Brief Articles

6-Anilinouracil-Based Inhibitors of *Bacillus subtilis* DNA Polymerase III: Antipolymerase and Antimicrobial Structure−Activity Relationships Based on Substitution at Uracil N3

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6-Anilinouracils (6-AUs) are dGTP analogues which selectively inhibit the DNA polymerase III of *Bacillus subtilis* and other Gram-positive bacteria. To enhance the potential of the 6-AUs as antimicrobial agents, a structure−activity relationship was developed involving substitutions of the uracil N3 position in two 6-AU platforms: 6-(3,4-trimethyleneanilino)uracil (TMAU) and 6-(3-ethyl-4-methylanilino)uracil (EMAU). Series of *N*3-alkyl derivatives of both 6-AUs were synthesized and tested for their ability to inhibit purified *B. subtilis* DNA polymerase III and the growth of *B. subtilis* in culture. Alkyl groups ranging in size from ethyl to hexyl enhanced the capacity of both platforms to bind to the polymerase, and with the exception of hexyl, they also significantly enhanced their antimicrobial potency. N3 substitution of the EMAU platform with more hydrophilic hydroxyalkyl and methoxyalkyl groups marginally enhanced anti-polymerase III activity but enhanced antibacterial potency severalfold. In sum, the results of these studies indicate that the ring N3 of 6-anilinouracils can tolerate substituents of considerable size and structural variety and, thus, can be manipulated to significantly enhance the antibacterial potency of this novel class of polymerase III-specific inhibitors.

Introduction

There is a rapidly growing crisis in the clinical management of life-threatening infectious disease caused by multi-antibiotic-resistant (MAR) strains of pathogenic Gram-positive (Gr+) bacteria.[1] Solving this problem will depend, in part, on the development of chemotherapeutic agents which selectively attack new bacterial targets. One such target is DNA polymerase III (pol III), an enzyme essential for the replication of the bacterial chromosome.[2−5] We have developed and characterized a novel class of 6-anilinouracils (6-AUs) specifically targeted to the pol III of Gr+ bacteria.[6−10] These agents act as Gr+ pol III-specific dGTP analogues. As shown in panel A of Figure 1, the 6-AU nucleus has two "domains": a base-pairing domain and an enzyme-specific, aryl domain. Figure 1B summarizes how these two domains combine to effect enzyme inhibition. The base-pairing domain of the molecule forms three H bonds with an unopposed template cytosine just distal to the DNA primer terminus. Simultaneously, the aryl substituent binds a unique aryl-specific site, or "receptor", within the enzyme's dNTP binding site and, thus, forms a nonproductive ternary complex of enzyme, inhibitor, and primer-template. It is the presence of this conserved aryl receptor in the active site region of the bacterial Gr+ pol IIIs that renders them and their respective hosts exquisitely sensitive to inhibition by the 6-AUs.[5,11]

We have sought to improve both the anti-pol III and antimicrobial potency of the 6-AUs. As part of this effort, we have exploited structure−activity relationships (SARs) involving the anilino moiety to develop two "platform" 6-AUs: 6-(3,4-trimethyleneanilino)uracil (TMAU, **4**) and 6-(3-ethyl-4-methylanilino)uracil (EMAU, **11**).[6,7,10,11] Although **4** and **11** display favorable potency versus the pol III target, they have relatively poor antibacterial potency. This deficiency has prompted us to further explore the effects of substitution at uracil N3—the only ring atom of the 6-AUs which can be substituted without negatively affecting inhibitor binding to pol III.[7] We have exploited compounds **4** and **11** and a model *Bacillus subtilis* system[10] to develop a broad-ranging SAR based on alkyl, hydroxyalkyl, and methoxyalkyl substitution of N3. The results, which are described below, indicate that N3 has significant potential as a site for enhancing the antimicrobial efficacy of these novel pol III-specific inhibitors.

Results

Synthesis and Properties of N3-Substituted 6-Anilinouracils. All N3-substituted 6-anilinouracils were prepared as described below. The syntheses of compounds **4**−**6**, **8**, **9**, and **11** have been reported previously.[7,12,13]

*N*3-Alkyl-6-anilinouracils were synthesized by the three-step sequence summarized in Scheme 1. First, the

