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Fluorocyclines. 2. Optimization of the C-9 Side-Chain for Antibacterial Activity and Oral Efficacy

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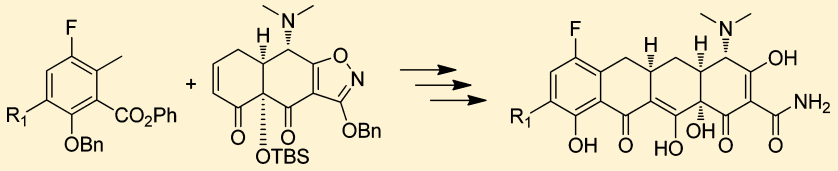
Supporting Information



ABSTRACT: Utilizing a fully synthetic route to tetracycline analogues, the C-9 side-chain of the fluorocyclines was optimized for both antibacterial activity and oral efficacy. Compounds were identified that overcome both efflux (*tet*(K), *tet*(A)) and ribosomal protection (*tet*(M)) tetracycline-resistance mechanisms and are active against Gram-positive and Gram-negative organisms. A murine systemic infection model was used as an oral efficacy screen to rapidly identify compounds with oral bioavailability. Two compounds were identified that exhibit both oral bioavailability in rat and clinically relevant bacterial susceptibility profiles against major respiratory pathogens. One compound demonstrated oral efficacy in rodent lung infection models that was comparable to marketed antibacterial agents.

INTRODUCTION

Widespread use of the current arsenal of approved antibacterial agents and the resulting development of bacterial resistance continues to necessitate the discovery and development of new antibiotics.[1−4] In the preceding paper (DOI: 10.1021/jm201465w),[25] we outlined the discovery of TP-434 (**1**),[5] a new broad-spectrum tetracycline analogue active against multidrug-resistant Gram-positive and Gram-negative pathogens that is currently undergoing phase 2 clinical trials in patients with complicated intra-abdominal infections (cIAI) as well as phase 1 trials to explore its potential for use as an oral therapy. This compound is a member of the broader class of 7-fluoro-6-demethyl-6-deoxytetracyclines which we have termed "fluorocyclines". Importantly, many of these compounds have been shown to overcome the two major bacterial resistance mechanisms identified for tetracyclines: (1) tetracycline-specific efflux pumps[6] (i.e., *tet*(A-E))[6a,b] and *tet*(K-L)[6c]) and (2) ribosomal protection (i.e., *tet*(M-O)).[7]

Traditionally, tetracyclines have been prepared by semisynthesis, a technique which is largely limited to electrophilic aromatic substitution reactions at the 7- and 9-positions of the tetracycline D-ring.[8] Recent examples of this include the 9-substituted glycylcyclines, such as tigecycline (**3**),[9] and the aminomethylcyclines, such as omadacycline (**4**),[10] both of which have shown improved activity for tetracycline-resistant organisms (Figure 1). More recently, we have extended the total synthetic methodology developed by Myers and coworkers[11−13] to the preparation of 8-azatetracyclines[14] and 8,9-pentacyclines,[15] generating completely novel analogues that are inaccessible via semisynthesis. While 7-fluorosancycline (**7a**, [9]R = H) has been prepared by fluorination of sancycline, the harsh conditions and subsequent low yields render the route impractical for generating sufficient quantities of material.[16] Furthermore, substitution at other ring positions on 7-fluorosancycline has not been reported. By employing a tandem Michael−Dieckmann condensation between a D-ring precursor **5** and enone **6**, the 7-fluorocyclines (**7**) can be readily accessed in sufficient quantities and with appropriate chemical protection to allow for rapid and efficient analoguing.

A particularly attractive aspect of the tetracyclines in general is their oral bioavailability (*F*) in humans.[17] In fact, almost all of the older tetracyclines can be administered orally, with minocycline and doxycycline having nearly 100% oral bioavailability.[18] One complication for drug discovery in the tetracycline class, however, is that oral bioavailability in rodents (rats and mice), the most commonly used and cost-effective species for infection models and pharmacokinetics, can be significantly lower than that in humans. For example, tetracycline (**2**) is generally reported to have oral bioavailability of 50−70% in humans,[19] whereas we have found the oral bioavailability to be only about 15% in rats and 6.3% in mice. Omadacycline appears to have a similar profile as it has very limited oral bioavailability in rats (0.7% *F*) and mice (1.5% *F*) but has been reported to have 33% oral bioavailability in man in a phase 2 clinical trial.[10] The C-9 glycylamide side chains found in tigecycline and **1** also

