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Fluorocyclines. 1. 7-Fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline: A Potent, Broad Spectrum Antibacterial Agent

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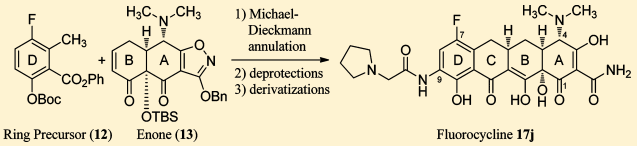
ABSTRACT: This and the accompanying report (DOI: 10.1021/jm201467r) describe the design, synthesis, and evaluation of a new generation of tetracycline antibacterial agents, 7-fluoro-9-substituted-6-demethyl-6-deoxytetracyclines ("fluorocyclines"), accessible through a recently developed total synthesis approach. These fluorocyclines possess potent antibacterial activities against multidrug resistant (MDR) Gram-positive and Gram-negative pathogens. One of the fluorocyclines, 7-fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline (**17j**, also known as **TP-434**, 50th Interscience Conference on Antimicrobial Agents and Chemotherapy Conference, Boston, MA, September 12−15, 2010, poster F1−2157), is currently undergoing phase 2 clinical trials in patients with complicated intra-abdominal infections (cIAI).



INTRODUCTION

Tetracyclines are a class of broad spectrum antibiotics first discovered in the mid-1940s.[2] They have been used to treat many bacterial infections caused by both Gram-positive and Gram-negative pathogens.[3] However, decades of widespread tetracycline use has resulted in significant bacterial resistance and has drastically decreased these agents' efficacy against a wide range of organisms.[4,5]

Two main mechanisms of tetracycline resistance have been reported to date: (1) active drug efflux (*tet*(A)−*tet*(D), and *tet*(K)−*tet*(L)), widely found in both Gram-positive and Gram-negative pathogens,[6,7] and (2) ribosomal protection (e.g., *tet*(M)−*tet*(O)), more commonly seen in Gram-positive organisms such as *Staphylococcus aureus* and *Streptococcus* spp.[8,9] Since the discovery of early generations of natural tetracyclines (chlorotetracycline (**1**),[10] oxytetracycline (**2**),[11] and tetracycline (**3**)[12,13]), a number of non-natural tetracycline antibiotics have been developed to combat tetracycline resistance. These synthetic tetracyclines include doxycycline (**4**),[14,15] minocycline (**5**),[16,17] and, more recently, tigecycline (**6**),[18−20] all derived from naturally occurring tetracycline intermediates via semisynthetic approaches (Figure 1).

Structure−activity relationships (SAR) of early generations of tetracycline analogues[21,22] and the recently solved tetracycline-30S ribosome cocrystal structure[23] indicate that the "northwest" region of the molecule is not directly involved in interactions with the ribosome and can be modified without substantial loss of activity. In contrast, the "southeast" portion is directly involved in an extensive H-bond network in the A-site of the bacterial ribosome and conservation of this H-bond network is essential to retain tight ribosomal binding. Structural modifications at C7 and C9 of the tetracycline D ring have emerged as one of the most promising approaches for improved antibacterial activity and led to the discovery and development of clinically important antibiotics like minocycline and tigecycline.[16−20] It has been observed that physicochemical properties such as the polarity and electronegativity of the C7 substituent can influence tetracycline's intrinsic antibacterial potency.[24,25] Additionally, as demonstrated by tigecycline, substitutions at C9 can dramatically improve both intrinsic antibacterial potency and activity against resistant pathogens expressing tetracycline-specific efflux and ribosomal protection mechanisms.[18−20] X-ray crystallorgraphy studies by Saenger et al. indicated that bulky substitution groups at C9 can render the Tet repressor protein ineffective.[26] Traditionally, substituents at C7 and C9 have been introduced through semisynthetic approaches and are largely limited to chemical groups that can be incorporated by direct electrophilic aromatic substitutions (e.g., halogenation and nitration) and through further modifications of these functional groups via a very limited set of chemical transformations.[14,16,25] The total synthesis approach recently developed by Myers et al.[27−29] and expanded by Tetraphase,[30,31] on the other hand, has the potential to introduce a more diverse set of substituents at more positions including C7, C8, and C9 on the D ring[27] as well as the ability to incorporate heterocyclic[27,30] and polycyclic ring systems[29,31] into the tetracycline scaffold. We therefore decided to explore new 7,9-disubstituted tetracycline analogues with diverse C7 substituents (e.g., fluoro, methoxy, trifluoromethoxy, trifluoromethyl, and cyano) coupled with a variety of C9 substituents (e.g., alkyl, aryl, amino, aminoalkyl, and amido). In addition to improved antibacterial activity, these new substituents and substitution patterns could potentially impart favorable

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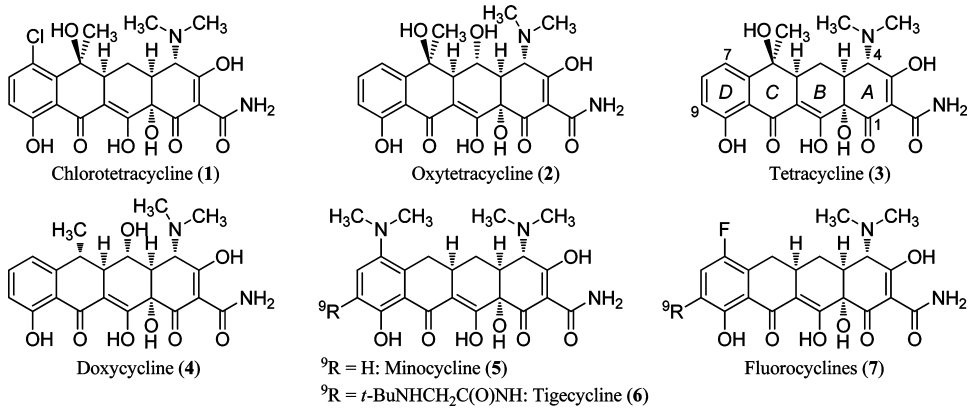
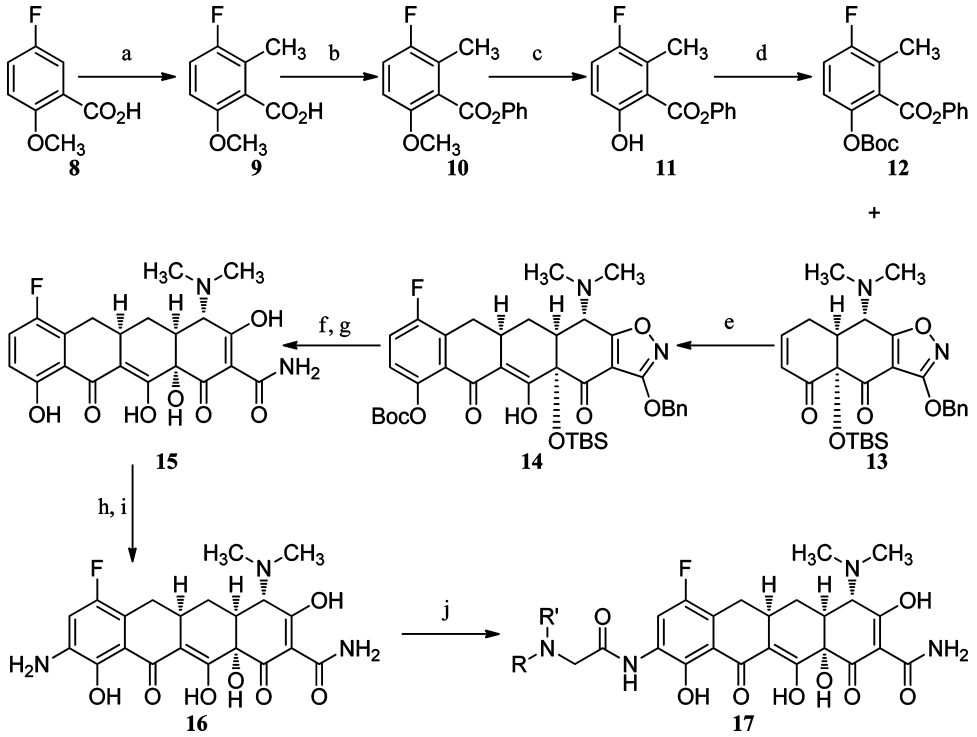


