Synthesis of Substituted 6-Anilinouracils and Their Inhibition of DNA Polymerase IIIC and Gram-Positive Bacterial Growth

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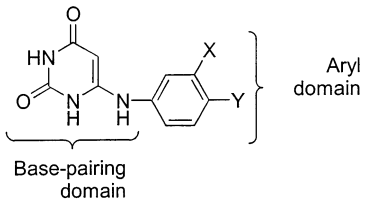
Certain substituted 6-anilinouracils are potent and selective inhibitors of Gram+ bacterial DNA polymerase IIIC (pol IIIC). In addition, analogues with 3-substituents in the uracil ring have potent antibacterial activity against Gram+ organisms in culture. In an attempt to find optimal anilino substituents for pol IIIC binding and optimal 3-substituents for antibacterial activity, we have prepared several series of 3-substituted-6-aminouracils and assayed their activity against pol IIIC from *Bacillus subtilis* and a panel of Gram+ and Gram− bacteria in culture. The 6-(3-ethyl-4-methylanilino) group and closely related substituent patterns maximized pol IIIC inhibition potency. Among a series of 3-(substituted-butyl)-6-(3-ethyl-4-methylanilino)uracils, basic amino substituents increased pol IIIC inhibition, but decreased antibacterial activity. The most potent antibacterials were simple hydroxybutyl and methoxybutyl derivatives, and hydrophobically substituted piperidinylbutyl derivatives.

Introduction

The last 15 years have witnessed a resurgence of bacterial and viral diseases worldwide. Gram-positive (Gram+) bacteria have been particularly problematic given the frequency of their involvement in nosocomial disease and their development of resistance to traditional antibiotics. Among the Gram+ organisms, *Staphylococcus aureus*, *Enterococcus fecalis*, *Enterococcus fecium*, and *Streptococcus pneumoniae* are of greatest concern. The future of clinical management of diseases caused by these Gram+ pathogens depends, in large part, on the development of effective chemotherapeutic agents that selectively attack new bacterial targets. One new target which has been validated recently in Gram+ organisms is DNA polymerase IIIC (pol IIIC), a DNA-dependent DNA polymerase which is specifically required for replicative DNA synthesis in these organisms.[1,2]

Validity of the Gram+ pol IIIC as an Antimicrobial Target. Pol IIIC is a member of one of three distinct classes (pols I, II, and III) of DNA polymerases found in Gram+ eubacteria.[3] Of the three enzymes, pol IIIC is clearly the most attractive antibiotic target, because it is essential for replication of the host chromosome. Inhibition of its activity prevents replicative DNA synthesis and, as a consequence, the host cell dies. Pol IIIC is essentially invariant among relevant Gram+ eubacteria, such as the model enzyme of *Bacillus subtilis* and those of *Staphylococcus aureus*, *Mycoplasma pulmonis*, and *Enterococcus fecalis*.[3] These Gram+ pol IIICs share a unique primary structure, distinct from the distantly related Gram− pol IIIEs and the recently described Gram+ pol IIIEs.[4] Most importantly, the pol IIICs strictly conserve structural features which are responsible for their unique sensitivity to 6-anilinouracils (AUs) and related compounds, the inhibitor class that is the subject of this work.

Gram+ pol IIICs share a unique capacity to bind certain AU compounds, via a guanine-like "base-pairing domain" and an enzyme-specific "aryl domain" (see pharmacophore structure). Through its base-pairing domain the molecule forms Watson−Crick-like hydrogen bonds with an unapposed cytosine residue in the template strand just distal to the DNA primer terminus; consequently its action is competitive with dGTP. Simultaneously, the aryl domain binds an aryl-specific "receptor" near the enzyme's active site, causing the formation of an inactive ternary complex of inhibitor, DNA, and pol IIIC.[5] Structure−activity relationship (SAR) studies have identified the 3-ethyl-4-methylanilino ("EMA") substituent pattern in AU compounds to be nearly optimal for binding with pol IIIC.[6,7]



We reported recently that inhibitory activity of AU compounds against pol IIIC and growth of Gram+ bacteria could be enhanced by introduction of substituents at the 3 position of the uracil ring. For example 3-(methoxyalkyl) (**1**) and 3-(hydroxyalkyl) derivatives (**2**) of 6-(3-ethyl-4-methylanilino)uracil (EMAU) showed significant activity against Gram+ bacteria in culture,[2] and two related 3-(4-hydroxybutyl) compounds showed activity against experimental *S. aureus* infection in mice.[8] Therefore, we have continued SAR and lead

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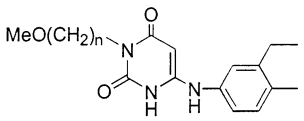
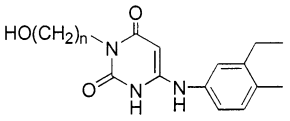
compound selection studies, and here report the effects of modification of the basic AU pharmacophore at the 3 and 6 positions of the uracil ring on pol IIIC inhibition and antibacterial activity in vitro.



1. a, n=2; b, n=3, c, n=4

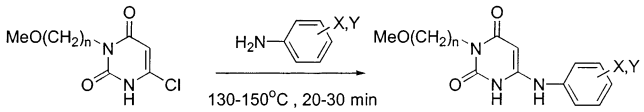


2. a, n=2; b, n=3, c, n=4

Selection of 3-(4-Methoxybutyl)- (1c) and 3-(4-Hydroxybutyl)-EMAUs (2c) as Lead Compounds and Synthetic Platforms. Simple 3-substituted EMAUs were found to possess potent pol IIIC inhibition and had enhanced antibacterial activity in vitro against Gram-positive bacteria.[2] To explore the components of the antibacterial pharmacophore, we undertook synthesis of derivatives modified in the 3 and 6 positions of the uracil ring.

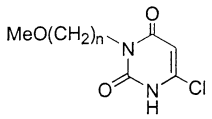
Validation of Rapid, Small Scale Synthetic "RS[3]" Methods. 3-Substituted EMAU compounds are prepared by fusion of a 6-chlorouracil with EMA at high temperature in the absence of solvent.[1] To explore the utility of this procedure to prepare large numbers of derivatives for screening purposes, we studied the reaction conditions required to convert a typical substrate 3-(3-methoxypropyl)-6-chlorouracil (**3b**) to AU compounds in small scales (Scheme 1). Heating mixtures of **3b** with EMA for as little as 10 min at 120 °C resulted in quantitative conversion to MP-EMAU, **1b**. However, optimal conditions for relatively unreactive anilines involved heating the 6-chlorouracil (2 mg) and 2 equiv of aniline in glass test tubes at 130−150 °C for 20−30 min. Typically, TLC showed consumption of **3b** and appearance of products (confirmed with authentic samples in several cases). Little or no decomposition products were noted. The cooled mixtures were dissolved directly in DMSO to give "20 mM" calculated stock solutions of products. Stock solutions were diluted into *B. subtilis* growth medium to give 2-fold serial dilutions from 100 to 1.57 μg/mL (see Experimental Section). In several cases the resulting antibacterial results were indistinguishable from those obtained with isolated, purified products. Heating of **3b** or anilines alone under the same conditions did not produce bioactive mixtures.

Scheme 1. Synthesis of 3-(Methoxyalkyl)-6-anilinouracils

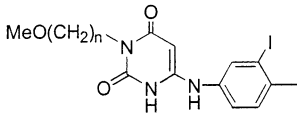


3b, n=3; **3c**, n=4 **1a-c, 9a-m**

This rapid, small scale synthesis "RS[3]" procedure was applied to reactions between **3b** and 40 substituted anilines in order to discover 6-anilino substituents that were equivalent or better than the EMA group in imparting antibacterial potency to the AU compounds. Anilino substituents ranged from alkyl and polyalkyl, halo and polyhalo, halo plus alkyl, alkoxy, and nitro, and involved substitution at 3, 4, and/or 5 positions. The products and results of screening against *B. subtilis* cultures are summarized in Table A of Supporting Information. Notably, the most potent antibacterials, i.e., compounds with lowest MIC, were **1b** and the corresponding 6-(3-iodo-4-methylanilino) analogue "MP−IMAU", with MICs of 3.13 and 1.57 μg/mL, respectively. Other derivatives with MIC < 10 μg/mL were 3,4-diMe, 3,4-diCl, 3-Et, 3,4-diBr, 3-Cl-4-Me, and 3-Me-4-Br compounds. Not surprisingly, the pattern of activity paralleled that for pol IIIC inhibition potency of the previously reported AU compounds, i.e., those without the 3 substituent.[6,7]

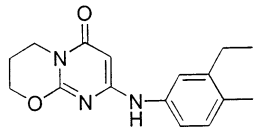


3. b, n=3; c, n=4



4. b, n=3; c, n=4

The RS[3] results prompted us to choose the 3-MP compounds as platforms for further SAR studies, for example by modifying the hydroxyl groups of the 3-hydroxyalkyl compounds **2**. However, attempted demethylation of the 3-methoxyalkyl compounds **1a** and **1b** resulted in their facile conversion to inactive byproducts. For example, **1b** gave a compound identified as the pyrimido-oxazine **5** (see Experimental Section). These byproducts also formed readily during attempts to acylate the hydroxyl groups of **2a** and **2b** with acetic anhydride or methanesulfonyl chloride. It is likely that these systems, which bear hydroxyl groups β and γ, respectively, to the pyrimidine ring, cyclize readily to the corresponding fused ring compounds. We were also unable to prepare the corresponding aminoethyl or aminopropyl derivatives because of their decomposition, likely also via cyclization to analogous fused ring compounds. For example, although 3-(2-azidoethyl)-EMAU was prepared successfully by direct synthesis from **2a** (PPh_3,NaN_3), attempted catalytic reduction gave not the expected aminoethyl derivative, but a byproduct whose ^{1}H NMR suggested it to be a pyrimidine-imidazoline (data not shown). The failure to modify these 3-substituents led us to explore the 3-(4-hydroxybutyl) group as a possible stable alternative for synthesis of 3-substituted-6-anilinouracils and related compounds.