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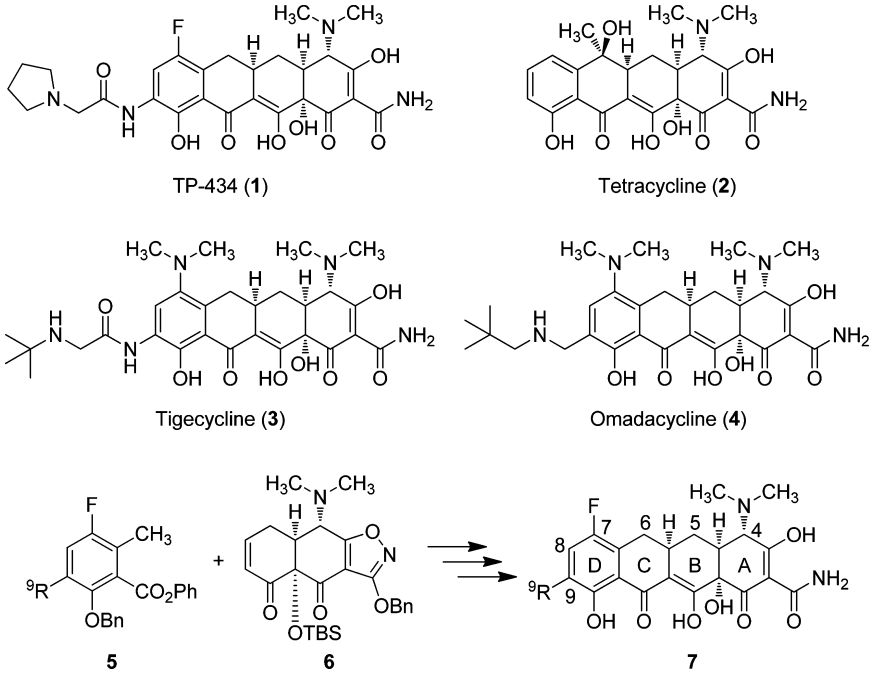

Figure 1. Tetracycline (**2**) and its semisynthetic (**3** and **4**) and fully synthetic (**1** and **7**) derivatives.

appear to impart low oral bioavailability in rats and mice, while data in humans has not been reported. As previously reported, the oral bioavailability of **1** appears to improve in larger animals, leading us to believe low bioavailability in rats may not adequately predict oral bioavailability in humans.[20] In this paper, we describe a detailed study of 9-substituted-7-fluorocyclines with two goals: (a) to explore a more diverse set of chemical space at the C-9 position and (b) to identify compounds with improved oral efficacy in lower animal species (rodents), enabling a more complete pharmacological and pharmacokinetic evaluation of compound activity in animal models.

RESULTS AND DISCUSSION

Chemistry. As described in the preceding paper,[25] many of the 7-F-9-amino derivatives were prepared from compound **8**. Treatment of **8** with either acid chlorides or carboxylic acids and 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) gave the 9-amido compounds **9** (Scheme 1). For the saturated heterocyclic compounds **9j−o** and **9s−u**, the side chains were introduced as the benzyloxycarbonyl (Cbz) protected amines and were deprotected under hydrogenation conditions to give the secondary amines **9j**, **9k**, **9s**, and **9u**. These compounds were further functionalized by reductive aminations with aldehydes or ketones to give compounds **9l−o** and **9t**. The 9-sulfonamides **10a−c** were prepared by reaction of **8** with the corresponding sulfonyl chlorides, while the 9-urea substituted fluorocycline **11** was generated from **8** upon treatment with methylisocyanate.

While derivatization of compound **8** as described above was effective in many cases, we found that reactions involving more forcing conditions, such as strong base or prolonged heating, led to significant decomposition. Additionally, it is known that epimerization of the C-4 dimethylamino group can occur in the pH range of 2−6.[21] Thus, we have developed chemistry to enable functionalization of the 9-position prior to deprotection to the final tetracycline compounds (Scheme 2). Aromatic compound **12** was nitrated, the phenol was protected with benzyl bromide, and the nitro group was reduced using sodium hydrosulfite to give compound **13** in 80% overall yield. Protection of the aniline by alkylation with allylbromide gave the diallylamino compound **14** in 89% yield. Other aniline protecting groups were explored but were either incompatible with the Michael−Dieckmann annulation conditions (i.e., mono- or di-Boc) or were not orthogonal (i.e., dibenzyl). The Michael−Dieckmann annulation was carried out by deprotonation of **14** with lithium diisopropylamide (LDA) at −78 °C, followed by addition of the enone **6** and subsequent addition of lithium bis-(trimethylsilyl)amide (LHMDS). Warming to −10 °C gave the fully protected intermediate **15** in moderate yield. The aniline intermediate **16** was then obtained in 71% yield upon treatment of compound **15** with $(Ph_3P)_4Pd$ and *N*,*N*-dimethylbarbituric acid.

Compound **16** was readily derivatized as outlined in Scheme 3. Acylation upon reaction with acid chlorides or with carboxylic acids mediated by HATU provided the protected amide intermediates **17**, which were desilylated with aqueous HF and hydrogenated to give the final compounds **9v−bb**. A palladium-mediated amination was carried out on compound **16** using 4,6-dichloropyrimidine as the coupling partner, Pd_2dba_3 and 9,9-dimethyl-4,5-bis-(diphenylphosphino)xanthenes (Xantphos) as catalysts, and K_3PO_4 as base.[22] Deprotection as above gave compound **18**. The 9-amino group of **16** was also functionalized via reductive aminations with aldehydes, acetic acid, and $Na(OAc)_3BH$ to give the protected intermediates **19**, which were deprotected as described above to yield the 9-aminoalkyl compounds **20a−e**.