Figure 1. Generations of tetracycline antibiotics.

Scheme 1. Synthesis of 7-Fluoro-9-aminoacetamido-6-demethyl-6-deoxytetracyclines[a]



[a]Reagents: (a) *s*-BuLi, TMEDA, THF, then CH_3I; (b) $(COCl)_2$, DMF, CH_2Cl_2, then PhOH, DMAP, Et_3N, 52% for two steps; (c) BBr_3, CH_2Cl_2; (d) Boc_2O, DMAP, CH_2Cl_2, 75% for two steps; (e) D ring precursor **12**, LDA, TMEDA, THF, then enone **13**, 35%; (f) aq HF, TFA, CH_3CN; (g) H_2, 10% Pd–C, dioxane–CH_3OH, 41% for two steps; (h) HNO_3, H_2SO_4; (i) H_2, 10% Pd–C, CH_3OH, 81% for two steps; (j) RR′$NCH_2C(O)Cl$ (HCl salt), DMF, or $BrCH_2C(O)Br$, Na_2CO_3, CH_3CN–DMPU, then RR′NH.

physicochemical[32,33] and pharmacokinetic–pharmacodynamic[34,35] properties into the new tetracycline analogues. This and the accompanying report (DOI: 10.1021/jm201467r)[44] describe the details of our studies on 7-fluoro-9-substituted-6-demethyl-6-deoxytetracyclines, the "fluorocyclines" (**7**), and the eventual discovery and development of one of the fluorocycline analogues, 7-fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline (**17j**),[1] as a potent and broad spectrum antibacterial agent. Details of the other 7-substituted tetracycline subclasses (such as 7-methoxy, 7-trifluoromethoxy, 7-trifluoromethyl, and 7-cyanotetracyclines) will be the subjects of future communications.

RESULTS AND DISCUSSION

Chemistry. Applying the total synthesis approach, a series of 7-fluoro-9-aminoacetamido-6-demethyl-6-deoxytetracyclines (**17**) were synthesized via enone **13**[27,36] and a D ring precursor **12**. As shown in Scheme 1, 2-methoxy-5-fluorobenzoic acid (**8**) was methylated by regioselective deprotonation[37] with *s*-BuLi in the presence of TMEDA in THF at −78 °C followed by the addition of methyl iodide to give 2-methoxy-5-fluoro-6-methylbenzoic acid (**9**), which was subsequently esterified to afford phenyl ester **10** in 52% overall yield. Demethylation of compound **10** with BBr_3 followed by protection with Boc_2O gave the desired D ring precursor **12** in 75% yield over two steps. The tandem Michael–Dieckmann annulation[27] was carried out by the deprotonation of **12** with LDA in the

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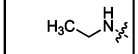
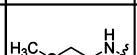
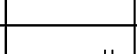
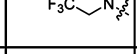
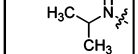
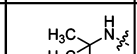
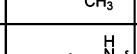
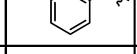
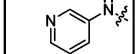
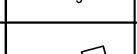
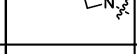
Table 1. In Vitro Antibacterial Activity of Fluorocycline Analogues

Compound	RR'N-	MIC (μg/mL)[a]												
		SA101	SA161[b]	SA158[c]	EF103	EF159[c]	SP106	SP160[c]	EC107	EC155[c]	AB110	PA111	EC108	KP109	KP153[c]
		29213	MRSA, *tet* (M)	*tet* (K)	29212	*tet* (M)	49619	*tet* (M)	25922	*tet* (A)	19606	27853	13047	13883	*tet* (A)
17a		0.0625	0.25	1	0.0625	0.125	0.0156	0.0156	0.25	16	2	8	0.5	1	8
17b		0.25	0.5	4	0.125	1	0.0156	0.0625	1	16	2	16	4	2	8
17c		1	2	4	2	4	0.5	1	16	>32	2	>32	>32	32	>32
17d		0.125	0.25	0.5	0.0625	0.0625	0.0156	0.0156	0.5	8	1	16	1	1	4
17e		0.125	0.25	0.0625	0.0625	0.125	0.0156	0.0156	0.25	2	0.5	16	1	1	2
17f		2	4	2	2	2	2	4	>32	>32	8	>32	>32	>32	>32
17g		4	>32	>32	8	>32	0.25	2	16	>32	8	>32	>32	32	>32
17h		0.0156	0.125	0.25	0.0156	0.0312	0.0156	0.0156	0.125	8	0.125	8	1	0.5	8
17i		0.125	0.25	0.5	0.0625	0.125	0.0156	0.0156	0.25	8	2	8	1	1	4
17j		0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	1	0.0312	8	0.125	0.125	0.5
17k		0.5	1	0.5	0.25	0.5	0.0156	0.0156	1	4	0.125	32	4	2	4
17l		4	8	8	8	8	0.5	1	32	>32	8	>32	>32	>32	>32
Tetracycline		0.125	64	32	16	64	0.25	32	1	>64	1	16	1	2	>64
Tigecycline		0.0625	0.125	0.125	0.0625	0.0625	0.0156	0.0156	0.125	1	0.5	16	0.25	0.25	1

