Synthesis and Antibacterial Activity of 3-Substituted-6-(3-ethyl-4-methylanilino)uracils

Chengxin Zhi,[†] Zheng-yu Long,[†] Andrzej Manikowski,[†] Neal C. Brown,[†] Paul M. Tarantino, Jr.,[†] Karsten Holm,[†] Edward J. Dix,[†] George E. Wright,*[,†] Kim A. Foster,[‡] Michelle M. Butler,[‡] William A. LaMarr,[‡] Donna J. Skow,[‡] Irina Motorina,[§] Serge Lamothe,[§] and Richard Storer[§,||]

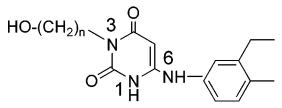
GLSynthesis Inc., One Innovation Drive, Worcester, Massachusetts 01605, Microbiotix Inc., One Innovation Drive, Worcester, Massachusetts 01605, and Shire BioChem Inc., Laval, Quebec, Canada

Received June 2, 2005

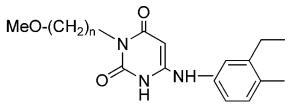
Numerous 3-substituted-6-(3-ethyl-4-methylanilino)uracils (EMAU) have been synthesized and screened for their capacity to inhibit the replication-specific bacterial DNA polymerase IIIC (pol IIIC) and the growth of Gram+ bacteria in culture. Direct alkylation of 2-methoxy-6-amino-4-pyrimidone produced the N3-substituted derivatives, which were separated from the byproduct 4-alkoxy analogues. The N3-substituted derivatives were heated with a mixture of 3-ethyl-4-methylaniline and its hydrochloride to effect displacement of the 6-amino group and simultaneous demethylation of the 2-methoxy group to yield target compounds in good yields. Certain intermediates, e.g. the 3-(iodoalkyl) compounds, were converted to a variety of (3-substituted-alkyl)-EMAUs by displacement. Most compounds were potent competitive inhibitors of pol IIIC (K_is 0.02−0.5 μM), and those with neutral, moderately polar 3-substituents had potent antibacterial activity against Gram+ organisms in culture (MICs 0.125−10 μg/mL). Several compounds protected mice from lethal intraperitoneal (ip) infections with *S.* aureus (Smith) when given by the ip route. A water soluble derivative, 3-(4-morpholinylbutyl)-EMAU hydrochloride, given subcutaneously, prolonged the life of infected mice in a dose dependent manner.

Introduction

The emergence of antibiotic-resistant Gram+ bacteria, notably *Staphylococcus aureus*, *Enterococcus fecalis*, *Enterococcus fecium*, and *Streptococcus pneumoniae*, has prompted development of new 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. Interference with pol IIIC function prevents the replication of the Gram+ host chromosome, thus killing the host.[1,2] Simple 3-(hydroxyalkyl) and 3-(methoxyalkyl) derivatives of 6-(3-ethyl-4-methylanilino)uracil (**1**, **2**) protected mice from an intraperitoneal (ip) *S. aureus* infection when also administered by the ip route.[2]



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



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

We recently reported that optimally 3-substituted 6-anilinouracils were potent inhibitors of pol IIIC from the Gram+ bacterium *Bacillus subtilis*, with strong selective antibacterial activity against a variety of Gram+ organisms.[3] However, among these derivatives, some lacked significant antibacterial activity despite their potent pol IIIC inhibition. Factors that may limit antibacterial activity could include lack of penetration of the cell wall or membrane, removal of compound by active efflux mechanisms, and alteration of the sensitivity of the target enzyme.

To learn more about the structure−antibacterial activity relationship and to select compounds for study in animal infection models, we undertook additional synthesis and screening programs among this class of compounds. Specifically, we have prepared additional derivatives of 6-(3-ethyl-4-methylanilino)uracil, "EMAU", the platform structure with the optimal substitution pattern for potent and selective inhibition of pol IIIC.[3,4] We have developed new methods for preparation of key intermediates to support the synthesis program. In addition, we report the inhibition of pol IIIC, SAR for in vitro antibacterial activity, and antibacterial activity in mice of selected compounds.

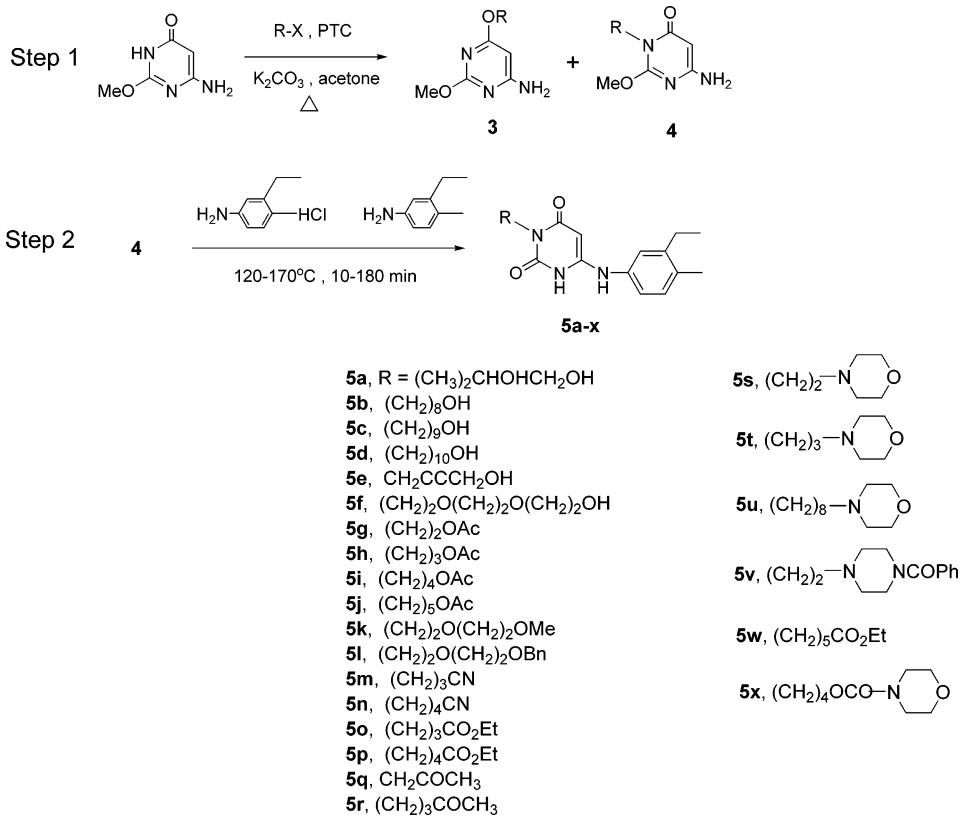
Results

Synthesis of Compounds. 3-Substituted-6-anilinouracils and related compounds have been prepared by conversion of *N*-alkylbarbituric acids to 6-chlorouracils followed by reaction with anilines or other amines.[1,3] This sequence, however, is not suitable for compounds with N3 substituents that are labile under the required strongly basic and acidic conditions. An alternative, two-step method has been developed, illustrated in Scheme 1. The first step is a phase transfer-catalyzed alkylation of 6-amino-2-methoxy-4-pyrimi-

* Author for correspondence: Phone 508 754−6700. Fax 508 754-7075. E-mail: george.wright@glsynthesis.com.
[†] GLSynthesis Inc.
[‡] Microbiotix Inc.
[§] Shire BioChem Inc.
[||] Present address: Laboratoires Idenix, 1682 Rue de la Valsière, 34189 Montpellier, France.

10.1021/jm050517r CCC: $30.25
Published on Web 10/12/2005

Scheme 1



done, similar to alkylations reported by Müller et al.[5] R−X is a substituted alkyl halide, and PTC is a phase transfer catalyst such as benzyltriethylammonium chloride (TBAC) or tetrabutylammonium bromide (TBAB), and acetone or acetonitrile is used as solvent. Reagents are heated at reflux for 10−72 h. Chromatography on silica gel with chloroform:methanol as eluent gives first the byproduct 6-amino-4-alkoxy-2-methoxypyrimidine **3** and then the desired 6-amino-2-methoxy-3-alkyl-4-pyrimidone **4**, in approximately equal yields. In a variation on this method, 6-amino-2-methoxy-4-pyrimidone is treated with sodium hydride in *N*,*N*-dimethylformamide (DMF) at 0 °C. Lithium bromide is added, the mixture is added dropwise to a solution of the alkylating agent in DMF at 50−80 °C, and the reaction mixture is stirred at 50−80 °C for 3−10 h. Workup by chromatography on silica gel gives first the O-alkylated isomers **3** followed by the N-alkylated isomers **4**.

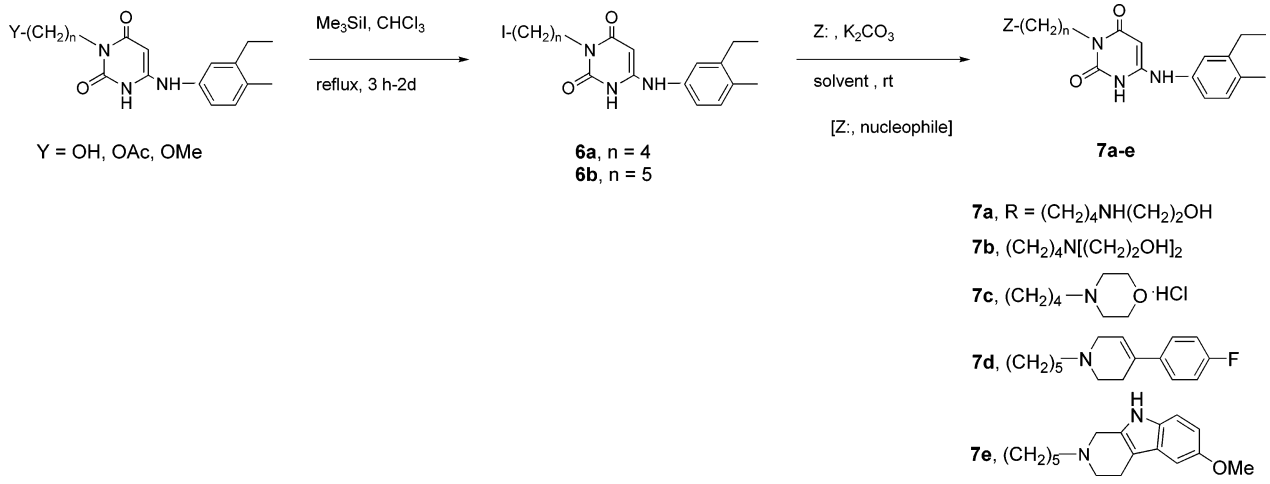
In the second step, a mixture of intermediate **4**, 3-ethyl-4-methylaniline hydrochloride, and a few drops of 3-ethyl-4-methylaniline (ca. 0.1−1 equiv) is heated at 120−170 °C for between 10 min to 3 h. After addition of water and extraction with chloroform, the residue is purified by chromatography on silica gel to give the target compounds, 3-alkyl-6-(3-ethyl-4-methylanilino)-uracils **5a**−**x**, in good yields (Scheme 1). This step not only effects displacement of the 6-amino group but also demethylation of the 2-methoxy group of **4**, yielding the desired uracil derivatives directly. Numerous examples of this method are presented in the Experimental Section and in Supporting Information.

Several useful intermediates for synthesis of 3-(substituted-alkyl)-EMAUs have been prepared. 3-(ω-Iodoalkyl) intermediates **6** were obtained in 80−95% yields by treatment of 3-(4-methoxybutyl), 3-(4-hydroxybutyl), 3-(4-acetoxybutyl), or 3-(5-hydroxypentyl)-EMAU with iodotrimethylsilane in dry chloroform (ref 3 and Scheme 2). Reaction of 3-(4-iodobutyl)- or 3-(5-iodopentyl)-EMAUs (**6a** or **6b**) with nucleophiles in the presence of potassium carbonate in a suitable solvent (acetone, acetonitrile, or DMF) and purification by chromatography on silica gel gave several candidate compounds (**7a**−**e**). Several ω-hydroxyalkyl derivatives (**8a**−**d**) were prepared by reduction of the corresponding esters or by dealkylation of corresponding ethers (Scheme 3). 3-(4-Aminobutyl)- and 3-(5-aminopentyl)-EMAUs (**9a** and **9b**) were prepared by reduction of the corresponding nitriles and acylated with carboxylic acid chlorides or sulfonyl chlorides to give 3-carboxamidoalkyl (**10a**−**c**) and 3-sulfonamidoalkyl derivatives (**11a**,**b**), respectively (Scheme 4). 3-(3-Carboxypropyl)-EMAU (**12**) was made by hydrolysis of the corresponding ester, **5o**.