9-Aminomethyl-7-fluorocyclines were prepared from 7-fluorocycline **7a** (Scheme 4). Thus, compound **7a** was treated with benzyl *N*-(hydroxymethyl)carbamate in TFA/CH_3SO_3H to provide the 9-methylamino compound **21**. Reductive amination with pivaldehyde and $Na(OAc)_3BH$ provided the alkylated compound **22**. Acylation of **21** with 2-*t*-butylaminoacetyl-chloride gave the amide **23**.

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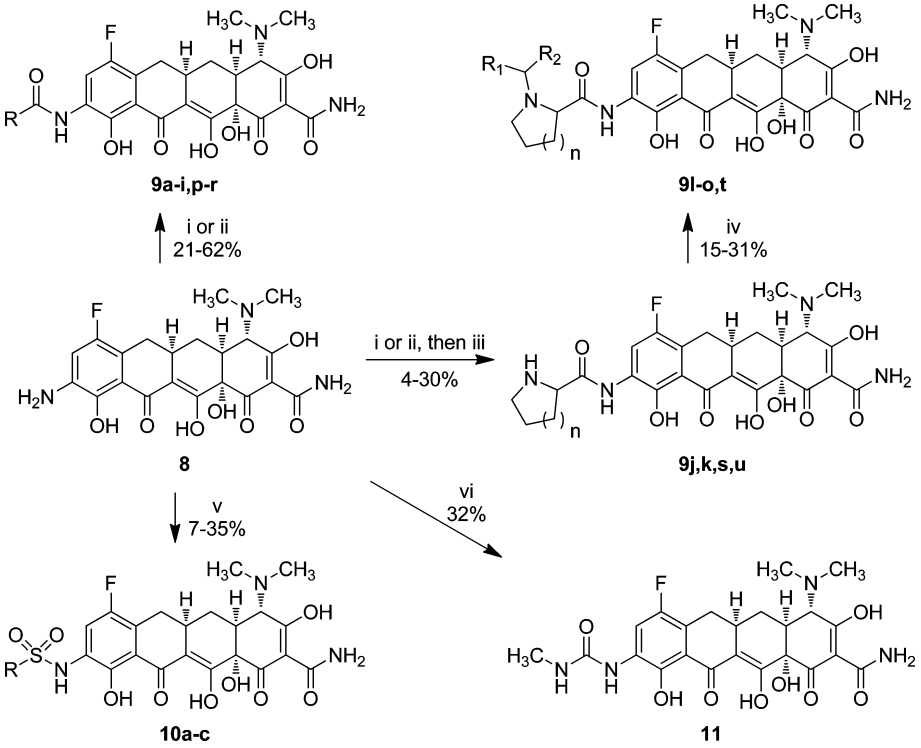
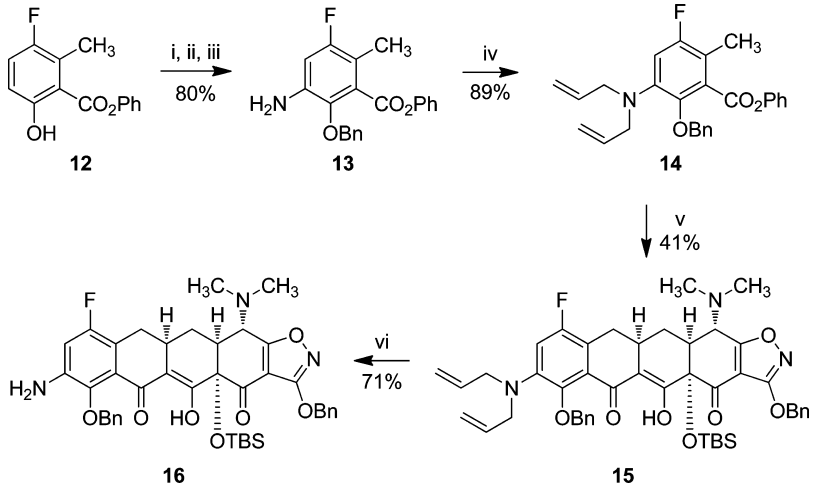
Scheme 1. Synthesis of 9-Amido-, 9-Sulfonamido-, and 9-Urea-7-fluorocyclines[a]



[a]Reagents and conditions: (i) for **9a**–**9k**, RCOCl, DMF or RCOCl, Na_2CO_3, THF; (ii) for **9p**–**s** and **9u**, RCO_2H, HATU, Et_3N, DMF; (iii) H_2, Pd/C, MeOH; (iv) R_1R_2CO, Et_3N, $Na(OAc)_3BH$, DMF; (v) RSO_2Cl, DIEA, THF; (vi) CH_3NCO, Et_3N, CH_2Cl_2.

Scheme 2. Alternate Synthesis of 9-Amino-7-fluorocyclines[a]



[a]Reagents and conditions: (i) HNO_3, *n*-Bu_4NBr, 1,2-dichloroethane, water; (ii) BnBr, K_2CO_3, KI, acetone, 56 °C; (iii) $Na_2S_2O_4$, THF, water, 10 °C to room temp; (iv) allylbromide, K_2CO_3, KI, NMP, 100 °C; (v) (a) LDA, TMEDA, −78 °C, (b) **6**, LHMDS, −78 °C to −10 °C; (vi) $(Ph_3P)_4Pd$, *N*,*N*-dimethylbarbituric acid, CH_2Cl_2, 35 °C.