[a]Strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA) unless otherwise noted. The first seven strains from the left are Gram-positive strains. The last seven strains are Gram-negative strains. Strains with "*tet*(A)", "*tet*(K)", or "*tet*(M)" noted underneath are tetracycline-resistant strains. SA, *Staphylococcus aureus*; EF, *Enterococcus faecalis*; SP, *Streptococcus pneumoniae*; EC, *Esherichia coli*; AB, *Acinetobacter baumannii*; PA, *Pseudomonas aeruginosa*; ECl, *Enterobacter cloacae*; KP, *Klebsiella pneumoniae*. [b]Obtained from Micromyx (Kalamazoo, MI). [c]Obtained from Marilyn Roberts' laboratory at the University of Washington.

presence of TMEDA in THF at −78 °C followed by the addition of enone **13**, yielding the pentacyclic intermediate **14** in 35% isolated yield (not optimized). Desilylation and Boc deprotection with aqueous HF followed by catalytic hydrogenation gave the desired 7-fluoro-6-demethyl-6-deoxytetracycline (**15**).[38] Nitration of compound **15** with nitric acid in sulfuric acid followed by catalytic hydrogenation afforded the aniline intermediate **16** in 81% yield over two steps. Acylation of aniline **16** with either aminoacetyl chlorides or bromoacetyl bromide followed by treatment with various amines afforded the desired 7-fluoro-9-aminoacetamido-6-demethyl-6-deoxytetracycline analogues **17** in moderate yields after preparative HPLC purification.

Biology. The fluorocyclines were evaluated for antibacterial activity against a panel of tetracycline-susceptible and tetracycline-resistant Gram-positive and Gram-negative bacterial strains. A large variety of amines on the C9 side chain were systematically explored. In general, a small, secondary or tertiary alkylamine is preferred (Table 1). Among the secondary alkylamines (**17a**, **17d**, and **17e**), potency against the *tet*(A) and *tet*(K) strains improved with increasing size. For the tertiary amines (**17h**−**17l**), *tet*(K) activity was generally good, while *tet*(A) activity was significantly improved for the pyrrolidinyl compound **17j** (**TP-434**). Alkylamines with weak basicity decreased potency, especially against Gram-negative organisms (**17b**, **17c**, and **17l**). Analogues with aromatic amines on the C9 side chain were substantially less active against most strains in the panel (**17f** and **17g**). Compounds bearing a cyclic alkylamine on the C9 side chain (**17i**−**17k**) had dramatically different potency depending on the ring size of the

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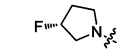
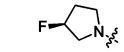
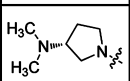
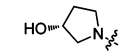
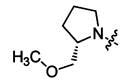
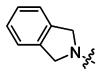
Table 2. In Vitro Antibacterial Activity of Fluorocycline Analogues

Compound	RR'N-	MIC (μg/mL)[a]												
		SA101	SA161[b]	SA158[c]	EF103	EF159[c]	SP106	SP160[c]	EC107	EC155[c]	AB110	PA111	ECl08	KP109	KP153[c]
		29213	MRSA, *tet* (M)	*tet* (K)	29212	*tet* (M)	49619	*tet* (M)	25922	*tet* (A)	19606	27853	13047	13883	*tet* (A)
17j		0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	0.0156	1	0.0312	8	0.125	0.125	0.5
17m		0.25	0.5	0.5	0.125	0.25	0.0156	0.0156	1	16	0.25	>32	8	4	8
17n		0.25	0.5	1	0.25	0.25	0.0156	0.0156	2	32	0.125	>32	4	4	32
17o		8	>32	>32	16	16	2	4	>32	>32	>32	>32	>32	>32	>32
17p		2	2	16	2	2	0.25	0.5	4	>32	32	>32	16	16	>32
17q		1	2	1	1	2	0.125	0.125	4	16	0.5	>32	16	8	16
17r		0.5	1	0.5	0.25	0.5	0.125	0.06	1	2	0.06	>32	4	2	4
17s		0.25	0.5	0.25	0.25	0.5	0.125	0.125	2	4	0.125	>32	32	4	32

[a]Strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA) unless otherwise noted. The first seven strains from the left are Gram-positive strains. The last seven strains are Gram-negative strains. Strains with "*tet*(A)", "*tet*(K)", or "*tet*(M)" noted underneath are tetracycline-resistant strains. SA, *Staphylococcus aureus*; EF, *Enterococcus faecalis*; SP, *Streptococcus pneumoniae*; EC, *Escherichia coli*; AB, *Acinetobacter baumannii*; PA, *Pseudomonas aeruginosa*; ECl, *Enterobacter cloacae*; KP, *Klebsiella pneumoniae*. [b]Obtained from Micromyx (Kalamazoo, MI). [c]Obtained from Marilyn Roberts' laboratory at the University of Washington.

amine. Compound **17j**, with a pyrrolidine on the C9 side chain, was the most potent analogue in this fluorocycline series, having MICs $\leq$ 1 μg/mL against Gram-negative bacteria including those that express the Tet(A) efflux protein. In contrast, the azetidine analogue **17i** and the piperidine analogue **17k** were 4- to 64-fold less potent than the pyrrolidine analogue **17j** against almost all bacterial strains in the panel.

A series of substituted pyrrolidines were then explored for potential potency improvement over compound **17j**. As shown in Table 2, fluoro-substitution on the pyrrolidine ring retained substantial Gram-positive activity (**17m** and **17n**) with almost no difference between the two diastereomers. Gram-negative activity, on the other hand, was substantially reduced relative to **17j**. Again, similar to compounds in Table 1, pyrrolidines with polar substituents (**17o**−**17q**) had dramatically decreased activity against most strains in the panel, especially Gram-negative organisms. Analogues with a bicyclic pyrrolidine (**17r** and **17s**) on the C9 side chain retained activity against the majority of Gram-positive strains in the panel. However, none of the substituted pyrrolidine analogues displayed improved activity over the unsubstituted pyrrolidine analogue **17j**.

Compound **17j** displayed broad spectrum activity against all Gram-negative bacteria in the panel except *P. aeruginosa*, as well as excellent in vitro activity against major Gram-positive pathogens, including methicillin-resistant *S. aureus* (Table 1). Further, compound **17j**'s activity was minimally affected by the presence of *tet*(M), *tet*(K), or *tet*(K). *S. aureus* SA161, *E. faecalis* EF159, and *S. pneumoniae* SP160 all express *tet*(M) and had MICs of ≥32 μg/mL for tetracycline, but compound **17j** maintained potency equivalent to isolates of the same species not harboring *tet*(M) (MIC of 0.0156 μg/mL). *S. aureus* SA158 contains *tet*(K) and had MICs of 32 μg/mL and 0.0156 μg/mL for tetracycline and **17j**, respectively. Both *E. coli* EC155 and *K. pneumoniae* KP153 carry *tet*(A) and had MICs of >64 μg/mL for tetracycline and MICs of 1 and 0.5 μg/mL for **17j**, respectively. As compared to tigecycline, compound **17j** was 4- to 16-fold more potent against *S. aureus*, *E. faecalis*, *E. coli* EC107, and *A. baumannii* AB110 and was equipotent to tigecycline against all other strains in the panel.