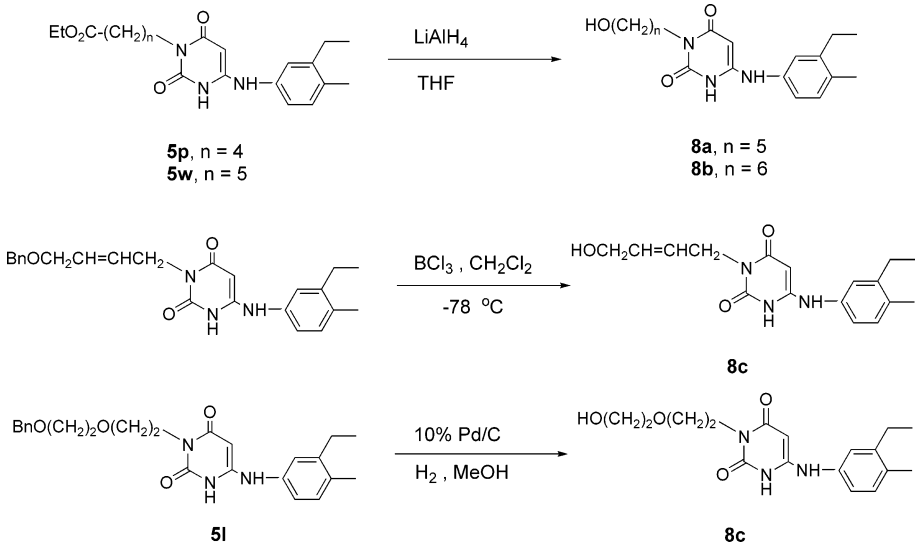
DNA Polymerase IIIC Inhibition. The EMAU derivatives were tested for inhibition of *B. subtilis* DNA polymerase IIIC using the "truncated" assay lacking the competitor dGTP, an assay which yields the K_i value of competitive inhibitors directly.[6] Many of the compounds were potent inhibitors of the enzyme, with K_i values ranging from 0.021 to 0.3 μM (Table 1). The common structural feature of the compounds is the 6-(3-ethyl-4-methylanilino)uracil core which optimizes binding of the class to the Gram+ pol IIIC enzymes.[4] The standard deviation of K_i values of inhibitors of pol IIIC is ca. 44%, indicating that the range of potencies of active 3-substituted EMAUs in Table 1 is small. [Other pol IIIC inhibitors with K_i values ranging between 0.024 and 73 μM lacked antibacterial activity (see Supporting Information)].

The chemical properties of N3 substituents had moderate to no effect on pol IIIC inhibition potency of

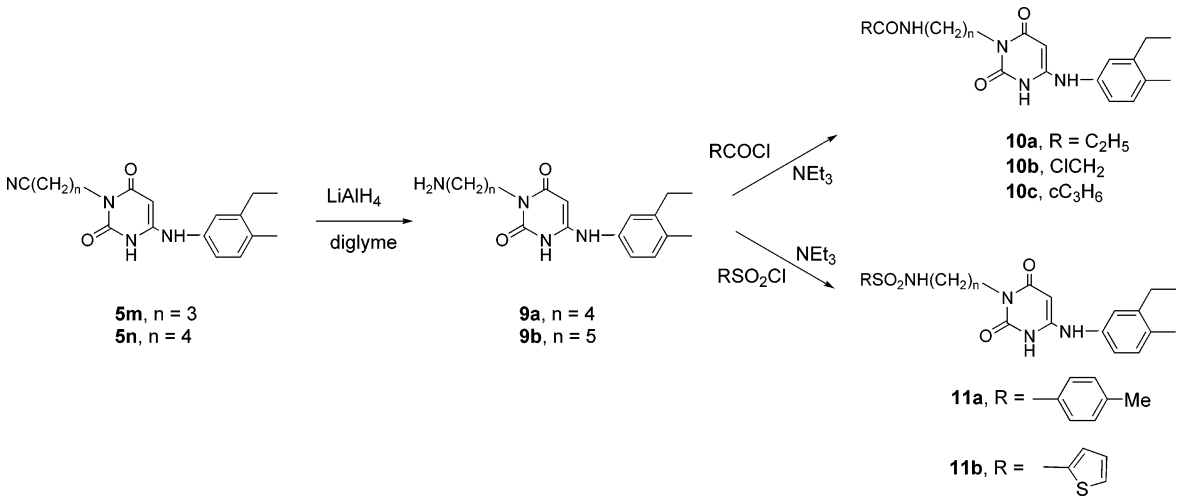
Scheme 2



Scheme 3

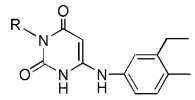


Scheme 4



the derivatives (Table 1). Within the homologous series, e.g. C_2-C_8 hydroxyalkyl, C_2-C_5 acetoxyalkyl, C_2-C_4 methoxyalkyl compounds, there was up to a 4-fold increase in potency with higher chain length. However, no increase was observed with the longer C_{9-10} chains of the hydroxynonyl and hydroxydecyl compounds, **5c** and **5d**. Compounds with polar (CN, CO_2Et), acidic (CO_2H, **12**), and basic (NH_2, NRH, NR_2) substituents, had K_i values that generally were between 0.02 and 0.1 μM. The most potent inhibitors were the most hydrophilic, i.e., the hydroxyalkyl tertiary amino compounds **7a** and **7b**. It appears that little change in binding affinity to the pol IIIC:DNA complex occurs after introduction of small substituents at N3, and the

Table 1. Pol IIIC Inhibition and Antibacterial Activity in Vitro of 3-Substituted-EMAUs



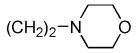
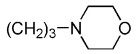
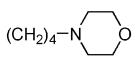
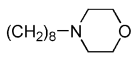
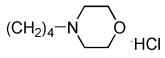
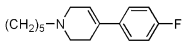
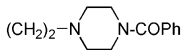
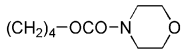
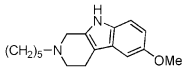
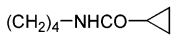
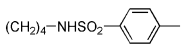
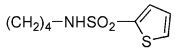
Cpd[ref]	R	K_i (μM)*	MIC (μg/ml)**							Serum effect (MIC+/MIC-)***	
		B.s. pol IIIC	*B.subtilis*	*S.aureus*	*S.a.* (Smith)	MRSA (B42876)	*E.faecalis*	*E.faecium*	VRE	*S.a.* (Smith)	*E.faecalis*
1a [1]	$(CH_2)_2OH$	0.2	<1.25	5	5	10	10	5	5		
1b [1]	$(CH_2)_3OH$	0.13	<1.25	2.5	5	5	5	2.5	2.5	5.6	
1c [3]	$(CH_2)_4OH$	0.055	<1.25	5	5	5	5	5	5	7.5	
8a	$(CH_2)_5OH$	0.043	<1.25	5	2.5	5	2.5	2.5	2.5	11.6	
5a	$(CH_2)_3CH(OH)CH_2OH$	0.096	10	20	20	>20	20	20	10		
8b	$(CH_2)_6OH$	0.049	<1.25	5	2.5	5	2.5	5	2.5	8.7	
5b	$(CH_2)_8OH$	0.026	1.25	2.5	2.5	2.5	2.5	2.5	2.5	21.3	
5c	$(CH_2)_9OH$	0.087	<1.25	20	2.5	20	2.5	2.5	2.5	>32	
5d	$(CH_2)_{10}OH$	0.051	<1.25	>20	>20	>20	>20	>20	>20		
8c	$CH_2CH{=}CHCH_2OH$	0.051	1.25	10	5	5	5	2.5	2.5	4.8	
5e	$CH_2C{\equiv}CCH_2OH$	0.068	5	10	5	10	10	10	10		
8d	$CH_2CH_2OCH_2CH_2OH$	0.125	<1.25	5	5	10	5	5	2.5	7.5	
5f	$(CH_2)_2O(CH_2)_2O(CH_2)_2OH$	0.087	2.5	10	10	>20	10	10	5		
5g	$(CH_2)_2OAc$	0.12	1.25	5	3.75	5	5	5	5		
5h	$(CH_2)_3OAc$	0.12	0.625	3.75	2.5	5	5	5	3.75		
5i	$(CH_2)_4OAc$	0.058	1.25	2.5	2.5	5	5	5	5		
5j	$(CH_2)_5OAc$	0.068	0.625	5	7.5	2.5	5	3.75	3.75		
2a [1]	$(CH_2)_2OMe$	0.127	<1.25	2.5	2.5	5	5	2.5	2.5	6.9	
2b [1]	$(CH_2)_3OMe$	0.12	<1.25	2.5	2.5	2.5	5	2.5	2.5	4.9	
2c [3]	$(CH_2)_4OMe$	0.088	<1.25	5	5	5	5	2.5	2.5	7.4	
5k	$CH_2CH_2OCH_2CH_2OMe$	0.045	2.5	5	2.5	5	5	5	5	5.5	
5l	$CH_2CH_2OCH_2CH_2OBn$	0.056	<1.25	20	20	20	5	2.5	5	>5.6	
5m	$(CH_2)_3CN$	0.14	2.5	5	2.5	5	5	5	5	5.4	
5n	$(CH_2)_4CN$	0.094	2.5	5	2.5	5	2.5	5	5	6	
12	$(CH_2)_3CO_2H$	0.057	>20	20	10	>20	>20	20	20		
5o	$(CH_2)_3CO_2Et$	0.1	<1.25	2.5	2.5	5	5	2.5	5	10	
5p	$(CH_2)_4CO_2Et$	0.076	<1.25	2.5	2.5	>20	5	<1.25	2.5	>12.2	
5q	CH_2COCH_3	0.3	2.5	10	10	10	20	10	20		
5r	$(CH_2)_3COCH_3$	0.061	<1.25	5	2.5	5	5	5	2.5		
7a	$(CH_2)_4NHCH_2CH_2OH$	0.028	>20	>20	>20	>20	>20	>20	>20		
7b	$(CH_2)_4N(CH_2CH_2OH)_2$	0.021	>20	>20	>20	>20	>20	>20	>20		
9a [3]	$(CH_2)_4NH_2\,HCl$	0.062	20	20	10	>20	>20	>20	>20		
9b	$(CH_2)_5NH_2\,HCl$	0.185	<1.25	5	10	10	>20	20	>20		
5s		0.09	<1.25	10	5	5	5	2.5	5	5.5	2.7
5t		0.11	2.5	10	10	10	10	5	5	2.9	1.6
13 [3]		0.048	2.5	10	5	10	5	2.5	5	2.3	3.2

Table 1. (Continued)

Cpd[ref]	R	K_i (μM)*	MIC (μg/ml)**							Serum effect (MIC+/MIC-)***	
		B.s. pol IIIC	*B.subtilis*	*S.aureus*	*S.a.* (Smith)	MRSA (B42876)	*E.faecalis*	*E.faecium*	VRE	*S.a.* (Smith)	*E.faecalis*
5u		0.076	2.5	10	10	20	10	10	5	>7.1	>12.8
7c		0.052	2.5	10	5	10	5	10	5	1.7	2
7d		0.098	5	10	7.5	7.5	7.5	7.5	5		
5v		0.077	2.5	10	10	10	10	5	5		
5x		0.071	<1.25	5	5	10	5	5	5		
7e		0.11	5	10	10	10	>20	20	5		
10a	$(CH_2)NHCOC_2H_5$	0.12	5	10	10	> 20	10	5	5		
10b	$(CH_2)_4NHCOCH_2Cl$	0.035	2.5	5	5	10	5	10	5		
10c		0.082	5	5	5	20	5	2.5	5	14.9	
11a		0.23	5	5	5	>20	5	5	5		
11b		0.075	2.5	>20	5	>20	5	2.5	5	13.7	
	norfloxacin	-	0.313	0.625	0.156	40	2.5	10	80	0.4	2.2
	ciprofloxacin	-	0.078	0.156	0.078	>20	0.625	5	20		
	vancomycin	-	0.313	0.313	0.313	0.625	0.625	1.25	>20		

*SD ±44%. **SD ±50%. ***MIC+ and MIC-, MIC values in the presence and absence, respectively, of added fetal calf serum.

significant activity of compounds bearing rather large 3-substituents, e.g. **7d**,**e**, demonstrates the high steric tolerance of substituents at this position in the drug: polymerase:DNA complex.