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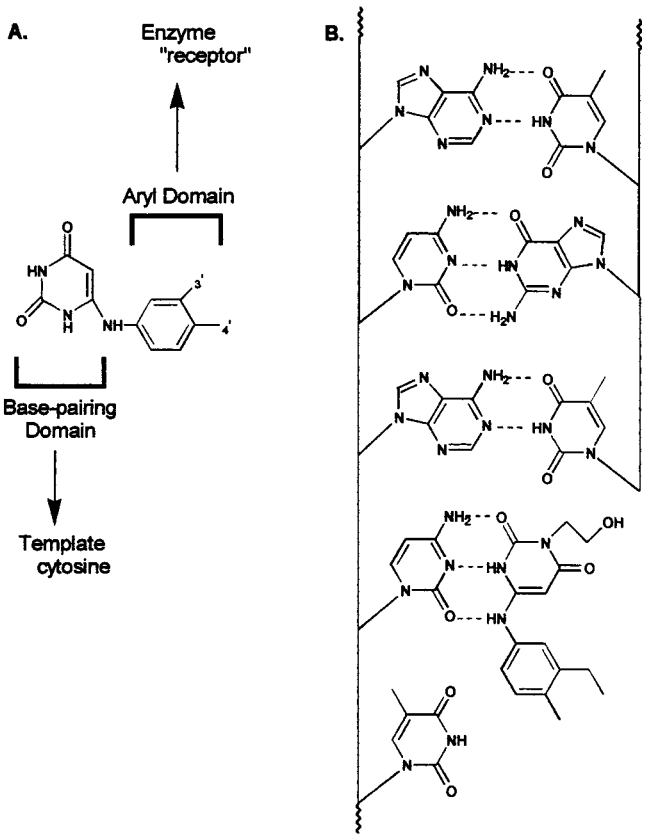


Figure 1. (A) 6-Anilinouracils have two essential domains: a base-pairing domain which binds template cytosine and an aryl domain which binds a specific aryl receptor in the target pol III. (B) Schematic summary of inhibitor bound to DNA. (The third essential component of the ternary complex, pol III, is not depicted.)

appropriate urea was reacted with diethyl malonate in refluxing sodium ethoxide/ethanol to form the substituted barbituric acid as described in the literature.[14] The *N*-alkylbarbituric acid was specifically chlorinated at C6 by refluxing in $POCl_3$ in the presence of water.[15] The final step employed the resulting substituted 6-chlorouracil and the appropriate aniline in refluxing 2-methoxyethanol as described previously.[13] Detailed descriptions of the yields and properties of all new inhibitors are provided in the Experimental Section.

*N*3-Hydroxyalkyl and *N*3-methoxyalkyl EMAUs were synthesized by the four-step sequence summarized in Scheme 2. 2-(Methoxyethyl)urea (**14**) and 2-(methoxypropyl)urea (**15**) were condensed with diethyl malonate in refluxing sodium ethoxide/ethanol to form the N-substituted barbituric acids.[14] The barbituric acids were selectively chlorinated at the 6 position using benzyltriethylammonium chloride and $POCl_3$ at 50 °C to give **18** and **19**.[16] Synthesis of the two methoxyalkyl EMAUs (**20**, **21**) employed the 6-chlorouracils and 3-ethyl-4-methylaniline, which were heated at 150 °C in the absence of solvent. Each of these compounds was converted to its respective hydroxyalkyl form (**22, 23**) using trimethylsilyl iodide (TMSI) in chloroform at room temperature. Yields and properties of these compounds are provided in the Experimental Section.

Effects of *N*3-Alkyl Substitution of TMAU (4) on Antipolymerase and Antimicrobial Activity. 1. Pol III Inhibition. We exploited **4** (TMAU) as the primary platform for alkyl substitution, generating derivatives **5**−**10**. The effects of these compounds on inhibition of *B. subtilis* pol III and on the growth of *B. subtilis* in culture are summarized in the upper half of Table 1**.** As the data in the left column indicate, none of the groups reduced affinity for pol III compared with the unsubstituted analogue **4**. Rather, with the exception of methyl, which was approximately neutral in its impact, larger groups significantly enhanced anti-pol III potency. The extent of enhancement did not vary strictly with increasing group size, although there was a trend in that direction between ethyl and butyl (**6**−**9**). Upon reaching the size of hexyl substitution (**10**), this trend began to reverse.

2. Inhibition of Bacterial Growth. As the data in the right-hand column of Table 1 indicate, alkyl substitution also significantly affected antimicrobial potency, but in a manner which differed somewhat from that seen for enzyme inhibition. With the exception of hexyl, which essentially destroyed the activity of **4**, all groups from methyl to butyl enhanced antimicrobial activity. Allyl and ethyl groups appeared to be optimal to increase potency.

