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7-Alkyl-N^2-substituted-3-deazaguanines. Synthesis, DNA polymerase III inhibition and antibacterial activity

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ABSTRACT

Several 2-anilino- and 2-benzylamino-3-deaza-6-oxopurines [3-deazaguanines] and selected 8-methyl and 8-aza analogs have been synthesized. 7-Substituted N^2-(3-ethyl-4-methylphenyl)-3-deazaguanines were potent and selective inhibitors of Gram+ bacterial DNA polymerase (pol) IIIC, and 7-substituted N^2-(3,4-dichlorobenzyl)-3-deazaguanines were potent inhibitors of both pol IIIC and pol IIIE from Gram+ bacteria, but weakly inhibited pol IIIE from Gram− bacteria. Potent enzyme inhibitors in both classes inhibited the growth of Gram+ bacteria (MICs 2.5–10 μg/ml), and were inactive against the Gram− organism *Escherichia coli*. Several derivatives had moderate protective activity in *Staphylococcus aureus*-infected mice.

The resurgence of infectious diseases caused by drug-resistant Gram+ organisms such as *Staphylococcus aureus, Enterococcus faecalis/faecium* and *Streptococcus pneumoniae* has stimulated discovery and development of new chemotherapeutic agents that selectively attack new bacterial targets. One new target has been validated recently in the low G:C family of Gram+ organisms, that is, DNA polymerase IIIC (pol IIIC), a DNA-dependent DNA polymerase which is specifically required for replicative DNA synthesis in these organisms. Inhibition of its activity prevents replicative DNA synthesis and, as a consequence, the host cell dies. Another recently described DNA polymerase in low G:C Gram+ eubacteria, DNA polymerase IIIE, bears close homology to its Gram− counterpart, also termed pol IIIE. The latter is the sole replicative enzyme in Gram− bacteria.[1,2] Gram+ pol IIICs share a unique capacity to bind a family of 'AU/PG' inhibitor compounds, via a guanine-like 'base-pairing domain' and an enzyme-specific 'aryl domain'. Early pol IIIC inhibitors were simple AU (6-anilinouracil) derivatives, which have two key structural features: (a) a substituted pyrimidine ring that permits base pairing to a pyrimidine in the DNA template; (b) a planar aryl ring at the 6-NH group.[3,4] Through its base-pairing domain, an AU 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 (Fig. 1).[5,6] Subsequent studies have shown that 3-(substituted-alkyl) groups in AU compounds, for example, HB-EMAU (**1**), can greatly enhance the antibacterial activity in vitro, and compounds with antibacterial activity in vivo have been described.[7]

Recently, we reported that novel 7-substituted N^2-(3-ethyl-4-methylphenyl)guanines (EMPGs, e.g., **2a**–**4a**) and 7-substituted

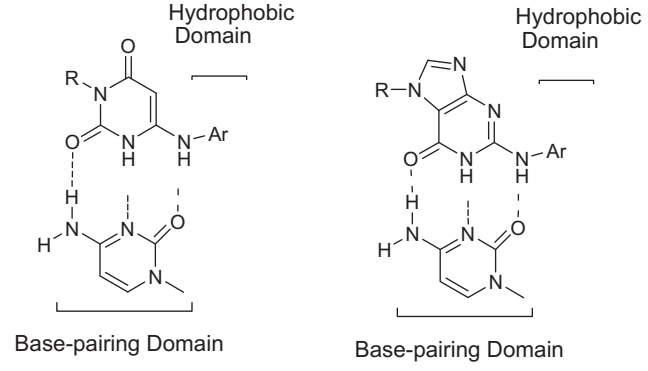

Figure 1. Mechanism of active site directed inhibition of bacterial DNA polymerase IIIs. Base-pairing with cytosine residues and hydrophobic (specificity) domains are illustrated for interaction of 3-substituted 6-anilinouracils (left) and 7-substituted guanines (right).