Antibacterial Activity in Vitro. The EMAU derivatives were screened for antibacterial activity against a panel of normal and antibiotic-resistant Gram+ bacteria and, as negative control, the Gram- organism *E. coli*. Table 1 summarizes the results for several related series of compounds that showed both potent enzyme inhibition and potent to moderate Gram+ antibacterial activity. (Results for many compounds that showed weak or no antibacterial activity are collected in Supporting Information.) The Gram− organism *E. coli* was resistant, as expected, to all compounds at the highest concentrations tested (data not shown).

Keeping in mind that reported MIC values may vary ±2-fold, 3-substituted-EMAU derivatives bearing hydroxyalkyl, methoxyalkyl, and acetoxyalkyl groups were most active. Derivatives with other neutral substituents, e.g. CN, CO_2R, retained good activity, but those with ionizable groups (CO_2H, NHR) were weaker. Amides and sulfonamides had intermediate activity. As found for the enzyme inhibitory activity, there was no direct steric effect of bulky 3-substituents in reducing antibacterial activity.

Among Gram+ strains, *B. subtilis* was more sensitive to active compounds than *S. aureus* and enterococcal strains. However, active compounds were uniformly active against all staphylococci and enterococci, irrespective of their sensitivity or resistance to known antibiotics. For example, MRSA B42876 and *E. fecium* are resistant to the fluoroquinolones norfloxacin and ciprofloxacin, and VRE, as expected, is resistant to vancomycin (Table 1).

Efficacy of antibiotics in vivo has generally been correlated to ratio of plasma concentration of free drug to MIC or "area under the curve" to MIC.[8] Because plasma protein binding can reduce free drug concentrations in vivo, and considering that the plasma concentration of an effective antibacterial drug should exceed MIC for some time, we retested several potent EMAU derivatives against two representative bacteria, *S. aureus* (Smith) and *E. faecalis*, in the presence of 50% fetal calf serum as a means of mimicking binding to plasma proteins. The results in Table 1 show that all compounds tested showed a "serum effect", i.e., higher MIC in the presence of serum, but the extents differed widely. Compounds with hydrophobic substituents had the greatest serum effect, whereas compounds with hydrophilic substituents, e.g. several morpholinylalkyl derivatives, had low serum effects. In particular, compound

Table 2. Effect of Ip Dosed Pol IIIC Inhibitors on Survival of *S. aureus* (Smith) ip-Infected Mice

compd (vehicle[a])	survivors ($n = 5$)[b] 10 mg/kg	20 mg/kg
controls (a or b)	0	-
vancomycin (a or b)	5	5
1c (a)	3	5
8d (a)	4	nd
5k (a)	2	nd
5m (a)	2	nd
13 (a)	2	4
7c (b)	4	5

[a] Dissolved in 10% DMSO/peanut oil (a) or phosphate buffered saline (PBS) (b). [b] Groups of five mice were infected ip with 10^8 CFU Smith strain in 0.5 mL of LB broth and dosed ip 15 min later; survivors were counted at 72 h post infection. nd, not done.

7c, the hydrochloride of 3-(*N*-morpholinylbutyl)-EMAU,[3] was a potent antibacterial compound with little effect of serum on its MIC values (Table 1). Because this compound was also highly water soluble, it became of interest to consider **7c** for parenteral testing in vivo.

In Vivo Studies in Mice. The choice of EMAU derivatives to evaluate for antibacterial activity in vivo could depend on several properties. Obviously the lowest MIC value is important, but the extent of protein binding, solubility in suitable vehicles, plasma half-life, and acute toxicity of test doses in a suitable animal species are important issues. In addition, breadth of activity against antibiotic-resistant organisms is a requirement for further development of an effective compound. We previously demonstrated activity of pol IIIC inhibitors in a mouse model of intraperitoneal (ip) infection with *S. aureus*.[2,7] Thus we selected the "best" antibacterial compounds from Table 1 to study further in this model.

Swiss-Webster mice were infected ip with 10^8 colony forming units (CFU) of *S. aureus* (Smith strain), and the survival was monitored for 72 h. Typically animals die within 10−18 h of the infection, and the positive control drug vancomycin, given ip or intravenously (iv), completely protects all animals for the 72 h observation period (Table 2). Test compounds were given by routes dependent upon both a suitable vehicle for that route and by results of dosing and distribution studies. Because of the poor water solubility of most compounds of Table 1, initial studies were done via the ip route with a vehicle consisting of 10% DMSO in peanut oil, a vehicle that we used previously to show efficacy of **1a** and **1b** and related compounds against ip *S. aureus* (Smith).[2] Table 2 summarizes the response of infected mice to ip doses of several pol IIIC inhibitors. At screening doses of 10 and 20 mg/kg, HB-EMAU (**1c**) protected 3/5 and 5/5 mice, respectively, consistent with its reported activity.[2] Potent antibacterials **7c** and **8d** protected 4/5 mice at 10 mg/kg, and **7c** completely protected mice at 20 mg/kg. Other potent enzyme inhibitors **5k** and **5m** were less effective, protecting 2/5 animals each. Compound **13** protected 4/5 animals at a dose of 20 mg/kg. The significant activity of the morpholinyl derivative **7c**, in contrast to the weaker activity of the hydrophobic compounds **5k** and **5m**, may be a result of the high water solubility of the former compound in peritoneal fluid, and, thus, its better distribution.

Pharmacokinetics of 7c. The combination of moderate antibacterial potency, low serum effect, good efficacy in the ip/ip mouse infection model, and high water solubility of **7c** prompted a detailed pharmacokinetic study of this compound. The uptake and distribution of **7c** in saline solution by the iv and subcutaneous (sc) routes in mice were compared with the corresponding parameters for **1c**. The latter compound is not sufficiently soluble in water or saline, however, and studies were done with **1c** dissolved in 30% aqueous HP-β-cyclodextrin (CDx). Before initiating pharmacokinetic studies, the acute toxicity of both compounds in their formulations was evaluated. Tail vein iv doses of **1c** higher than 20 mg/kg and sc doses higher than 60 mg/kg were lethal to mice, but sc doses of **7c** up to 400 mg/kg were well tolerated by mice.

Table 3. Pharmacokinetic Parameters of **1c** and **7c** in Mice

	1c[a] (mg/kg)			**7c**[b] (mg/kg)				
	iv	sc		iv	sc			po
	20	20	60	20	20	200	400	200
C_{max} (μg/mL)[c]	53	5.1	13	27.4	4.6	15.1	24.1	7.3
T_{max} (min)	-	15	15	-	5	30	30	60
$t_{1/2}$ (min)	33	29.3	nd	39	149	132	373	111
AUC (μg·min/mL)	288	466.5	1214	234	406	1613	3042	922
(0−t) (min)	(60)	(120)	(120)	(120)	(180)	(180)	(180)	(180)

[a] 2 mg/mL in 30% HP-β-cyclodextrin in water. [b] 4 mg/mL in PBS. [c] Calculated with Win-Nonlin software.

HPLC was used to measure the time-dependence of plasma concentrations of **1c** and **7c** following dosing by several routes (see Experimental Section for details). The results of Table 3 show that sc injection of **7c** resulted in dose-dependent increases in C_{max}, apparent plasma half-life, and exposure (area under the curve, AUC). Comparison between properties of 20 mg/kg doses of **7c** given iv and sc suggests complete uptake of the compound from the sc route and prolongation of the apparent elimination half-life, $t_{1/2}$. Oral dosing of **7c** resulted in about half the peak concentration compared with sc dosing (Table 3). Although the CDx formulation of **1c** permitted dosing at 20 mg/kg iv and 60 mg/kg sc, the low plasma concentrations and short $t_{1/2}$ of this compound are likely responsible for lack of efficacy of either regimen in ip infections of mice with *S. aureus* (Smith) (data not shown).

In addition to the peak corresponding to the parent compound **7c**, a second major peak in the HPLC of plasma samples from **7c**-dosed animals was observed at 4.17 min. The peak paralleled the rise and fall of parent compound and was a greater proportion of parent peak after oral than parenteral dosing. Fractions enriched in the second peak were concentrated and subjected to MS with an ion trap instrument. The metabolite had M + 1 = 361, compared with the M + 1 = 387 for **7c**, and both gave a MS2 peak at M + 1 = 300. On the basis of the likelihood that oxidation of the morpholine ring of **7c** would result in the 3-oxo intermediate, followed by elimination of acrolein, it was predicted that the metabolite was 3-[4-(2-hydroxyethyl)-aminobutyl]EMAU, **7a**. This prediction was proved by comparison of the HPLC and MS properties of the metabolite with those of authentic **7a**.

Antibacterial Efficacy of 7c in Vivo. Efficacy studies of **7c** were carried out in *S. aureus* (Smith) ip infected mice, based on the findings of the pharmacokinetic studies. The results of single oral (po) and sc doses are summarized in Figure 1. A po dose of 400 mg/

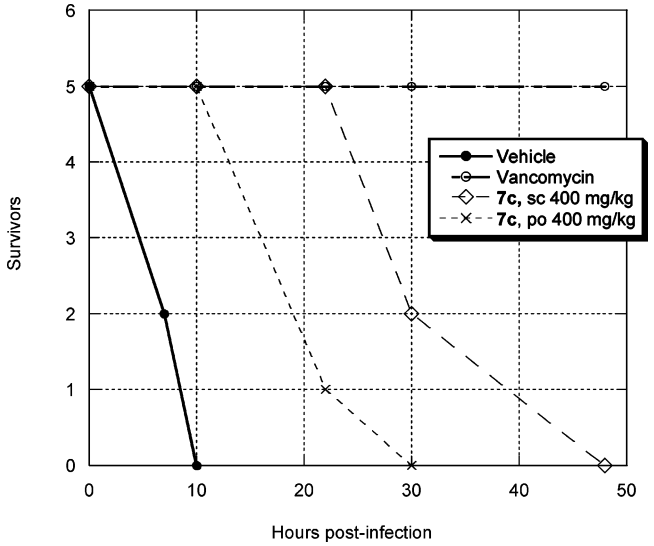


Figure 1. Comparison of oral (po) and sc dosing on antibacterial efficacy of **7c** in *S. aureus* (Smith) ip-infected mice. Vancomycin was given ip at 10 mg/kg.