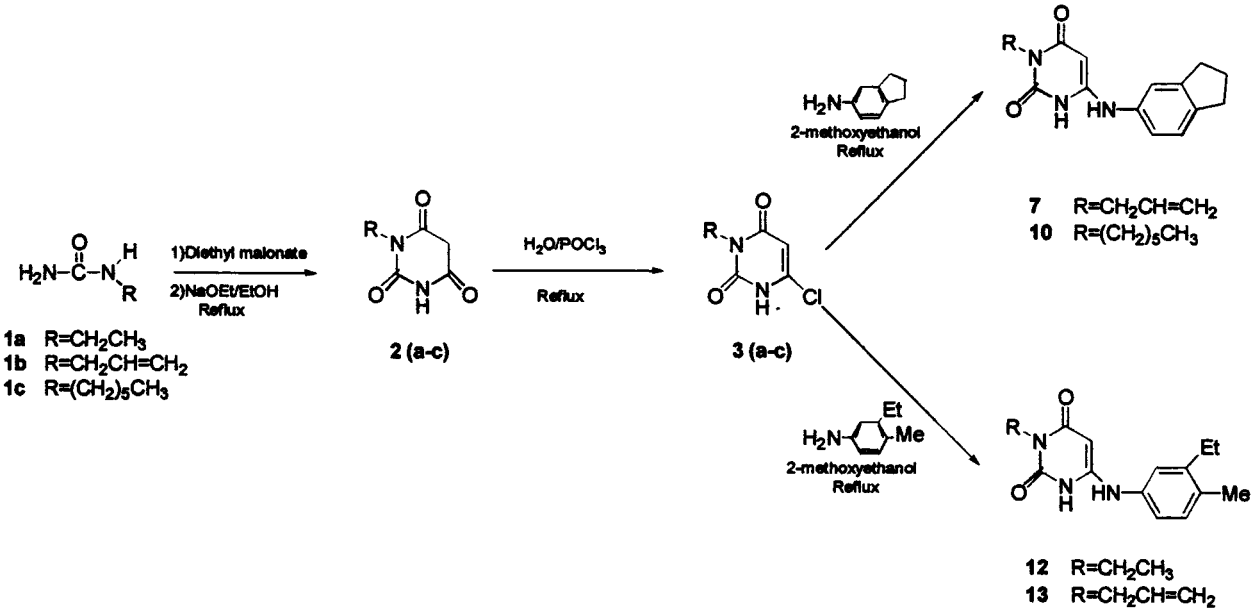
Effects of *N*3-Alkyl Substitution of EMAU (11) on Antipolymerase and Antimicrobial Activity. We sought to determine if the biological effects of *N*3-alkyl substitution of platform **4** also applied more generally with another 6-AU platform. We therefore targeted 6-(3-ethyl-4-methylanilino)uracil (EMAU, **11**),[13] one of the most potent pol III inhibitors. N3 of **11** was substituted with ethyl and allyl, the two substituents which increased the antibacterial activity of the compound **4** platform. The properties of the resulting compounds, **12** (ethyl) and **13** (allyl), are summarized in the middle section of Table 1.

As the data clearly indicate, both ethyl and allyl enhanced the antipolymerase and antibacterial potency of **11** as they did with **4**. The enhancement of both parameters was slightly more pronounced than it was for **4.** Antipolymerase potency of **12** and **13** increased ∼5−8-fold relative to **11**, compared with increases of ∼3.2−3.8-fold for the analogous derivatives of **4**. The enhancement of antibacterial activity of **12** and **13** was approximately double that seen with the analogous derivatives of **4** (i.e., ∼20-fold vs ∼10-fold).