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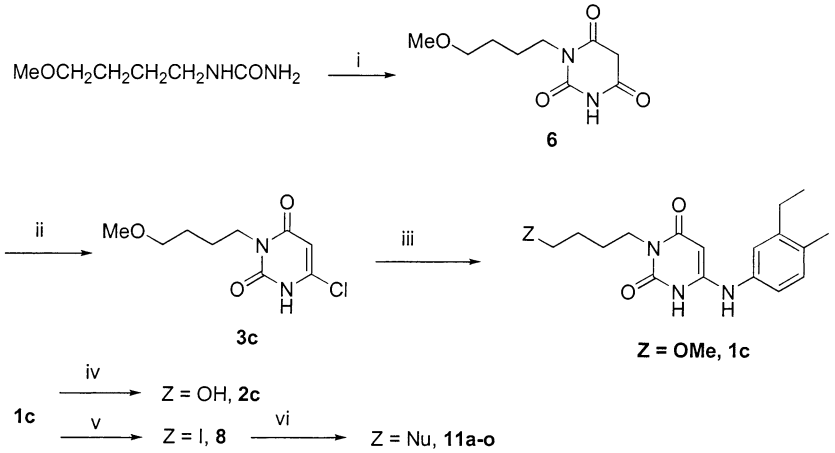
Results

Synthesis of "MB" and "HB" 6-Anilinouracils. A previously developed[1] method was improved to give the key intermediate **3c**. The ring synthesis method illustrated in Scheme 2 required synthesis of *N*-(4-methoxybutyl)urea. Methylation of 1,3-propanediol with iodomethane gave 3-methoxy-1-propanol, which reacted with *N*-bromosuccinimide in the presence of triphenylphosphine to give 1-bromo-3-methoxypropane.[9] This compound reacted with potassium cyanide in aqueous

Scheme 2. Synthesis of **3c** and 3-Substituted-6-(3-ethyl-4-methylanilino)Uracils[a]



[a] Reagents and conditions: i, diethyl malonate, NaOMe, MeOH, reflux. ii, $BnEt_3NCl$, $POCl_3$, 50 °C; iii. EMA, 150 °C, 30 min. iv, Me_3SiI, $CHCl_3$, rt. v, Me_3SiI, $CHCl_3$, reflux. vi, K_2CO_3, Me_2CO, rt.

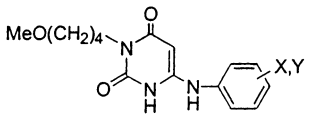
Table 1. Pol IIIC Inhibition and Antibacterial Activity of 3-(4-Methoxybutyl)-6-anilinouracils

		K_i, μM	MIC, μg/mL							
Cpd	Anilino subst	*B.subtilis* pol IIIC	*B.subtilis*	*S.aureus*	*S.aureus* (Smith)	MRSA (B42876)	*E.fecalis*	*E.fecium*	VRE	*E.coli*
1c	**3-Et-4-Me**	**0.088**	**<1.25**	**5**	**5**	**5**	**5**	**2.5**	**2.5**	**>20**
4	**3-I-4-Me**	**0.31**	**5**	**10**	**5**	**5**	**10**	**10**	**5**	**>20**
9a	**3,4-Me_2**	**0.55**	**2.5**	**20**	**10**	**10**	**10**	**20**	**10**	**>20**
9b	**3-CH=CH_2-4-Me**	**0.56**	**<1.25**	**5**	**5**	**10**	**10**	**5**	**10**	**>20**
9c	**3-C(Me)=CH_2-4-Me**	**0.565**	**2.5**	**20**	**10**	**>20**	**20**	**20**	**20**	**>20**
9d	**3-Cl-4-Me**	**0.042**	**<1.25**	**10**	**5**	**>20**	**5**	**5**	**5**	**>20**
9e	**3-Br-4-Me**	**0.129**	**2.5**	**5**	**2.5**	**5**	**5**	**5**	**2.5**	**>20**
9f	**3-CH_2Cl-4-Me**	**0.04**	**5**	**20**	**20**	**20**	**>20**	**>20**	**>20**	**>20**
9g	**3-CH_2SCN-4-Me**	**0.06**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
9h	**3-CH_2CN-4-Me**	**1.02**	**20**	**>20**	**20**	**>20**	**>20**	**20**	**>20**	**>20**
9i	**3-CH_2SH-4-Me**	**0.18**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
9j	**3-CHClMe-4-Me**	**6.47**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
9k	**3-Ac-4-Me**	**3.26**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
9l	**3-CH_2OH-4-Me**	**20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
9m	**3-CH(OH)Me-4-Me**	**2.1**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
10	**3,4-Cl_2Bn**	**0.08**	**3.75**	**10**	**5**	**20**	**5**	**5**	**5**	**>20**
2c	**3-Et-4-Me [3-HB]**	**0.063**	**<1.25**	**5**	**5**	**5**	**5**	**2.5**	**2.5**	**>20**
7	**3-I-4-Me [3-HB]**	**0.067**	**5**	**10**	**5**	**5**	**5**	**5**	**2.5**	**>20**
	ciprofloxacin		**0.078**	**0.156**	**0.078**	**>20**	**0.625**	**5**	**20**	**0.313**
	vancomycin		**0.313**	**0.313**	**0.313**	**0.625**	**0.625**	**1.25**	**>20**	**>20**

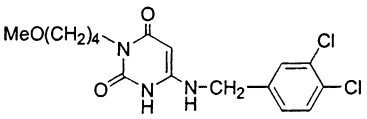
methanol to give 4-methoxybutyronitrile, which was reduced by lithium aluminum hydride in diethyl ether to afford 4-methoxybutylamine, purified by distillation, in high yield. Treatment of 4-methoxybutylamine as the hydrochloride with potassium cyanate in water gave *N*-(4-methoxybutyl)urea in high yield.[10] Reaction of the urea with diethyl malonate in the presence of sodium methoxide gave 1-(4-methoxybutyl)barbituric acid (**6**), and its selective 6-chlorination with $POCl_3$ in the presence of benzyltriethylammonium chloride at 50 °C gave the key intermediate 3-(4-methoxybutyl)-6-chlorouracil, **3c**.

Compound **3c** reacted readily with EMA and 3-iodo-4-methylaniline at high temperatures to give **1c** and **4c**, respectively. These compounds were demethylated by treatment with iodotrimethysilane in chloroform at room temperature to afford the desired 4-hydroxybutyl derivatives **2c** and **7.** The products were accompanied by byproducts whose relative yield increased during prolonged treatment with iodotrimethysilane and at higher reaction temperature (see Experimental Section). The byproduct from **2c** was identified as the 4-iodobutyl compound **8**. Although a nuisance when 4-hydroxybutyl compounds are the desired products, the 4-iodobutyl compounds ("IB-AUs") are excellent intermediates to prepare substituted derivatives (see below). In addition, the 3-(4-hydroxybutyl) group of **2c** and **7** was more stable to acids and bases than the hydroxyethyl and hydroxypropyl groups. Compounds **2c** and **7** had high potency against pol IIIC and Gram+ bacterial growth (Table 1), and both compounds, given intraperitoneally (ip), protected mice from ip challenge with *S. aureus*.[8]

RS^3 with 3c. Availability of the 6-chlorouracil intermediate **3c** provided further opportunities to apply the RS^3 procedure to give candidate antibacterials with diverse 6 substituents. Three groups of compounds were prepared. Thirty eight anilines, some identical to those described above but others synthesized herein for this purpose, 30 benzylamines and related amines, and 18 arylalkylamines of various structures were heated with **3c** as described above. Results of assay of the reaction mixtures against *B. subtilis* pol IIIC and a panel of seven Gram+ and one Gram− (*E. coli*) bacteria are summarized in Tables B−D (Supporting Information). Several MB-AUs **9** with substituent patterns similar to EMA were potent inhibitors of pol IIIC, but none was a better antibacterial compound than **2c** (Table B). As in the case of the MP compounds described above, no



9a-m



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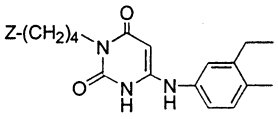
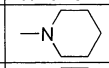
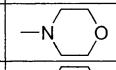
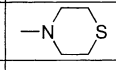
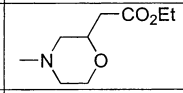
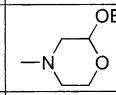
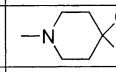
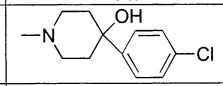
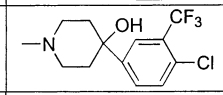
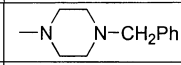
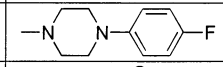
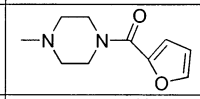
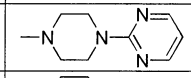
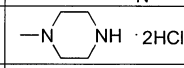