Biology. The in vitro antibacterial activities of all analogues were determined for a panel of Gram-positive (e.g., *Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram-negative (e.g., *Escherichia coli* and *Klebsiella pneumoniae*) bacteria. Initially, a set of 7-fluorocyclines was prepared and assayed in order to explore the structure−activity relationships (SAR) of the C-9 glycylamido and aminomethyl side chains (Table 1). The unsubstituted 9-amino-7-fluorocycline **8** showed comparable minimum inhibitory concentrations (MICs) to tetracycline for the tetracycline-susceptible strains but had significantly improved activity for the strains bearing *tet*(M) and *tet*(K) resistance mechanisms. Addition of the *N*-*t*-butylglycylamido side chain (**9a**) resulted in further improvement in all of the tetracycline-resistant strains, including *tet*(A). Activity was comparable to tigecycline for the Gram-positive strains but was 2−6-fold less potent for the Gram-negative strains. Removal of the carbonyl (**20a**) gave a 4−8-fold loss in activity across all strains, but activity similar to compound **8** was retained. Further removal of the secondary amino group to an alkylamino side-chain (**20c**–**e**) resulted in improved activity for

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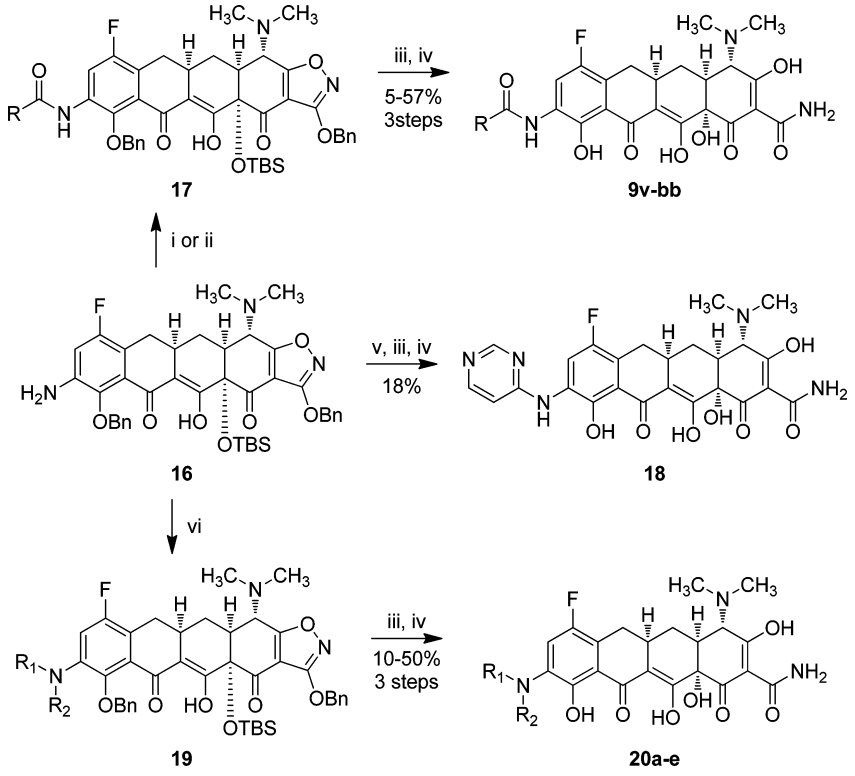
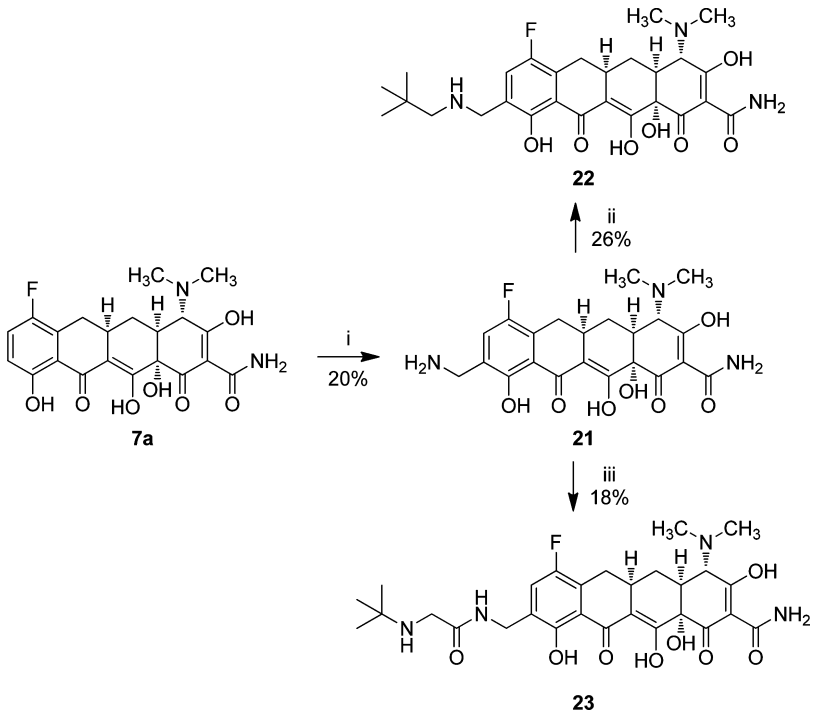
Scheme 3. Alternate Synthesis of 9-Amino-7-fluorocyclines[a]