Ribosomal inhibition by compound **17j** and tetracycline was characterized in an in vitro *E. coli* coupled transcription/translation assay[39] where inhibition of translation of the reporter gene encoding firefly luciferase was detected by luminescence. Compound titrations were tested, and 50% inhibition of total luminescence (IC_{50}) was determined. In a representative assay (Figure 2), compound **17j** displayed 7-fold greater potency than tetracycline (IC_{50} of 0.62 μM (0.39 μg/mL) vs 4.58 μM (2.2 μg/mL)).

The excellent in vitro activity of compound **17j** extended to promising in vivo efficacy in a number of animal infection models, including a murine septicemia model challenged with a SHV-family[40] extended-spectrum β-lactamase producing *E. coli* also encoding a tetracycline-specific *tet*(B) efflux gene (EC133) and a neutropenic thigh model challenged with MRSA containing the ribosomal protection gene *tet*(M) (SA191). As shown in Table 3, compound **17j** administered intravenously had a PD_{50} of 1.3 mg/kg in the septicemia model, compared to 3.5 mg/kg for tigecycline in the same model. The MIC for both

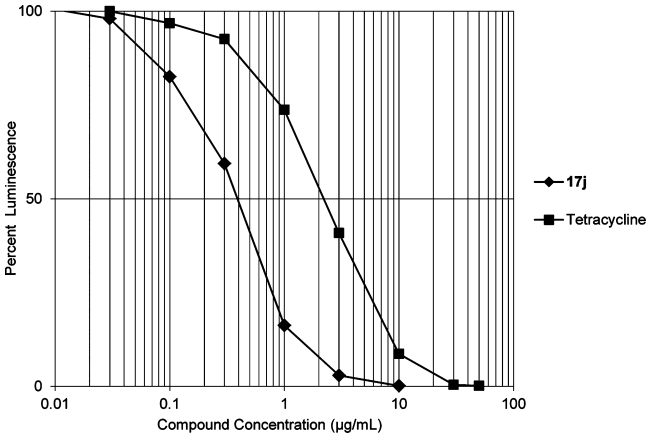


Figure 2. Transcription–translation activity of compound **17j** and tetracycline.

17j and tigecycline against the challenging strain was 0.125 μg/mL. Table 3 also shows the comparison of compound **17j** (MIC = 0.25 μg/mL) to tigecycline (MIC = 0.25 μg/mL) and vancomycin (MIC = 1 μg/mL) in the neutropenic thigh model where the reduction in bacterial burden is used to measure antibacterial activity. Compound **17j** demonstrated a one-log bacterial burden reduction at an IV dose of 0.6 mg/kg and a three-log reduction at 3 mg/kg, while doses required for the two comparators to reach the same levels of bacterial reductions were 3 mg/kg and 17.3 mg/kg for tigecycline and 0.75 mg/kg and 10 mg/kg for vancomycin, respectively.

Selected rat pharmacokinetic (PK) parameters of compound **17j** are summarized in Table 4. When dosed intravenously, **17j** had an AUC of 1.766 μg·hr/mL, lower than tetracycline but 70% greater than tigecycline. Its clearance was similar to tetracycline but 50% slower than tigecycline. All three compounds had similar elimination half-lives of 4–5 h. These PK characteristics of compound **17j** most likely contributed to its higher in vivo efficacy than tigecycline as data in Table 3 has shown. The oral bioavailability was poor in rats for compound **17j** but was only moderate for tetracycline in the same species. Of note, the oral bioavailability of tetracycline in man is 60–80%,[41] suggesting that lower mammals may not model this parameter well. In fact, we later found compound **17j** to have better oral bioavailability in higher mammals.[42]

CONCLUSIONS

We have designed and synthesized a series of fluorocycline analogues using the total synthesis approach. These fluorocyclines possess potent, broad spectrum antibacterial activities against multidrug resistant (MDR) Gram-positive and Gram-negative pathogens. Optimization of the amino group on the C9 side chain indicated that a small alkylamine is particularly preferred for increased antibacterial activity. This led to the discovery of the highly potent fluorocycline **17j**, 7-fluoro-9-pyrrolidinoacetamido-6-demthyl-6-deoxytetracycline. In addition to its potent, broad spectrum in vitro antibacterial activities, compound **17j** also displayed potent ribosomal inhibition, promising in vivo efficacy in animal infection models and desirable pharmacokinetic properties in rats. Compound **17j** was selected for further preclinical studies and demonstrated favorable pharmacological and toxicological profiles. Following IND filing, compound **17j** has successfully completed phase 1 single- and multiple-ascending dose studies in man. Currently, compound **17j** is being evaluated for treatment of adult community-acquired intra-abdominal infections in a global phase 2 trial.

EXPERIMENTAL SECTION

Chemistry. All commercially available solvents, including anhydrous solvents, and reagents were used without further purification. All reactions under dry conditions were performed under nitrogen atmospheres. ^{1}H NMR (nuclear magnetic resonance) spectra were recorded on a 400 MHz JEOL ECX-400 spectrometer. Thin layer chromatography (TLC) analysis was performed on Merck silica-gel 60 F_{254} and visualized under UV light. Flash chromatography was performed on Merck silica gel 60 (40–43 μm). Purity of tested compounds was determined to be ≥95% by reverse phase analytical HPLC/MS analysis (high performance liquid chromatography/mass spectrometry) performed on a Waters Alliance system (column, SunFire C18, 5 μm, 4.6 mm × 50 mm; solvent A, water with 0.1% formic acid; solvent B, acetonitrile with 0.1% formic acid; MS detector, Waters 3100). Reverse phase preparative HPLC was performed on a Waters Autopurification system with mass-directed fraction collection (for final compounds: column, Polymerx RP-1 100A, 10 μm, 150 mm × 21.20 mm; flow rate, 20 mL/min; solvent A, water with 0.05 N HCl; solvent B, acetonitrile. For intermediates: column, SunFire Prep C18 OBD, 5 μm, 19 mm × 50 mm; flow rate, 20 mL/min; solvent A, water with 0.1% formic acid; solvent B, acetonitrile with 0.1% formic acid).

3-Fluoro-6-methoxy-2-methylbenzoic Acid (9). To a THF (5 mL) solution of 5-fluoro-2-methoxybenzoic acid (500 mg, 2.94 mmol, 1 equiv) cooled at −78 °C was added *s*-BuLi (4.60 mL, 1.40 M/THF, 6.44 mmol, 2.2 equiv) and TMEDA (0.97 mL, 6.47 mmol, 2.2 equiv). The reaction was stirred at −78 °C for 2 h. Methyl iodide (1.10 mL, 17.64 mmol, 6 equiv) was added dropwise. The reaction was allowed to warm to 25 °C over 1 h and stirred at 25 °C for 1 h. Aqueous NaOH (6 N, 20 mL) was added. The resulting mixture was extracted with *t*-butyl methyl ether (20 mL × 2). The aqueous layer was acidified with aqueous HCl (6 N) to pH 1 and extracted with EtOAc (20 mL × 4). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to give compound **9** (510 mg, crude, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (dd, J = 9.8, 8.5 Hz, 1 H), 6.75 (dd, J = 9.8, 3.7 Hz, 1 H), 3.86 (s, 3 H), 2.34 (d, J = 2.4 Hz, 3 H). MS (ESI) m/z 185.12 (M + H).