11a-o

Table 2. Pol IIIC Inhibition and Antibacterial Activity of 3-(4-Substituted-butyl)-6-(3-ethyl-4-methylanilino)uracils

		K_i, μM	MIC, μg/mL							
Cpd	subst	*B.subtilis* pol IIIC	*B.subtilis*	*S.aureus*	*S.aureus* (Smith)	MRSA (B42876)	*E.fecalis*	*E.fecium*	VRE	**E.coli**
1c	**-OMe**	**0.088**	**<1.25**	**5**	**5**	**5**	**5**	**2.5**	**2.5**	**>20**
2c	**-OH**	**0.063**	**<1.25**	**5**	**5**	**5**	**5**	**5**	**5**	**>20**
8	**-I**	**0.14**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
11a	**-N_3**	**0.12**	**<1.25**	**2.5**	**2.5**	**>20**	**5**	**<1.25**	**2.5**	**>20**
11b	**-$NH3^+$ Cl^-**	**0.062**	**20**	**20**	**10**	**>20**	**>20**	**>20**	**>20**	**>20**
11c		**0.028**	**10**	**20**	**40**	**40**	**20**	**20**	**10**	**>20**
11d		**0.048**	**2.5**	**10**	**5**	**10**	**5**	**2.5**	**5**	**>20**
11e		**0.068**	**5**	**20**	**10**	**20**	**5**	**5**	**5**	**>20**
11f		**0.026**	**5**	**20**	**20**	**20**	**>20**	**10**	**>20**	**>20**
11g		**0.043**	**2.5**	**10**	**20**	**20**	**10**	**20**	**10**	**>20**
11h		**0.067**	**20**	**20**	**20**	**20**	**20**	**10**	**10**	**>20**
11i		**0.025**	**5**	**5**	**5**	**5**	**5**	**5**	**10**	**>20**
11j		**0.032**	**1.25**	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**	**2.5**	**>20**
11k		**0.022**	**20**	**20**	**10**	**10**	**10**	**10**	**5**	**>20**
11l		**0.07**	**5**	**10**	**20**	**20**	**10**	**5**	**5**	**>20**
11m		**0.043**	**5**	**5**	**5**	**20**	**5**	**10**	**10**	**>20**
11n		**0.043**	**2.5**	**10**	**10**	**10**	**5**	**5**	**5**	**>20**
11o		**0.03**	**20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**	**>20**
	ciprofloxacin		**0.078**	**0.156**	**0.078**	**>20**	**0.625**	**5**	**20**	**0.313**
	vancomycin		**0.313**	**0.313**	**0.313**	**0.625**	**0.625**	**1.25**	**>20**	**>20**

surprises were discovered based on previously reported enzyme inhibition results.[6,7] Among the 6-benzylamino compounds, MB−BAUs, only 3,4-diCl (compound **10**), 3-Br-4-F, and 3-Cl-4-F derivatives had K_i values below 1 μM or MICs below 10 μg/mL (Table C, Supporting Information). Related monohalo and dihalo and methyl BAUs had K_i values between 1 and 10 μM, but these and all other substituted BAUs were devoid of antibacterial activity. MB products obtained from diverse amines, including heteroarylamines, heteroarylmethylamines, and tryptamines, were devoid of enzyme inhibitory or antibacterial activity (Table D, Supporting Information).

Synthesis and Activity of 3-Substituted-6-anilinouracils. Compounds closely related to **2c** were synthesized and characterized. In the first series, modifications were made in the anilino substituents to produce compounds of series **9** (Table 1) similar to the 3-ethyl-4-methyl pattern of **2c**. In the second group of compounds, substitutions in the 3-(4-hydroxybutyl) group of **2c** were made to give compounds of series **11**. The 4-iodo group of **8** was readily displaced by various nucleophiles in the presence of potassium carbonate in a polar, aprotic solvent at room temperature to give compounds **11** (Table 2). The 3-(4-aminobutyl) derivative **11b** was prepared by reduction of the azido compound **11a** with hydrogen over 10% palladium on carbon in methanol. Details of the synthesis and characterization of all compounds are presented in the Experimental Section.

Results of enzyme and antibacterial assays of the compounds are summarized in Tables 1 and 2 and compared with activity of the reference compound HBEMAU (**2c**) and antibiotics ciprofloxacin and vancomy-

cin. HB-EMAU is a potent inhibitor of pol IIIC, with K_i value of 0.063 μM, and an effective Gram+ antibacterial, with MIC values of 2.5−5 μg/mL. (*B. subtilis* is generally the most sensitive of the Gram+ organisms.) Ciprofloxacin potently inhibited growth of all organisms with the exception of MRSA B42876, a methicillin-resistant strain of *S. aureus* with cross-resistance to the fluoroquinolones. Vancomycin was selective for all Gram+ organisms except the vancomycin-resistant strain of *E. fecalis*. As expected, none of the 6-anilinouracils inhibited growth of the Gram− organism *E. coli*.

Quantitative SAR studies suggested that optimal 6-anilinouracil:pol IIIC interaction required in the anilino ring a hydrophobic group in the 3-position of the size of Et or I and a hydrophobic group in the 4 position of the size of Me.[7] Some of the derivatives shown in Table 1 were assayed to determine if subtle changes in these positions would enhance enzyme binding and/or antibacterial activity. 3-(4-Methoxybutyl) derivatives **9d** (3-Cl-4-Me), **9f** (3-CH_2Cl-4-Me), and **9g** (3-CH_2SCN-4-Me) were as potent pol IIIC inhibitors as **1c** (3-Et-4-Me), but only **9d** was equivalent in antibacterial activity. Although the 3-Br (**9e**) and 3-I (**4c**) analogues were somewhat weaker as pol IIIC inhibitors, they were nearly as potent antibacterials as **1c**. The closely related 3-vinyl (**9b**) and 3-isopropenyl (**9c**) compounds were weaker in both assays. Compounds with substituents of similar size in the 3 position, but with electron-attracting or hydrophilic properties were considerably weaker pol IIIC inhibitors and largely devoid of antibacterial activity.

Modification of the uracil 3 position provided a series of highly potent pol IIIC inhibitors, but with wide variation in antibacterial activity (Table 2). Simple changes in the OMe group of MB-EMAU (**1c**) to OH (**2c**), I (**8**), N_3 (**11a**), and NH_3^+ (**11b**) did not alter pol IIIC affinity; however, the I and NH_3^+ derivatives were weak or inactive as antibacterials. A series of tertiary aminobutyl derivatives, consisting of piperidinyl, morpholinyl, and piperazinyl compounds, showed enhanced pol IIIC inhibition, ranging from 2-fold to 4-fold. The narrow range of K_i values for these derivatives and the lack of steric limit for bulky substituents in several of them suggest that these groups interact minimally with enzyme or DNA primer:template. In contrast to the increase in pol IIIC inhibition, many of these compounds had only modest antibacterial activity or were inactive. For example, the hydrophilic morpholinyl compounds **11f** and **11g** and the piperazinyl compounds **11k**−**n** were weak antibacterials, and the piperazinylbutyl compound **11o** was inactive. Among a series of 4-hydroxy-4-phenylpiperidinyl compounds, the 4-Cl-phenyl derivative (**11i**) was almost as active as **1c**, and the 3-CF_3-4-Cl-phenyl derivative (**11j**) was the most potent antibacterial among the 6-anilinouracils. That the most hydrophilic analogues have the weakest antibacterial activity despite their potent inhibition of pol IIIC suggests that uptake by the bacteria may limit access to the target enzyme.

Discussion

The prototype pol IIIC inhibitors MB-EMAU (**1c**) and HB-EMAU (**2c**) demonstrate potent and selective antibacterial activity against Gram+ organisms. An extensive study showed that the compounds inhibited the growth of a wide range of clinical isolates of *S. aureus*, *E. fecalis*, and *E. fecium* in the range of 4−16 μg/mL.[11] These compounds were equally active against drug-sensitive and drug-resistant strains and were bactericidal against MRSA and both vancomycin-sensitive and vancomycin-resistant strains of *E. fecalis*. Although two analogues (**2c** and **7**) protected mice from intraperitoneal challenge with *S. aureus* when administered at 10 mg/kg by the same route,[8] attempted treatment by the intravenous or subcutaneous route was hampered by the low water solubility of the compounds. Compound **2c** was solubilized in 30% β-hydroxypropylcyclodextrin in physiological saline, but the compound still did not protect mice when given in single doses intravenously or subcutaneously in this vehicle (data not shown).

Results of assay of several series of substituted 6-aminouracils have led us to conclude that the 6-(3-ethyl-4-methylanilino)uracil (EMAU) scaffold provides optimal binding affinity to the target enzyme pol IIIC (Table 1, and see Supporting Information). Using this scaffold we have found that 3 substituents, preferably substituted alkyl groups, increase antibacterial activity against Gram+ organisms. Simple hydroxyalkyl and methoxyalkyl[1,2] and more complex aminoalkyl substituents can increase pol IIIC inhibition potency (Table 2), but do not consistently increase antibacterial potency. Indeed, the most potent 3-substituted-butyl EMAU derivative **11j** has a bulky, hydrophobically substituted piperidinyl group. In the case of 6-benzylaminouracils, the 3,4-dichlorobenzyl substitution pattern of **10** provided optimal binding to pol IIIC and moderate antibacterial activity (Table 1) among a limited series of 6-benzylamino and other 6-aminouracils (see Supporting Information).