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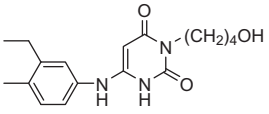
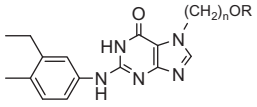
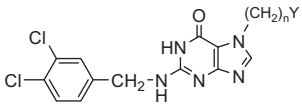
N^2-(3,4-dichlorobenzyl)guanines (DCBGs, e.g., **5a**–**7a**) were potent inhibitors of pol IIIC, and that the DCBGs were also active against pol IIIE species from both Gram+ and, with lower potency, Gram− bacteria.[8] Importantly, both classes inhibited the enzymes by the same active site-directed, competitive mechanism previously established for 'classical' AU inhibitors.[8] Based on the similarity of the structures of the AU and PG inhibitors bound to a cytosine residue in DNA (Fig. 1), we expanded our exploration into other heterocycles, such as 3-deazapurines and their 8-substituted derivatives. Such compounds would be expected to be DNA polymerase inhibitors and possess antibacterial activity. In this paper, we report the synthesis, structure determination, and in vitro biological activity of these compounds. Modest antibacterial activity of several compounds was observed in a *S. aureus* infection model in mice.



1 (HB-EMAU)



2a (n=4,R=H)
3a (n=5,R=H)
4a (n=5,R=Me)

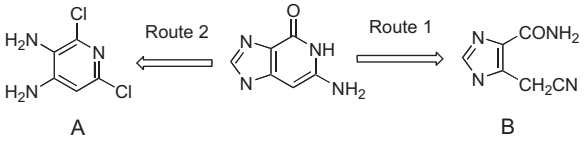


5a (n=4,Y=OH)
6a (n=5,Y=OH)
7a (n=4,Y=OMe)

8a (n=5, Y=OMe)
9a (n=5,Y=OAc)
10a (n=4, Y=N-morpholinyl)

Structure–activity studies of pol III inhibition by substituted guanines has shown that the hydrogen bonding groups at positions 1, 2, and 6 are required for activity.[8] Additionally, the 3-ethyl-4-methylphenyl or 3,4-dichlorobenzyl groups provided optimal hydrophobic groups for binding to pol IIIC and pol IIIC/E, respectively. Substituents at the 7-position were preferable to the 9 position in both cases (cf. compds. **2a** vs **2b** and **7a** vs **7b**) for optimal pol affinity (Table 2).[8] Thus, we decided to evaluate isosteric analogs by removing the 3-N atom of the guanine ring to create 3-deazapurines and the analogous 8-methyl-3-deazapurines and 8-aza-3-deazapurines (Table 1). The synthesis and comparative enzyme and bacterial inhibition activity of these modified compounds are presented here.

Two approaches were considered to synthesize 3-deazaguanine pol inhibitors: start from the imidazole B and build the pyridine ring (Route 1),[9] which gave little flexibility in modifying the imidazole ring structure, or start from the pyridine A and build the imidazole ring (Route 2).[10]

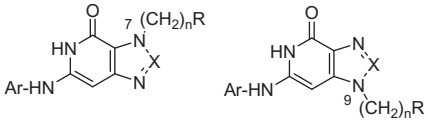


We chose the latter approach, starting with 3,4-diamino-2,6-dichloropyridine (Scheme 1).[11] This compound was cyclized to 4,6-dichloropyrido[4,5-c]imidazole (**11**), and the latter compound was treated with sodium benzoxide to afford 2-benzyloxy-7-chloropyrido[4,5-c]imidazole (**12**). Alkylation of **9** separately with 1,4-dibromobutane and 1,5-dibromopentane gave mixtures of the 7-alkylated products **13a** and **14a** and 9-alkylated products **13b** and **14b**, respectively. The mixtures were treated with sodium methoxide or other nucleophiles such as morpholine to give easily separable isomers **15a**–**18a** and **15b**–**18b**. To confirm the isomeric structures NOESY ^{1}H NMR was applied to a pair of products. The spectrum of **15a** revealed a crosspeak between N–CH_2 and O6–CH_2 protons, but **15b** did not. In addition, the N–CH_2 chemical shift of the 3-isomer **15a** was greater than that of the 5-isomer **15b**, consistent with findings for analogous 7 and 9 substituted purine nucleosides.[12] Thus, the 3-alkylated intermediate was assigned as the major isomer in all cases, in a ratio **a**:**b** of ca. 2.5:1 (from ^{1}H NMR). Fusion of the purified 7-chloro compounds with 3-ethyl-4-methylaniline or 3,4-dichlorobenzylamine simultaneously displaced the 7-chloro group and removed the 2-benzyl group to afford 3- and 5-alkyl-7-(substituted-amino)-2-oxopyrido[4,5-c]imidazoles (i.e., 7- and 9-alkyl-2-(N-substituted)-3-deazaguanines) **19a**–**25a** and **19b**–**25b**, respectively (Scheme 1).[13]

