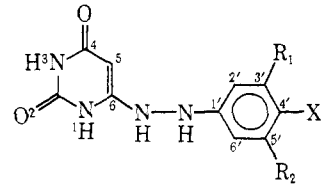


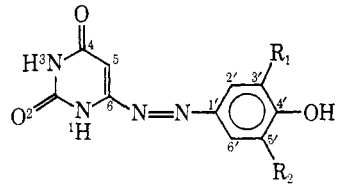
Table II. Nmr Data for 6-(Phenylhydrazino)uracils



Compd	X	R_1	R_2	Chemical shifts, δ, ppm[a] 1,3-H	5-H	6-NH	1-NH	2′,6′-H	3′,5′-H	Other
2	H	H	H	10.44	4.68	8.37	7.90	7.32	6.84[b]	
3	Me	H	H	10.23	4.62	8.20	7.73	7.10	6.70	CH_3, 2.25
4	Et	H	H	10.32	4.63	8.27	7.78	7.14	6.74	CH_2, 2.55; CH_3, 1.17
5	F	H	H	10.41	4.65	8.33	7.91	(6.98 m)		
6	Cl	H	H	10.42	4.62	8.36	8.08	6.81	7.32	
7	Br	H	H	10.39	4.62	8.35	8.08	6.75	7.42	
8	NO_2	H	H	10.36	4.60	8.51	8.97	6.91	8.24	
9	SO_2Me	H	H	10.45	4.60	8.51	8.69	6.92	7.78	CH_3, 3.14
16	OH	H	H	10.27	4.67	8.22	7.43	6.73	6.73	4′-OH, 8.92
17	OH	Me	H	10.31	4.67	8.23	7.32	(6.57 m)[c]		3′-CH_3, 2.13
18	OH	Cl	H	10.30	4.62	8.27	7.59	(6.74 m)[c]		
19	OH	Br	H	10.33	4.62	8.32	7.58	(6.82 m)[c]		
20	OH	Me	Me	10.33	4.63	8.11	7.27	6.37		3′,5′-CH_3, 2.15
21[d]	H	H	H	10.42	4.63	8.60		7.35	6.92[b]	1′-NCH_3, 3.10

[a] All spectra were determined in DMSO-d_6. [b] 3′,4′,5′-H (multiplet). [c] 2′,5′,6′-H (complex multiplet). [d] 1′-NMe.

Table III. 6-(Phenylazo)uracils



Compd	R_1	R_2	Yield, %	Mp, °C, dec	Analyses	Formula	Chemical shifts, δ, ppm[a,b] 1,3-H	5-H	2′-H	5′-H	6′-H	CH_3
12	Me	H	66	267–272	C, H, N	$C_{11}H_{10}N_4O_3$	11.04, 11.31	6.03	7.80	7.06	7.83	2.25
13	Cl	H	67	282–285	C, H, N	$C_{10}H_7N_4O_3Cl \cdot 0.5H_2O$	11.06, 11.34	6.13	8.02	7.22	7.92	
14	Br	H	56	272–276	C, H, N	$C_{10}H_7N_4O_3Br$	11.04, 11.37	6.12	8.16	7.21	7.94	
15	Me	Me	97	275	C, H, N	$C_{12}H_{12}N_4O_3$	10.95, 11.31	6.02	7.72		7.72	2.30

[a] All spectra were determined in DMSO-d_6. [b] Compound 11 (HPUra): δ (DMSO-d_6) 11.40 (1,3-H), 6.10 (5-OH), 8.00 (2′,6′-H), 7.06 (3′,5′-H).[6]