The challenges in developing the family of pol IIIC inhibitors as antibacterials include improved potency at the target enzyme and whole bacteria levels—MIC values of 1 μg/mL or less are desirable, water solubility to facilitate parenteral formulations, metabolic stability to prolong blood levels of active compound, and enhanced oral bioavailability. In this paper we have addressed the first of these properties, extending two series of inhibitors in the search for more potent pol IIIC inhibitors and antibacterials in vitro.

Experimental Section

Materials. Most reagent chemicals and solvents were obtained from commercial sources. 3-Acetyl-4-methylaniline and 3-(1-hydroxyethyl)-4-methylaniline[12] and 3-ethyl-4-methylaniline[7] were prepared as described. Other substituted anilines, benzylamines, and related amines were available commercially, except as noted. NMR spectra were obtained in Me_2SO-d_6 solutions, unless indicated otherwise, with a Bruker Avance 300 instrument; chemical shifts are in ppm from internal TMS, and *J* values were as expected. Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by the Microanalysis Laboratory, University of Massachusetts, Amherst. Values for C, H, and N were within 0.4% of calculated values except as noted.

Cyclization of 2b. Iodotrimethylsilane (Me_2SiI) (132 mg, 0.66 mmol) was added to a stirred solution of **2b** (100 mg, 0.33 mmol) in dry $CHCl_3$. The reaction mixture was stirred at room temperature overnight, until disappearance of starting material. MeOH and Na_2SO_3 were then added to the brown-purple solution. After being stirred at room temperature for 10 min,

the mixture was filtered and the solvent was removed. The residue was purified by chromatography on silica gel with 2−5% MeOH in $CHCl_3$ as eluent to give 80 mg (85%) of **5**, mp 223−224 °C. ^{1}H NMR: 1.12 (t, 3H, CH_3), 2.11 (m, 2H, CH_2), 2.20 (s, 3H, $PhCH_3$), 2.56 (q, 2H, $PhCH_2$), 3.69 (t, 2H, NCH_2), 4.29 (t, 2H, OCH_2), 5.26 (s, 1H, 5-H), 7.05 (d, 1H, PhH), 7.27 (s, 1H, PhH), 7.46 (d, 1H, PhH), 9.11 (s, 1H, NH). Anal. ($C_{16}H_{19}N_3O_2 \cdot 0.25H_2O$) C, H, N.

***N*-(4-Methoxybutyl)urea. a. 4-Methoxy-1-butyronitrile.** A solution of 1-bromo-3-methoxypropane[9] (54 g, 353 mmol) in MeOH (40 mL) was added dropwise to a stirred solution of KCN (27 g, 415 mmol) in 50 mL of water and 50 mL of MeOH at room temperature. The mixture was heated at reflux for 20 h, cooled to room temperature, and treated with 100 mL of water. The mixture was extracted with EtOAc (4 × 100 mL), and the extracts were dried over $MgSO_4$. After removal of the solvent, the residue was distilled to give 30 g (86%) of 4-methoxy-1-butyronitrile as a colorless oil, bp 168−169 °C. ^{1}H NMR ($CDCl_3$): 1.92 (m, 2H, CH_2), 2.48 (t, 2H, CH_2CN), 3.37 (s, 3H, OCH_3), 3.50 (t, 2H, OCH_2). **b. 4-Methoxybutylamine Hydrochloride.** A solution of 4-methoxy-1-butyronitrile (29 g, 292 mmol) in 50 mL of anhydrous Et_2O was added dropwise to a stirred solution of $LiAlH_4$ (13 g) in 150 mL anhydrous Et_2O at 0 °C. After the mixture was stirred for 1 h at room temperature, 15% aqueous NaOH (50 mL) was added dropwise. The reaction mixture was filtered, and the solid was washed with Et_2O. The ether phase was separated, and the aqueous phase was extracted with Et_2O and $CHCl_3$. After the combined organic extracts were dried over $MgSO_4$, the solvents were removed and the residue was dissolved in 30 mL of MeOH, which was neutralized with concentrated aqueous HCl to give 33 g (81%) of 4-methoxybutylamine hydrochloride. ^{1}H NMR: 1.58 (m, 4H, 2 × CH_2), 2.80 (m, 2H, NCH_2), 3.25 (s, 3H, OCH_3), 3.33 (t, 2H, OCH_2) and 7.91 (br s, 3H, NH_3). **c. 4-Methoxybutylurea.** A mixture of 4-methoxybutylamine hydrochloride (5.0 g, 35.8 mmol) and KNCO (2.9 g, 35.8 mmol) in 50 mL of water was heated at reflux for 4 h. The water was evaporated at reduced pressure, EtOH (100 mL) was added, and the suspension was heated. The warm mixture was filtered, and the filtered solid was washed with hot EtOH. Concentration and cooling of the filtrate gave 5 g (96%) of 4-methoxybutylurea.[10] Crystallization from ethyl acetate gave white crystals, mp 86−88 °C. ^{1}H NMR ($CDCl_3$): 1.60 (m, 4H, 2 × CH_2), 3.17 (m, 2H, NCH_2), 3.33 (s, 3H, OCH_3), 3.40 (t, 2H, OCH_2), 4.60 (br s, 2H, NH_2) and 5.01 (br s, 1H, NH).

1-(4-Methoxybutyl)barbituric Acid, 6. Clean Na (1.6 g, 70 mmol) was added slowly to 60 mL of absolute EtOH. When all the Na had dissolved, *N*-(4-methoxybutyl)urea (4.0 g, 27.4 mmol) and diethyl malonate (4.4 g, 27.5 mmol) were added, and the mixture was heated at reflux for 6 h. The mixture was allowed to cool, and concd aq HCl was added until the solution was acidic. The solvent was evaporated at reduced pressure, and EtOH (100 mL) was added to the residue with heating. The hot mixture was filtered, and the filtered solid was washed with EtOH. Concentration of the filtrate gave 5.1 g (87%) of 1-(4-methoxybutyl)barbituric acid as a light yellow oil. ^{1}H NMR ($CDCl_3$): 1.64 (m, 4H, 2 × CH_2), 3.33 (s, 3H, OCH_3), 3.41 (t, 2H, NCH_2), 3.64 (s, 2H, 5-H), 3.88 (t, 2H, OCH_2) and 8.94 (s, 1H, 3-H). Anal. ($C_9H_{14}N_2O_4$) H,N. C, calcd. 50.46; found 51.17.

6-Chloro-3-(4-methoxybutyl)uracil, 3c. A stirred mixture of 1-(4-methoxybutyl)barbituric acid (5.0 g, 23.3 mmol) and benzyltriethylammonium chloride (11 g, 48.3 mmol) in $POCl_3$ (50 mL) was heated at 50 °C for 3 h. The reaction mixture was cooled to room temperature and evaporated to dryness in vacuo. The residue was carefully quenched with 60 g of ice chips and maintained at 0 °C. The solution was extracted with $CHCl_3$ (4 × 60 mL), and the combined organic extract was dried over Na_2SO_4 and the solvent evaporated. Crystallization of the residue from EtOH gave 3.8 g (70%) of product as white crystals, mp 145−147 °C. The filtrate was evaporated, and the residue was purified by chromatography on silica gel with 1−2% MeOH in $CHCl_3$ as eluent to give an additional 0.9 g (17%) of product. ^{1}H NMR ($CDCl_3$): 1.58−1.80 (m, 4H, 2 × CH_2), 3.32(s, 3H, OCH_3), 3.38 (t, 2H, NCH_2), 3.92 (t, 2H, OCH_2), 5.84 (s, 1H, 5-H) and 9.70 (s, 1H, 1-H). Anal. ($C_9H_{13}ClNO_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-ethyl-4-methylanilino)uracil, 1c. A stirred mixture of **3c** (3.5 g, 15 mmol) and 3-ethyl-4-methylaniline (4.5 g, 33.3 mmol) was heated at 150 °C for 30 min. The mixture was cooled to room temperature, and the mass was crystallized from EtOH to give 3.6 g (72%) of product as white crystals, mp 186−188 °C. The filtrate was evaporated, and the residue was purified by chromatography on silica gel with 2−4% MeOH in $CHCl_3$ as eluent to give an additional 1.15 g (23%) of product. ^{1}H NMR: 1.14 (t, 3H, $PhCH_2CH_3$), 1.50 (m, 4H, 2 × CH_2), 2.24 (s, 3H, $PhCH_3$), 2.57 (q, 2H, CH_2-Ar), 3.21 (s, 3H, OCH_3), 3.32 (t, 2H, NCH_2), 3.68 (t, 2H, OCH_2), 4.72 (s, 1H, 5-H), 6.92−7.17 (m, 3H, PhH), 8.10 (s, 1H, 6-NH), 10.40 (s, 1H, 1-H). Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-iodo-4-methylanilino)uracil, 4c. This compound was prepared by the same procedure as above using 3-iodo-4-methylaniline. Yield, 88%, mp 214−216 °C. ^{1}H NMR: 1.47 (m, 4H, 2 × CH_2), 2.33 (s, 3H, $PhCH_3$), 3.20 (s, 3H, OCH_3), 3.31 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_2), 4.72 (s, 1H, 5-H), 7.16 (d, 1H, PhH), 7.32 (d, 1H, PhH), 7.63 (s, 1H, PhH), 8.24 (s, 1H, 6-NH), 10.62 (s, 1H, 1-H). Anal. ($C_{16}H_{20}IN_3O_3$) C, H, N.

