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Improved synthesis of antibacterial 3-substituted 6-anilinouracils

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Abstract—3-Substituted 6-anilinouracils, presently the most promising class of inhibitors of the bacterial DNA polymerase in Gram-positive bacteria, have been prepared by a general and straightforward three-step procedure starting from a readily available 1-benzyloxymethyl-protected derivative of 6-chlorouracil.

Bacteria are able to overcome antibiotic pressure by a variety of smart mechanisms that facilitate the selection of resistant organisms. Antibiotic-resistant Gram-positive bacteria represent a growing challenge in present day healthcare: the efficacy of powerful drugs such as the β-lactams and the macrolides is continually decreasing due to the emergence of antibiotic-resistant bacteria.[1] These pathogens carry resistance genes that spread in the bacterial population across geographic and interspecies boundaries.[2] Development of resistance is the inevitable result of antibiotic use, and limits efficacy and life span of every antibiotic. Correct antibiotic use will slow down resistance, but cannot prevent it. Only the persistent discovery and development of new antibiotics will guarantee future therapy. Established antibiotics utilize a limited set of validated mechanisms.[3] Therefore, new structural classes of antibiotics that address novel and valid targets are urgently needed. Here, we report our progress in the novel class of anilinouracils, inhibitors of the Gram-positive DNA polymerase IIIC.[4]

Anilinouracils and other substituted uracils have found widespread applications as pharmaceutical drugs and drug candidates, as have substituted nucleotide and nucleoside analogues in general. Appropriately substituted 6-chlorouracils are important intermediates in the synthesis of several biologically active compound classes such as antivirals,[5] antimalarial agents,[6] adenosine receptor ligands,[7] anti-cancer agents,[8] and compounds targeting delayed-type hypersensitivity.[9] In addition, 6-chlorouracils are themselves important starting materials for the synthesis of 6-anilinouracils, presently the most promising lead structure for targeting the Gram-positive DNA polymerase IIIC.[10] DNA polymerase IIIC is the polymerase required for the replication of chromosomal DNA in Gram-positive bacteria with low G+C content,[11] and thus represents a highly interesting target for therapy of some of the microorganisms most commonly associated with nosocomial infections today.

In a recent project we were interested in synthesizing a series of 3-substituted 6-anilinouracils for the purpose of investigating the role of the 3-substituent in the structure–activity relationship (SAR) of anilinouracils.[4] Existing methods for the synthesis of such derivatives are rather cumbersome, involving four linear steps, in which the 3-substituent is introduced at the very beginning of the sequence (Scheme 2). Consequently only a handful of such derivatives have been described so far.[10] This methodology is less suitable for use in a discovery setting, where a high-throughput synthesis and late-stage structural variation would be preferred to rapidly elaborate SAR. Our goal was to develop a synthetic strategy which is operationally simple, short, high yielding, and amenable to be run on parallel synthesis equipment. The result is an improved synthesis of 3-substituted 6-anilinouracils by a three-step strategy using an easily available precursor (Scheme 1).

Traditionally, 3-alkyl-6-chlorouracils are prepared by a three-step synthetic route[7,10] (Scheme 2) starting from an appropriately substituted alkylamine, which will eventually form the 3-substituent in the final product.

Keywords: Antibacterials; DNA polymerase IIIC; Gram-positive bacteria; Resistance; Anilinouracils.
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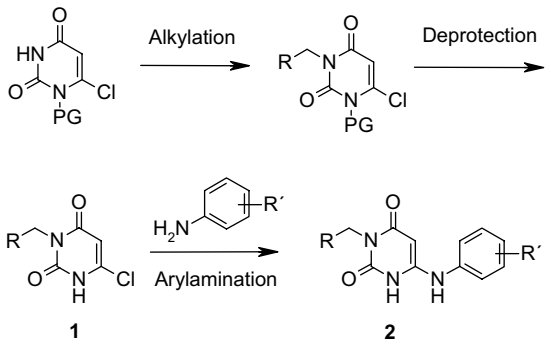
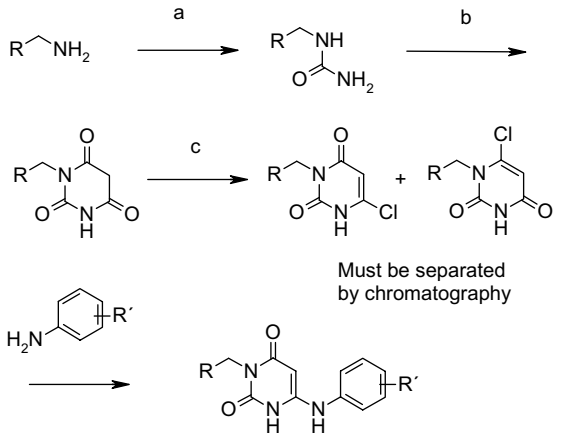