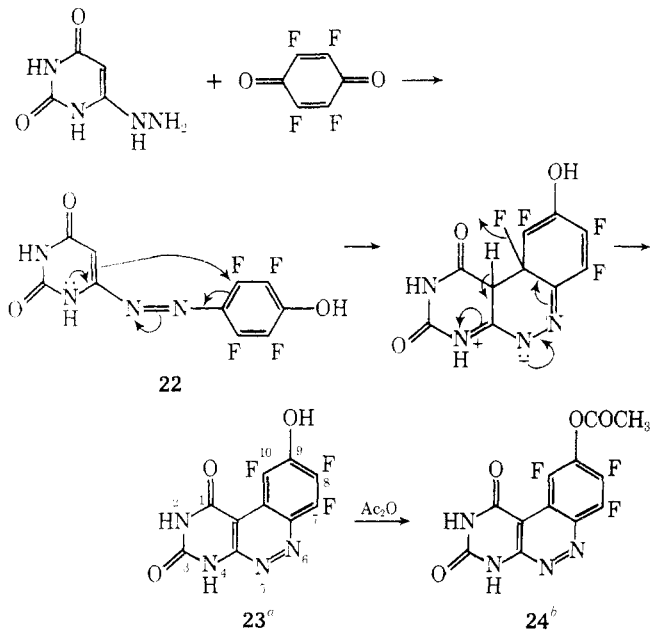
ly deshielded by the fluorines in the benzo ring. Compound **24**, the acetate ester of **23**, exhibited, in addition to two merged NH's, a singlet at δ 1.97 (CH_3CO). The order of ^{19}F chemical shifts (DMSO-d_6, external CF_3CO_2H) appears reasonable, considering the "substituents" on this part of the molecule, although the large "meta" coupling constant, $J_{8,10}$ = 15.0 Hz, in **23** appeared anomalous. The usual order of such couplings is 0–7 Hz.[8] However, in one example, the 3,5 coupling in 4-amino-2-bromo-3,5,6-trifluoronitrobenzene is 14.9 Hz.[9] Formation of the acetate **24** brings the meta coupling to a normal value. The mass spectra of both **23** and **24** confirm the structural assignment of **23**, large molecular ions being observed in both cases.

Biology. The compounds synthesized for this study were examined for their capacity to inhibit DNA polymerase III, the target of arylhydrazinopyrimidine action[3-7] (*cf.* Experimental Section for details of assay). The results of these experiments are tabulated in Table IV. All para-substituted 6-(phenylhydrazino)uracils, with the exception of compounds **8** and **9**, were equally inhibitory to the enzyme and were reversed specifically by dGTP; the di-substituted compounds **12–14** were somewhat less potent, and all other compounds were inactive.

Discussion

Two observations suggest that the inhibitory action of H_2-HPUra (**16**) is derived, in part, from an ability to mimic dGTP in its capacity to base pair specifically with cytosine residues in template DNA.[5,6] First, dGTP specifically reverses the inhibitory effect of **16**. Second, nmr analysis indicates that **16** pairs specifically *via* hydrogen bonding with cytosine. The action of the drug is not wholly explained, however, by its ability to pair with template cytosine residues. Indeed, 6-aminouracil (**1**) and 6-hydrazinouracil (**10**), although capable of hydrogen bonding to

Scheme II



22

23[a] 24[b]

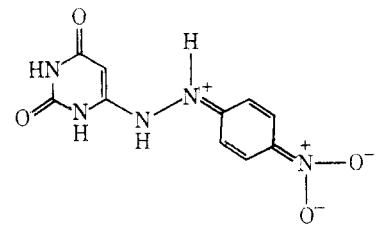
[a]Mass spectrum M^+ 284; ^1H nmr δ 11.8, 12.7; ^{19}F nmr δ_7 51.7 ($J_{7,8}$ = 18.2 Hz), δ_8 60.6 ($J_{7,10}$ = 15.0 Hz), δ_{10} 71.2 ppm ($J_{8,10}$ = 15.0 Hz). *Anal.* C, H, N, F. [b]Mass spectrum M^+ 326; ^1H nmr δ 11.7, 1.97; ^{19}F nmr δ_7 49.8 ($J_{7,8}$ = 17.1 Hz), δ_8 58.2 ($J_{7,10}$ = 17.1 Hz), δ_{10} 61.0 ppm ($J_{8,10}$ = 4.2 Hz).

cytosine, are not inhibitors of DNA polymerase III (N. C. Brown, unpublished observations); a phenyl ring is essential for enzyme inhibition.