3-(4-Hydroxybutyl)-6-(3-ethyl-4-methylanilino)uracil, 2c. Me_3SiI (2.3 mL, 16 mmol) was added to a stirred solution of **1c** (1.3 g, 4 mmol) in dry $CHCl_3$ (60 mL). The reaction mixture was stirred at room temperature until disappearance of starting material (TLC). MeOH (10 mL) and Na_2SO_3 (0.5 g) were added to the brown-purple solution. After stirring at room temperature for 10 min, the mixture was filtered, and the solvent was removed in vacuo. The residue was purified by chromatography on silica gel with 1−7% MeOH in $CHCl_3$ as eluent to give, first, 3-(4-iodobutyl)-6-(3-ethyl-4-methylanilino)uracil, **8** (21%) (see below). ^{1}H NMR: 1.14 (t, 3H, $PhCH_2CH_3$), 1.54−1.78 (m, 4H, 2 × CH_2), 2.24 (s, 3H, $PhCH_3$), 2.57 (q, 2H, $PhCH_2$), 3.29 (t, 2H, ICH_2), 3.72 (t, 2H, NCH_2), 4.73 (s, 1H, 5-H), 6.92−7.15 (m, 3H, PhH), 8.15 (s, 1H, 6-NH), 10.45 (s, 1H, 1-H). Anal. ($C_{17}H_{22}IN_3O_2$) C, H, N. The second eluate was **2c** (710 mg, 57%), mp 211−212 °C. ^{1}H NMR: 1.14 (t, 3H, $PhCH_2CH_3$), 1.38−1.50 (m, 4H, 2 × CH_2), 2.24 (s, 3H, $PhCH_3$), 2.57 (q, 2H, $PhCH_2$), 3.34 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_2), 4.38 (t, 1H, OH), 4.72 (s, 1H, 5-H), 6.92−7.17 (m, 3H, PhH), 8.08 (s, 1H, 6-NH), 10.38 (s, 1H, 1-H). Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

3-(4-Hydroxybutyl)-6-(3-iodo-4-methylanilino)uracil, 7. This compound was prepared by demethylation of **4c**, by the same procedure as described above, in 48% yield, mp 184−185 °C. ^{1}H NMR: 1.30−1.57 (m, 4H, 2 × CH_2), 2.35 (s, 3H, $PhCH_3$), 3.37 (t, 2H, NCH_2), 3.68 (t, 2H, OCH_2), 4.38 (br s, 1H, OH), 4.73 (s, 1H, 5-H), 7.17 (d, 1H, PhH), 7.32 (d, 1H, PhH), 7.63 (s, 1H, PhH), 8.25 (s, 1H, 6-NH), 10.60 (s, 1H, 1-H). Anal. ($C_{15}H_{18}IN_3O_3 \cdot 0.5H_2O$) C, H, N.

3-(4-Iodobutyl)-6-(3-ethyl-4-methylanilino)uracil, 8. Me_3SiI (7.1 mL, 50 mmol) was added to a stirred solution of **1c** (3.31 g, 10 mmol) in dry $CHCl_3$. The reaction mixture was stirred at reflux overnight until disappearance of starting material (TLC). MeOH and Na_2SO_3 were then added to the brown-purple solution. After stirring at room temperature for 10 min, the mixture was filtered and the solvent was removed. Crystallization of the residue from EtOH gave 3.6 g (84%) of product as a white solid, mp 216−217 °C. The filtrate was evaporated, and the residue was purified by chromatography on silica gel with 1−2% MeOH in $CHCl_3$ as eluent to give an additional 0.4 g (9.4%) of product. See above for properties.

3-(4-Methoxybutyl)-6-anilinouracils. General Procedure. Compound **3c** (465 mg, 2 mmol) and the aniline (2 equiv) were mixed well in a test tube and stirred at 145 °C for 8−15 min under N_2. The reaction mixture typically solidifies after a few min. The reaction was cooled to room temperature, and the solid was crystallized from EtOH or purified by column chromatography on silica gel to obtain the product.

3-(4-Methoxybutyl)-6-(3,4-dimethylanilino)uracil, 9a. Reaction with 3,4-dimethylaniline for 15 min yielded 540 mg (85%) of product as an off-white powder after crystallization, mp 210−212 °C. ^{1}H NMR: 1.47 (m, 4H, 2 × CH_2), 2.18 (s, 3H, $PhCH_3$), 2.21 (s, 3H, $PhCH_3$), 3.02 (s, 3H, OCH_3), 3.30 (t, 2H, NCH_2), 3.65 (t, 2H, OCH_2), 4.69 (s, 1H, 5-H), 6.90 (dd, 1H, PhH), 6.96 (d, 1H, PhH), 7.13 (d, 1H, PhH), 8.01 (s, 1H, 6-NH), 10.35 (s, 1H, 1-H). Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-vinyl-4-methylanilino)uracil, 9b. A mixture of **3c** (46.5 mg, 0.2 mmol) and 3-(1-hydroxyethyl)-4-methylaniline (60.5 mg, 4 mmol) was heated at 150 °C for 15 min. The mixture was purified by chromatography on silica gel with 1−1.5% MeOH in $CHCl_3$ as eluent to give 30 mg (45%) of **9b** as an off-white powder, mp 155 °C (dec). ^{1}H NMR: 1.47 (m, 4H, 2 × CH_2), 2.29 (s, 3H, $PhCH_3$), 3.20 (s, 3H, OCH_3), 3.28 (t, 2H, NCH_2), 3.69 (t, 2H, OCH_3), 4.66 (s, 1H, 5-H), 5.30 (d, 1H, vinyl), 5.62 (d, 1H, vinyl), 6.88 (dd, 1H, vinyl), 6.95 (dd, 1H, PhH), 7.12 (d, 1H, PhH), 7.28 (d, 1H, PhH), 8.10 (s, 1H, 6-NH), 10.50 (s, 1H, 1-H). Anal. ($C_{18}H_{23}N_3O_3$) H,N. C, calcd. 65.63; found 65.12.

3-(4-Methoxybutyl)-6-(3-isopropenyl-4-methylanilino)-uracil, 9c. Reaction with 3-isopropenyl-4-methylaniline for 15 min yielded 357 mg (52%) of product as a light yellow powder after crystallization, mp 186−188 °C. ^{1}H NMR: 1.42 (m, 4H, 2 × CH_2), 1.98 (s, 3H, C=CCH_3), 2.21 (s, 3H, $PhCH_3$), 3.18 (s, 3H, OCH_3), 3.28 (t, 2H, NCH_2), 3.62 (t, 2H, OCH_3), 4.71 (s, 1H, 5-H), 4.81 (d, 1H, =CH), 5.20 (d, 1H, =CH), 6.90 (d, 1H, PhH), 7.00 (dd, 1H, PhH), 7.17 (d, 1H, PhH), 8.11 (s, 1H, 6-NH), 10.44 (s, 1H, 1-H). Anal. ($C_{19}H_{25}N_3O_3$·0.55 H_2O) C, H, N.

3-(4-Methoxybutyl)-6-(3-chloro-4-methylanilino)uracil, 9d. Reaction with 3-chloro-4-methylaniline for 15 min yielded 527 mg (78%) of product as a light yellow powder after crystallization, mp 220−222 °C. ^{1}H NMR: 1.45 (m, 4H, 2 × CH_2), 2.27 (s, 3H, $PhCH_3$), 3.18 (s, 3H, OCH_3), 3.27 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_2), 4.76 (s, 1H, 5-H), 7.08 (dd, 1H, PhH), 7.23 (d, 1H, PhH), 7.31 (d, 1H, PhH), 8.31 (s, 1H, 6-NH), 10.60 (s, 1H, 1-H). Anal. ($C_{16}H_{20}ClN_3O_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-bromo-4-methylanilino)uracil, 9e. Reaction with 3-bromo-4-methylaniline for 15 min yielded 535 mg (70%) of product as a light yellow powder after crystallization, mp 218−220 °C. ^{1}H NMR: 1.44 (m, 4H, 2 × CH_2), 2.29 (s, 3H, $PhCH_3$), 3.18 (s, 3H, OCH_3), 3.26 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_2), 4.75 (s, 1H, 5-H), 7.12 (dd, 1H, PhH), 7.32 (d, 1H, PhH), 7.39 (d, 1H, PhH), 8.31 (s, 1H, 6-NH), 10.62(s, 1H, 1-H). Anal. ($C_{16}H_{20}BrN_3O_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-chloromethyl-4-methylanilino)-uracil, 9f. Compound **9l** (166.5 mg, 0.5 mmol) was treated as in the preparation of **9j** to give the product (109 mg, 62%) as a white solid, mp 196−198 °C. ^{1}H NMR: 1.45 (m, 4H, 2 × CH_2), 2.32 (s, 3H, $PhCH_3$), 3.18 (s, 3H, OCH_3), 3.27 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_2), 4.73 (s, 1H, 5-H), 4.76 (s, 2H, $ClCH_2$), 7.08 (dd, 1H, PhH), 7.21 (d, 1H, PhH), 7.23 (d, 1H, PhH), 8.20 (s, 1H, 6-NH), 10.47 (s, 1H, 1-H). Anal. ($C_{17}H_{22}ClN_3O_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-thiocyanomethyl-4-methylanilino)uracil, 9g. KSCN (48.5 mg, 0.5 mmol) was added to a solution of **9f** (35.2 mg, 0.1 mmol) in EtOH (5 mL). The mixture was heated at reflux for 6 h, the solvent was removed in vacuo, and the residue was applied to a silica gel column. Elution with 2−3% MeOH in $CHCl_3$ yielded the product (27 mg, 71%) as a white solid, mp 170−172 °C. ^{1}H NMR: 1.45 (m, 4H, 2 × CH_2), 2.31 (s, 3H, CH_3), 3.18 (s, 3H, OCH_3), 3.28 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_3), 4.38 (s, 2H, $NCSCH_2$), 4.83(s, 1H, 5-H), 7.08 (d, 1H, PhH), 7.19 (s, 1H, PhH), 7.22 (d, 1H, PhH), 8.22 (s, 1H, 6-NH), 10.35 (s, 1H, 1-H). Anal. ($C_{18}H_{22}N_4O_3S$) C,H. N, calcd. 14.96; found 13.52.