Phenyl 3-Fluoro-6-methoxy-2-methylbenzoate (10). Oxalyl chloride (0.95 mL, 11.10 mmol, 4 equiv) and dry DMF (0.1 mL) were

Table 3. In Vivo Efficacy of Compound 17j (IV)[a]

model	strain		17j	tigecycline	vancomycin
murine septicemia	EC133 *tet* (B)	MIC (μg/mL)	0.125	0.125	NT
		PD_{50} (mg/kg)	1.3	3.5	NT
neutropenic thigh	SA191 *tet* (M) (MRSA)	MIC (μg/mL)	0.25	0.25	1
		dose at 1 log reduction (mg/kg)	0.6	3	0.75
		dose at 3 log reduction (mg/kg)	3	17.3	10

[a]SA, *Staphylococcus aureus*; EC, *Esherichia coli*. EC133 was obtained from Clinical Microbiology Institute, Wilsonnillw, OR, and SA191 was obtained from Vivisource, Waltham, MA. NT, not tested.

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Table 4. Rat PK Parameters of Compound 17j and Comparators[a]

compd	dosage route (mg/kg)	CLs (L/h/kg)	V_z (L/kg)	T1/2 (L/kg)	C_{max} (hr)	AUC_{last}	% F (%)
17j	IV (1)	0.564	3.2	4.0	0.812	1.766	
	PO (10)			6.9	0.045	0.295	1.7
tetracycline	IV (1)	0.542	1.2	4.6	2.664	3.083	
	PO (10)			5.2	0.791	4.536	14.9
tigecycline	IV (1)	0.929	6.12	4.6	0.428	1.052	
	PO (10)			3.98	0.0278	0.107	1.0

[a]Measured in Sprague–Dawley rats (n = 3). Animals were fasted overnight (minimum of 12 h) and given a single oral (10 mg/kg) or IV (1 mg/kg) dose followed by a sampling scheme for 24 h. Sterile water was used as vehicle. PK parameters were calculated by noncompartmental analysis using WinNonlin.

added to compound **9** (510 mg, 2.77 mmol, 1 equiv) in dry CH_2Cl_2 (15 mL). The reaction was stirred at 25 °C for 1 h and concentrated. The resulting solid was redissolved in dry CH_2Cl_2 (15 mL). Phenol (520 mg, 5.50 mmol, 2 equiv), DMAP (670 mg, 5.60 mmol, 2 equiv), and triethylamine (1.90 mL, 13.90 mmol, 7 equiv) were added. The reaction was stirred at 25 °C for 12 h and concentrated. The residue was redissolved in EtOAc (100 mL). The solution was washed with aqueous NaOH (1 N, 100 mL × 1), water (100 mL × 1), and brine (50 mL × 1), dried (Na_2SO_4), and concentrated. Flash chromatography on silica gel (40:1 hexanes/EtOAc) yielded the desired product **10** (400 mg, 52% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.41 (m, 2 H), 7.31–7.24 (m, 3 H), 7.08 (dd, J = 9.2, 9.2 Hz, 1 H), 6.77 (dd, J = 9.2, 3.7 Hz, 1 H), 3.88 (s, 3 H), 2.36 (d, J = 2.3 Hz, 3 H). MS (ESI) m/z 261.12 (M + H).

Phenyl 3-Fluoro-6-hydroxy-2-methylbenzoate (11). BBr_3 (1.85 mL, 1 M/CH_2Cl_2, 1.85 mmol, 1.2 equiv) was added to compound **10** (400 mg, 1.54 mmol, 1 equiv) in CH_2Cl_2 (10 mL) at −78 °C. The reaction was warmed from −78 to 25 °C over 1.5 h and quenched with saturated aqueous $NaHCO_3$ (100 mL). The reaction mixture was extracted with EtOAc (100 mL × 1, 50 mL × 2). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to yield compound **11** (360 mg, crude, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.66 (s, 1 H), 7.50–7.44 (m, 2 H), 7.36–7.31 (m, 1 H), 7.26–7.18 (m, 3 H), 6.86 (dd, J = 9.3, 4.9 Hz, 1 H), 2.60 (d, J = 2.4 Hz, 3 H). MS (ESI) m/z 245.11 (M − H).

Phenyl 6-{[(*tert*-Butoxy)carbonyl]oxy}-3-fluoro-2-methylbenzoate (12). Boc_2O (350 mg, 1.60 mmol, 1.1 equiv) and DMAP (20 mg, 0.16 mmol, 0.1 equiv) were added to compound **11** (360 mg, 1.46 mmol, 1 equiv) in CH_2Cl_2 (8 mL). The reaction was stirred at 25 °C for 1.5 h and concentrated. Flash chromatography on silica gel (35:1 hexanes/EtOAc) yielded the desired compound **12** (400 mg, 75% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.41 (m, 2 H), 7.31–7.23 (m, 3 H) 7.18 (dd, J = 8.8, 8.7 Hz, 1 H), 7.10 (dd, J = 8.8, 4.4 Hz, 1 H), 2.41 (d, J = 2.3 Hz, 3 H), 1.44 (s, 9 H). MS (ESI) m/z 345.18 (M − H).

(1*R*,3*S*,4*S*,11*S*)-8-(Benzyloxy)-11-[(*tert*-butyldimethylsilyl)oxy]-4-(dimethylamino)-19-fluoro-12-hydroxy-10,14-dioxo-6-oxa-7-azapentacyclo[11.8.0.0.^{3,11}0.^{5,9}0^{15,20}]henicosa-5(9),7,12,15(20),16,18-hexaen-16-yl *tert*-Butyl Carbonate (14). A THF solution (6 mL) of compound **12** (487 mg, 1.40 mmol, 2 equiv) was added to a THF solution (5 mL) of LDA (6.30 mL, 10 wt % in hexane, 4.20 mmol, 6 equiv) and TMEDA (1.70 mL, 11.20 mmol, 16 equiv) at −78 °C. The reaction was stirred at −78 °C for 5 min. A THF solution of enone **13** (339 mg, 0.70 mmol, 1 equiv) was added to the reaction mixture dropwise. The reaction was warmed from −78 to 25 °C over 1 h and quenched with saturated NH_4Cl (100 mL). The reaction mixture was extracted with EtOAc (50 mL × 3). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to yield the crude product. Preparative reverse phase HPLC purification yielded compound **14** as a yellow solid (185 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.67 (s, 1 H), 7.51–7.46 (m, 2 H), 7.39–7.29 (m, 3 H), 7.21 (dd, J = 8.9, 8.9 Hz, 1 H), 7.03 (dd, J = 8.9, 4.0 Hz, 1 H), 5.34 (s, 2 H), 3.93 (d, J = 10.4 Hz, 1 H), 3.30–3.21 (m, 1 H), 3.10–3.00 (m, 1 H), 2.57–2.41 (m, 3 H), 2.48 (s, 6 H), 2.17–2.12 (m, 1 H), 1.53 (s, 9 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.12 (s, 3 H). MS (ESI) m/z 735.45 (M + H).