Scheme 1. New synthetic strategy for the preparation of 3-substituted 6-anilinouracils.



Scheme 2. Traditional synthesis of anilinouracils by chlorination of barbituric acid derivatives. Reagents: (a) KOCN, HCl, H_2O; (b) Diethyl malonate, NaOEt, EtOH, reflux; (c) $POCl_3$, heating.

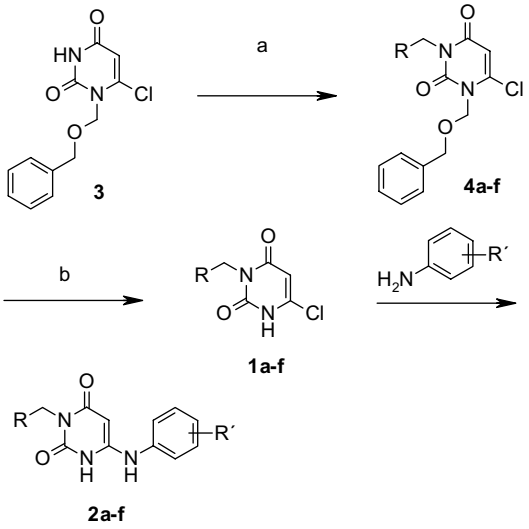
In the first step the amine is converted to the corresponding urea by treatment with aqueous hydrochloric acid and potassium cyanate, and the resulting urea is then cyclized to an N1-substituted barbituric acid using either (a) a base-promoted cyclization employing diethyl malonate and sodium ethoxide in refluxing ethanol,[10] or (b) an acid-promoted cyclization using malonic acid and acetic anhydride in glacial acetic acid at 90 °C.[7,12] Both reactions share certain disadvantages: they are incompatible with many functional groups, thus limiting the scope of the reaction to derivatives insensitive to either acid/acid anhydrides or bases. Additionally, the acidic cyclization is often accompanied by the formation of the corresponding 5-acetylated barbituric acid in significant amounts,[13] thus lowering overall yields and complicating isolation of the desired product. In the third step of this synthetic sequence the barbituric acid is transformed into the desired 3-alkyl-6-chlorouracil using phosphoryl chloride at elevated temperature.[7,10] In principle, two regioisomers can be formed in this reaction (Scheme 2), but inherent selectivity often favors the formation of the desired isomer. Again, conditions are rather harsh, and we were concerned about the safety issues relating to the very exothermic quenching of the reaction mixtures resulting from these reactions, especially on a larger scale. The final step consists of a nucleophilic replacement of the chlorine under thermal conditions with an aniline.[10] Wright et al. addressed some of these shortcomings in a much improved two-step synthesis via alkylation of 2-methoxy-6-amino-4-pyrimidone and following one-pot arylamination and demethylation.[14] The yield and regioselectivity in the first step is generally poor, however, and a demanding chromatographic separation is usually involved, making this method less useful for medium-throughput medicinal chemistry.

For medicinal chemistry purposes, the ideal synthetic access to 3-substituted 6-anilinouracils optimized would be by N3-alkylation of a preformed 6-chlorouracil. Depending on the alkylating agent, the steric and electronic characteristics of the uracil ring system favor alkylation of N-1 in most cases, although with varying degrees of selectivity. We took advantage of this fact by employing 1-benzyloxymethyl-6-chlorouracil **3**, easily prepared from commercially available 6-chlorouracil, as starting material (Scheme 3).[15]

1-Benzyloxymethyl-6-chlorouracil **3** can be alkylated with alkyl halides in DMF using cesium carbonate as the base in good yields (Scheme 3).