3-(4-Methoxybutyl)-6-(3-cyanomethyl-4-methylanilino)-uracil, 9h. Reaction of KCN (32 mg, 0.5 mmol) and **9f** (35 mg, 0.1 mmol), as described for the preparation of **9g**, yielded the product (20 mg, 60%) as a white solid, mp 200−202 °C. ^{1}H NMR: 1.45 (m, 4H, 2 × CH_2), 2.24 (s, 3H, CH_3), 3.17 (s, 3H, OCH_3), 3.28 (t, 2H, NCH_2), 3.67 (t, 2H, OCH_2), 3.98 (s, 2H, $NCCH_2$), 4.76 (s, 1H, 5-H), 7.06 (dd, 1H, PhH), 7.18 (d, 1H, PhH), 7.22 (d, 1H, PhH), 8.30 (s, 1H, 6-NH), 10.50(s, 1H, 1-H). Anal. ($C_{18}H_{22}N_4O_3$) C,H; N, calcd. 16.36, found 15.52.

3-(4-Methoxybutyl)-6-(3-mercaptomethyl-4-methylanilino)uracil, 9i. Reaction of $NaSH·H_2O$ (25.5 mg, 0.5 mmol) and **9f** (35 mg, 0.1 mmol), as described for the preparation of **9g**, yielded the product (24 mg, 70%) as a white solid, mp 198−200 °C. ^{1}H NMR: 1.45 (m, 4H, 2 × CH_2), 2.25 (s, 3H, CH_3), 3.17 (s, 3H, OCH_3), 3.28 (t, 2H, NCH_2), 3.67 (t, 2H, SCH_2), 3.76 (t, 2H, OCH_2), 4.76 (t, 1H, SH), 4.84 (s, 1H, 5-H), 7.00 (m, 2H, 2 × PhH), 7.16 (d, 1H, PhH), 8.17 (s, 1H, 6-NH), 10.38 (s, 1H, 1-H). Anal. ($C_{17}H_{23}SN_3O_3$·0.5H_2O) C,H. N, calcd 11.72; found 10.45.

3-(4-Methoxybutyl)-6-[3-(1-chloroethyl)-4-methylanilino]uracil, 9j. Ph_3P (157 mg, 0.6 mmol) was added to a solution of **9m** (174 mg, 0.5 mmol) in CCl_4 (2 mL) and MeCN (2 mL) at room temperature. The mixture was stirred at room temperature for 2 h and at 40 °C for 0.5 h. The solvents were removed in vacuo, and the residue was purified by silica gel chromatography. Elution with 2−3% MeOH in $CHCl_3$ yielded 128 mg (70%) of **9j** as a white solid, mp 190−192 °C. ^{1}H NMR: 1.44 (m, 4H, 2 × CH_2), 1.76 (d, 3H, CCH_3), 2.32 (s, 3H, $PhCH_3$), 3.20 (s, 3H, OCH_3), 3.27 (t, 2H, NCH_2), 3.66 (t, 2H, OCH_2), 4.70 (s, 1H, 5-H), 5.47 (q, 1H, ClCH), 7.06 (dd, 1H, PhH), 7.18 (d, 1H, PhH), 7.32 (d, 1H, PhH), 8.20 (s, 1H, 6-NH), 10.48 (s, 1H, 1-H). Anal. ($C_{18}H_{24}ClN_3O_3$) C, H, N.

3-(4-Methoxybutyl)-6-(3-acetyl-4-methylanilino)uracil, 9k. Reaction with 3-acetyl-4-methylaniline for 8 min yielded 518 mg (75%) of product as an off-white powder after crystallization, mp 182−184 °C (dec). ^{1}H NMR: 1.47 (m, 4H, 2 × CH_2), 2.08 (s, 3H, $COCH_3$), 2.39 (s, 3H, $PhCH_3$), 3.20 (s, 3H, OCH_3), 3.28 (t, 2H, NCH_2), 3.68 (t, 2H, OCH_2), 4.75 (s, 1H, 5-H), 7.29 (m, 2H, 2 × PhH), 7.59 (s, 1H, PhH), 8.30 (s, 1H, 6-NH), 10.62 (s, 1H, 1-H). Anal. ($C_{18}H_{23}N_3O_4$·0.35 H_2O) C, H, N.

3-(4-Methoxybutyl)-6-(3-hydroxymethyl-4-methylanilino)uracil, 9l. Reaction with 3-hydroxymethyl-4-methylaniline for 10 min yielded 333 mg (50%) of product as a light yellow powder after crystallization, mp 190−192 °C (dec). ^{1}H NMR: 1.45 (m, 4H, 2 × CH_2), 2.16 (s, 3H, $PhCH_3$), 3.18 (s, 3H, OCH_3), 3.26 (t, 2H, NCH_2), 3.66 (d, 2H, OCH_2), 4.45 (d, 2H, $PhCH_2$), 4.76 (s, 1H, 5-H), 5.19 (t, 1H, OH), 6.96 (d, 1H, PhH), 7.10 (d, 1H, PhH), 7.20 (s, 1H, PhH), 8.11 (s, 1H, 6-NH), 10.33 (s, 1H, 1-H). Anal. ($C_{17}H_{23}N_3O_3$·0.25H_2O) C, H, N.

3-(4-Methoxybutyl)-6-[3-(1-hydroxyethyl)-4-methylanilino]uracil, 9m. A solution of **9k** (345 mg, 1 mmol) in MeOH (10 mL) was treated 0 °C with $NaBH_4$ (228 mg, 6 mmol). The temperature was raised to room temperature, and the mixture was stirred for 2 h. The reaction mixture was cooled in an ice−water bath, and aqueous NH_4Cl (10 mL) was added slowly. After stirring the mixture for 0.5 h at room temperature, the solid was filtered and washed with MeOH (3 × 5 mL). The combined filtrate was concentrated, and the residue was applied to a silica gel column and eluted with 6% MeOH in $CHCl_3$ to yield 330 mg (95%) of **9m** as a white solid, mp 204−206 °C. ^{1}H NMR: 1.27 (d, 3H, CCH_3), 1.47 (m, 4H, 2 × CH_2), 2.24 (s, 3H, $PhCH_3$), 3.20 (s, 3H, OCH_3), 3.30 (t, 2H, NCH_2), 3.69 (t, 2H, OCH_2), 4.79 (s, 1H, 5-H), 4.87 (m, 1H, PhCH), 5.17 (d, 1H, OH), 6.95 (dd, 1H, PhH), 7.10 (d, 1H, PhH), 7.29 (d, 1H, PhH), 8.16 (s, 1H, 6-NH), 10.37 (s, 1H, 1-H). Anal. ($C_{18}H_{25}N_3O_4$ ·0.25H_2O) C, H, N.

3-(4-Methoxybutyl)-6-(3,4-dichlorobenzylamino)uracil, 10. A mixture of **3c** (111 mg, 0.5 mmol) and 3,4-dichlorobenzylamine (444 mg, 2.5 mmol) was heated at 125 °C for 4 h. The reaction mixture was cooled to room temperature and dissolved in 20 mL of $CHCl_3$. The solution was extracted with 2 × 20 mL of 0.1 M aqueous HCl, and the $CHCl_3$ layer was evaporated to leave a solid. This solid was stirred in EtOAc and filtered to give 125 mg (69%) of product as a white powder, mp 226−229 °C. ^{1}H NMR: 1.46 (m, 4H, 2 × CH_2), 3.19 (s, 3H, OCH_3), 3.28 (t, 3H, NCH_2), 3.64 (t, 2H, OCH_2), 4.29 (d, $BnCH_2$), 4.51 (s, 1H, 5-H), 6.68 (t, 1H, 6-NH), 7.31 (dd, 1H, PhH), 7.60 (d, 1H, PhH), 7.62 (d, 1H, PhH), 10.40 (s, 1H, 1-H). Anal. ($C_{16}H_{19}Cl_2N_3O_3$) C, H, N.

3-(Substituted-butyl)-EMAUs. General Procedure. A mixture of **8**, K_2CO_3, and the nucleophile in an appropriate solvent (Me_2CO, MeCN, DMF) was stirred at room temperature. Once the reaction was complete (TLC), the solution was concentrated in vacuo and water was added. The mixture was extracted with $CHCl_3$, and the extracts were dried over Na_2SO_4. After removal of solvent, the residue was purified by chromatography on silica gel using a mixture of MeOH:$CHCl_3$ as eluent to give the product. In all cases, proton NMR resonances characteristic of the 3-ethyl-4-methylphenyl group were observed (e.g., see compounds **1c** and **2c**).