(1*R*,3*S*,4*S*,11*S*)-8-(Benzyloxy)-4-(dimethylamino)-19-fluoro-11,12,16-trihydroxy-6-oxa-7-azapentacyclo-[11.8.0.0.^{3,11}0.^{5,9}0^{15,20}]henicosa-5(9),7,12,15(20),16,18-hexaene-10,14-dione. Aqueous HF (3 mL, 48%) and TFA (4 mL) were added to a CH_3CN solution (7 mL) of **14** (210 mg, 0.29 mmol) in a polypropylene tube at 25 °C. The reaction was stirred at 25 °C for 18 h. The resulting mixture was poured into an aqueous solution of K_2HPO_4 (21 g, dissolved in 150 mL water). The mixture was extracted with EtOAc (50 mL × 3). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to yield the title compound (180 mg, crude). ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.64 (s, 1 H), 11.47 (s, 1 H), 7.49–7.45 (m, 2 H), 7.39–7.32 (m, 3 H), 7.14 (dd, J = 9.2, 8.8 Hz, 1 H), 6.77 (dd, J = 9.2, 4.3 Hz, 1 H), 5.36 (s, 2 H), 3.68 (d, J = 3.7 Hz, 1 H), 3.09 (dd, J = 15.6, 4.6 Hz, 1 H), 3.02–2.92 (m, 1 H), 2.84–2.79 (m, 1 H), 2.49 (s, 6 H), 2.34–2.22 (m, 1 H), 2.09–2.02 (m, 1 H), 1.55–1.44 (m, 1 H). MS (ESI) m/z 521.30 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (15). Palladium on carbon (35 mg, 10 wt %) was added to a CH_3OH/dioxane solution (4 mL/4 mL) of the above crude product (180 mg). The reaction was purged with hydrogen and stirred under a hydrogen atmosphere (1 atm, balloon) at 25 °C for 1 h. The reaction mixture was filtered through a small Celite pad. The Celite pad was washed with methanol (5 mL × 3). The combined filtrates were concentrated to yield the crude product, which was purified by preparative reverse phase HPLC to yield compound **15** (51 mg, yellow solid, 41% for 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (dd, J = 9.2, 9.2 Hz, 1 H), 6.80 (dd, J = 9.2, 4.3 Hz, 1 H), 4.09 (br s, 1 H), 3.14 (dd, J = 15.0, 4.6 Hz, 1 H), 3.04 (s, 3 H), 2.96 (s, 3 H), 3.09–2.91 (m, 2 H), 2.31–2.18 (m, 2 H), 1.68–1.56 (m, 1 H). MS (ESI) m/z 433.28 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-nitro-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide. A mixture of HNO_3 (8.5 μL, 69%) and H_2SO_4 (0.5 mL) was added to a H_2SO_4 solution (1 mL) of compound **15** (51 mg, 0.12 mmol) at 0 °C. The reaction was stirred at 0 °C for 30 min. The reaction mixture was added dropwise to vigorously stirred diethyl ether (60 mL). The suspension was filtered through a small Celite pad. The Celite pad was washed with more diethyl ether (5 mL × 4). The Celite pad was then eluted with CH_3OH until the eluent became colorless. The yellow CH_3OH eluent was collected and concentrated under reduced pressure to afford the crude product of the title compound. ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (d, J = 8.5 Hz, 1 H), 4.09 (br s, 1 H), 3.50–2.97 (m, 3 H), 3.04 (s, 3 H), 2.96 (s, 3 H), 2.46–2.36 (m, 1 H), 2.29–2.20 (m, 1 H), 1.71–1.59 (m, 1 H). MS (ESI) m/z 478.20 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-9-Amino-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (16). Palladium on carbon (12 mg, 10 wt %) was added to a CH_3OH solution (4 mL) of the above crude product. The reaction was purged with hydrogen and stirred under a hydrogen atmosphere (1 atm, balloon) at 25 °C for 2 h. The

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catalyst was filtered off with a small Celite pad. The Celite pad was washed with methanol (5 mL × 3). The combined filtrates were concentrated to yield the crude product, which was purified by preparative reverse phase HPLC to yield the desired compound **16** (43 mg, yellow solid, 81% for 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.43 (d, J = 8.5 Hz, 1 H), 4.11 (br s, 1 H), 3.22−3.16 (m, 1 H), 3.15−3.08 (m, 1 H), 3.06−2.95 (m, 1 H), 3.04 (s, 3 H), 2.96 (s, 3 H), 2.40−2.31 (m, 1 H), 2.28−2.21 (m, 1 H), 1.71−1.59 (m, 1 H). MS (ESI) m/z 448.24 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-9-[2-(*tert*-Butylamino)acetamido]-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17e). 2-*t*-Butylaminoacetylchloride hydrochloride (4.2 mg, 0.022 mmol, 2 equiv) was added to a DMF solution (0.1 mL) of compound **16** (5 mg, 0.011 mmol, 1 equiv) at 25 °C. The reaction was stirred at 25 °C for 30 min. The reaction mixture was diluted with 0.05 N aqueous HCl (2 mL) and purified by preparative reverse phase HPLC to yield compound **17e** as a yellow solid (3.9 mg, bis HCl salt, 56%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 11.0 Hz, 1 H), 4.11 (br s, 1 H), 4.09 (s, 2 H), 3.22−2.86 (m, 3 H), 3.05 (s, 3 H), 2.97 (s, 3 H), 2.33−2.20 (m, 2 H), 1.69−1.57 (m, 1 H), 1.42 (s, 9 H). MS (ESI) m/z 561.39 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-9-[2-(Azetidin-1-yl)acetamido]-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17i). Anhydrous Na_2CO_3 (16 mg, 0.15 mmol, 5.5 equiv) was added to an anhydrous DMPU/acetonitrile (0.2 mL, 3:1, v/v) solution of compound **16** (12 mg, 0.027 mmol, 1 equiv). Bromoacetyl bromide (2.8 μL, 0.032 mmol, 1.2 equiv) was added. The reaction was stirred at 25 °C for 10 min, followed by the addition of azetidine (36 μL, 0.54 mmol, 20 equiv). The reaction mixture was stirred at 25 °C for 2 h, concentrated under reduced pressure, acidified with HCl (0.5 N in methanol, 0.7 mL), and added dropwise to vigorously stirred diethyl ether (10 mL). The resulting suspension was filtered through a small Celite pad. The Celite pad was washed with more diethyl ether (5 mL × 4) and eluted with methanol until the eluent became colorless. The yellow CH_3OH eluent was collected and concentrated under reduced pressure to afford the crude product, which was purified by preparative reverse phase HPLC to yield compound **17i** as a yellow solid (2.0 mg, bis HCl salt, 12%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (d, J = 11.0 Hz, 1 H), 4.41−4.31 (m, 2 H), 4.32 (s, 2 H), 4.24−4.13 (m, 2), 4.08 (br s, 1 H), 3.18−2.86 (m, 3 H), 3.03 (s, 3 H), 2.95 (s, 3 H), 2.71−2.57 (m, 1 H), 2.54−2.42 (m, 1 H), 2.33−2.16 (m, 2 H), 1.69−1.57 (m, 1 H). MS (ESI) m/z 545.20 (M + H).