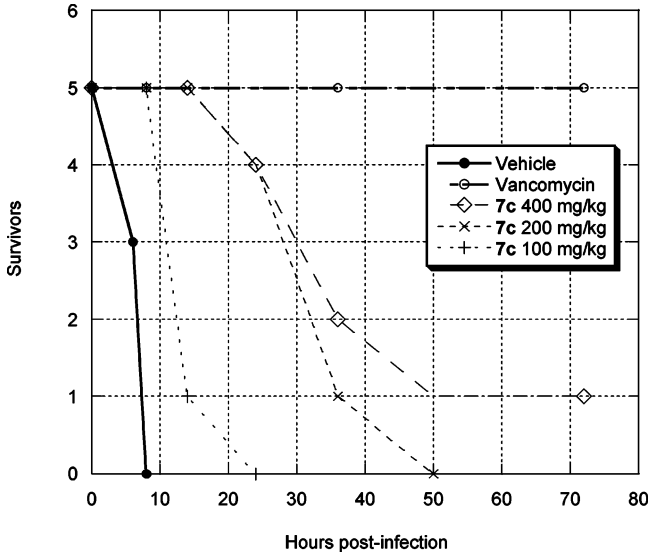


Figure 2. Effect of sc doses of **7c** on survival of *S. aureus* (Smith) ip-infected mice. Vancomycin was given ip at 10 mg/kg.

kg prolonged the life of mice about 3-fold relative to untreated animals, but a sc dose of 400 mg/kg significantly prolonged the life of the mice, although it was not curative. Subcutaneously dosed animals lived an average of 30 h, and untreated and orally dosed animals lived an average of only 6 and 18 h, respectively. The vancomycin-treated mice survived past the 48 h observation period. A dose−response to single sc doses of **7c** is observed in the results of Figure 2. Doses of 200 and 400 mg/kg were clearly more effective in prolonging life than 100 mg/kg, but again complete cures were not obtained.

The inability of **7c** to provide cures may be a result of its moderate MIC (Table 1) but may also result from its rapid conversion to the weaker hydroxyethylamino analogue **7a** in vivo. Thus, although the high solubility and low protein binding of **7c** were major advantages of the compound, its rapid conversion to a relatively inactive compound limits its promise as an antibacterial drug.

Discussion

Many 3-substituted EMAU derivatives are potent inhibitors of Gram+ pol IIIC, and some of these compounds have potent activity against Gram+ bacteria in culture. Together with our previous work[1,3] and that of other groups,[9,10] the present results confirm the targeted nature of 6-anilinouracils and related compounds as inhibitors of Gram+ DNA polymerase IIIC and the potential of this class of compounds for development as antibacterial agents.

Potency of 6-anilinouracils as inhibitors of pol IIIC is largely dictated by the structure of the 6-anilino group. The 3-ethyl-4-methylanilino group provides maximum enzyme affinity,[3,4] while the 3-substituents often enhance that activity, but to a limited extent (Table 1). However, potent pol IIIC inhibition is not always translated into potent antibacterial activity. Derivatives with highly polar or charged substituents show high enzyme inhibition potency but reduced or absent antibacterial activity. Derivatives with hydrophobic or less polar substituents show both potent enzyme and antibacterial activity. However, derivatives with hydrophobic substituents showed the highest apparent protein binding, a property that would reduce the free concentration of drug in plasma. In a simple antibacterial screen in mice, representative compounds with moderately polar substituents (**1c**, **8d**) were more potent than those with hydrophobic 3 substituents (**5k**, **5m**) in protecting animals from lethal *S. aureus* infection (Table 2). Paradoxically, the morpholinyl compound **7c** was highly active in this model (Table 2). Compound **7c** had a moderate level of antibacterial activity and low "serum effect" (Table 1), and because it was highly water soluble, it was studied by parenteral dosing in vivo. The compound was readily dissolved in saline and was well absorbed by the sc route, but less so by the oral route (Table 3). A comparison of single 400 mg/kg doses of **7c** in *S. aureus* (Smith) infected mice showed greater delay of death after sc than oral dosing (Figure 1). In addition, a consistent increase in delay of death was observed with increasing sc doses of **7c** (Figure 2). Demonstrating activity in vivo of the better antibacterial analogues of Table 1 which are *not* water soluble, however, will require development of formulations better suited for parenteral dosing.

Experimental Section

Materials. Most reagent chemicals and solvents were obtained from commercial sources. 3-Ethyl-4-methylaniline was prepared as described.[4] Preparation of several starting materials and reference compounds was published previously.[1,3] Proton NMR spectra were obtained in Me_2SO-d_6 solution with a Bruker Avance 300 spectrometer, unless indicated otherwise. Chemical shifts (δ) are in ppm from internal TMS. Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were done by the Microanalysis Laboratory, University of Massachusetts, Amherst. Values for C, H, and N were within 0.4% of calculated values except as noted. MS and LC-MS were obtained with a ThermoFinnigan LCQ Advantage instrument. HR-MS data were obtained with a FAB source on a Kratos MS50TCTA instrument equipped with a peak matching unit. General synthesis methods were also applied to preparation of compounds listed in Supporting Information.

General Method for the Preparation of 3-Substituted EMAUs. Step 1. 6-Amino-2-methoxy-3-substituted-4-pyrimidones. A mixture of 6-amino-2-methoxy-4-pyrimidone (1 equiv), potassium carbonate (1.2−2 equiv), benzyltriethylammonium chloride (0.2−1 equiv) and alkylating agent (1−5 equiv) in Me_2CO or MeCN was heated at 50−100 °C for 10−72 h. After cooling to room temperature, the insoluble salts

were removed by filtration and the solvent was evaporated. The residue was purified by chromatography on silica gel with $CHCl_3$:MeOH as eluent to give both 6-amino-2-methoxy-4-alkoxypyrimidine and 6-amino-2-methoxy-3-substituted-4-pyrimidone. Typical examples are shown below.

6-Amino-2-methoxy-3-(3-cyanopropyl)-4-pyrimidone, 4a. 4-Bromo-1-butyronitrile gave 6-amino-2-methoxy-4-(3-cyanopropoxy)pyrimidine (**3a**), which eluted first in 27% yield, Mp: 121−123 °C, followed by 6-amino-2-methoxy-3-(3-cyanopropyl)-4-pyrimidone (**4a**) (9.73 g, 66%), isolated as a white solid, Mp: 139−141 °C. **3a**: ^{1}H NMR: 1.95 (quintet, 2H), 2.50 (t, 2H), 3.75 (s, 3H), 4.21 (t, 2H), 5.38 (s, 1H), 6.23 (s, 2H). Anal. ($C_9H_{12}N_4O_2$) C, H, N. **4a**: ^{1}H NMR: 1.78 (m, 2H, CH_2), 2.50 (t, 2H, CH_2CN) 3.84 (t, 2H, CH_2N), 3.88 (s, 3H, CH_3O), 4.83 (s, 1H, 5-H), 6.44 (s, 2H, NH_2). Anal. ($C_9H_{12}N_4O_2$) C, H, N.

6-Amino-2-methoxy-3-(4-acetoxybutyl)-4-pyrimidone, 4b. Using 4-acetoxybutyl bromide the O4-isomer **3b** was obtained in 35% yield, Mp: 71−73 °C, and **4b** was obtained in 54% yield as a white solid, Mp: 135−136 °C. **3b**: ^{1}H NMR: 1.68 (m, 4H), 2.00 (s, 3H), 3.74 (s, 3H), 4.03 (t, 2H), 4.16 (t, 2H), 5.35 (s, 1H), 6.58 (s, 2H). Anal. ($C_{11}H_{17}N_3O_4$) C, H, N. **4b**: ^{1}H NMR: 1.52 (m, 4H, 2×CH_2), 2.0 (s, 3H, CH_3CO) 3.76 (t, 2H, CH_2O), 3.88 (s, 3H, CH_3N), 4.0 (t, 2H, CH_2O), 4.82 (s, 1H, 5-H), 6.41 (s, 2H, NH_2). Anal. ($C_{11}H_{17}N_3O_4$) C, H, N.

6-Amino-2-methoxy-3-(8-hydroxyoctyl)-4-pyrimidone, 4c. 8-Bromooctanol gave the O4 isomer **3c** in 32% yield, Mp: 86−87 °C, and **4c** in 34% yield, Mp: 96−97 °C. **3c**: ^{1}H NMR: 1.18−1.50 (m, 10 H, 5×CH_2), 1.64 (m, 2H, CH_2), 3.35 (m, 2 H, CH_2OH), 3.75 (t, 2 H, CH_2N), 4.13 (t, 2 H, CH_2O), 4.31 (t, 1H, OH), 5.38 (s, 1H, 5-H), 6.55 (br, 2 H, NH_2). Anal. ($C_{13}H_{23}N_3O_3$) C, H, N. **4c**: ^{1}H NMR: 1.14−1.58 (m, 12 H, 6×CH_2), 3.35 (m, 2 H, CH_2OH), 3.73 (t, 2 H, CH_2N), 3.88 (s, 3 H, CH_3O), 4.30 (t, 1 H, OH), 4.82 (s, 1 H, 5-H), 6.34 (br, 2 H, NH_2). Anal. ($C_{13}H_{23}N_3O_3$) C, H, N.

6-Amino-2-methoxy-3-[(4-ethoxycarbonyl)butyl]-4-pyrimidone, 4d. Ethyl 5-bromovalerate gave the O4 isomer **3d** in 34% yield, Mp: 69−71 °C, and **4d** in 37% yield, Mp: 75−76 °C. **3d**: ^{1}H NMR: 1.17 (t, 3 H, C*H*$_3$CH$_2$), 1.58−1.64 (m, 4 H, 2×CH_2), 2.33 (t, 2 H, CH_2CO_2), 3.74 (s, 3 H, CH_3O), 4.04 (q, 2 H, CH_2O), 4.14 (t, 2 H, CH_2O), 5.35 (s, 1 H, C_5−H), 6.60 (br, 2 H, NH_2). Anal. ($C_{12}H_{19}N_3O_4$) C, H, N. **4d**: ^{1}H NMR 1.17 (t, 3 H, C*H*$_3$CH$_2$), 1.47 (m, 4 H, 2×CH_2), 2.30 (t, 2 H, CH_2CO_2), 3.73 (t, 2 H, CH_2N), 3.87 (s, 3 H, CH_3O), 4.04 (q, 2 H, CH_2O), 4.81 (s, 1 H, 5-H), 6.39 (br, 2 H, NH_2). Anal. ($C_{12}H_{19}N_3O_4$) C, H, N.

Step 2. 3-Substituted-6-(3-ethyl-4-methylanilino)uracils. A stirred mixture of the 6-amino-2-methoxy-3-alkyl-4-pyrimidone **4** (1 equiv), 3-ethyl-4-methylaniline hydrochloride (1.1−1.5 equiv), and a few drops of 3-ethyl-4-methylaniline was heated at 120−170 °C for 10−180 min. After cooling to room temperature, the residue was either dissolved in MeOH:$CHCl_3$, or water was added and the mixture was extracted with $CHCl_3$. The combined organic layers were dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel with MeOH:$CHCl_3$ as eluent to give target compounds.

3-(4-Acetoxybutyl)-6-(3-ethyl-4-methylanilino)uracil, 5i. A stirred mixture of **4b** (15 g, 59 mmol), 3-ethyl-4-methylaniline hydrochloride (12.1 g, 75 mmol), and 3-ethyl-4-methylaniline (4.0 g, 29 mmol) was heated in an oil bath at 160 °C for 15 min. After cooling to room temperature, the residue was dissolved in 1:1 MeOH:$CHCl_3$, and the solution was evaporated with silica gel. The material was placed atop a silica gel column and eluted with 4% MeOH in $CHCl_3$ to give crude product. Trituration with 1:1 Me_2CO:Et_2O gave colorless crystals of product (17.8 g, 84%). Mp: 190−191 °C. ^{1}H NMR: 1.14 (t, 3H, C*H*$_3$CH$_2$Ar), 1.53 (m, 4H, 2×CH_2), 2.0 (s, 3H, CH_3CO), 2.24 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 3.71 (t, 2H, CH_2O),3.99 (t, 2H, CH_2N), 4.73 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.43 (s, 1H, NH). Anal. ($C_{19}H_{25}N_3O_4$) C, H, N.

3-(4-Ethoxycarbonylbutyl)-6-(3-ethyl-4-methylanilino)-uracil, 5p. A mixture of **4d** (608 mg, 2.26 mmol), 3-ethyl-4-methylaniline hydrochloride (430 mg, 2.5 mmol), and a few drops of 3-ethyl-4-methylaniline was heated at 160 °C for 3 h. Chromatography gave 632 mg (75% yield) of product. Crystallization from EtOH gave white crystals. Mp: 185−186 °C. ^{1}H NMR: 1.11−1.19 (m, 6H, 2×CH_3), 1.49 (m, 4H, 2×CH_2), 2.24 (s, 3H, CH_3Ar), 2.30 (t, 2H, CH_2CO_2Et), 2.57 (q, 2H, CH_2Ar), 3.69 (t, 2H, CH_2N), 4.04 (q, 2H, CO_2CH_2), 4.72 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.16 (s, 1H, NH), 10.48 (s, 1H, NH). Anal. ($C_{20}H_{27}N_3O_4$) C, H, N.