Several lines of evidence, in fact, suggest the active participation of the enzyme. For example, DNA polymerase III is the only H_2-HPUra-sensitive enzyme of the three DNA polymerases present in drug-sensitive bacteria. Further, H_2-HPUra-resistant forms of DNA polymerase III are demonstrable in appropriate drug-resistant mutant strains.[7,10] Finally, the results of studies with specific template–primer combinations and experiments involving gel filtration of enzyme–DNA–drug mixtures indicate that the enzyme, in the presence of **16**, is sequestered as part of a catalytically inactive protein–drug–DNA complex.[5,10-12] Since the phenyl ring is an important component for H_2-HPUra activity, the effects of chemical modification of this part of the molecule should provide insight into the mode of action of **16** and, indirectly, information about the active site of DNA polymerase III.

The enzyme inhibition data presented in Table IV for the series of H_2-HPUra analogs allow several generalizations regarding the relationship of drug structure and function. First, active compounds are reversed specifically by dGTP and not by any other deoxyribonucleotide, suggesting a mechanism of action in common with H_2-HPUra. Second, inhibition does not require the presence of a substituent on the phenyl ring. Third, the presence of a simple alkyl group or halogen in the para position has no significant effect on inhibitor potency, *i.e.*, compounds **3**–**7** are equipotent with **16**. Disubstituted compounds **17**–**19** show somewhat diminished activity while the trisubstituted compound **20** is inactive. This decrease of inhibitory activity with increasing number of substituents suggests a steric effect on interaction of the phenyl ring with a possible enzyme binding site.

The unexpected lack of activity of several monosubstituted compounds (**8**, **9**, **21**) suggests that electronic and/or conformational effects are critical to drug action. The electron-withdrawing groups in **8** (p-NO_2) and **9** (p-SO_2Me) would be expected to increase the electron density in the phenyl ring, although such an effect would not seem likely in **21** where the 1′-N is substituted by methyl. However, both types of substitution may alter the conformational mobility of the hydrazino N–N bond. Electron-withdrawing groups in **8** and **9** probably render the phenyl ring and its attached NH coplanar because of the resonance effect, as depicted below.



Certainly, the large downfield shifts of 1′-NH protons in these compounds (Table II) are in agreement with such extensive π-electron delocalization. Since the uracil ring must also be nearly coplanar with the 6-NH group for optimal hydrogen bonding to cytosine (a consequence of delocalization of the 6-N electron pair), two structural alterations may ensue: (1) the 6-N proton will be forced out of the plane of the uracil ring, thus eliminating proper drug–cytosine pairing, or (2) the two rings and their attached NH's will be mutually perpendicular in order to minimize overlap between π orbitals on adjacent nitrogens. The structural rigidity in the latter model could prohibit effective interaction of the phenyl ring with the enzyme. That the *N*-methyl group of **21** may also reduce conformational mobility about the hydrazino group is speculative. It certainly does not destroy the hydrogen-bonding capacity of the uracil ring:**21** and cytosine pair normally in DMSO-d_6

Table IV. Effect of 6-(Phenylhydrazino)uracils on the Activity of DNA Polymerase III

	% inhibition[a] at					
Compd	12.5 μM	25 μM	50 μM	100 μM	50 μM + mM dGTP[b]	50 μM + mM dATP,[b,c] dCTP, or TTP
2	37	53	67	81	14	69
16	53	68	81	89	21	80
3	41	69	81	89	18	80
4	37	54	72	82	13	80
5	35	49	67	79	10	68
6	43	61	76	87	23	75
7	41	62	75	84	12	73
8				0–1		
9				0		
17	18	30	37	56		
18	26	54	72	77		
19	20	35	51	68		
20				0		
21[d]				0		
23				0		

[a]The standard assay and reduction of drugs with dithionite were as described in the Experimental Section; compounds were used within 5 min of dithionite treatment. [b]The concentration of deoxyribonucleoside triphosphate was increased from 25 μM to 1 mM. [c]In experiments performed with mM TTP, [^{3}H]dCTP was used as the labeled substrate at a concentration of 10 μM and a specific activity of 100 cpm/pmol. [d]This compound did not require dithionite treatment.