A similar approach was used to prepare 8-methyl-3-deazaguanines (Scheme 2). 3,4-Diamino-2,6-dichloropyridine was treated with acetic acid and polyphosphoric acid[14] to afford 4,6-dichloro-8-methylimidazo[4,5-c]pyridine (**26**), and treatment of **26** with sodium benzoxide gave the intermediate **27**. Alkylation of **27** with 1,4-dibromobutane gave a 2.1:1 mixture of 7 and 9 bromobutyl isomers **28a** and **28b**. Methanolysis of the latter compounds gave the methoxybutyl derivatives **29a** and **29b**, and fusion of these with 3,4-dichlorobenzylamine gave, after concomitant debenzylation, the candidate inhibitors **30a** and **30b**.

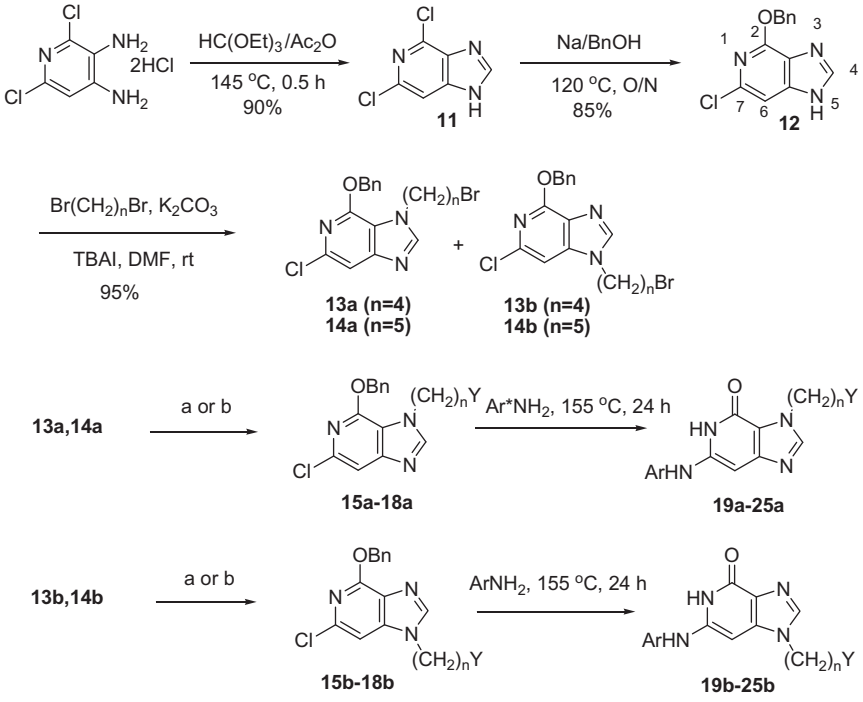
To access the 8-aza-3-deaza series 3,4-diamino-2,6-dichloropyridine was cyclized with sodium nitrite in dilute hydrochloric acid to afford the key intermediate 8-aza-2,6-dichloroimidazo[4,5-c]pyridine (**31**) (Scheme 3). The latter compound was subjected to a sequence similar to that in Scheme 2, that is, conversion to the 6-methoxy intermediate **32**, alkylation to the 7/9 isomer mixture **33a**/**33b**, and methanolysis to give the

Table 1
Isosteric pol III inhibitors



Compd.	X	Ar	7 or 9	*n*	R
19a	CH	EMP[a]	7	4	OMe
19b	‘	EMP	9	4	OMe
20a	‘	EMP	7	5	OMe
21a	‘	EMP	7	4	*N*-Morpholinyl
21b	‘	EMP	9	4	*N*-Morpholinyl
22a	‘	DCB[b]	7	4	OMe
22b	‘	DCB	9	4	OMe
23a	‘	DCB	7	5	OMe
24a	‘	DCB	7	4	*N*-Morpholinyl
24b	‘	DCB	9	4	*N*-Morpholinyl
25a	‘	DCB	7	5	*N*-Morpholinyl
30a	CMe	DCB	7	4	OMe
30b	‘	DCB	9	4	OMe
35a	N	DCB	7	5	OMe

[a] 3-Ethyl-4-methylphenyl.
[b] 3,4-Dichlorobenzyl.