3-(4-Azidobutyl)-6-(3-ethyl-4-methylanilino)uracil, 11a. A mixture of **8** (150 mg, 0.35 mmol) and NaN_3 (200 mg, 3 mmol) in DMF (30 mL) was heated at 70−80 °C for 7 h. The solution was concentrated in vacuo, and water (25 mL) was added. The mixture was extracted with $CHCl_3$ (3 × 40 mL), and the combined organic layers were dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by chromatography on silica gel using 3−5% MeOH in $CHCl_3$ as eluent, to give 110 mg (92%) of product, mp 209−210 °C. ^{1}H NMR: 1.15 (t, 3H, $PhCH_2CH_3$), 1.53 (m, 4H, 2 × CH_2), 2.23 (s, 3H, $PhCH_3$), 2.57 (q, 2H, $PhCH_2$), 3.33 (t, 2H, N_3CH_2), 3.70 (t, 2H, NCH_2), 4.73 (s, 1H, 5-H), 6.92−7.18 (m, 3H, PhH), 8.11 (s, 1H, 6-NH), 10.40 (s, 1H, 1-H). Anal. ($C_{17}H_{22}N_6O_2$) C, H, N.

3-(4-Aminobutyl)-6-(3-ethyl-4-methylanilino)uracil Hydrochloride, 11b. A mixture of **11a** (100 mg, 0.3 mmol) and 10% Pd/C (45 mg) in MeOH (40 mL) and $CHCl_3$ (10 mL) was stirred under H_2 at 35 psi for 14 h at room temperature. The mixture was filtered, and the catalyst was washed with MeOH. The combined filtrates were concentrated, and the residue was purified by chromatography on silica gel with 10−20% MeOH in $CHCl_3$ as eluent, to give 78 mg (84%) of 3-(4-aminobutyl)-6-(3-ethyl-4-methylanilino)uracil. This product was dissolved in a mixture of MeOH and $CHCl_3$, and a solution of 4.0 M HCl in dioxane (4 mL) was added. The mixture was stirred at room temperature for 0.5 h, and the solvents were removed to give the hydrochloride as a white solid, mp 218−219 °C. ^{1}H NMR: 1.11 (t, 3H, $PhCH_2CH_3$), 1.50 (m, 4H, 2 × CH_2), 2.21 (s, 3H, $PhCH_3$), 2.57 (q, 2H, $PhCH_2$), 2.78 (m, 2H, NH_3CH_2), 3.72 (t, 2H, NCH_2), 4.75 (s, 1H, 5-H), 6.92−7.15 (m, 3H, PhH), 7.86 (br s, 3H, NH_3), 8.89 (s, 1H, 6-NH), 10.76 (s, 1H, 1-H). Anal. ($C_{17}H_{25}ClN_4O_2$) C, H, N.

3-[4-(1-Piperidyl)butyl]-6-(3-ethyl-4-methylanilino)-uracil, 11c. The general method with piperidine gave the product in 76% yield, mp 202−204 °C. ^{1}H NMR: 1.10 (t, 3H, $PhCH_2CH_3$), 1.35−1.62 (m, 10H, 5 × CH_2), 2.23−2.33 (m, 9H, 3 × NCH_2, $PhCH_3$), 2.56 (q, 2H, $PhCH_2$), 3.72 (m, 2H, NCH_2), 4.73 (s, 1H, 5-H), 6.90−7.14 (m, 3H, PhH), 8.32 (s, 1H, 6-NH), 10.48 (s, 1H, 1-H). Anal. ($C_{22}H_{32}N_4O_2$) C, H, N.

3-[4-(1-Morpholinyl)butyl]-6-(3-ethyl-4-methylanilino)-uracil, 11d. The general method with morpholine gave the product in 72% yield, mp 225−226 °C. ^{1}H NMR: 1.12 (t, 3H, $PhCH_2CH_3$), 1.30−1.52 (m, 4H, 2 × CH_2), 2.20−2.30 (m, 7H, CH_2NCH_2, $PhCH_3$), 2.55 (q, 2H, $PhCH_2$), 3.30 (m, 2H, CH_2N), 3.56 (m, 4H, CH_2OCH_2), 3.68 (m, 2H, NCH_2), 4.72 (s, 1H, 5-H), 6.90−7.14 (m, 3H, PhH), 8.04 (s, 1H, 6-NH), 10.38 (s, 1H, 1-H). Anal. ($C_{21}H_{30}N_4O_3$) C, H, N.

3-[4-(1-Thiomorpholinyl)butyl]-6-(3-ethyl-4-methylanilino)uracil, 11e. The general method with thiomorpholine gave the product in 77% yield, mp 228−229 °C. ^{1}H NMR: 1.10 (t, 3H, $PhCH_2CH_3$), 1.30−1.51 (m, 4H, 2 × CH_2), 2.18 (s, 3H, $PhCH_3$), 2.45 (m, 8H, 2 × NCH_2CH_2S), 2.55 (q, 2H, $PhCH_2$), 3.30 (m, 2H, NCH_2), 3.62 (m, 2H, NCH_2), 4.71 (s, 1H, 5-H), 6.90−7.12 (m, 3H, PhH), 8.10 (s, 1H, 6-NH), 10.03 (s, 1H, 1-H). Anal. ($C_{20}H_{27}SN_4O_2$) C, H, N.

3-{4-[3-(Ethoxycarbonylmethyl)-1-morpholinyl]butyl}-6-(3-ethyl-4-methylanilino)uracil, 11f. The general method gave the product in 56% yield, mp 160−161 °C. ^{1}H NMR: 1.15 (m, 6H, 2 × CH_3), 1.30−1.50 (m, 4H, 2 × CH_2), 1.68 (t, 1H, NCH), 1.82 (t, 1H, NCH), 2.20 (s, 3H, $PhCH_3$), 2.30−2.62 (m, 7H, CH_2N, $PhCH_2$, NCH, CO_2CH_2), 2.70 (d, 1H, NCH), 3.68 (m, 5H, NCH_2, OCH_2, OCH), 4.06 (q, 2H, $COCH_2$), 4.72 (s, 1H, 5-H), 6.92−7.13 (m, 3H, PhH), 8.04 (s, 1H, 6-NH), 10.42 (s, 1H, 1-H). Anal.($C_{25}H_{36}N_4O_5$) C, H, N.

3-{4-[(3-Ethoxy-1-morpholinyl]butyl}-6-(3-ethyl-4-methylanilino)uracil, 11g. The general method gave the product in 51% yield, mp 162−163 °C. ^{1}H NMR: 1.12 (m, 6H, 2 × CH_3), 1.31−1.50 (m, 4H, 2 × CH_2), 1.82−2.24 (m, 5H, NCH_2, $PhCH_3$), 2.38−2.60 (m, 6H, NCH_2, $PhCH_2$), 3.42 (m, 2H, OCH_2), 3.70 (m, 4H, NCH_2, OCH_2), 4.54 (m, 1H, CHO), 4.73 (s, 1H, 5-H), 6.92−7.12 (m, 3H, PhH), 8.06 (s, 1H, 6-NH), 10.42 (s, 1H, 1-H). Anal. ($C_{23}H_{34}N_4O4$) C, H, N.

3-{4-[(4-Hydroxy-4-phenyl)-1-piperidinyl]butyl}-6-(3-ethyl-4-methylanilino)uracil, 11h. The general method gave the product in 67% yield, mp 203−204 °C. ^{1}H NMR: 1.10 (t, 3H, $ArCH_2CH_3$), 1.36−1.42 (m, 6H, 3 × CH_2), 1.90 (t, 2H, CH_2), 2.20 (s, 3H, $PhCH_3$), 2.30−2.72 (m, 8H, 3 × CH_2, $PhCH_2$), 3.30 (s, 1H, OH), 3.68 (t, 2H, NCH_2), 4.73 (s, 1H, 5-H), 6.91−7.14 (m, 3H, PhH), 7.20 (t, 1H, PhH), 7.31 (t, 2H, PhH), 7.48 (d, 2H, PhH), 8.33 (s, 1H, 6-NH), 10.66 (s, 1H, 1-H). Anal. ($C_{28}H_{36}N_4O_3$) C, H, N.

3{[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]butyl}-6-(3-ethyl-4-methylanilino)uracil, 11i. The general method gave the product in 64% yield, mp 235 °C (dec). ^{1}H NMR: 10.48 (s, 1H, 1-H), 8.22 (s, 1H, 6-NH), 7.50 (d, 2H, PhH), 7.31 (d, 2H, PhH), 6.90−7.14 (m, 3H, PhH), 4.73 (s, 1H, 5-H), 3.70 (t, 2H, NCH_2), 2.30−2.72 (m, 8H, 3 × NCH_2, $PhCH_2$), 2.20 (s, 3H, $PhCH_3$), 1.90 (t, 2H, CH_2), 1.37−1.62 (m, 6H, 3 × CH_2), 1.10 (t, 3H, $PhCH_2CH_3$). Anal. ($C_{28}H_{35}ClN_4O_3$) C,H; N, calcd 10.96, found 10.37.