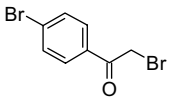
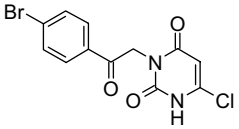
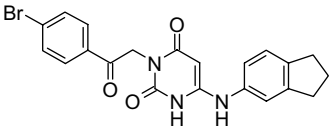
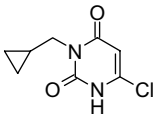
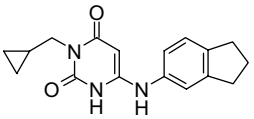
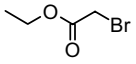
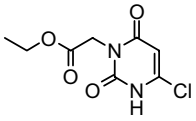
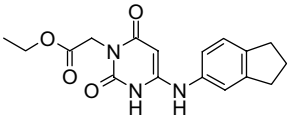
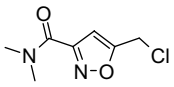
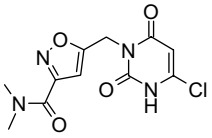
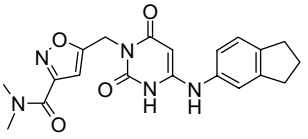
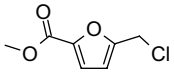
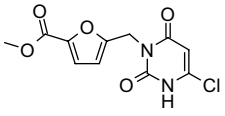
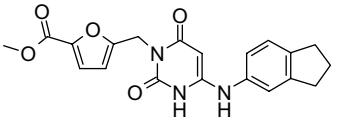
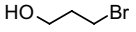
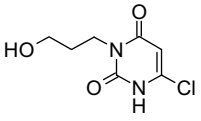
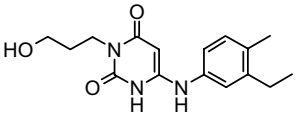
Deprotection of derivatives **4a**–**f** is achieved with non-aqueous TFA under conditions that selectively hydrolyze the benzyloxymethyl group without affecting esters and other hydrolytically sensitive groups. The resulting 3-substituted 6-chlorouracils **1a**–**f** can conveniently be isolated by crystallization. In the final step of the reaction, the aniline moiety is introduced by nucleophilic substitution (Scheme 3) as performed in the traditional synthesis.

By this synthetic methodology, a large number of structurally diverse anilinouracils **2** were prepared, many of which were previously synthetically inaccessible (representative examples are shown in Table 1). Compound



Scheme 3. Novel and improved synthesis of antibacterial anilinouracils. Reagents: (a) RCH_2X, Cs_2CO_3, DMF; (b) TFA, heating.

Table 1. Synthesis of 3-alkylated 6-chlorouracils **1** and their corresponding 3-alkyl-6-anilinouracils **2**

Entry	Starting material	Chlorouracil 1	Yield (%)[a]	Anilinouracil 2	Yield (%)
1		 **1a**	94	 **2a**	88
2		 **1b**	73	 **2b**	68
3		 **1c**	96	 **2c**	78
4		 **1d**	68	 **2d**	51
5		 **1e**	78	 **2e**	37
6		 **1f**	67	 **2f**	49

[a] Overall yield for the alkylation and deprotection steps.

2f (Table 1, entry 6), a known DNA polymerase IIIC inhibitor,[10] was synthesized by the new method for the purpose of comparison (the published synthesis involves five synthetic steps).