[a]Reagents and conditions: (i) for **9v–z**, RCOCl, Et_3N, THF; (ii) for **9aa–bb**, RCO_2H, HATU, Et_3N, THF or CH_2Cl_2; (iii) aq HF, 1,4-dioxane or CH_3CN; (iv) H_2, Pd/C, MeOH; (v) 4,6-dichloropyrimidine, Pd_2dba_3, Xantphos, K_3PO_4, 1,4-dioxane, 80 °C; (vi) R_1CHO, AcOH, $Na(OAc)_3BH$, CH_2Cl_2 or DCE.

Scheme 4. Synthesis of 9-Aminomethyl-7-fluorocyclines[a]



[a]Reagents and conditions: (i) $HOCH_2NHCO_2Bn$, TFA, CH_3SO_3H; (ii) *t*-BuCHO, AcOH, $Na(OAc)_3BH$, DMF; (iii) *t*-$BuNHCH_2COCl$·HCl, DMF.

the *S. aureus* strains, but further reduction in potency for both the *S. pneumoniae* and Gram-negative strains. Moving to the smaller and more polar dimethylamino analogue **20b** gave somewhat improved MICs for the Gram-negative strains but reduced activity for the *S. aureus* strains. The omadacycline-type side chain was also prepared and assayed (**22**), yielding a

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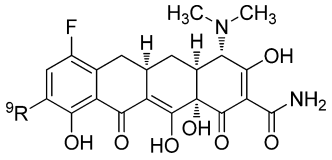
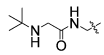
Table 1. In Vitro Antibacterial Activity of 9-Substituted-7-fluorocyclines



MIC (μg/mL)

		S. aureus			*S. pneumoniae*		*E. coli*		*K. pneumoniae*
Cmpd	^{9}R	Wild type[a]	*tet*(M)[b]	*tet*(K)[c]	Wild type[d]	*tet*(M)[c]	Wild type[e]	*tet*(A)[c]	Wild type[f]
8	NH_2	0.25	2	2	0.25	4	2	32	4
9a		0.25	0.25	0.063	0.016	0.016	0.25	2	1
20a		1	4	1	0.13	0.25	2	16	8
20b	$(CH_3)_2N$-	1	4	4	0.25	4	4	32	8
20c	*n*-PrNH-	0.5	0.5	0.5	1	4	32	>32	>32
20d		0.25	0.5	0.13	2	4	>32	>32	>32
20e		0.25	2	0.25	4	16	>32	>32	>32
22		2	4	2	0.25	0.25	4	16	8
23		16	>32	16	0.5	4	32	>32	>32
1		0.016	0.016	0.016	0.016	0.016	0.016	1	0.125
Tigecycline		0.063	0.13	0.063	0.016	0.016	0.13	0.5	0.125
Omadacycline		0.5	2	0.25	0.031	0.125	2	16	2
Tetracycline		1	>32	32	0.25	32	2	>32	4

[a]ATCC 13709. [b]Obtained from Micromyx (Kalamazoo, MI). [c]Obtained from Marilyn Roberts' lab at the University of Washington. [d]ATCC 49619. [e]ATCC 25922. [f]ATCC 13883.

similar overall profile to omadacycline, albeit with a 2−8-fold decrease in potency. The presence of the benzylic amine did result in improved Gram-negative and *S. pneumoniae* activity relative to similar aniline compounds (**20c−e**) although not nearly to the extent as the glycylamido side chain in **9a**, **1**, or tigecycline. The combination of the tigecycline- and omadacycline-type side chains (**23**) led to significant loss in activity across all strains. Overall from this initial study it appeared that a more polar side chain (i.e., small amino group, amide, or secondary amine) was a key factor in obtaining Gram-negative and *S. pneumoniae* activity, whereas the presence of a larger group was the greatest factor in determining *S. aureus*, and in particular, *tet*(M) activity.

Because the amide bond appeared to be important for good broad-spectrum activity, we prepared and assayed a set of amide isosteres to further probe this space (Table 2). The parent benzamide compound **9b** retained balanced activity across the tetracycline-sensitive and resistant *S. aureus* strains but lacked significant activity for the *S. pneumoniae* and Gram-negative strains. The more polar sulfonamide compounds (**10a−c**) were able to pick up the *S. pneumoniae* activity, with the most polar methyl analogue **10c** also having good activity for the two tetracycline-sensitive Gram-negative strains. Unfortunately, this compound had poor activity for the two strains with efflux resistance mechanisms, *tet*(K) and *tet*(A). The urea analogue **11** showed similar activity to **10c**, although the activity for all of the resistant strains was further reduced. The pyrimidine analogue **18** had fairly similar Gram-positive activity to the benzamide **9b** and the sulfonamide **10a**, while the Gram-negative activity was improved for the tetracycline-sensitive Gram-negative strains. The compound still lacked activity for the *tet*(A) strain.