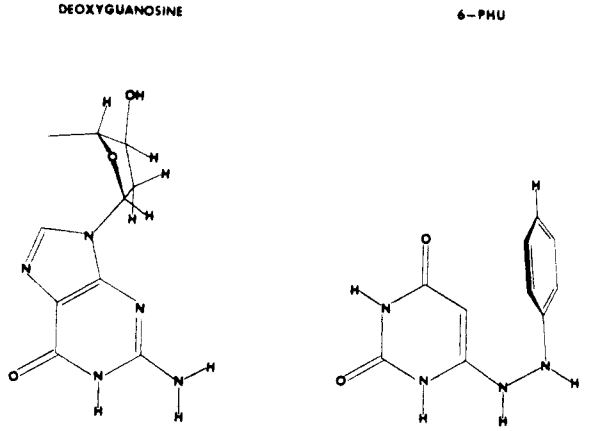


Figure 2. Structures of deoxyguanosine (partial) and an "active" conformation of 6-(phenylhydrazino)uracil (6-PHUra).

(G. E. Wright, unpublished observation). Perhaps the methyl group prohibits pairing to *template* cytosine or destabilizes a drug-enzyme complex.

One rationalization of the above data is that conformational mobility of the phenyl ring and the hydrazino N-N bond is critical to drug action. Indeed, such mobility may permit the enzyme to induce a drug conformation in which the phenyl ring occupies a position (relative to its base-pairing sites) analogous to the position of the deoxyribose ring of dGTP (*cf.* Figure 2). The model shown in Figure 2 suggests the phenyl ring as the most likely site of drug-enzyme interaction. Furthermore, the diminished activity of multiply substituted compounds (**17–20**) is consistent with a steric inhibition at this binding site.

Collectively, the data presented above suggest that inhibition of DNA polymerase III by 6-(phenylhydrazino)-uracils requires a structure which permits (1) hydrogen bonding to template cytosine and (2) binding of the phenyl ring with the enzyme. The exact nature of this drug-template-enzyme binding is unknown; we are attempting to elucidate this process by continuing synthesis in this series and by examining directly the interaction of drug, template, and enzyme.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by HetChem Co., Harrisonville, Mo.; unless otherwise noted, these agree to within ±0.4% with calculated values. The analysis of compound **23** was done by PCR, Inc., Gainesville, Fla. Nuclear magnetic resonance spectra were determined on a JEOL C60-HL instrument, field sweep, external lock mode, at ambient (*ca.* 23°) temperature. All nmr spectra were obtained in DMSO-d_6 solutions, and chemical shifts are reported in parts per million (δ) from internal TMS. Mass spectra (70 eV) were taken on a DuPont 21-490 instrument *via* heated probe inlet. DNA polymerase III was prepared from *B. subtilis* NB841 and purified as described in ref 6.

All chemicals were commercially available with the exception of *p*-ethylphenylhydrazine hydrochloride (from reduction[13] of diazotized *p*-ethylaniline) and chloro-[14] and bromo-*p*-benzoquinones[15] (from chromic acid oxidation[16] of the respective hydroquinones).

6-(*p*-Hydroxyphenylazo)uracils 12–15.† A solution of the *p*-benzoquinone (1.4 mmol) in warm ethanol (1 ml) was added *in toto* to a stirred suspension of compound **10** (100 mg, 0.7 mmol) in 5 ml of 10% hydrochloric acid at room temperature. The mixture, which immediately turned bright orange, was stirred for 30 min. The product was filtered, washed with water and ethanol, and allowed to dry in air. The azo compounds were crystallized from 10% acetic acid in dimethylformamide or purified by column chromatography on silica gel G and eluted with 10% methanol in chloroform.

6-(Phenylhydrazino)uracils. (a) Reaction of 6-Aminouracil (1) with Substituted Phenylhydrazines, 2–9 and 21. A mixture of 1 (0.5 g, 0.004 mol), the phenylhydrazine (0.008 mol), and acetic acid (0.5 g, 0.0083 mol) was heated to reflux in 15 ml of H_2O. [When phenylhydrazine hydrochlorides were used, a mixture of the salt and 1 was heated to reflux in water, and a neutralizing volume of 1 *N* NaOH (8.0 ml) was added through the condenser; the acetic acid was then added in the same manner.] After 3 hr the reaction mixture was filtered hot and allowed to come to room temperature. After 12 hr at 0–4° the precipitated product was harvested by filtration, washed with water, and dried in air. The products were generally beige-colored powders (except for **8** which is dark brown) and were free from starting material.