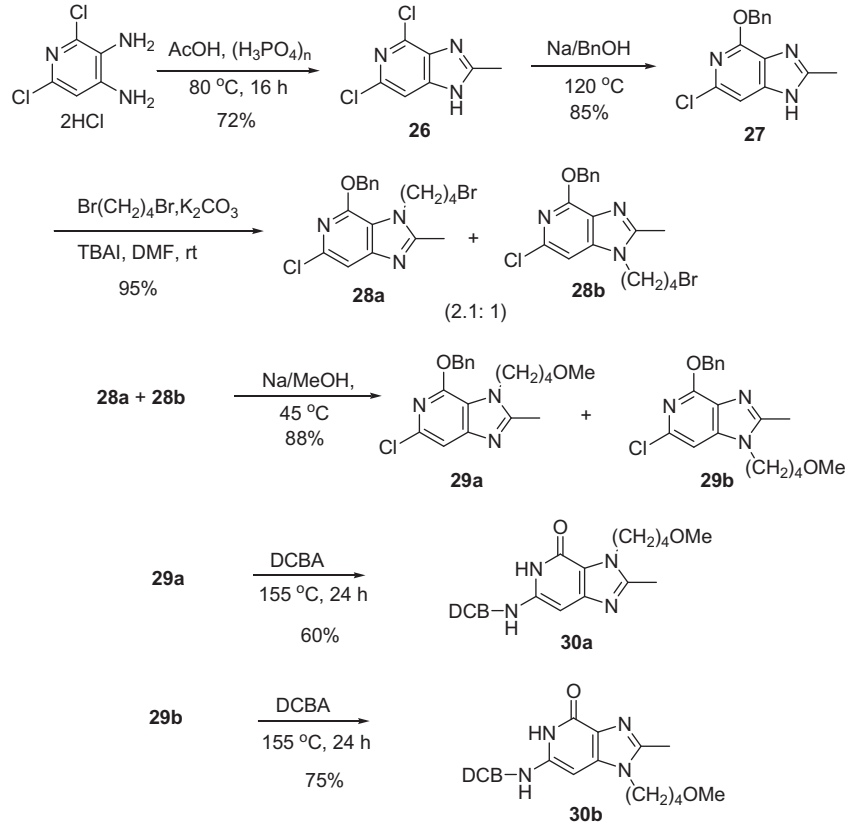


a. Na/MeOH, 45°C (Y = OMe)