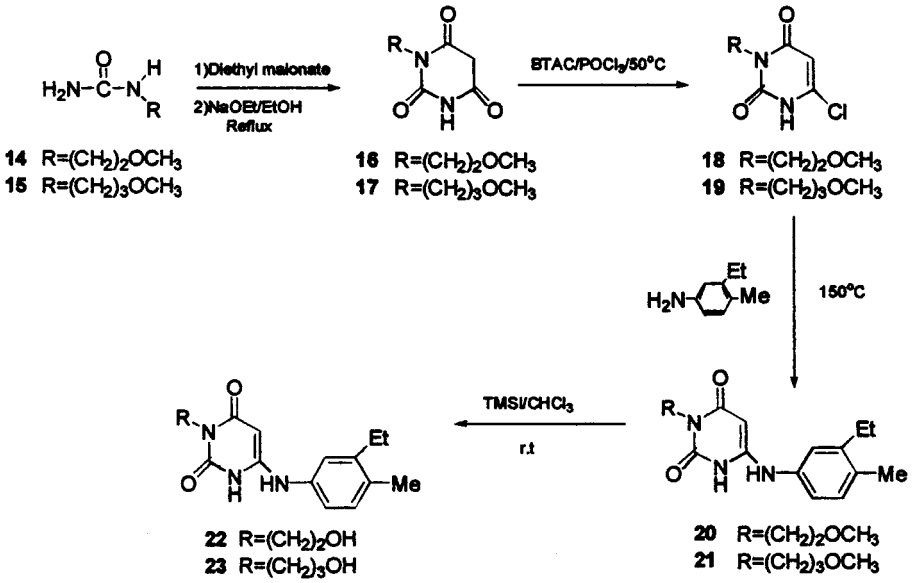
Effects of *N*3-Methoxyalkyl and *N*3-Hydroxyalkyl Substitution of EMAU on Antipolymerase and Antimicrobial Activity. To probe the tolerance of N3 for substituents more hydrophilic than alkyl, we exploited the EMAU (**11**) platform and the method summarized in Scheme 2 to synthesize four relevant derivatives: *N*3-(2-hydroxyethyl) (**22**), *N*3-(3-hydroxypropyl) (**23**), and their respective methoxy derivatives (**20** and **21**). The antipolymerase and antimicrobial activities of each of these compounds are summarized in the bottom four rows of Table 1.

At the level of the isolated pol III, none of the four substituents enhanced inhibitory potency as effectively as *N*3-ethyl (**12**) or *N*3-allyl (**13**). Hydroxyethyl (**22**) and methoxyethyl (**20**) groups were the most effective, enhancing potency approximately 2-fold, while the effect of the hydroxypropyl (**23**) and methoxypropyl (**21**) groups was essentially neutral. The effect of the four substituents on antimicrobial activity was both positive

Scheme 1. Synthesis of *N*3-Alkyl-Substituted 6-Anilinouracils



Scheme 2. Synthesis of *N*3-(Methoxyalkyl)- and *N*3-(Hydroxyalkyl)-6-anilinouracils[a]



[a] BTAC, benzyltriethylammonium chloride; TMSI, trimethylsilyl iodide.

and considerably more profound than their respective effects on anti-pol activity. Potency increases relative to **11** ranged between 20-fold for the methoxypropyl derivative **21** and approximately 10-fold for the other three derivatives.

Discussion

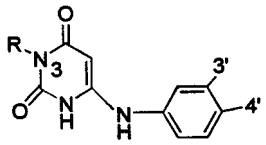
The main purpose of this work was to explore substitution to enhance the antimicrobial potency of the 6-AU inhibitor molecule for Gr+ bacteria. The results of our previous SARs on the isolated pol III[7,13] effectively excluded manipulation of all sites except one—the uracil N3 position. As the results in Table 1 indicate, the N3 position has shown considerable promise as a target for rational substitution of the inhibitor nucleus. In sum, our results make four significant points.

First, as the SAR for the TMAU (**4**) platform indicates, the N3 position can accommodate groups ranging widely in size. Groups larger than methyl enhance pol III binding, but with the exception of butyl (**9**), the enhancement varies no more than 2-fold through hexyl. These results suggest that the N3 position in the inhibitor−enzyme complex must approximate an enzyme surface or space which can accommodate hydrophobic groups that vary considerably in size.

Second, the results, albeit limited only to two related platforms, suggest that the effect of a given *N*3-alkyl substituent on antimicrobial and anti-pol III activity is likely to be relatively independent of the structure of the inhibitor's anilino moiety. Substitution of the TMAU (**4**) and EMAU (**11**) platforms with ethyl or allyl enhanced, in a similar fashion, the anti-pol III and antimicrobial activity of both.

Third, the methoxyalkyl and hydroxyalkyl groups, like the less polar alkyls of comparable size (i.e., ethyl (**12**) and allyl (**13**)), also have a positive impact on

Table 1. Antipolymerase and Antimicrobial Activity of 6-Anilinouracils



compd no.	R	3′-R, 4′-R	K_i (μM)[a]	MIC (μM)[b]
4	H	$-(CH_2)_3-$	1.20	30
5	CH_3	$-(CH_2)_3-$	1.34	5
6	CH_2CH_3	$-(CH_2)_3-$	0.31	3
7	$CH_2CH{=}CH_2$	$-(CH_2)_3-$	0.38	3
8	$(CH_2)_2CH_3$	$-(CH_2)_3-$	0.31	11
9	$(CH_2)_3CH_3$	$-(CH_2)_3-$	0.09	3
10	$(CH_2)_5CH_3$	$-(CH_2)_3-$	0.24	>100
11	H	Et, Me	1.00	30
12	CH_2CH_3	Et, Me	0.12	1.5
13	$CH_2CH{=}CH_2$	Et, Me	0.21	1.5
20	$(CH_2)_2OCH_3$	Et, Me	0.58	3
21	$(CH_2)_3OCH_3$	Et, Me	1.00	1.5
22	$(CH_2)_2OH$	Et, Me	0.59	3
23	$(CH_2)_3OH$	Et, Me	0.73	3