(b) Reduction of 6-(Phenylazo)uracils with Sodium Dithionite. The azo compound (0.1 mmol) was dissolved in 5 ml of 50 m*M* NaOH previously purged with N_2 and stirred with 1 ml of 1 *M* sodium dithionite freshly prepared in nitrogen-purged water. The solution, which turned light pink, was acidified with acetic acid, and a straw-colored precipitate separated. The product was filtered, washed with water, and stored in a vacuum desiccator over P_2O_5.

Purification of 6-(Phenylhydrazino)uracils. Crystallization from hot water containing excess sodium dithionite (to decolorize) gave 30–60% recoveries of pure phenylhydrazinouracils. Alternatively, quantitative recoveries of nearly colorless materials were obtained by reducing crude phenylhydrazinouracils in 1 *N* NaOH with excess aqueous sodium dithionite and acidifying with acetic acid. The precipitated products were filtered, washed repeatedly with water, and dried *in vacuo*. Products were characterized by elemental (Table I) and nmr (Table II) analyses.

9-Hydroxy-7,8,10-trifluoropyrimido[4,5-*c*]cinnoline-1,3-dione (23). The reaction conditions between **10** and tetrafluoro-*p*-benzoquinone were identical with those described above for 6-(*p*-hydroxyphenylazo)uracils. An 88% yield of bright yellow product was obtained: mp 321–323° dec. *Anal.* ($C_{10}H_3N_4O_3F_3$) H, N; C: calcd, 42.25; found, 41.72; F: calcd, 20.05; found, 19.35.

Acetate Ester of 23. Ice (5 g) and 1 ml of acetic anhydride were added to a 5-ml solution of **23** (200 mg, 0.7 mmol) in 1 *N* NaOH. The dark red mixture was shaken for 5 min, and an additional 0.5 ml of acetic anhydride was added. Shaking was continued for 10 min and the dark yellow suspension was filtered with suction. The light brown product was washed with water giving 160 mg (49%) of **24**; the nmr and mass spectra of **24** (Scheme II) were commensurate with the proposed structure.

DNA Polymerase Assay. In the standard assay the reaction mixture contained, in a volume of 0.05 ml, 20% glycerol, 10 m*M* magnesium acetate, 50 m*M* Tris-HCl (pH 7.6), 400 μg/ml of DNAse-treated[17] calf thymus DNA (Worthington Biochemicals), 25 μM each of dATP, dCTP, and dGTP, 10 μM [^{3}H]TTP (New England Nuclear; 130 cpm/pmol), 5 m*M* dithiothreitol, and 0.1 units of DNA polymerase III. Samples were incubated at 30° for 5 min, and the reaction was terminated by addition of 0.5 ml of 0.5 *M* NaOH containing 40 μg/ml of denatured salmon sperm DNA. The samples were chilled in ice and mixed with 0.5 ml of cold 20% trichloroacetic acid containing 20 m*M* sodium pyrophosphate. The collection and counting of the acid-insoluble material have been described.[3] One unit of enzyme activity catalyses the incorporation of 1 nmol of deoxyribonucleotide into an acid-insoluble product in 5 min at 30° under the standard conditions discussed above.

Reduction of Inhibitors for Assay. A 5 m*M* solution of uracil derivative in 100 m*M* NaOH was mixed at 30° with an equal volume of 100 m*M* sodium dithionite. In all cases this method immediately and quantitatively reduced the inhibitors to their colorless hydrazino forms. Sodium dithionite at concentrations up to 2.5 m*M* did not inhibit the activity of DNA polymerase III in standard assay conditions.