3-(4,5-Dihydroxypentyl)-6-(3-ethyl-4-methylanilino)-uracil, 5a. Yield: 79%. Mp: 189 °C. ^{1}H NMR: 1.12 (t, 3H, $ArCH_2CH_3$), 1.30−1.68 (m, 4H, CH_2CH_2), 2.13 (s, 3H, $ArCH_3$), 2.56 (q, 2H, $ArCH_2$), 3.12−3.30 (m, 3H, CH_2O, CHO), 3.70 (t, 2H, NCH_2), 4.42 (t, 2H, 2×OH), 4.76 (s, 1H, 5-H), 6.90−7.14 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.35 (s, 1H, NH). Anal. ($C_{18}H_{25}N_3O_4$) C, H, N.

3-(8-Hydroxyoctyl)-6-(3-ethyl-4-methylanilino)uracil, 5b. Yield: 78%. Mp: 155−157 °C. ^{1}H NMR: 1.14 (t, 3H, C*H*$_3$CH$_2$Ar), 1.20−1.30 (m, 8H, 4×CH_2), 1.37−1.52 (m, 4H, 2×CH_2), 2.21 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 3.35 (m, 2H, CH_2O), 3.64 (t, 2H, CH_2N), 4.30 (t, 1H, OH), 4.69 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.05 (s, 1H, NH), 10.35 (s, 1H, NH). Anal. ($C_{21}H_{31}N_3O_3$) C, H, N.

3-(9-Hydroxynonyl)-6-(3-ethyl-4-methylanilino)uracil, 5c. Yield: 74%. Mp: 139−141 °C. ^{1}H NMR: δ 1.14 (t, 3 H, C*H*$_3$CH$_2$Ar), 1.25 (m, 10 H, 5×CH_2), 1.35−1.51 (m, 4 H, 2×CH_2), 2.24(s, 3 H, CH_3Ar), 2.57 (q, 2 H, CH_2Ar), 3.29−3.39 (m, 2H, CH_2OH), 3.66 (t, 2 H, CH_2N), 4.33 (t, 1 H, OH), 4.72 (s, 1 H, 5-H), 6.92−7.15 (m, 3 H, Ar), 8.09 (br, 1 H, NH), 10.39 (s, 1 H, NH). Anal. ($C_{22}H_{33}N_3O_3$) C, H, N.

3-(10-Hydroxydecyl)-6-(3-ethyl-4-methylanilino)uracil, 5d. Yield: 94% yield. Mp: 105−106 °C. ^{1}H NMR: δ 1.14 (t, 3 H, C*H*$_3$CH$_2$Ar), 1.24 (m, 12 H, 6×CH_2), 1.30−1.50 (m, 4 H, 2×CH_2), 2.23(s, 3 H, CH_3Ar), 2.57 (q, 2 H, CH_2Ar), 3.36 (m, 2 H, C*H*$_2$OH), 3.66 (t, 2 H, CH_2N), 4.32 (t, 1 H, OH), 4.71 (s, 1 H, C_5−H), 6.92−7.12 (m, 3 H, Ar), 8.07 (br, 1 H, NH), 10.37 (s, 1 H, NH). Anal. ($C_{23}H_{35}N_3O_3$) C, H, N.

3-(4-Hydroxy-2-butynyl)-6-(3-ethyl-4-methylanilino)uracil, 5e. Yield: 41%. Mp: 152 °C (dec). ^{1}H NMR: 1.14 (t, 3H, $ArCH_2CH_3$), 2.23 (s, 3H, $ArCH_3$), 2.57 (q, 2H, $ArCH_2$), 4.02 (d, 2H, OCH_2), 4.47 (m, 2H, NCH_2), 4.72 (s, 1H, 5-H), 5.12 (t, 1H, OH), 6.93−7.13 (m, 3H, ArH), 8.19 (S, 1H, NH), 10.60 (s, 1H, NH). HRMS: calcd for $C_{17}H_{20}N_3O_3$ (M + 1), 314.1505; observed, 314.1519.

3-{2-[2-(2-Hydroxyethoxy)ethoxy]ethyl}-6-(3-ethyl-4-methylanilino)uracil, 5f. Yield: 72%. Mp: 118−119 °C. ^{1}H NMR: 1.14 (t, 3H, CH_3C), 2.24 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 3.32−3.50 (m, 10H, 5×CH_2O), 3.87 (t, 2H, CH_2N), 4.56 (t, 1H, OH), 4.72 (s, 1H, 5-H), 6.92−7.16 (m, 3H, ArH), 8.16 (s, 1H, NH), 10.50 (s, 1H, NH). Anal. ($C_{19}H_{27}N_3O_5$) C, H, N.

3-(2-Acetoxyethyl)-6-(3-ethyl-4-methylanilino)uracil, 5g. Yield: 73%. Mp: 187−188 °C (from MeOH). ^{1}H NMR: 1.14 (t, 3H, CH_3C), 1.94 (s, 3H, CH_3CO), 2.24 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 3.95 (t, 2H, NCH_2), 4.14 (t, 2H, OCH_2), 4.73 (s, 1H, 5-H), 6.95−7.15 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.47 (s, 1H, NH). Anal. ($C_{17}H_{21}N_3O_4$) C, H, N.

3-(3-Acetoxypropyl)-6-(3-ethyl-4-methylanilino)uracil, 5h. Yield: 54%. Mp: 184−186 °C (from MeOH). ^{1}H NMR: 1.14 (t, 3H, CH_3C), 1.81 (quin, 2H, CH_2), 1.98 (s, 3H, CH_3CO), 2.24 (s, 3H, CH_3Ar), 2.55 (q, 2H, CH_2C), 3.78 (t, 2H, CH_2N), 3.98 (t, 2H, CH_2O), 4.73 (s, 1H, 5-H), 6.95−7.14 (m, 3H, ArH), 8.09 (s, 1H, NH), 10.41 (s, 1H, NH). Anal. ($C_{18}H_{23}N_3O_4$) C, H, N.

3-(5-Acetoxypentyl)-6-(3-ethyl-4-methylanilino)uracil, 5j. Yield: 81% yield. Mp: 173−175 °C (from MeOH). ^{1}H NMR: 1.14 (t, 3H, CH_3C), 1.29 (quin, 2H, CH_2), 1.54 (m, 4H, 2×CH_2), 2.00 (s, 3H, CH_3CO), 2.24 (s, 3H, CH_3Ar), 2.55 (q, 2H, CH_2C), 3.68 (t, 2H, CH_2N), 3.97 (t, 2H, CH_2O), 4.73 (s, 1H, 5-H), 6.95−7.14 (m, 3H, ArH), 8.06 (s, 1H, NH), 10.37 (s, 1H, NH). Anal. ($C_{20}H_{27}N_3O_4$) C, H, N.

3-[2-(2-Methoxyethoxy)ethyl]-6-(3-ethyl-4-methylanilino)uracil, 5k. Yield: 74%. Mp: 160−161 °C. ^{1}H NMR: δ 1.13 (t, 3H, C*H*$_3$CH$_2$), 2.24 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 3.22 (s, 3H, CH_3O), 3.37 (m, 2H, CH_2O), 3.49 (m, 4H, 2×CH_2O), 3.85 (t, 3H, CH_2N), 4.70 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.40 (s, 1H, NH). Anal. ($C_{18}H_{25}N_3O_4$) C, H, N.

3-[2-(2-Benzyloxyethoxy)ethyl]-6-(3-ethyl-4-methylanilino)uracil, 5l. Yield: 72%. Mp: 151−152 °C. ^{1}H NMR: 1.13 (t, 3H, C*H*$_3$CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.57 (q, 2H, CH$_2$Ar), 3.53 (m, 6H, 3×CH$_2$), 3.88 (t, 2H, CH$_2$N), 4.47 (s, 2H, PhCH$_2$), 4.72 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 7.25−7.36 (m, 5H, PhH), 8.16 (s, 1H, NH), 10.49 (s, 1H, NH). Anal. (C$_{24}$H$_{29}$N$_3$O$_2$) C, H, N.

3-(3-Cyanopropyl)-6-(3-ethyl-4-methylanilino)uracil, 5m. Yield: 81% yield. Mp: 265−267 °C. ^{1}H NMR: 1.14 (t, 3H, C*H*$_3$CH$_2$Ar), 1.79 (m, 2H, CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.50 (t, 2H, C*H*$_2$CN), 2.57 (q, 2H, CH$_2$Ar), 3.79 (t, 2H, CH$_2$N), 4.74 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.47 (s, 1H, NH). Anal. (C$_{17}$H$_{20}$N$_4$O$_2$) C, H, N.

3-(4-Cyanobutyl)-6-(3-ethyl-4-methylanilino)uracil, 5n. Yield: 78%. Mp: 217−218 °C. ^{1}H NMR: 1.14 (t, 3H, CH$_3$C), 1.50−1.61 (m, 4H, 2×CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.58 (m, 4H, C*H*$_2$CN, CH$_2$Ar), 3.72(t, 2H, CH$_2$N), 4.73 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.45 (s, 1H, NH). Anal. (C$_{18}$H$_{22}$N$_4$O$_2$) C, H, N.

3-(3-Ethoxycarbonylpropyl)-6-(3-ethyl-4-methylanilino)uracil, 5o. Yield: 92%. Mp: 171−173 °C. ^{1}H NMR: 1.16 (m, 6H, 2×CH$_3$), 1.75 (m, 2H, CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.27 (t, 2H, CH$_2$CO$_2$Et), 2.57 (q, 2H, CH$_2$Ar), 3.72 (t, 2H, CH$_2$N), 4.03 (q, 2H, CO$_2$CH$_2$), 4.72 (s, 1H, C$_5$−H), 6.92−7.16 (m, 3H, ArH), 8.09 (s, 1H, NH), 10.40 (s, 1H, NH). Anal. (C$_{19}$H$_{25}$N$_3$O$_4$) C, H, N.

3-(2-Oxopropyl)-6-(3-ethyl-4-methylanilino)uracil, 5q. Yield: 59%. Mp: 212 °C (dec). ^{1}H NMR: 1.14 (t, 3H, ArCH$_2$C*H*$_3$), 2.14 (s, 3H, ArCH$_3$), 2.24 (s, 3H, CH$_3$CO), 2.56 (q, 2H, ArCH$_2$), 4.54 (s, 2H, NCH$_2$), 4.72 (s, 1H, C$_5$−H), 6.94−7.16 (m, 3H, ArH), 8.20 (s, 1H, NH), 10.59 (s, 1H, NH). Anal. (C$_{16}$H$_{19}$N$_3$O$_3$) H,N. C, calcd 63.77; found 64.43.

3-(4-Oxopentyl)-6-(3-ethyl-4-methylanilino)uracil, 5r. Yield: 32%. Mp: 223−225 °C. ^{1}H NMR: 1.17 (t, 3 H, C*H*$_3$CH$_2$Ar), 1.90 (t, 2 H, CH$_2$), 2.09(s, 3 H, CH$_3$CO), 2.28 (s, 3 H, CH$_3$Ar), 2.45 (t, 2 H, CH$_2$CO), 2.58 (q, 2 H, CH$_2$Ar), 3.87 (t, 2 H, CH$_2$N), 5.09 (s, 1 H, 5-H), 6.89−7.11 (m, 3 H, Ar), 7.46 (br, 1 H, NH), 10.12 (s, 1 H, NH). Anal. (C$_{18}$H$_{23}$N$_3$O$_3$) C, H, N.