The following compounds were prepared similarly to compound **17e** or **17i**.

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-[2-(ethylamino)acetamido]-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17a). ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (d, J = 10.8 Hz, 1 H), 4.00 (s, 1 H), 3.99 (s, 2 H), 3.10−2.87 (m, 11 H), 2.32−2.12 (m, 2 H), 1.59−1.51 (m, 1 H), 1.26 (t, J = 7.2 Hz, 3 H). MS (ESI) m/z 533.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-{2-[(2-methoxyethyl)amino]acetamido}-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17b). ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 11.0 Hz, 1 H), 4.12 (s, 2 H), 4.09 (s, 1 H), 3.72−3.67 (m, 2 H), 3.43 (s, 3 H), 3.19−2.92 (m, 1 1 H), 2.35−2.18 (m, 2 H) 1.71−1.58 (m, 1 H). MS (ESI) m/z 563.23 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-{2-[(2,2,2-trifluoroethyl)amino]acetamido}-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17c). ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 11.0 Hz, 1 H), 4.22 (s, 2 H), 4.14−4.05 (m, 3 H), 3.18−2.84 (m, 9 H), 2.34−2.17 (m, 2 H), 1.70−1.57 (m, 1 H). MS (ESI) m/z 587.28 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-{2-[(propan-2-yl)amino]acetamido}-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17d). ^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (d, J = 11.0 Hz, 1 H), 4.08 (s, 2 H), 4.01−3.89 (m, 1 H), 3.50−3.42 (m, 1 H), 3.20−2.84 (m, 9 H), 2.30 (at, J = 14.7 Hz, 1 H), 2.23−2.15 (m, 1 H), 1.70−1.58 (m, 1 H), 1.37 (d, J = 6.7 Hz, 6 H). MS (ESI) m/z 547.25 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-[2-(phenylamino)acetamido]-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17f). ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (d, J = 10.4 Hz, 1 H), 7.38−7.34 (m, 2 H), 7.10−7.06 (m, 3 H), 4.17 (s, 2 H), 4.10 (s, 1 H), 3.18−2.99 (m, 11 H), 2.29 (dd, J = 15.6, 15.6 Hz, 1 H), 2.25 (ddd, J = 14.8, 5.2, 2.8 Hz, 1 H), 1.66 (ddd, J = 14.8, 14.8, 14.8 Hz, 1 H). MS (ESI) m/z 581.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-{2-[(pyridin-3-yl)amino]acetamido}-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17g). ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (d, J = 11.2 Hz, 1 H), 8.16 (d, J = 2.4 Hz, 1 H), 8.06 (d, J = 5.2 Hz, 1 H), 7.85−7.78 (m, 2 H), 4.27 (s, 2 H), 4.11 (s, 1 H), 3.18−2.98 (m, 9 H), 2.32−2.21 (m, 2 H), 1.70−1.60 (m, 1 H). MS (ESI) m/z 582.2 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-[2-(dimethylamino)acetamido]-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17h). ^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (d, J = 11.0 Hz, 1 H), 4.24 (s, 2 H), 4.09 (s, 1 H), 3.14−2.93 (m, 1 5 H), 2.24−2.18 (m, 2 H), 1.65 (dt, J = 13.4, 11.6 Hz, 1 H). MS (ESI) m/z 533.17 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-[2-(pyrrolidin-1-yl)acetamido]-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17j). ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (d, J = 11.0 Hz, 1 H), 4.33 (s, 2 H), 4.10 (s, 1 H), 3.83−3.72 (m, 2 H), 3.25−2.89 (m, 1 2 H), 2.32−2.00 (m, 6 H), 1.69−1.56 (m, 1 H). MS (ESI) m/z 559.39 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-[2-(piperidin-1-yl)acetamido]-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17k). ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (d, J = 11.0 Hz, 1 H), 4.19 (s, 2 H), 4.09 (s, 1 H), 3.65−3.58 (m, 2 H), 3.19−2.92 (m, 1 0 H), 2.34−2.18 (m, 2 H), 2.02−1.79 (m, 6 H), 1.69−1.50 (m, 2 H). MS (ESI) m/z 573.35 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-[2-(morpholin-4-yl)acetamido]-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17l). ^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (d, J = 11.0 Hz, 1 H), 4.28 (s, 2 H), 4.03−4.00 (m, 2 H), 3.94−3.81 (m, 2 H), 3.68-.3.55 (m, 2 H), 3.20−2.88 (m, 1 2 H), 2.36−2.18 (m, 2 H), 1.71−1.57 (m, 1 H). MS (ESI) m/z 575.37 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-{2-[(3*R*)-3-fluoropyrrolidin-1-yl]acetamido}-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17m). ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 11.0 Hz, 1 H), 5.54−5.31 (m, 1 H), 4.39−4.20 (m, 2 H), 4.09−4.01 (m, 1 H), 3.40−3.30 (m, 2 H), 3.09−2.89 (m, 1 2 H), 2.50−2.34 (m, 2 H), 2.34−2.25 (m, 1 H), 2.24−2.16 (m, 1 H), 1.71−1.58 (m, 1 H). MS (ESI) m/z 577.32 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-{2-[(3*S*)-3-fluoropyrrolidin-1-yl]acetamido}-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17n). ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (d, J = 10.4 Hz, 1 H), 5.57−5.37 (m, 1 H), 4.47−4.33 (m, 2 H), 4.15−3.87 (m, 2 H), 3.72−3.40 (m, 1 H), 3.17−2.83 (m, 1 2 H), 2.55−2.34 (m, 2 H), 2.33−2.18 (m, 2 H), 1.69−1.57 (m, 1 H). MS (ESI) m/z 577.37 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-{2-[(3*R*)-3-(dimethylamino)pyrrolidin-1-yl]acetamido}-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17o). ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (d, J = 10.7 Hz, 1 H), 4.08 (s, 1 H), 4.00−3.91 (m, 2 H), 3.09−2.57 (m, 1 8 H), 3.26−3.18 (m, 3 H), 2.49−2.34 (m, 2 H), 2.35−2.06 (m, 2 H), 1.72−1.59 (m, 1 H). MS (ESI) m/z 602.37 (M + H).