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Synthesis and Biological Activity of Selected 2-Substituted 6-(β-D-Ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones

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Several 2-substituted 6-(β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones have been prepared by condensation of the appropriate silylated heterocyles with 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl bromide and the subsequent removal of blocking groups from the carbohydrate moiety with methanolic ammonia. The site of ribosylation was established by comparison of the uv spectra of the nucleosides with that of an appropriate model compound. The anomeric configuration was determined by pmr spectroscopy. These nucleosides have been evaluated for their inhibitory activity against leukemia L1210 and *Escherichia coli* cells *in vitro* and for their effects on leukemia L1210 growth *in vivo*. Among these compounds the 2-methyl, 2-ethyl, and 2-propyl derivatives markedly inhibited the *in vitro* growth of both cell types, the inhibitory concentrations ranging from 5×10^{-7} to 8×10^{-4} *M*. Only the 2-methyl derivative was significantly active against leukemia L1210 *in vivo* with a dose of 200 mg/kg/day × 5, causing a 31% increase in the life span of the tumor-bearing mice.

The characterization[1] of uric acid ribonucleoside isolated[2] from bovine erythrocytes as 3-(D-ribofuranosyl)uric acid has generated interest in the chemical synthesis[3,4] of bicyclic heterocyclic nucleosides with the glycosidic linkage on a nitrogen atom in the pyrimidine ring rather than in the five-membered ring portion of the molecule. 3-(β-D-Ribofuranosyl)adenosine (isoadenosine) was prepared and found[5] to inhibit the growth of various tumor cell lines *in vitro* and *in vivo*, as well as exhibiting some activity against adeno III virus in culture. This interest has been further stimulated by the recent isolation[6] of 7-(D-ribosyl)pyrazolo[3,4-*d*]pyrimidine-4,6-dione (oxoallopurinol ribonucleoside) from the urine of patients treated with allopurinol and the report[7] that oxoallopurinol ribonucleoside, presumably as the corresponding 5′-phosphate derivative, inhibits pyrimidine biosynthesis *de novo*. These findings have prompted us to synthesize a number of 2-substituted 6-(β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones and to study their biological effects.[8,9]

Results and Discussion

Chemistry. The trimethylsilylation of 2-substituted oxazolo[5,4-*d*]pyrimidin-7-ones[10] with hexamethyldisilazane in the presence of a catalytic amount of ammonium sulfate was accomplished in excellent yield. These silyl derivatives (**1a–f**) were subsequently condensed[11] with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in benzene at reflux temperature in the presence of mercuric oxide–mercuric bromide to furnish good yields of the blocked nucleosides **2a–f** as syrups. Thin-layer chromatography of these syrups revealed the presence of only one nucleoside in each reaction mixture.

Deacetylation of **2a–f** was accomplished with methanolic ammonia to furnish the nucleosides **3a–f** (Table I). That complete deblocking had occurred without ring opening of the labile oxazole ring† was established by uv and pmr spectroscopy. The site of ribosylation was established as N_6 by a comparison of the uv spectral data (Table II) obtained for **3a** and the data reported[12] for 2,6-dimethyloxazolo[5,4-*d*]pyrimidin-7-one. The anomeric configuration of the deblocked nucleosides could not be assigned on the basis of pmr spectral data (Table II) since the peaks assigned to the anomeric proton ($H_{1'}$) of **3b** and **3f** revealed a coupling constant ($J_{1',2'}$) of a magnitude which precluded an unequivocal anomeric assignment.[13] Therefore, the isopropylidene derivatives **4a** and **4b** were prepared from **3b** and **3f** using a standard procedure,[14] and the 5′-*O*-tosyl-2′,3′-*O*-isopropylidene derivative **6** was prepared by tosylation of **4a** (Table I). The pmr spectra of **4a, 4b,** and **6** revealed singlets for their respective anomeric protons, which now allowed an unequivocal assignment of the β configuration to these nucleosides (Table III). This anomeric assignment is further substantiated by utilizing a recently reported[15] criterion for determining anomeric configuration for β-D-ribonucleosides. The difference between the chemical shifts of the two methyl signals for the 2′,3′-*O*-isopropylidene group is between 0.18 and 0.22 ($\Delta\delta$) for β-D-riboncleosides and 0.0 and 0.10 ($\Delta\delta$) for α-D-ribonucelosides. The pmr spectral data in Table III corroborate the β assignment for the nucleosides reported herein.

†The oxazole ring is, however, susceptible to ring opening. This was established by the frequent pmr spectroscopic monitoring of a reaction mixture of the 2-ethyl nucleoside derivative **3b** dissolved in NaOD; at least two additional compounds can be formed.