Optimal yields are achieved with activated alkylating agents such as alpha-bromo esters and ketones (Table 1, entries 1 and 3), or with halomethyl-heterocycles (Table 1, entry 4), but the reaction is not limited to such substrates. Non-activated alkylating agents also react well, as demonstrated by the synthesis of 3-hydroxypropyl-6-chlorouracil in 67% combined yield for the alkylation and deprotection (Table 1, entry 6); such reactions generally require longer reaction times. The general synthetic sequence, as illustrated by the synthesis of 3-(cyclopropylmethyl)-6-(2,3-dihydro-1H-inden-5-ylamino)uracil **2b**, is included in the Reference section.[16]

The 3-substituent has a significant effect on the antibacterial potency of anilinouracils. Small substituents such as substituted alkyl or heteroarylalkyl seem to be preferred. The antibacterial effect correlates well with target activity (Table 2), except for compounds in which very polar functionality prevents the compound from passively penetrating the bacterial cell wall and membrane (sulfonic acids and certain amines and carboxylic acids). The most potent compound of this series is **2b**, which in terms of antibacterial potency is equipotent or superior to the known anilinouracil **2f** against all tested organisms.

Still, despite good in vitro antibacterial properties, **2b** exhibited no efficacy in vivo at a dose of 100 mg/kg after intraperitoneal application in a *S. aureus* lethal challenge model 30 min post infection with 10^6 CFU/mouse.

Table 2. DNA polymerase IIIC target activity and in vitro antibacterial activity against selected pathogens for compounds **2a–f**

Compound	DNA Pol IIIC inhibition IC_{50}[a] (μM)	MIC *S. aureus* 133[b] (μg/mL)	MIC *S. pneumoniae* 1707/4[b,c] (μg/mL)	MIC *E. faecalis* ICB 27159[b,c] (μg/mL)
2a	>13.0	>256	>256	>256
2b	0.3	4	4	16
2c	1.5	16	16	64
2d	1.2	32	16	32
2e	2.0	32	16	32
2f	0.3	8	8	16

[a] DNA Pol IIIC activity was assayed using Pol. IIIC from *S. aureus* (see Ref. 4).
[b] MIC values were determined by the broth microdilution method with an inoculum of 5×10^5 CFU/mL in BHI medium.
[c] Bovine serum (10%) was added to the medium, incubation was performed under microaerophilic conditions.

We speculate that poor pharmaceutical properties, especially poor aqueous solubility, are the primary reasons for the lack of efficacy.[17]

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16. 1-(Benzyloxy)methyl-6-chloro-3-cyclopropylmethyl-uracil **4b**: 1-(benzyloxy)methyl-6-chlorouracil **3** (1.70 g, 6.4 mmol) was dissolved in dry DMF (15 mL) under argon and cesium carbonate (4.15 g, 12.7 mmol) was added. The resulting reaction mixture was stirred for 15 min, whereupon bromomethylcyclopropane (680 μL, 7.0 mmol) was added. The reaction mixture was stirred at rt for 3 h, the solvent was removed in vacuo, and the crude product was dissolved in DCM (150 mL) and washed with brine (3× 50 mL). The organic phase was dried (Na_2SO_4) and evaporated in vacuo to give the product **4b** as a solid, yield 1.76 g (86%). 6-Chloro-3-cyclopropylmethyluracil **1b**: a suspension of 1-(benzyloxy)methyl-6-chloro-3-cyclo-propyl-methyl-uracil **4 b** (600 mg, 1.9 mmol) was suspended in TFA (30 mL) and heated at reflux for 45 min. The reaction mixture was cooled to rt and evaporated in vacuo, whereupon MeOH (10 mL) was added and subsequently removed in vacuo. Ethylacetate (5 mL) was added, and the sample was left overnight to crystallize. The solid was filtered off, washed with ether and dried in vacuo to give **1b** as colorless crystals, yield 320 mg (85%). 3-Cyclopropylmethyl-6-(2,3-dihydro-1H-inden-5-ylamino)uracil **2b**: a mixture of 6-Chloro-3-(cyclo-propylmethyl)uracil **1b** (120 mg, 0.6 mmol) and 5-aminoindane (320 mg, 2.4 mmol) was heated under argon at 160 °C for 30 min. The reaction mixture was cooled to rt, 2-propanol (5 mL) was added, and the resulting precipitate was filtered off and dried to give a crude product, which was purified by reverse-phase preparative HPLC to give the product **2b** as a solid, yield 123 mg (68%).
17. The aqueous solubility of **2b** is 3.3 mg/L.