3-[2-(*N*-Morpholinyl)ethyl]-6-(3-ethyl-4-methylanilino)-uracil, 5s. Yield: 75%. Mp: 221−223 °C. ^{1}H NMR: 1.14 (t, 3H, ArCH$_2$C*H*$_3$), 2.23 (s, 3H, ArCH$_3$), 2.40 (m, 4H, CH$_2$NCH$_2$), 2.58 (q, 2H, ArCH$_2$), 3.24 (m, 2H, CH$_2$N), 3.47 (m, 4H, CH$_2$OCH$_2$), 3.82 (m, 2H, NCH$_2$), 4.72 (s, 1H, 5-H), 6.90−7.18 (m, 3H, ArH), 8.14 (S, 1H, NH), 10.48 (s, 1H, NH). Anal. (C$_{19}$H$_{26}$N$_4$O$_3$) C, H, N.

3-[3-(*N*-Morpholinyl)propyl]-6-(3-ethyl-4-methylanilino)uracil, 5t. Yield: 78%. Mp: 220−222 °C. ^{1}H NMR: 1.16 (t, 3H, CH$_3$C), 1.68 (m, 2H, CH$_2$), 2.23 (s, 3H, ArCH$_3$), 2.22−2.38 (m, 6H, 3xNCH$_2$), 2.60 (q, 2H, ArCH$_2$), 3.59 (m, 4H, CH$_2$OCH$_2$), 3.78 (t, 2H, NCH$_2$), 4.75 (s, 1H, 5-H), 6.95−7.18 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.40 (s, 1H, NH). Anal. (C$_{20}$H$_{28}$N$_4$O$_3$·0.5H$_2$O) C, H, N.

3-[8-(*N*-Morpholinyl)octyl]-6-(3-ethyl-4-methylanilino)-uracil, 5u. Yield: 47%. Mp: 175−176 °C. ^{1}H NMR: 1.12 (t, 3H, ArCH$_2$C*H*$_3$), 1.20−1.52 (m, 12H, 6×CH$_2$), 2.20−2.32 (m, 7H, 2×CH$_2$, ArCH$_3$), 2.56 (q, 2H, ArCH$_2$), 3.30 (m, 2H, CH$_2$), 3.54 (m, 4H, 2×CH$_2$), 3.70 (t, 2H, NCH$_2$), 4.74 (s, 1H, 5-H), 6.90−7.14 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.35 (s, 1H, NH). Anal. (C$_{25}$H$_{38}$N$_4$O$_3$) C, H, N.

3-[2-(4-Benzoylpiperazinyl)ethyl]-6-(3-ethyl-4-methylanilino)uracil, 5v. Yield: 81%. Mp: 104−106 °C. ^{1}H NMR: 1.16 (t, 3H, ArCH$_2$C*H*$_3$), 2.24 (s, 3H, ArCH$_3$), 2.42−2.60 (m, 6H, NCH$_2$ × 2, ArCH$_2$), 3.30 (m, 4H, NCH$_2$ × 2), 3.59 (m, 2H, CH$_2$N), 3.83 (t, 2H, NCH$_2$), 4.72 (s, 1H, 5-H), 6.92−7.45 (m, 8H, ArH), 8.12 (S, 1H, NH), 10.45 (s, 1H, NH). Anal. (C$_{26}$H$_{31}$N$_5$O$_3$·0.75H$_2$O) C, H, N.

3-[(5-Ethoxycarbonyl)pentyl]-6-(3-ethyl-4-methylanilino)uracil, 5w. Yield: 75%. Mp: 160−161 °C. ^{1}H NMR: 1.16 (t, 6 H, 2×C*H*$_3$CH$_2$Ar), 1.22 (m, 2 H, CH$_2$), 1.42−1.52 (m, 4 H, 2×CH$_2$), 2.21 (s, 3 H, CH$_3$Ar), 2.24(t, 2 H, CH$_2$COO), 2.57 (q, 2 H, CH$_2$Ar), 3.64 (t, 2 H, CH$_2$N), 4.02 (q, 2 H, CH$_2$O), 4.69 (s, 1 H, 5-H), 6.92−7.15 (m, 3 H, Ar), 8.08 (br, 1 H, NH), 10.39 (s, 1 H, NH). Anal. (C$_{21}$H$_{29}$N$_3$O$_4$) C, H, N.

3-[4-(*N*-Morpholinylcarbonyloxy)butyl]-6-(3-ethyl-4-methylanilino)uracil, 5x. Yield 82%. Mp: 211−212 °C. ^{1}H NMR: 1.10 (t, 3H, ArCH$_2$C*H*$_3$), 1.55 (m, 4H, CH$_2$CH$_2$), 2.18 (s, 3H, ArCH$_3$), 2.56 (q, 2H, ArCH$_2$), 3.30 (m, 4H, 2×CH$_2$), 3.54 (m, 4H, 2×CH$_2$), 3.71 (t, 2H, NCH$_2$), 4.03 (t, 2H, CH$_2$COO), 4.74 (s, 1H, 5-H), 6.92−7.14 (m, 3H, ArH), 8.16 (s, 1H, NH), 10.45 (s, 1H, NH). Anal. (C$_{22}$H$_{30}$N$_4$O$_5$) C, H, N.

3-(4-Hydroxybutyl)-6-(3-ethyl-4-methylanilino)uracil, 1c. A stirred suspension of the acetoxy compound **5i** (10.5 g, 24 mmol) in MeOH (150 mL) was treated with concentrated NH$_4$OH (150 mL) at room temperature. After 30 min all solid dissolved, and the solution was stirred for 72 h. The solvent was removed, and the solid was coevaporated three times with MeOH and filtered from MeOH to give the product as a colorless solid (9.0 g, 97%), identical with an authentic sample.[3]

3-[2-(2-Hydroxyethoxy)ethyl]-6-(3-ethyl-4-methylanilino)uracil, 8d. A mixture of the benzyl ether **5l** (150 mg, 0.35 mmol) and 10% Pd/C (60 mg) in MeOH (40 mL) was stirred at room temperature under an atmosphere of H$_2$ for 12 h. 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 2−4% MeOH in CHCl$_3$ as eluent, to give 109 mg (92% yield) of product. Crystallization from EtOH gave white crystals. Mp: 153−154 °C. ^{1}H NMR: 1.14 (t, 3H, C*H*$_3$CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.57 (q, 2H, CH$_2$Ar), 3.53−3.40 (m, 6H, 3×CH$_2$), 3.87 (t, 2H, CH$_2$N), 4.56 (t, 1H, OH), 4.72 (s, 1H, 5-H), 6.92−7.16 (m, 3H, ArH), 8.16 (s, 1H, NH), 10.50 (s, 1H, NH). Anal. (C$_{17}$H$_{23}$N$_3$O$_4$) C, H, N

3-(5-Hydroxypentyl)-6-(3-ethyl-4-methylanilino)uracil, 8a. Method a. A solution of 1.0 M LiAlH$_4$ in tetrahydrofuran (THF) (1.5 mL) was added dropwise to a stirred solution of the ester **5p** (160 mg, 0.43 mmol) in anhydrous THF (30 mL) at room temperature. After stirring for 20 min, MeOH (5 mL) was added dropwise, and the solvents were removed. EtOH was added, the mixture was filtered, and the solid was washed carefully with EtOH. The solvent was removed, and the residue was purified by chromatography on silica gel with 2−4% MeOH in CHCl$_3$ as eluent to give 141 mg (99% yield) of product. Crystallization from 50% aqueous EtOH gave white crystals. Mp: 198−199 °C. ^{1}H NMR: 1.14 (t, 3H, C*H*$_3$CH$_2$Ar), 1.20−1.30 (m, 2H, CH$_2$), 1.37−1.52 (m, 4H, 2×CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.57 (q, 2H, CH$_2$Ar), 3.34 (t, 2H, CH$_2$O), 3.67 (t, 2H, CH$_2$N), 4.35 (t, 1H, OH), 4.72 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.18 (s, 1H, NH), 10.48 (s, 1H, NH). Anal. (C$_{18}$H$_{25}$N$_3$O$_3$) C, H, N. **Method b.** A suspension of the acetoxy compound **5j** (12.0 g, 32.1 mmol) in a mixture of MeOH (300 mL) and concentrated NH$_4$OH (200 mL) was stirred at room temperature for 65 h. Evaporation of solvents gave 9.2 g (86%) of product as a white powder, identical with the sample prepared by method a.

3-(6-Hydroxyhexyl)-6-(3-ethyl-4-methylanilino)uracil, 8b. Reduction of the ester **5w** by method a used above for **8a** gave the product in 94% yield. Mp: 145−147 °C. ^{1}H NMR: 1.14 (t, 3H, CH$_3$CH$_2$Ar), 1.17−1.48 (m, 8H, 4×CH$_2$), 2.24 (s, 3H, CH$_3$Ar), 2.57 (q, 2H, CH$_2$Ar), 3.34 (m, 2H, CH$_2$O), 3.67 (t, 2H, CH$_2$N), 4.35 (t, 1H, OH), 4.72 (s, 1H, C$_5$−H), 6.92−7.15 (m, 3H, ArH), 8.08 (s, 1H, NH), 10.39 (s, 1H, NH). Anal. (C$_{19}$H$_{27}$N$_3$O$_3$·0.25H$_2$O) C, H, N.

3-(3-Carboxypropyl)-6-(3-ethyl-4-methylanilino)uracil, 12. A mixture of the ester **5o** (80 mg, 0.22 mmol) and NaOH (10 mg, 0.25 mmol) in 10 mL of water and 10 mL of MeOH was stirred at reflux for 3 h. Evaporation of solvents and mixing the residue with 6 N hydrochloric acid gave a precipitate, which was filtered and washed with water. Drying gave 68 mg (92% yield) of product as white crystals. Mp: 229−230 °C. ^{1}H NMR: 1.14 (t, 3H, C*H*$_3$CH$_2$Ar), 1.17 (m, 2H, CH$_2$), 2.17 (t, 2H, C*H*$_2$CO$_2$H), 2.24 (s, 3H, CH$_3$Ar), 2.57 (q, 2H, CH$_2$Ar), 3.72 (t, 2H, CH$_2$N), 4.73 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.42 (s, 1H, NH), 10.80 (s, 1H, NH), 12.05 (s, 1H, CO$_2$H). Anal. (C$_{17}$H$_{21}$N$_3$O$_4$·0.25H$_2$O) C, H, N.

3-(4-Hydroxy-2-butenyl)-6-(3-ethyl-4-methylanilino)uracil, 8c. A solution of 1.0 M BCl$_3$ in CH$_2$Cl$_2$ (3 mL) was added dropwise during 10 min to a stirred solution of 3-(4-benzyloxy-2-butenyl)-6-(3-ethyl-4-methylanilino)uracil (200 mg; see Sup-

porting Information) in CH_2Cl_2 (8 mL) at −78 °C under a N_2 atmosphere. The reaction mixture was stirred at −78 °C for 5 h, whereupon the reaction was quenched by a cautious addition of MeOH (5 mL) and 10% ammonia in MeOH (5 mL). The mixture was allowed to warm to room temperature, and the insoluble portion was filtered and washed with CH_2Cl_2:MeOH (4:1, 4 × 25 mL). The solvents were removed, and the residue was purified by chromatography on silica gel with 10−25% MeOH in CH_2Cl_2 as eluent to give 118 mg (72% yield) of product. Mp: 185−186 °C. ^{1}H NMR: 1.16 (t, 3H, $ArCH_2CH_3$), 2.22 (s, 3H, $ArCH_3$), 2.59 (q, 2H, $ArCH_2$), 4.15 (m, 2H, NCH_2), 4.38 (m, 2H, OCH_2), 4.66 (t, 1H, OH), 4.72 (s, 1H, C_5−H), 5.32 (m, 1H, =CH), 5.60 (m, 1H, =CH), 6.93−7.13 (m, 3H, ArH), 8.12 (s, 1H, NH), 10.46 (s, 1H, NH). Anal. ($C_{17}H_{21}N_3O_5$·0.25H_2O) C, H, N.