Expanding on this SAR information, a series of substituted aryl and heteroaryl amides was prepared with the goal of increasing polarity to improve Gram-negative activity (Table 3). As with the benzamide **9b**, all of the compounds except **9i** had good activity against *S. aureus*, including the *tet*(M) and *tet*(K) strains, while none of the compounds exhibited significant antibacterial activity for the Gram-negative strains tested. The main differences were seen in the *S. pneumoniae* activity. Substitution at the 2-position (**9e**) or the 3-position (**9c**, **9d**, **9f**) was not beneficial for activity. In contrast to the corresponding 2- and 3-substituted compounds **9e** and **9f**, the 4-dimethylamino analogue **9g** had improved activity for both *S. pneumoniae* strains. All of the heteroaryl compounds had some activity for *S. pneumoniae*, with the more polar analogues (**9i**, **9v**, **9x−z**) having 2−3 dilution more potent activity than the less polar compounds (**9h**, **9w**). Interestingly, the 2-pyridyl compound **9v** and the isosteric 2-thiazolyl analogue **9z** were significantly more potent against *S. aureus* than the 3-pyridyl compound **9i**.

On the basis of results from the heteroaryl amides, we next explored a series of saturated heterocyclic amides (Table 4).

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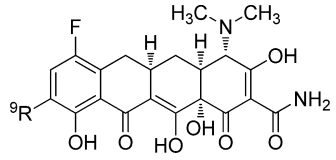
Table 2. In Vitro Antibacterial Activity of 7-Fluorocyclines with Amide Bond Replacements at C9

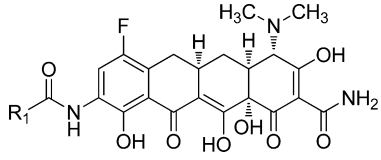


MIC (μg/mL)

		S. aureus			*S. pneumoniae*		*E. coli*		*K. pneumoniae*
Cmpd	^{9}R	Wild type[a]	*tet*(M)[b]	*tet*(K)[c]	Wild type[d]	*tet*(M)[c]	Wild type[e]	*tet*(A)[c]	Wild type[f]
9b	PhCONH-	0.25	0.25	0.5	16	16	>32	>32	>32
10a	$PhSO_2NH$-	0.5	1	0.5	0.25	0.25	32	>32	>32
10b		0.5	1	0.5	0.25	1	>32	>32	>32
10c	CH_3SO_2NH-	0.13	0.25	4	0.016	0.016	0.25	>32	2
11	$CH_3NHCONH$-	0.25	2	16	0.031	0.5	1	>32	4
18		0.25	2	2	0.063	0.5	2	>32	8

[a]ATCC 13709. [b]Obtained from Micromyx (Kalamazoo, MI). [c]Obtained from Marilyn Roberts' lab at the University of Washington. [d]ATCC 49619. [e]ATCC 25922. [f]ATCC 13883.

Table 3. In Vitro Antibacterial Activity of 9-Heteroarylamido-7-fluorocyclines



MIC (μg/mL)

		S. aureus			*S. pneumoniae*		*E. coli*		*K. pneumoniae*
Cmpd	R_1	Wild type[a]	*tet*(M)[b]	*tet*(K)[c]	Wild type[d]	*tet*(M)[c]	Wild type[e]	*tet*(A)[c]	Wild type[f]
9b	Ph	0.25	0.25	0.5	16	16	>32	>32	>32
9c	3-CF_3Ph	0.25	0.5	0.13	>32	>32	>32	>32	>32
9d	3-MeOPh	0.13	0.25	0.13	>32	>32	>32	>32	>32
9e	2-$(CH_3)_2$NPh	0.13	0.13	0.063	32	>32	>32	>32	>32
9f	3-$(CH_3)_2$NPh	0.13	0.13	0.13	>32	>32	>32	>32	>32
9g	4-$(CH_3)_2$NPh	0.063	0.063	0.063	2	8	>32	>32	>32
9h	2-Thiophenyl	0.13	0.25	0.5	16	16	>32	>32	>32
9i	3-Pyridyl	2	4	16	1	2	>32	>32	>32
9v	2-Pyridyl	0.13	0.13	0.13	4	8	>32	>32	>32
9w		0.25	0.5	0.5	8	8	>32	>32	>32
9x		0.25	0.5	0.5	2	2	>32	>32	>32
9y		0.5	0.5	1	1	2	>32	>32	>32
9z		0.063	0.13	0.063	2	4	>32	>32	>32

[a]ATCC 13709. [b]Obtained from Micromyx (Kalamazoo, MI). [c]btained from Marilyn Roberts' lab at the University of Washington. [d]ATCC 49619. [e]ATCC 25922. [f]ATCC 13883.