[a] All K_i values for inhibition of *B. subtilis* pol III are the average of three independent experiments performed as described in the Experimental Section; average standard deviation for all values was 20.3%. [b] MICs (minimum inhibitory concentrations) against *B. subtilis* represent the average of three independent experiments performed as described in the Experimental Section; average standard deviation for all values was ±15.3%.

inhibitor−pol III binding. However, their impact is considerably less, ranging from none for methoxypropyl (**21**) to approximately 2-fold for hydroxyethyl and methoxyethyl (i.e., **22** and **20**). This reduction in potency relative to that seen with a comparably sized alkyl group is consistent with the reduced affinity expected between the polar hydroxy and methoxy substituents and the hydrophobic enzyme space hypothesized above.

Fourth, the effect of N3 substitution on antibacterial potency with *B. subtilis* in culture (i.e., MIC) is, with the exception of *n*-hexyl, positive. However, it differs in several significant respects from that observed with the isolated enzyme. First, the effect does not vary as predictably with respect to size or extent. For example, the methyl group, which has no significant effect on the anti-pol III potency of **4**, lowers the MIC more than 6-fold, and *n*-hexyl, which increases anti-pol III potency of **4**, essentially destroys its antimicrobial activity. Similarly, in the case of **11**, the hydroxyalkyl and methoxyalkyl substituents, which enhanced anti-pol III potency less than 2-fold, increased antimicrobial potency between 10- and 20-fold.

We have considered two possible explanations for these apparent discrepancies between the effect of the N3 substituent on affinity for the isolated target pol III in vitro and growth of its host bacterial cell in vivo. The first envisions the possibility that a given N3 substituent changes the 6-AU molecule such that it acts not only on its specific pol III target but nonspecifically on another bacterial target(s). The second, simpler, explanation assumes that the N3 primarily affects access of the platform to its target rather than changes its target specificity—i.e., by facilitating inhibitor transport through the bacterial cell wall and membrane. We favor the latter transport model for two reasons. First, it readily explains the lack of antimicrobial efficacy of the very potent pol III inhibitor, *N*3-hexyl TMAU (**10**). Second, the results of assays of the effect of relevant *N*3-alkyl TMAUs on pol III-dependent DNA and RNA synthesis in intact, growing *B. subtilis*[17] indicate that they retain their specificity for inhibition of DNA synthesis.

Experimental Section

Analyses. All new compounds were fully characterized by ^{1}H NMR and elemental analysis (C, H, N). Proton NMR spectra were obtained at 300 MHz with a Varian Unity 300 instrument, and results are consistent with the proposed structures. Elemental analyses were performed by the Microanalysis Laboratory, University of Massachusetts, Amherst, MA, and agree to within ±0.4% of calculated values unless otherwise noted. Melting points were determined on a Mel-temp apparatus and are uncorrected.

Barbituric Acids 2a and 2b. All *N*-alkyl-substituted barbituric acids were synthesized by condensing diethyl malonate with the substituted urea as described in the literature.[14] Properties of ethyl-,[18] allyl-,[19] and hexylbarbituric acids[20] have been previously reported.

***N*3-Alkyl-6-chlorouracils 3a and 3b.** The substituted barbituric acids were reacted with $POCl_3$ in the presence of H_2O to yield the *N*3-substituted 6-chlorouracils, as described in the literature.[15] Crude products were isolated and identified by TLC and NMR but not purified. Isolated products were reacted directly in the next step.

Synthesis of N3-Substituted 6-Anilinouracils and Relevant Intermediates. The synthesis of 6-anilinouracils employed 6-chlorouracil and the appropriate aniline in refluxing 2-methoxyethanol as described previously.[13] 3-Ethyl-4-methylaniline was synthesized as described.[6] Syntheses of 6-(3,4-trimethyleneanilino)uracil (TMAU) and 6-(3-ethyl-4-methylanilino)uracil (EMAU) were previously reported.[6]