3-(4-Aminobutyl)-6-(3-ethyl-4-methylanilino)uracil Hydrochloride, 9a. A solution of 0.5 M $LiAlH_4$ in diglyme (3 equiv) was added dropwise to a stirred solution of the nitrile **5m** (1 equiv) in anhydrous diglyme at room temperature. The reaction mixture was stirred at room temperature until disappearance of the starting material (TLC). MeOH was added dropwise, and the solvents were removed. EtOH was added, the mixture filtered, and the solid washed with EtOH. The solvent was removed, and the residue was purified by chromatography on silica gel with $CHCl_3$:MeOH as eluent, to give 3-(4-aminobutyl)-6-(3-ethyl-4-methylanilino)uracil (91% yield). This compound was dissolved in $CHCl_3$ and MeOH, and a solution of 4 M HCl in dioxane was added. The mixture was stirred at room temperature for 1 h, and the solvents were removed to give the hydrochloride **9a** as a white solid, identical with an authentic sample.[3]

3-(5-Aminopentyl)-6-(3-ethyl-4-methylanilino)uracil Hydrochloride, 9b. Reduction ofthe nitrile **5n** and conversion to the hydrochloride, as described for **9a**, gave the product in 64% yield as a hygroscopic white solid. ^{1}H NMR: 1.12 (t, 3H, CH_3CH_2Ar), 1.25−1.70 (m, 6H, 3×CH_2), 2.21 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 2.76 (m, 2H, CH_2NH_2), 3.68 (t, 2H, CH_2N), 4.75 (s, 1H, 5-H), 6.92−7.15 (m, 3H, ArH), 8.30 (br, 3H, NH_3), 9.60 (s, 1H, NH), 10.95 (s, 1H, NH). HRMS: calcd for $C_{18}H_{27}N_4O_2$ (M + 1), 331.2134; observed, 331.2139.

General method for the Preparation of 3-(Carboxamidoalkyl)-6-(3-ethyl-4-methylanilino)uracils. The appropriate acyl chloride (1.2−3.3 equiv) was added to a solution of **9a** or **9b** (100 mg, 1 equiv) in dry 1,2-dichloroethane or EtOH. Triethylamine (2.4−10 equiv) was added, and the solution was stirred at room temperature for 3−3.5 h. The solvent was removed in vacuo, and the residue was applied to a silica gel column. Products were eluted with MeOH:$CHCl_3$.

3-[4-(Propionamido)butyl]-6-(3-ethyl-4-methylanilino)-uracil, 10a. Yield: 63%. Mp: 214−215 °C (from MeOH). ^{1}H NMR: 0.98 (t, 3H, CH_3C), 1.14 (t, 3H, CH_3), 1.36, 1.47 (quin, 4H, 2×CH_2), 2.04 (q, 2H, CH_2C), 2.25 (s, 3H, CH_3Ar), 2.58 (q, 2H, CH_2NH), 3.02 (q, 2H, CH_2NH), 3.68 (t, 2H, CH_2N), 4.73 (s, 1H, C_5−H), 6.95−7.15 (m, 3H, ArH), 7.74 (t, 1H, $NHCH_2$), 8.10 (s, 1H, NH), 10.41 (s, 1H, NH). Anal. ($C_{20}H_{28}N_4O_3$) C, H, N.

3-[4-(Chloroacetamido)butyl]-6-(3-ethyl-4-methylanilino)uracil, 10b. Yield: 50%. Mp: 191−192 °C (from MeOH). ^{1}H NMR: 1.14 (t, 3H, CH_3C), 1.38−1.49 (m, 4H, 2×CH_2), 2.24 (s, 3H, CH_3Ar), 2.55 (q, 2H, CH_2N), 3.09 (q, 2H, CH_2C), 3.69 (t, 2H, CH_2NH), 4.03 (s, 2H, CH_2Cl), 4.73 (s, 1H, C_5−H), 6.95−7.15 (m, 3H, ArH), 8.07 (s, 1H, NH), 8.19 (t, 1H, $NHCH_2$), 10.37 (s, 1H, NH). Anal. ($C_{19}H_{25}ClN_4O_3$) C, H, N.

3-{4-[(Cyclopropanecarboxamido]butyl}-6-(3-ethyl-4-methylanilino)uracil, 10c. Yield: 66%. Mp: 224−225 °C. ^{1}H NMR: 0.64 (m, 4H, $(CH_2)_2$), 1.13 (t, 3H, CH_3Ar), 1.35 (quin, 2H, CH_2), 1.48 (m, 3H, CH_3C), 2.23 (s, 3H, CH_3Ar), 2.55 (q, 2H, CH_2N), 3.03 (q, 2H, CH_2C), 3.69 (t, 2H, CH_2NH), 4.72 (s, 1H, C_5−H), 6.93 (d, 1H, CH), 6.97−7.14 (m, 3H, ArH), 8.02 (t, 1H, $NHCH_2$), 8.10 (s, 1H, NH), 10.41 (s, 1H, NH). Anal. ($C_{21}H_{28}N_4O_3$) C, H, N.

General Method for the Preparation of 3-(Sulfonamidoalkyl)-6-(3-ethyl-4-methylanilino)uracils. The appropriate sulfonyl chloride (1.1 equiv) was added to a solution of **9a** or **9b** (100 mg, 1 equiv) in EtOH (10 mL) containing triethylamine (2.5 equiv) or dry pyridine (10 mL) at room temperature. The mixture was stirred at room temperature or at 50 °C for 3−15 h. The reaction mixture was concentrated under vacuum, and the residue was applied to a silica gel column. Elution with MeOH:$CHCl_3$ yielded products which were crystallized from MeOH or Me_2CO.

3-[4-(4-Toluenesulfonamido)butyl]-6-(3-ethyl-4-methylanilino)uracil, 11a. Yield: 33%. Mp: 197−199 °C. ^{1}H NMR: 1.14 (t, 3H), 1.32 (quintet, 2H), 1.44 (quintet, 2H), 2.03 (s, 3H), 2.37 (s, 3H), 2.58 (q, 2H), 2.68 (q, 2H), 3.63 (t, 2H), 4.71 (s, 1H), 6.92 (d, 1H), 6.97 (s, 1H), 7.14 (d, 1H), 7.38 (d, 2H), 7.50 (t, 1H), 7.65 (d, 2H), 8.10 (s, 1H), 10.41 (s, 1H). Anal. ($C_{24}H_{30}N_4O_4S$·0.5H_2O) C, H, N.

3-[4-(2-Thiophenesulfonamido)butyl]-6-(3-ethyl-4-methylanilino)uracil, 11b. Yield: 52%. Mp: 183−185 °C. ^{1}H NMR: 1.14 (t, 3H), 1.35 (quintet, 2H), 1.47 (quintet, 2H), 2.23 (s, 3H), 2.56 (q, 2H), 2.79 (q, 2H), 3.65 (t, 2H), 4.72 (s, 1H), 6.93 (dd, 1H), 6.98 (d, 1H), 7.17 (m, 2H), 7.56 (d, 2H), 7.80 (t, 1H), 7.90 (d, 2H), 8.10 (s, 1H), 10.42 (s, 1H). Anal. ($C_{21}H_{26}N_4O_4S_2$) C, H, N.

3-(5-Iodopentyl)-6-(3-ethyl-4-methylanilino)uracil, 6b. A stirred suspension of the hydroxy compound **8a** (3.4 g, 10.3 mmol) in $CHCl_3$ (100 mL) was treated with iodotrimethylsilane (4 equiv) at room temperature. The solid disappeared to give a yellow solution. The solution was heated under reflux for 14 h, and after cooling to room temperature, the reaction was quenched with saturated aqueous Na_2SO_3. The mixture was extracted with $CHCl_3$ (3 × 50 mL), and the combined organic extracts were evaporated under reduced pressure to give a white slurry. The solid was filtered, washed with water and acetone, and dried to give 4.3 g (95%) of product. Mp: 201−203 °C (from MeOH). ^{1}H NMR: 1.14 (t, 3H, CH_3C), 1.32−1.48 (m, 6H, 3×CH_2), 1.77 (quin, 2H, CH_2), 2.24 (s, 3H, CH_3Ar), 2.54 (q, 2H, CH_2C), 3.27 (t, 2H, CH_2N), 3.68 (t, 2H, CH_2I), 4.73 (s, 1H, C_5−H), 6.95−7.14 (m, 3H, ArH), 8.06 (s, 1H, NH), 10.37 (s, 1H, NH). Anal. ($C_{18}H_{24}IN_3O_2$) C, H, N.

General Method for the Reaction of 3-(Iodoalkyl)-EMAUs with Nucleophiles. A mixture of **6a** or **6b**, K_2CO_3, and nucleophile in a suitable solvent (Me_2CO, MeCN, DMF) was stirred at room temperature. Once the reaction was complete, as monitored by 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 MeOH:$CHCl_3$ as eluent to give the product.

3-{[4-(2-Hydroxyethyl)amino]butyl}-6-(3-ethyl-4-methylanilino)uracil, 7a. Yield: 77%. Mp: 140 °C (dec). ^{1}H NMR: 1.10 (t, 3H, CH_3CH_2Ar), 1.58 (m, 4H, 2×CH_2), 2.18 (s, 3H, CH_3Ar), 2.56 (q, 2H, CH_2Ar), 2.91 (m, 4H, 2×CH_2N), 3.30 (s, 1H, NH), 3.60 (m, 2H, CH_2O), 3.73 (t, 2H, CH_2N), 4.72 (s, 1H, 5-H), 5.22 (t, 1H, OH), 6.88−7.12 (m, 3H, ArH), 8.22 (s, 1H, NH), 10.40 (br s, 1H, NH). HRMS: calcd for $C_{19}H_{29}N_4O_3$ (M + 1), 361.2240; found 361.2236.

3-{[4-Bis(2-hydroxyethyl)amino]butyl}-6-(3-ethyl-4-methylanilino)uracil, 7b. Yield: 42%. ^{1}H NMR: 1.12 (t, 3H, CH_3CH_2Ar), 1.51 (m, 4H, 2×CH_2), 2.21 (s, 3H, CH_3Ar), 2.57 (q, 2H, CH_2Ar), 3.0 (m, 6H, 3×CH_2N), 3.62−3.78 (m, 6H, 2×CH_2O, CH_2N), 4.72 (s, 1H, 5-H), 4.90 (br, 2H, OH), 6.92−7.15 (m, 3H, ArH), 8.10 (s, 1H, NH), 10.40 (br s, 1H, NH). HRMS: calcd for $C_{21}H_{33}N_4O_4$ (M + 1), 405.2502; found 405.2488.

3-{5-[1-(4-(4-Fluorophenyl)-1,2,3,6-tetrahydropyridinyl)]-pentyl}-6-(3-ethyl-4-methylanilino)uracil, 7d. Yield: 76%. ^{1}H NMR (400 MHz, DMSO-d_6): 1.1 (t, 3H, $ArCH_2CH_3$), 1.25−1.3 (m, 2H, CH2), 1.49−1.55 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 2.2 (s, 3H, $ArCH_3$), 2.53 (q, 2H, $ArCH_2CH_3$), 2.63−2.72 (m, 2H, CH_2), 3.12−3.25 (m, 3H, CHN and NCH_2), 3.66−3.7 (m, 4H, 2xNCH_2), 3.95 (m, 1H, CHN), 4.69 (s, 1H, C5−H), 6.13 (s, 1H, CH), 6.90−6.94 (m, 2H, ArH), 7.13 (d, 1H, ArH), 7.17−7.21 (m, 2H, ArH), 7.49−7.51 (m, 2H, ArH), 8.07 (s, 1H, NH), 9.44 (s, 1H, NH). HRMS: calcd for $C_{29}H_{36}FN_4O_2$, 491.2822; found 491.2835.