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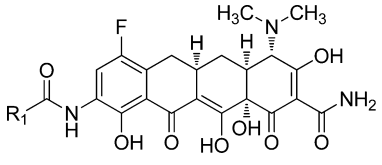
For the most part, these compounds had significantly improved antibacterial activity, especially for the *S. pneumoniae* and the Gram-negative strains tested. One exception was the *tet*(A) *E. coli* strain for which only compound **9bb** had an MIC of 1 μg/mL or less. Ring size was a key factor in potency, with antibacterial activity improving as ring size was reduced (**9bb** > **9l** > **9t**). The stereochemistry of the α-position of the amide side chain was also important, with the (*S*)-stereochemistry providing the best activity in each case. For the 4- and 5-membered ring compounds, the *N*-methyl amines (**9bb**, **9l**) were more potent than the corresponding *N*-H amines (**9aa**, **9j**), while the opposite was seen for the 6-membered ring

Table 4. In Vitro Antibacterial Activity of 9-Heterocyclicamido-7-fluorocyclines



MIC (μg/mL)

		S. aureus			*S. pneumoniae*		*E. coli*		*K. pneumoniae*
Cmpd	R_1	Wild type[a]	*tet*(M)[b]	*tet*(K)[c]	Wild type[d]	*tet*(M)[c]	Wild type[e]	*tet*(A)[c]	Wild type[f]
9aa		0.25	0.13	0.5	0.16	0.031	0.25	16	1
9bb		0.016	0.016	0.016	0.016	0.016	0.016	1	0.063
9j		0.25	0.5	0.5	0.016	0.016	1	16	1
9k		2	2	4	0.13	0.5	4	32	8
9l		0.13	0.25	0.13	0.016	0.016	0.25	8	2
9m		2	2	2	0.25	0.25	2	16	4
9n		0.031	0.13	0.031	0.016	0.016	0.13	4	NT
9o		0.5	1	0.25	0.016	0.016	2	8	NT
9p		0.5	2	1	0.063	0.063	4	>32	8
9q		0.13	0.5	0.063	0.016	0.016	2	>32	16
9r		1	1	1	0.5	0.5	>32	>32	>32
9s		0.5	1	0.25	0.031	0.063	0.5	8	2
9t		2	8	1	0.13	0.25	0.5	>32	2
9u		2	2	2	0.5	0.5	4	>32	16

[a]ATCC 13709. [b]Obtained from Micromyx (Kalamazoo, MI). [c]Obtained from Marilyn Roberts' lab at the University of Washington. [d]ATCC 49619. [e]ATCC 25922. [f]ATCC 13883.

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compounds (**9s** vs **9t**). Substitution on the nitrogen was further explored in the pyrrolidine series. Here, the *N*-ethyl compound **9n** was 1−2 dilutions more potent than **9l**, while the *N*-isopropyl compound **9o** was less active than either compound. Finally, several fluorine substituted compounds were prepared (**9p**−**r**) in order to explore the effect of changes in the basicity of the nitrogen atom on activity. For the monofluoro compound **9q**, the Gram-positive activity remained similar to the parent compound **9l**, while the Gram-negative activity was clearly reduced. The corresponding diastereomer **9p** was somewhat less potent at all strains tested. The difluoro compound **9r** was the least potent compound in the series, in particular for the Gram-negative strains. Once again, the overall data suggests that a more polar or more basic compound gives better antibacterial activity, particularly for Gram-negative bacteria.

We chose to screen and rank compounds for oral bioavailability using a mouse systemic infection model challenged with *S. aureus* ATCC 13709. Six mice per group were dosed at either 3 mg/kg intravenously (IV) or 30 mg/kg orally (PO), and survival was recorded at 48 h post dose. To validate this screening paradigm, three control tetracyclines were tested in the efficacy screen and oral bioavailability was assessed in mice and rats (Table 5). Tetracycline, with 6.3% *F* in mice and 14.7% *F* in rats, was used as a positive control and provided 100% survival after both IV and oral administration in the mouse efficacy model. As negative controls, tigecycline (below level of quantitation (BLQ) in mice and 1.1% *F* in rats) and omadacycline (1.5% in mice and 0.7% *F* in rats) both provided minimal protection after oral administration (0% and 33% survival, respectively) while affording 100% survival when dosed IV in the efficacy screen. As additional validation for the screen, oral PD_{50}s were determined for tetracycline (6.9 mg/kg) and omadacycline (30.3 mg/kg), the results of which appear to correlate well with the oral bioavailability data.

Table 5. Oral Efficacy Screen Results

	S. aureus[a]	survival (%)		PD_{50}	% *F*	% *F*
compd	MIC (μg/mL)	IV, 3 mg/kg	PO, 30 mg/kg	PO (mg/kg)	mouse	rat
9bb	0.016	100	83	14.3		4.6
9j	0.25	100	0			
9l	0.13	100	100			5.7
9n	0.031	67	40			
9q	0.13	83	33			
9v	0.13	0	50	>30		
9z	0.063	17	0			
10c	0.13	100	50	>30		
11	0.25	83	33	>30		
20d	0.25	17	33	>60		
tetracycline	1	100	100	6.9	6.3	14.9
tigecycline	0.063	100	0		BLQ	1.1
omadacycline	0.5	100	33	30.3	1.5	0.7

[a]ATCC 13709.