**(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-{2-[(3*R*)-3-hydroxypyrrolidin-1-yl]acetamido}-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carbox-

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amide (17p). ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (d, J = 11.0 Hz, 1 H), 4.62−4.54 (m, 1 H), 4.48−4.24 (m, 2 H), 4.08 (s, 1 H), 3.99−3.69 (m, 3 H), 3.50−3.40 (m, 1 H), 3.17−2.90 (m, 9 H), 2.44−2.11 (m, 4 H), 2.10−2.00 (m, 1 H), 1.69−1.56 (m, 1 H). MS (ESI) m/z 575.27 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-{2-[(2*S*)-2-(methoxymethyl)pyrrolidin-1-yl]-acetamido}-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17q). ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 11.0 Hz, 1 H), 4.54 (d, J = 16.5 Hz, 1 H), 4.26 (d, J = 15.9 Hz, 1 H), 4.09 (s, 1 H), 3.95−3.81 (m, 2 H), 3.81−3.75 (m, 1 H), 3.69−3.62 (m, 1 H), 3.35 (s, 3 H), 3.23−2.92 (m, 9 H), 2.35−2.04 (m, 6 H), 1.91−1.80 (m, 1 H), 1.71−1.59 (m, 1 H). MS (ESI) m/z 603.35 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-(2-{octahydrocyclopenta[*c*]pyrrol-2-yl}-acetamido)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17r). ^{1}H NMR (400 MHz, CD_3OD, 2:1 mixture of diastereomers) δ 8.25 (d + d, J = 11.0 Hz, 1 H), 4.29, 4.24 (s + s, 2 H), 4.08 (s + s, 1 H), 4.01−3.92 (m + m, 3 H), 3.20−2.62 (m + m, 1 3 H), 2.35−2.16 (m + m, 3 H), 1.83−1.46 (m + m, 5 H). MS (ESI) m/z 599.36 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-9-[2-(2,3-Dihydro-1*H*-isoindol-2-yl)-acetamido]-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (17s). ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (d, J = 11.0 Hz, 1 H), 7.41 (s, 5 H), 4.50−4.37 (m, 2 H), 4.05 (s, 1 H), 3.95−3.81 (m, 2 H), 3.40−3.37 (m, 1 H), 3.24−3.15 (m, 3 H), 3.10−2.70 (m, 9 H), 2.36−2.25 (m, 1 H), 2.25−2.16 (m, 1 H), 1.72−1.59 (m, 1 H). MS (ESI) m/z 607.34 (M + H).

In Vitro Coupled *E. coli* Transcription/Translation Assay. Antitranslational activity (IC_{50} values) was assessed in an *E. coli* in vitro coupled transcription/translation assay (TnT)[39] with a firefly luciferase readout (cat. no. L1020, Promega, Madison, WI). Reactions were run at a volume of 20 μL in Costar black 96-well assay plates (Costar cat. no. 3915) for 60 min at 37 °C. The reaction was stopped by placing on ice for 5 min, followed by addition of 25 μL of luciferase assay substrate (cat. no. E1500, Promega, Madison, WI). Plates were read on a LUMIStar Optima (BMG Labtech, Ortenberg, Germany) with gain set to 3600, 0.2 s read, 0 s between wells. Percent luminescence was plotted against inhibitor concentration with 50% inhibition versus untreated controls marked as the IC_{50} value.

Susceptibility Testing. Compound stocks were prepared and serially diluted in sterile deionized water. Minimal inhibitory concentration (MIC) determinations were performed in liquid medium in 96-well microtiter plates according to the methods described by the Clinical and Laboratory Standards Institute (CLSI).[43] Cation-adjusted Mueller Hinton broth was obtained from BBL (cat. no. 212322, Becton Dickinson, Sparks, MD), prepared fresh and kept at 4 °C prior to testing. Defibrinated horse blood (cat. no. A0432, PML Microbiologicals, Wilsonville, OR) was used to supplement medium, as appropriate. All test methods met acceptable standards based on recommended quality control ranges for all comparator antibiotics and the appropriate ATCC quality control strains.

Mouse Systemic Infection Studies. *E. coli* EC133 was grown overnight with shaking in brain heart infusion (BHI) broth at 37 °C. Five hours prior to initiation of infection, a 1:10 dilution was performed into fresh BHI and the culture was allowed to grow for 5 h. The 5 h culture was subsequently diluted into 5% hog gastric mucin to achieve a bacterial inoculum that would result in 0% survival in infected mice by 48 h postinfection (generally (1−3) × 10^6 colony-forming units (CFU)/mouse in a volume of 0.5 mL). CD-1 mice (group size of six) were inoculated intraperitoneally (IP) and received treatment via intravenous (IV) injection 1 h postinfection. After 24 h, percent survival was calculated and the dose (mg/kg) affecting 50% survival, the protective dose 50% (PD_{50}), was reported along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software).

Mouse Thigh Infection Studies. Mice were rendered neutropenic through two consecutive IP injections of cyclophosphamide of 150 and 100 mg/kg on days −4 and −1, respectively. Mice were infected with approximately 5 × 10^5 CFU/mL of bacteria (SA191 *tet*(M)) in a 0.1 mL volume into the right thigh. At 1.5 h postinfection, mice received treatment via IV injection. One group of infected mice were euthanized and thighs were excised and processed for bacterial CFU and serves as T = 0 controls. Twenty-four hours post-treatment, the remaining mice were euthanized, thighs aseptically removed, weighed, homogenized, serially diluted, and CFUs per gram of thigh tissue were calculated. The amount of test article required to achieve 1 and 3 $\log_{10}$ reductions from 24 h control thighs was determined.

PK Analyses. Pharmacokinetic (PK) parameters were determined in Sprague−Dawley rats (n = 3). Animals were fasted overnight (minimum of 12 h) and given a single oral (10 mg/kg) or IV dose (1 mg/kg) followed by a sampling scheme for 24 h. Plasma and dosing solution concentrations were determined by TurboIonspray LC/MS-MS analysis using appropriate standard curves. PK parameters were calculated by noncompartmental analysis using WinNonlin.

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ABBREVIATIONS USED

ATCC, American Type Culture Collection; AUC, area under curve; aq, aqueous; BHI, brain heart infusion; CFU, colony-forming units; cIAI, complicated intra-abdominal infection; Cl, clearance; C_{max}, maximum plasma concentration; CLSI, Clinical and Laboratory Standards Institute; DMAP, 4-(dimethylamino)pyridine; ESI, electrospray ionization; %*F*, percent oral bioavailability; HPLC, high performance liquid chromatography; IP, intraperitoneal; IV, intravenous; LDA, lithium diisopropylamide; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; PD_{50}, dose at which 50% protection was observed; PK, pharmacokinetic; PO, oral; $T_{1/2}$, elimination half-life; TBS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; TMEDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; V_z, volume of distribution

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