3-[5-(6-Methoxy-2,3,4,9-tetrahydro-1*H*-β-carboline-2-yl)-pentyl]-6-(3-ethyl-4-methylanilino)uracil, 7e. Yield: 36%. ^{1}H NMR (400 MHz, CD_3OD): 1.20 (t, 3H, $ArCH_2CH_3$), 1.42 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 1.81 (m, 2H, CH_2), 2.29 (s, 3H, $ArCH_3$), 2.63 (q, 2H, $ArCH_2CH_3$), 2.94−3.03 (m, 4H, NCH_2 and CH_2), 3.31 (m, 2H, NCH_2), 3.79 (s, 3H, OCH_3), 3.87 (m, 2H, NCH_2), 4.13 (m, 2H, NCH_2), 4.88 (s, 1H, C5−H), 6.74 (dd, 1H, ArH), 6.93−7.0 (m, 3H, ArH), 7.15−7.2 (m, 2H, ArH). HRMS: calcd for $C_{30}H_{38}N_5O_3$, 516.2975; found 516.2965.

3-[4-(*N*-Morpholinyl)butyl]-6-(3-ethyl-4-methylanilino)-uracil hydrochloride, 7c. A solution of 3-[4-(*N*-morpholinyl)-butyl]-6-(3-ethyl-4-methylanilino)uracil[3] (**13**) (772 mg, 2 mmol) in 5% MeOH:$CHCl_3$ (20 mL) was treated with 1 mL of 4.0 M HCl in dioxane. The mixture was stirred at room temperature for 1 h. After removal of the solvent, the residue was dried in vacuo to provide the product in 98% yield. ^{1}H NMR: 1.16 (t, 3H, $ArCH_2CH_3$), 1.48−1.55 (m, 4H, CH_2CH_2), 2.26 (s, 3H, $ArCH_3$), 2.60 (q, 2H, $ArCH_2$), 3.12 (m, 4H, $NCH_2 \times 2$), 3.38 (m, 2H, CH_2N), 3.80 (m, 4H, CH_2OCH_2), 3.90 (m, 2H, NCH_2), 4.72 (s, 1H, C5−H), 6.93−7.15 (m, 3H, ArH), 8.75 (s, 1H, NH), 10.58 (s, 1H, NH), 10.75 (s, 1H, NH^+). HRMS: calcd for $C_{21}H_{31}N_4O_3$(M-HCl+1), 387.2396; found 387.2391.

DNA Polymerase Assays. DNA polymerase IIIC (pol IIIC) of *B. subtilis* was the homogeneous recombinant protein expressed and prepared as described previously.[11] The enzyme was assayed in a 96-well plate format by using activated calf thymus DNA as a substrate according to the method of Barnes and Brown.[12] Briefly, enzyme was added to a buffered mixture containing Mg^{2+}, dithiothreitol, glycerol, dATP, dCTP, dTTP, [^{3}H]dTTP, and activated calf thymus DNA. Serial dilutions of compounds (in DMSO) were added to the plates. Assays were initiated by the addition of 0.025−0.06 units of enzyme (1 unit is the amount required to incorporate 250 pmol of [^{3}H]dTMP under standard assay conditions), 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 sequentially with dilute aqueous HCl and EtOH, dried, and counted in a liquid scintillation counter. Apparent inhibition constants (K_i values) were obtained directly in this "truncated" assay (lacking the competitor dGTP),[6] and had average standard deviation of ±44%.

Bacterial Strains. The standard panel for antibacterial screening included *S. aureus* 25923, *S. aureus* 13709 (Smith), *E. faecalis* 29212 and *E. faecium* 19434, all purchased from the American Type Culture Collection (ATCC, Manassas, VA). Methicillin-resistant *S. aureus* (MRSA B42876) and vancomycin-resistant *E. faecium* (VRE) are clinical isolates provided by Dr. Richard Ellison, University of Massachusetts Medical School. *B. subtilis* (BD54) is a standard laboratory strain. *E. coli* (J-53) was provided by Dr. Martin Marinus, University of Massachusetts Medical School.

Determination of Minimum Inhibitory Concentrations. Minimum inhibitory concentrations (MIC) of compounds were measured using a modification of the microdilution method outlined by the National Committee for Clinical Laboratory Standards (NCCLS guidelines M7-A4, vol. 17, 1997). Log-phase bacterial cultures were grown in Luria broth (LB) for *B. subtilis* and staphylococci, and in brain-heart infusion broth (BHIB) for enterococci. Diluted cultures were seeded into 96-well plates (200 μL/well) at a concentration of $(3-7) \times 10^5$ colony forming units (CFU)/mL. Each compound was tested in duplicate assays by diluting stock solutions 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 with shaking, and bacterial growth was determined by measuring optical density (600 nm, 1 cm path length) in a microplate reader. MIC values for antimicrobial drug-treated cultures are the lowest concentrations of test compounds at which growth was not apparent (less than 25% of the DMSO control), and reported values varied ±2-fold. Experiments to determine serum effects were carried out in the presence of 50% fetal calf serum added to the growth medium.

Experiments in Mice. Pathogen-free Swiss-Webster mice (males, 20−24 g) were purchased from Taconic Farms (Germantown, NY). The animals were housed at the University of Massachusetts Medical School (UMMS) Animal Medicine facility. All animal experiments were approved by the UMMS institutional animal care and use committee. Mice were allowed free access to food and water throughout the study.

Pharmacokinetics. Compound **1c** was dissolved in a 30% solution of CDx in water. Compound **7c** was dissolved in phosphate buffered saline (PBS), and the pH was adjusted to neutral with aqueous KOH prior to dosing. Mice were given **1c** or **7c** as an iv bolus of 20 mg/kg by tail vein in a volume of 5 mL/kg. Blood samples were taken from groups of three animals before and 2, 5, 15, 30, 60, and 120 min after dosing. For sc injection, mice were given drug in single doses of 20 and 60 mg/kg (**1c**) or 20, 200, and 400 mg/kg (**7c**). Blood samples were taken from groups of three animals before and 5, 15, 30, 60, 120, and 180 min after dosing. (A 360 min sample was included for the animals receiving 400 mg/kg). For po dosing, mice were given drug in a single dose of 200 mg/kg by gavage. Blood samples were taken before and 15, 30, 60, 120, and 180 min after dosing. At the specified time-points, mice were anesthetized with halothane, and blood was collected by cardiac puncture and placed in heparinized tubes. Plasma was harvested by centrifugation at 13 000 rpm in a Microfuge 18 Centrifuge (Beckman Instruments) and stored at −20 °C until analyzed.

A 0.3 mL aliquot of each plasma sample was transferred to a microcentrifuge tube, and an equal volume of MeCN was added. The samples were mixed well by vortexing and centrifuged to precipitate protein. The supernatant of each sample was transferred to a new tube and evaporated in a SpeedVac evaporator. The residue was suspended in 200 μL of MeCN:H_2O (20:80) for analysis by HPLC. The HPLC system was a Varian Prostar System (Varian Analytical Instruments, Walnut Creek, CA) equipped with two model PS-210 pumps, a model 410 autosampler, a model 384 UV−Visible variable wavelength detector, and a computer using the Star Chromatography Workstation (5.52). The samples were analyzed on a 5 μm C18 reverse phase column, 4.6 × 150 mm (Microsorb, Varian). The mobile phase consisted of MeCH:H_2O:NEt_3:AcOH (25:74.7:0.2:0.1) at a flow rate of 1 mL/min and a detection wavelength of 282 nm. The concentration of **1c** or **7c** was determined by comparison of peak areas with those from a standard curve generated from plasma samples spiked with compound. The linear range was 1 to 40 μg/mL.

Mass spectra were measured on a ThermoFinnigan LCQ Advantage ion trap instrument by APC ionization. Samples were introduced by infusion from solutions in water or by HPLC in the above conditions.

Antibacterial Efficacy. *S. aureus* (Smith) was grown at 37 °C to log-phase in LB medium, and the CFU were determined using a nomogram relating CFU to optical density at 600 nm. Bacteria were washed in fresh cold LB and injected ip as a suspension in 0.5 mL of LB, ca. 10^8 CFU/animal. Groups of five or 10 mice were treated at 15 min postinfection and/or various times postinfection with vehicle, test compound in vehicle, or vancomycin hydrochloride (Vancocin, Lilly) in saline. Mice were returned to their cages and monitored for mortality for up to 72 h.

Acknowledgment. This work was supported by small business grant AI41260 from the National Institutes of Health and by Shire BioChem Inc., Laval, Canada.

Supporting Information Available: Syntheses, pol IIIC inhibition, and antibacterial activity of additional compounds; elemental analyses. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Tarantino, P. M., Jr.; Zhi, C.; Gambino, J. J.; Wright, G. E.; Brown, N. C. 6-Anilinouracil-based Inhibitors of *Bacillus subtilis* DNA Polymerase III: Antipolymerase and Antimicrobial Structure−Activity Relationships Based on Substitution at Uracil N3. *J. Med. Chem*. **1999**, *42*, 2035−2040.

(2) Tarantino, P. M., Jr.; Zhi, C.; Wright, G. E.; Brown, N. C. Inhibitors of DNA Polymerase III as Novel Antimicrobial Agents against Gram-positive Eubacteria. *Antimicrob. Agents Chemother*. **1999**, *43*, 1982−1987.

(3) Zhi, C.; Long, Z.-Y.; Gambino, J.; Xu, W.-C.; Brown, N. C.; Barnes, M.; Butler, M.; LaMarr, W.; Wright, G. E. Synthesis of Substituted 6-Aminouracils and Their Inhibition of DNA Polymerase IIIC and Gram-positive Bacterial Growth. *J. Med. Chem.* **2003**, *46*, 2731−2739.

(4) Wright, G. E.; Gambino, J. J. Quantitative Structure−Activity Relationships of 6-Anilinouracils as Inhibitors of *Bacillus Subtilis* DNA Polymerase III. *J. Med. Chem*. **1984**, *27*, 181−185.

(5) Müller, C. E.; Deters, D.; Dominik, A.; Pawlowski, M. Synthesis of Paraxanthine and Isoparaxanthine Analogues (1,7- and 1,9-Substituted Xanthine Derivatives). *Synthesis* **1998**, 1428−1436.

(6) Wright, G. E.; Brown, N. C. Inhibition of *Bacillus subtilis* DNA Polymerase III by Arylhydrazinopyrimidines: Novel Properties of 2-Thiouracil Derivatives. *Biochim. Biophys. Acta* **1976**, *432*, 37−48.

(7) Wright, G. E.; Brown, N. C. DNA polymerase III: A new target for antibiotic development. *Curr. Opin. Anti-inf. Invest. Drugs* **1999**, *1*, 45−48.

(8) Gunderson, B. W.; Ross, G. H.; Ibrahim, K. H.; Rotschafer, J. C. What Do We Really Know About Antibiotic Pharmacodynamics? *Pharmacotherapy* **2001**, *21*, 302s−318s.

(9) Ali, A.; Aster, S. D.; Graham D. W.; Patel, G. F.; Taylor, G. E.; Tolman, R. L.; Painter, R. E.; Silver, L. L.; Young, K.; Ellsworth, K.; Geissler, W.; Harris, G. S. Design and Synthesis of Novel Antibacterial Agents with Inhibitory Activity against DNA Polymerase III. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2185−2188.

(10) Kuhl, L.; Svenstrup, N.; Ladel, C.; Otteneder, M.; Binas, A.; Schiffer, G.; Brands, M.; Lampe, T.; Ziegelbauer, K.; Rübsamen-Waigmann, H.; Haebich, D.; Ehlert, K. Biological Characterization of Novel Inhibitors of the Gram-Positive DNA Polymerase IIIC Enzyme. *Antimicrob. Agents Chemother*. **2005**, *49*, 987−995.

(11) Hammond, R.; Barnes, M.; Mack, S.; Mitchener, J.; Brown, N. *Bacillus subtili*s DNA polymerase III: complete sequence, overexpression, and characterization of the *polC* gene. *Gene* **1991**, *98*, 29−36.

(12) Barnes, M. H.; Brown, N. C. Antibody to *B. subtilis* DNA polymerase III: use in enzyme purification and examination of homology among replication-specific DNA polymerases. *Nucl. Acids Res*. **1979**, *6*, 1203−1219.

JM050517R