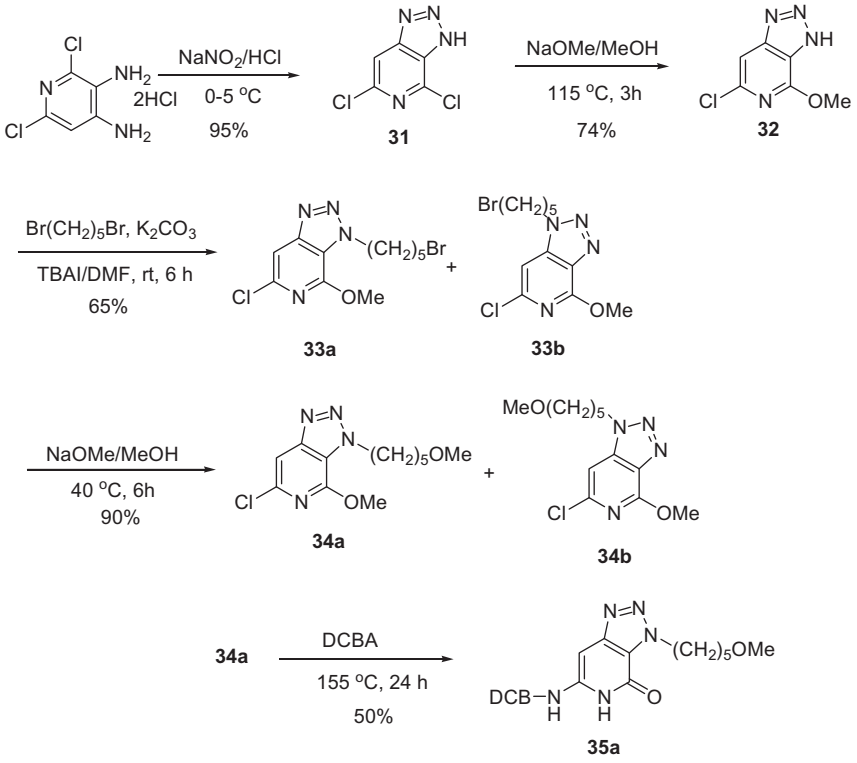
b. Morpholine/CH_2Cl_2, rt (Y = N-morpholinyl)

* Ar = 3-ethyl-4-methylphenyl (EMP), 3,4-dichlorobenzyl (DCB)

Scheme 1.



Scheme 2.



Scheme 3.

Table 2
Pol IIIC and pol IIIE inhibition

Compd.	K_i (μM)[a]		
	B. s. pol IIIC	*B. s.* pol IIIE	*E. c.* pol IIIE
1[b]	0.063	117	in[c]
EMPGs:[b]			
2a	0.47	in	nt[d]
2b	in	in	nt
3a	0.28	318	nt
4a	0.26	394	nt
3-deazaEMPGs:			
19a	1.5	in	nt
19b	in	in	nt
20a	0.69	490	in
21a	1.1	in	nt
21b	50	in	nt
DCBGs:[b]			
5a	0.19	0.063	86
6a	0.052	0.091	27
7a	0.19	0.37	56
7b	in	in	in
8a	0.07	0.088	47
9a	0.052	0.058	13.8
10a	0.051	0.047	26
3-deazaDCBGs:			
22a	0.19	0.37	56
23a	0.16	0.42	81
24a	0.094	0.2	nt
24b	50	54	nt
30a	1.25	2.5	426
3a	0.96	1.05	444

[a] K_i values were determined by measuring incorporation of [^{3}H]TMP into activated DNA incubated with dATP, dCTP and [^{3}H]TTP, that is, lacking the competitor dGTP (see Wright and Brown in Ref. 15).
[b] From Ref. 8.
[c] Inactive (<10% inhibition) at highest conc. tested.
[d] Not tested.

Table 3
Antibacterial activity (MICs)

Compd.	MIC (μg/ml)[a]							
	B. subtilis	*S. aureus*	*S. a.* (Smith)	MRSA1090	*E. fecalis*	*E. fecium*	VRE	*E. coli* J53
1	<1.25	5	5	5	5	5	5	>80
EMPGs:								
2a	15	30	40	30	20	20	10	>80
2b	>80	>80	>80	>80	>80	>80	>80	>80
3a	20	20	20	20	10	10	5	>80
4a	1.25	5	5	2.5	5	10	5	>80
3-deazaEMPGs:								
19a	2.5	10	10	10	10	10	10	>80
19b	>80	>80	>80	>80	>80	>80	>80	>80
20a	1.25	2.5	2.5	2.5	>80	2.5	>80	>80
21a	10	40	40	30	>80	20	>80	>80
21b	>80	>80	>80	>80	>80	>80	>80	>80
DCBGs:								
5a	15	30	25	30	10	7.5	3.75	>80
6a	10	20	5	10	5	10	5	>80
7a	1.4	3.7	3.7	1.25	2.5	2.5	2.5	>80
7b	>80	>80	>80	>80	>80	>80	>80	>80
8a	1.4	3.7	3.7	1.25	2.5	2.5	2.5	>80
9a	1.25	2.5	1.25	1.25	1.25	1.25	5	>80
10a	10	20	40	40	10	10	5	>80
3-deazaDCBGs:								
22a	0.625	5	3.75	2.5	5	5	2.5	>80
23a	1.25	2.5	1.25	2.5	5	1.25	5	>80
24a	7.5	20	10	20	20	15	10	>80
24b	>40	>40	>40	>40	>40	>40	>40	>40
25a	10	20	20	20	20	20	15	>80
30a	6.25	60	20	5	80	80	20	80
30b	>80	>80	>80	>80	>80	>80	>80	>80
35a	2.5	10	7.5	7.5	10	10	10	>80
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
Linezolid	0.625	1.25	2.5	1.25	1.25	2.5	1.25	>80