3{[4-(3-Trifluoromethyl-4-chlorophenyl)-4-hydroxy-1-piperidinyl]butyl}-6-(3-ethyl-4-methylanilino)uracil, 11j. The general method gave the product in 54% yield, mp 158−160 °C. ^{1}H NMR: 1.10 (t, 3H, $PhCH_2CH_3$), 1.50 (m, 6H, 3 × CH_2), 1.88(t, 2H, CH_2), 2.10 (s, 3H, $PhCH_3$), 2.24−2.38 (m, 4H, CH_2NCH_2), 2.52 (q, 2H, $PhCH_2$), 3.40 (m, 2H, NCH_2), 3.68 (m, 2H, NCH_2), 4.72 (s, 1H, 5-H), 5.11 (s, 1H, OH), 6.90−7.14 (m, 3H, PhH), 7.68−7.90 (m, 3H, PhH), 8.14 (s, 1H, 6-NH), 10.36 (s, 1H, 1-H). Anal. ($C_{29}H_{34}ClF_3N_4O_3 \cdot 0.5H_2O$) C, H, N.

3-[4-(4-Benzyl-1-piperazinyl)butyl]-6-(3-ethyl-4-methylanilino)uracil, 11k. K_2CO_3 (276 mg, 2 mmol) and 1-benzylpiperazine (317 mg, 1.8 mmol) were added to a mixture of **8** (638 mg, 1.5 mmol) in 60 mL of MeCN. The mixture was stirred at room temperature for 16 h. Once the reaction was complete (TLC), the mixture was concentrated in vacuo. Water (20 mL) was added, and the mixture was extracted with $CHCl_3$ (2 × 50 mL). The extracts were dried over Na_2SO_4, the solvent was removed, and the residue was purified by chromatography on silica gel using 5% MeOH in $CHCl_3$ as eluent to give 545 mg (78%) of product, mp 205−206 °C. ^{1}H NMR: 1.10 (t, 3H, $PhCH_2CH_3$), 1.26−1.50 (m, 4H, 2 × CH_2), 2.16−2.38 (m, 9H, 3 × NCH_2, $PhCH_3$), 2.52 (q, 2H, $PhCH_2$), 3.28 (m, 4H, 2 × NCH_2), 3.40 (m, 2H, NCH_2), 3.64 (t, 2H, NCH_2), 4.70 (s, 1H, 5-H), 6.88−7.32 (m, 8H, PhH), 8.15 (s, 1H, 6-NH), 10.38 (s, 1H, 1-H). Anal. ($C_{28}H_{37}N_5O_2 \cdot 0.75H_2O$) C, H, N.

3-{4-[(4-Fluorophenyl)-1-piperazinyl]butyl}-6-(3-ethyl-4-methyylanilino)uracil, 11l. The general method gave the product in 57% yield, mp 188−189 °C. ^{1}H NMR: 1.14 (t, 3H, $PhCH_2CH_3$), 1.42−1.54 (m, 4H, 2 × CH_2), 2.21 (s, 3H, $PhCH_3$), 2.33 (m, 2H, NCH_2), 2.55 (m, 2H, NCH_2), 2.59 (q, 2H, $PhCH_2$), 2.99 (m, 4H, 2 × NCH_2), 3.30 (m, 2H, NCH_2), 3.71 (t, 2H, NCH_2), 4.73 (s, 1H, 5-H), 6.93−7.15 (m, 7H, PhH), 8.11 (s, 1H, 6-NH), 10.41 (s, 1H, 1-H), Anal. ($C_{27}H_{34}FN_5O_2$) C, H; N, calcd 14.60; found 14.15.

3-{4-[4-(2-Furoyl)-1-piperazinyl]butyl}-6-(3-ethyl-4-methylanilino)uracil, 11m. The general method gave the product in 68% yield, mp181−182 °C. ^{1}H NMR: 1.15 (t, 3H, $PhCH_2CH_3$), 1.40−1.50 (m, 4H, 2 × CH_2), 2.24 (s, 3H, $PhCH_3$), 2.30−2.45 (m, 4H, 2 × NCH_2), 2.58 (q, 2H, $PhCH_2$), 3.33 (m, 4H, 2 × CH_2N), 3.68 (m, 4H, 2 × NCH_2), 4.73 (s, 1H, 5-H), 6.62 (s, 1H, furyl-H), 6.96 (m, 3H, 2 × PhH, furyl-H), 7.15 (m, 1H, PhH), 7.83 (s, 1H, furyl-H), 8.12 (s, 1H, 6-NH), 10.42 (s, 1H, 1-H). Anal. ($C_{26}H_{33}N_5O_4 \cdot 0.25H_2O$) C, H, N.

3-{4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl}-6-(3-ethyl-4-methyylanilino)uracil, 11n. The general method gave the product in 62% yield, mp 199−201 °C. ^{1}H NMR: 1.14 (t, 3H, $PhCH_2CH_3$), 1.45−1.51 (m, 4H, 2 × CH_2), 2.22 (s, 3H, $PhCH_3$), 2.39 (m, 4H, 2 × NCH_2), 2.60 (q, 2H, $PhCH_2$), 3.32 (m, 2H,

NCH_2), 3.70 (m, 6H, 3 × NCH_2), 4.73 (s, 1H, 5-H), 6.61 (t, 1H, pyrimidine-H), 6.93−7.15 (m, 3H, PhH), 8.12 (s, 1H, 6-NH), 8.38 (m, 2H, pyrimidine-H), 10.41 (s, 1H, 1-H). Anal. ($C_{25}H_{33}N_7O_2$) C, H, N.

3-[4-(1-Piperazinyl)butyl]-6-(3-ethyl-4-methylanilino)-uracil dihydrochloride, 11o. Treatment of **8** with *N*-Boc-piperazine gave 3-{4-[4-(*tert*-butoxycarbonyl)piperazino]butyl}-6-(3-ethyl-4-methylanilino)uracil in 62% yield, mp 215−216 °C (dec). ^{1}H NMR: consistent. A solution of this intermediate (2.4 g) in a mixture of $CHCl_3$ and MeOH (3:1, 20 mL) was treated with 50 mL of a solution of 4.0 M HCl in dioxane, and the mixture was stirred at room temperature for 4 h. After removal of the solvent, the residue was washed with Et_2O and dried in vacuo to give the product as a colorless solid in 94% yield, mp 210 °C (dec). ^{1}H NMR: 1.16 (t, 3H, $PhCH_2CH_3$), 1.50−1.70 (m, 4H, 2 × CH_2), 2.22 (s, 3H, $PhCH_3$), 2.60 (q, 2H, $PhCH_2$), 3.18 (m, 4H, 2 × NCH_2), 3.50 (m, 6H, 3 × NCH_2), 3.72 (t, 2H, NCH_2), 4.72 (s, 1H, 5-H), 6.93−7.15 (m, 3H, PhH), 8.50 (s, 1H, 6-NH), 10.38 (s, 3H, NH_2^+, NH^+), 10.58 (s, 1H, 1-H). Anal. ($C_{21}H_{32}Cl_2N_5O_2 \cdot 2.5H_2O$) C, H, N.

DNA Polymerase Assays. DNA polymerase IIIC (pol IIIC) of *Bacillus subtilis* was the homogeneous recombinant protein expressed and purified as described previously.[13] The enzyme was assayed in 96-well plates with the use of activated calf thymus DNA as a substrate according to the method of Barnes and Brown.[14] Briefly, enzyme was added to a buffered mixture containing Mg^{2+}, dithiothreitol, glycerol, dATP, dCTP, dTTP, [^{3}H]dTTP, and activated calf thymus DNA. Assays were initiated by the addition of 0.025−0.06 units of enzyme (1 unit is the amount required to incorporate 250 pmoles of [^{3}H]dTMP in a standard assay), incubated for 10 min at 30 °C, and terminated by the addition of a trichloroacetic acid−sodium pyrophosphate solution. Precipitated labeled DNA was collected on glass fiber filter plates, and the plates were washed with dilute aqueous HCl followed by EtOH, dried, and counted in a liquid scintillation counter. Serial dilutions of compounds (in DMSO) were added to the plates before enzyme addition. Apparent inhibition constants (K_i values) are obtained directly in this "truncated" assay, i.e., lacking the competitor dGTP, as described previously.[15,16] Compounds are assayed in duplicate; reported values typically vary ±43%.

Bacterial Strains. The standard screening panel included *S. aureus* 25923, *S. aureus* 13709 (Smith) and *E. fecalis* 29212, all purchased from the American Type Culture Collection (ATCC, Manassas, VA). Methicillin-resistant *S. aureus* (MRSA B42876), *E. fecium*, and vancomycin-resistant *E. fecium* (VRE) are clinical isolates provided by the University of Massachusetts Medical School. *B. subtilis* (BD54) is a standard laboratory strain.[17] *E. coli* (J-53) was provided by Prof. Martin Marinus, University of Massachusetts Medical School.

Minimum Inhibitory Concentrations (MIC). Antibacterial activity was measured as the minimum inhibitory concentration (MIC) of test compound required to prevent bacterial growth. Log-phase bacterial cultures were grown in Luria broth. Diluted bacteria cultures were seeded into 96-well plates (200 μL/well) from stocks containing (3−7) × 10^5 colony forming units/mL. Each compound was tested in duplicate assays by diluting stock solutions of compound in DMSO in 2-fold serial dilutions to give five concentrations from 20 to 1.25 μg/mL plus an untreated control culture. Each well, including the DMSO controls, contained a final concentration of 1% DMSO. Plates were incubated at 37 °C for 16 to 24 h, and bacterial growth was determined by measuring optical density (600 nm, 1 cm path length) in a microplate reader. MIC values are the average of the lowest concentration of test compound, in μg/mL, at which growth was not apparent (<25% of the DMSO control optical density). Reported MIC values typically vary 2-fold.

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Supporting Information Available: Tables of biological activity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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