3-Allyl-6-(3,4-trimethyleneanilino)uracil (7). A stirred mixture of 3-allyl-6-chlorouracil (2.5 g, 13.3 mmol) and 5-aminoindane (3.75 g, 26.6 mmol) was heated at reflux in 80 mL of 2-methoxyethanol for 17 h under nitrogen. After cooling to room temperature, the product was crystallized from 80% acetic acid to give 2.53 g (67% yield) of 3-allyl-6-(3,4-trimethyleneanilino)uracil (**7**) as yellow crystals: mp 205−208 °C; ^{1}H NMR (DMSO-d_6) δ 2.04 (m, 2H, $CH_2\mathbf{CH}_2CH_2$), 2.86 (m, 4H, $\mathbf{CH}_2CH_2\mathbf{CH}_2$), 4.30 (d, 2H, CH_2N), 4.75 (s, 1H, C_5-H), 5.12 (dd, 2H, CH_2=), 5.80 (m, 1H, CH=), 7.11 (m, 3H, Ar-H), 8.18 (s, 1H, NH), 10.45 (s, 1H, NH). Anal. ($C_{16}H_{17}N_3O_2$) C,H,N.

3-Hexyl-6-(3,4-trimethyleneanilino)uracil (10). This compound was prepared by the same procedure as above. Crystallization was from ethanol: mp 232−235 °C; yield 55%; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.25 (s, 6H, $(CH_2)_3$), 1.42 (t, 2H, $\mathbf{CH}_2CH_2N$), 2.02 (m, 2H, $CH_2\mathbf{CH}_2CH_2$), 2.83 (m, 4H, $\mathbf{CH}_2CH_2\mathbf{CH}_2$), 3.67 (t, 2H, CH_2N), 4.71 (s, 1H, C5-H), 7.07 (m, 3H, Ar-H), 8.11 (s, 1H, NH), 10.38 (s, 1H, NH). Anal. ($C_{19}H_{25}N_3O_2\cdot 0.125H_2O$) C,H,N.

3-Ethyl-6-(3-ethyl-4-methylanilino)uracil (12). A stirred mixture of 3-ethyl-6-chlorouracil (174 mg, 1.0 mmol) and 3-ethyl-4-methylaniline (341 mg, 2.5 mmol) was heated at reflux in 6.0 mL of 2-methoxyethanol for 8 h. After the mixture cooled to room temperature, precipitated product was filtered and crystallized from ethanol to give 230 mg (85% yield) of 3-ethyl-6-(3-ethyl-4-methylanilino)uracil (**12**) as white crystals: mp 280−282 °C; ^{1}H NMR (DMSO-d_6) δ 1.05 (t, 3H, $\mathbf{CH}_3CH_2N$), 1.16 (t, 3H, $\mathbf{CH}_3CH_2$), 2.25 (s, 3H, CH_3Ar), 2.58 (q, 2H, $\mathbf{CH}_2CH_3$), 3.78 (q, 2H, $N\mathbf{CH}_2CH_3$), 4.76 (s, 1H, C_5-H), 7.06 (m, 3H, Ar-H), 8.09 (s, 1H, NH), 10.40 (s, 1H, NH). Anal. ($C_{15}H_{19}N_3O_2$) C,H,N.

3-Allyl-6-(3-ethyl-4-methylanilino)uracil (13). This compound was prepared by the same procedure as above: mp 253−254 °C; yield 88%; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $\mathbf{CH}_3CH_2$), 2.30 (s, 3H, CH_3Ar), 2.62 (q, 2H, $\mathbf{CH}_2CH_3$), 4.49 (d, 2H, CH_2N), 5.10 (s, 1H, C_5-H), 5.15 (dd, 2H, CH_2=), 5.87 (m, 1H, CH=), 6.40 (s, 1H, NH), 7.05 (m, 3H, Ar-H), 9.22 (s, 1H, NH). Anal. ($C_{16}H_{19}N_3O_2$) C,H,N.

(2-Methoxyethyl)urea (14).[21] (2-Methoxyethyl)amine (15.0 g, 0.2 mol) was neutralized with concentrated hydrochloric acid

to give (2-methoxyethyl)amine hydrochloride, which was treated with potassium cyanate (16.2 g, 0.2 mol) in 100 mL of water. After heating at reflux for 4 h, the mixture was evaporated in vacuo. Ethanol (150 mL) was added, and the residue was heated. The warm mixture was filtered, and the filtered solid was washed with hot ethanol. Concentration and cooling of the filtrate gave 22.2 g (94% yield) of **14**. Recrystallization from ethyl acetate gave colorless needles: mp 74−76 °C; ^{1}H NMR (DMSO-d_6) δ 3.11 (m, 2H, CH_2N), 3.24 (s, 3H, CH_3O), 3.31 (t, 2H, CH_2O), 5.45 (s, 2H, NH_2), 5.95 (s, 1H, NH).