[a] Minimum Inhibitory Concentration; averages of at least two independent experiments.

methoxybutyl derivatives **34a** and **34b**. Fusion of **34a** with 3,4-dichlorobenzylamine afforded the 8-aza-3-deaza compound **35a**.

Evaluation of the isosteres for enzyme inhibition and antibacterial activity in vitro and in vivo was carried out.[15] Results of assays of the isosteres against bacterial DNA polymerases are summarized in Table 2, where they are compared with activity of the corresponding guanine derivatives. All 7-substituted guanines and isosteric compounds, EMP and DCB types alike, were potent inhibitors of Gram+ pol IIIC, with K_i values of 1 μM or less, several compounds being as active as the prototype HB-EMAU (**1**). All of the 9-isomers were much less active or inactive (only representative ones are reported in Table 2). Among the active isosteres, DCB compounds were also potently active against Gram+ pol IIIE. In addition, several 7-substituted DCB compounds were weakly active against Gram− pol IIIE, a result consistent with weak inhibition of this enzyme by DCBG-related compounds (see Table 2).[8]

Results of antibacterial assays in culture of the 7-substituted isosteres and their guanine counterparts vs. a panel of Gram+ organisms and one Gram− organism (*Escherichia coli*) are summarized in Table 3. As reference compounds, HB-EMAU (**1**) and the antibiotics ciprofloxacin, vancomycin and linezolid are included. The sources of bacterial strains and methods for determination of minimum inhibitory concentrations (MIC) in 96-well plates were as previously described.[7] HB-EMAU (**1**) is an effective Gram+ antibacterial with MIC values of 2.5-5 μg/ml against various organisms, but inactive against the Gram− bacterium *E. coli*.[4,7] Ciprofloxacin potently inhibited growth of all organisms with the exception of MRSA 1090, a methicillin-resistant strain of *S. aureus* with cross-resistance to the fluoroquinolones. Vancomycin was active against all Gram+ organisms except VRE, the vancomycin-resistant strain of *E. faecalis*, and linezolid was active against all Gram+ organisms.

Antibacterial activity of isosteric 3-deaza compounds against Gram+ organisms generally paralleled the potency of 7 versus 9 substituted derivatives against the DNA polymerases, consistent with the activity of corresponding 7 and 9 substituted EMPGs and DCBGs (Table 2). The 7-methoxyalkyl side chains of **4a**, **7a** and **8a** imparted ca. eight-fold increased activity compared with the 7-hydroxyalkyl compounds **2a**, **3a**, **5a** and **6a**. The 7-acetoxypentyl compound **9a** was a potent antibacterial, but the corresponding 7-morpholinylpentyl compound **10a** was weak. 3-Deaza isosteres of DCBGs had activities similar to those of the DCBGs and **1**. Substitutions at the 8-position with nitrogen or a methyl group resulted in 2- to 10-fold loss of activity compared with the 8-H counterparts.

Several analogs were tested by the intraperitoneal (IP) route for efficacy in protecting mice from IP infection with *S. aureus* (Smith). The results of Table 4 show that, with the exception of **8a**, all the analogs had moderate activity, and the control drug daptomycin had highest activity. There was no obvious difference between activity of the purines **4a** and **8a** and their 3-deaza analogs **20a** and **23a**. A clear dose-response was observed with **9a** (7-acetoxypentyl-DCBG), consistent with its potent antibacterial activity in vitro (Table 3).