(3-Methoxypropyl)urea (15).[22] (3-Methoxypropyl)urea was prepared by the same procedure as above: mp 76−78 °C; yield 90%; recrystallization from ethyl acetate gave colorless needles; ^{1}H NMR (DMSO-d_6) δ 1.57 (m, 2H, CH_2), 2.98 (m, 2H, CH_2N), 3.21 (s, 3H, CH_3O), 3.31 (t, 2H, CH_2O), 5.39 (s, 2H, NH_2), 5.92 (s, 1H, NH).

***N*-(3-Methoxyethyl)barbituric Acid (16).** Sodium (5.75 g, 0.25 mol) was dissolved in 150 mL of superdry ethanol. (2-Methoxyethyl)urea (11.8 g, 0.1 mol) and diethyl malonate (16.0 g, 0.1 mol) were added, and the mixture was refluxed for 6 h. The mixture was allowed to cool, and concentrated hydrochloric acid was added until the solution was acidic. After evaporation at reduced pressure, ethanol (150 mL) was added, and the residue was heated. The hot mixture was filtered, and the filtered solid was washed with hot ethanol. Concentration and cooling of the filtrate gave 16.5 g (88.6% yield) of **16**: mp 91−92 °C; recrystallization from ethanol gave white crystals; ^{1}H NMR ($CDCl_3$) δ 3.37 (s, 3H, CH_3), 3.61 (t, 2H, CH_2O), 3.66 (s, 2H, CH_2), 4.11 (t, 2H, CH_2N), 9.38 (s, 1H, NH). Anal. ($C_7H_{10}N_2O_4$) C,H,N.

***N*-(3-Methoxypropyl)barbituric Acid (17).** This compound was prepared by the same procedure as above: yield 86%; recrystallization from ethanol gave white crystals; mp 90−92 °C; ^{1}H NMR ($CDCl_3$) δ 1.89 (m, 2H, **CH**$_2$$CH_2N$), 3.30 (s, 3H, CH_3), 3.45 (t, 2H, CH_2O), 3.64 (s, 2H, C5-CH_2), 3.97 (t, 2H, CH_2N), 8.41 (s, 1H, NH). Anal. ($C_8H_{12}N_2O_4$) C,H,N.

3-(2-Methoxyethyl)-6-chlorouracil (18). A stirred mixture of *N*-(3-methoxyethyl)barbituric acid (1.5 g, 8.1 mmol) and benzyltriethylammonium chloride (BTAC) (3.7 g, 16.2 mmol) in phosphorus oxychloride (25 mL) was heated at 50 °C for 2 h. The reaction mixture was cooled to room temperature and evaporated to dryness in vacuo. The residue was carefully quenched with 40 g of ice chips at 0 °C, and the mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. After removal of the solvent, 1.35 g (82% yield) of **18** was obtained as a white precipitate: ^{1}H NMR ($CDCl_3$) δ 3.36 (s, 3H, CH_3), 3.64 (t, 2H, CH_2O), 4.15 (t, 2H, CH_2N), 5.87 (s, 1H, C_5−H), 9.77 (s, 1 H, NH). Anal. ($C_7H_9N_2O_3$-Cl) C,H,N.

3-(3-Methoxypropyl)-6-chlorouracil (19). This compound was prepared by the same procedure as above: yield 80%; ^{1}H NMR ($CDCl_3$) δ 1.88 (m, 2H, CH_2**CH**$_2CH_2$), 3.29 (s, 3H, CH_3O), 3.42 (t, 2H, CH_2O), 3.97 (t, 2H, CH_2N), 5.82 (s, 1H, C_5-H), 10.59 (s, 1H, NH). Anal. ($C_8H_{11}N_2O_3Cl$) C,H,N

3-(2-Methoxyethyl)-6-(3-ethyl-4-methylanilino)uracil (20). A stirred mixture of **18** (205 mg, 1.0 mmol) and 3-ethyl-4-methylaniline (271 mg, 2.0 mmol) was heated at 150 °C for 15 min. After cooling to room temperature, the residue was chromatographed on silica gel with chloroform/methanol (97/3−95/5) as eluent to give 280 mg (92% yield) of **20**. Crystallization from ethanol gave white crystals: mp 226−227 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, **CH**$_3$$CH_2$), 2.24 (s, 3H, CH_3-Ar), 2.57 (q, 2H, **CH**$_2$$CH_3$), 3.23 (s, 3H, CH_3O), 3.43 (t, 2H, CH_2O), 3.88 (t, 2H, CH_2N), 4.72 (s, 1H, C5-H), 7.05 (m, 3H, Ar-H), 8.17 (s, 1H, NH),10.45 (s, 1H, NH). Anal. ($C_{16}H_{21}N_3O_3$·$0.75H_2O$) C,H,N.

3-(3-Methoxypropyl)-6-(3-ethyl-4-methylanilino)-uracil (21). This compound was prepared by the same procedure as above (yield 88%). Crystallization from ethanol gave white crystals: mp 218−220 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, **CH**$_3$$CH_2$), 1.72 (m, 2H, CH_2**CH**$_2CH_2$), 2.25 (s, 3H, CH_3Ar), 2.58 (q, 2H, **CH**$_2$$CH_3$), 3.22 (s, 3H, CH_3O), 3.34 (t, 2H, CH_2O), 3.75 (t, 2H, CH_2N), 4.74 (s, 1H, C_5-H), 7.05 (m, 3H, Ar-H), 8.12 (s, 1H, NH), 10.42 (s, 1H, NH). Anal. ($C_{17}H_{23}N_3O_3$) C,H,N.

3-(2-Hydroxyethyl)-6-(3-ethyl-4-methylanilino)uracil (22). Trimethylsilyl iodide (TMSI) (0.3 mL, 2.1 mmol) was added to a stirred solution of **20** (152 mg, 0.5 mmol) in dry chloroform (15 mL). The reaction mixture was stirred at room temperature until disappearance of the starting material (about 12 h). Methanol (10 mL) and 0.5 g of sodium sulfite were then added to the brown-purple solution. After stirring at room temperature for 30 min, the mixture was filtered and the solvent was removed. The residue was purified by chromatography on silica gel with chloroform/methanol (90/10) as eluent to give 130 mg (90% yield) of **22**. Crystallization from ethanol/water gave white crystals: mp 243−244 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, **CH**$_3$$CH_2$), 2.24 (s, 3H, CH_3Ar), 2.58 (q, 2H, **CH**$_2$$CH_3$), 3.44 (m, 2H, CH_2O), 3.78 (t, 2H, CH_2N), 4.72 (t, 2H, OH, 1H, C_5-H), 7.05 (m, 3H, Ar-H), 8.12 (s, 1H, NH), 10.41 (s, 1H, NH). Anal. ($C_{15}H_{19}N_3O_3$) C,H,N.

3-(3-Hydroxypropyl)-6-(3-ethyl-4-methylanilino)-uracil (23). TMSI (0.2 mL, 1.41 mmol) was added to a stirred solution of **21** (95 mg, 0.3 mmol) in dry chloroform (10 mL). After the mixture stirred for 5 h at room temperature, methanol (10 mL) and 0.5 g of sodium sulfite were then added to the brown-purple solution. After stirring at room temperature for 30 min, the mixture was filtered and the solvent was removed. The residue was purified by chromatography on silica gel with chloroform/methanol (97/3−90/10) as eluent to give 51 mg (56% yield) of **23**: ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, **CH**$_3$$CH_2$), 1.63 (m, 2H, CH_2**CH**$_2CH_2$), 2.24 (s, 3H, CH_3Ar), 2.57 (q, 2H, **CH**$_2$$CH_3$), 3.40 (m, 2H, CH_2O), 3.74 (t, 2H, CH_2N), 4.42 (t, 1H, OH), 4.73 (s, 1H, C5-H), 7.05 (m, 3H, Ar-H), 8.12 (s, 1H, NH), 10.45 (s, 1H, NH). Anal. ($C_{16}H_{21}N_3O_3$) C,H,N.

Enzyme Assay. *B. subtilis* DNA pol III was a homogeneous recombinant protein expressed and purified as described previously.[23] Pol III was assayed using activated calf thymus DNA as described,[24] and apparent inhibitor constants (K_i's) of the 6-AUs were determined as previously described[25] using a truncated assay in the absence of the competitor dGTP.

Determination of Minimal Inhibitory Concentration (MIC). The test organism was *B. subtilis* BD54, a standard, penicillin-sensitive laboratory strain.[26] A log-phase culture of BD54 was diluted to a concentration of 10^4 cells/mL in Luria broth,[23] and 0.5 mL of this suspension was distributed to each of 48 wells of a sterile microtiter plate. Inhibitors were dissolved in DMSO and were added to wells at concentrations ranging from 0 (control) to 100 μM. The final concentration of DMSO in all wells was adjusted to 1%. Plates were incubated for 24 h at 37 °C, and growth was assessed by visual inspection. MIC is defined as the lowest concentration of inhibitor at which bacterial growth was not apparent. The growth of the test organism was not affected by the presence of 1% DMSO in the medium.

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