We have synthesized and identified 7 and 9 regioisomers of isosteric analogs of antibacterial 2-substituted guanines, specifically 3-deaza, 8-methyl-3-deaza and 8-aza-3-deazaguanines. Isosteres of both the EMP and DCB classes with substitution exclusively at the 7-position of the heterocycle were potent inhibitors of bacterial DNA polymerase IIIC, an enzyme unique to Gram+ bacteria. DCB analogs also were potent inhibitors of DNA polymerase IIIE

Table 4
Antibacterial activity in mice[a]

Treatment, IP 10 ml/kg	IP dose, mg/kg	% Survivors at 24 h
Vehicle[b]	—	0
Daptomycin[c]	10	100
1	100	80
4a	30	20
‘	100	40
20a	30	40
8a	30	20
9a	10	30
‘	30	50
‘	60	80
23a	100	40

[a] Groups of 10 mice were infected IP with *S. a.* (Smith) and treated IP 15 min post-infection as described.
[b] 10% DMSO/peanut oil.
[c] 5% dextrose/water.

from Gram+ bacteria, but weak inhibitors of the DNA polymerase IIIE from the Gram− *E. coli* (Table 2). Some isosteric compounds were potent antibacterials against Gram+ bacteria (Table 3), generally paralleling their activity against the Gram+ DNA polymerases, but weaker than the corresponding guanines. However, the isosteres showed weak activity against experimental *S. aureus* infection in mice (Table 4), consistent with their moderate in vitro potencies and low solubilities.

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Supplementary data

Supplementary data (details of syntheses and characterization of new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.093.

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13. All 9-isomers of isosteres were isolated and tested, but were weakly active as enzyme inhibitors (Table 2) and inactive in antibacterial tests (results not shown).
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15. Recombinant pol IIIC and pol IIIE of *Bacillus subtilis* (B.s.) were prepared according to Hammond, R.; Barnes, M.; Mack, S.; Mitchener, J.; Brown, N. Gene. 1991, 98, 29, and Ref. 2, respectively. *E. coli* (*E. c.*) pol IIIE was the three subunit core enzyme purchased from Enzyco. The enzymes were assayed with activated calf thymus DNA according to the method described by Barnes, M. H.; Brown, N. C. Nucl. Acids Res. 1979, 6, 1203. Apparent inhibition constants (K_i values) were obtained directly in a truncated assay lacking the competitor dGTP [Wright, G. E.; Brown, N. C. *Biochim. Biophys. Acta* 1976, 432, 37.] or conventionally by variation of the concentration of dGTP. The standard panel for determination of Minimum Inhibitory Concentration (MIC) values included *S. aureus* 25923, *S. aureus* 13709 (Smith) and *E. faecalis* 29212, all purchased from the American Type Culture Collection (ATCC, Manassas, VA). Methicillin-resistant *S. aureus* (MRSA 1094), *E. faecium* 19434, and vancomycin-resistant *E. faecium* (VRE) are clinical isolates provided by the University of Massachusetts Medical School. *B. subtilis* BD54 [Love, E.; D'Ambrosio, J.; Brown, N. C.; Dubnau, D. Molec. Gen. Genet. 1976, 144, 313.] is a standard laboratory strain. *E. coli* (J-53) was provided by Professor Martin Marinus, University of Massachusetts Medical School. Measurement of Minimum Inhibitory Concentration (MIC) was done as described,[7] by incubating bacteria at 37 °C for 16–24 h in the presence of two-fold serial dilutions of test compounds. Compounds were dissolved in and diluted from DMSO stocks, and all assays contained 1% DMSO. MIC values are the lowest concentrations of test compounds at which bacterial growth was not apparent (<25% of the DMSO control, based on optical density at 600 nm). 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 studies. *S. aureus* (Smith) was grown at 37 °C to log-phase in Luria Broth (LB), and the colony forming units (CFU) were determined using a nomogram relating CFU to optical density at 600 nm. Bacteria were washed in fresh cold broth, and given by intraperitoneal (IP) injection to mice as a suspension in 0.5 mL of broth. Groups of five or ten mice were treated via the IP route 15 min post-infection with vehicle, test compound in vehicle, or daptomycin (Cubicin®, Cubist) in saline at 30 mg/Kg. Mice were returned to their cages and monitored for mortality for 24 h.