Compounds were selected based on both antibacterial activity and chemical diversity and screened in the mouse sepsis model. Neither the heteroaryl amides (**9v**, **9z**) nor the alkylamine (**20d**) exhibited substantial efficacy in either the IV or PO arm. Both the sulfonamide (**10c**) and the urea (**11**) analogues provided good protection by IV administration but had only modest oral efficacy. The two heterocyclic amides (**9bb**, **9l**) performed the best in the efficacy screen, providing nearly complete protection in both the IV and PO arms. On the basis of these results, several other pyrrolidinyl amides (**9j**, **9n**, **9q**) were also screened. All three compounds showed reasonable efficacy following IV dosing. Despite differing only by the amino substituent (NH or N-Et vs N-Me) or by a fluorine atom on the ring, none of the compounds afforded greater than 40% survival when dosed orally. To ensure against false negatives, PD_{50}s were determined for several of the compounds. All of the compounds that showed less than 50% survival in the screen had PD_{50}s greater than 30 mg/kg. Compound **9bb**, with 83% survival in the oral screen, had a PD_{50} of 14.3 mg/kg. Compounds **9bb** and **9l** were selected for pharmacokinetic (PK) analysis in rats and were found to have oral bioavailabilities of 4.6% and 5.7%, respectively. While these values would generally be considered low, they are significantly better than the glycylcyclines and omadacycline. It is possible that oral bioavailability for these compounds will improve in higher mammals as has been observed for tetracycline, omadacycline, and **1**.

On the basis of the efficacy and oral bioavailability data, compounds **9bb** and **9l** were advanced for further profiling against panels of recently isolated respiratory pathogens (Table 6). Linezolid and levofloxacin were screened as relevant standard of care therapies for respiratory infections. For methicillin-resistant *S. aureus* (MRSA), both compounds had superior MIC_{90} values as compared to linezolid, although **9l** distinguished itself with an MIC_{90} value of 0.13 μg/mL. Most of these strains were resistant to levofloxacin. Against *S. pneumoniae*, **9bb** and **9l** were very potent, with MIC_{90} values of 0.016 μg/mL, while both marketed agents had MIC_{90} values of 1 μg/mL. The two tetracycline analogues were both superior to linezolid against *Hemophilis influenzae*, with MIC_{90} values of 0.5 and 0.25 μg/mL vs 16 μg/mL for linezolid. Levofloxacin was more potent, with an MIC_{90} value of 0.031 μg/mL. Compounds **9l** and **9b** were also screened against *Streptococcus pyogenes* and *Moraxella catarrhalis* and were both found to have MIC_{90} values of 0.016 μg/mL against these species, significantly more potent than linezolid and levofloxacin. Compounds were tested for activity against *Acinetobacter baumannii*, an opportunistic multidrug-resistant Gram-negative pathogen and cause of nosocomial infections including pneumonia.[23] Against a panel of 25 *A. baumannii* isolates, both **9b** and **9l** had MIC_{90} values of 1 vs 32 μg/mL for levofloxacin. Overall, compound **9l** was more potent against almost all species and appeared to be able to provide broader coverage across these species than the two marketed comparator antibiotics.

Compound **9l** was profiled in two neutropenic mouse lung infection models with *tet*(M) resistant strains (Figure 2A,B). In the MRSA lung infection model, **9l** (MIC = 0.25 μg/mL) exhibited similar efficacy to linezolid (MIC = 2 μg/mL) with a 1.99 vs 2.46 $\log_{10}$ reduction in colony forming units (CFUs)/g lung tissue when dosed orally at 50 mg/kg. In the IV arm, **9l** had greater efficacy than linezolid (3.03 vs 1.29 $\log_{10}$ reduction in CFU/g lung tissue). With an even larger advantage in MIC for the *S. pneumoniae* lung infection model, **9l** (MIC ≤ 0.016 μg/mL) showed greater efficacy than linezolid (MIC = 0.5 μg/mL) in both the PO and IV arms of the study. Two immunocompetent lung infection models were also examined. In an *S. pneumoniae* mouse lung infection model (Figure 2C), compounds were dosed orally at 30 mg/kg, with **9l** (MIC ≤ 0.008 μg/mL) exhibiting a 4.75 $\log_{10}$ reduction in CFU/g lung

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Table 6. MIC_{50} and MIC_{90} Values for 9l and 9bb

species	MIC (μg/mL)	**9l**	**9bb**	linezolid	levofloxacin
S. aureus (MRSA, n = 32)	MIC_{50}	0.031	0.031	2	16
	MIC_{90}	0.13	1	4	>32
	MIC range	0.016−1	0.016−4	2−4	0.25−>32
S. pneumoniae (n = 19)	MIC_{50}	0.016	0.016	1	1
	MIC_{90}	0.016	0.016	1	1
	MIC range	0.016−0.016	0.016−0.016	0.5−1	0.5−1
H. influenzae (n = 14)	MIC_{50}	0.25	0.063	8	0.016
	MIC_{90}	0.5	0.25	16	0.031
	MIC range	0.031−0.5	0.016−0.5	8−16	0.016−0.031
S. pyogenes (n = 14)	MIC_{50}	0.016	0.016	1	0.5
	MIC_{90}	0.016	0.016	2	2
	MIC range	0.016−0.016	0.016−0.016	1−2	0.25−2
M. catarrhalis (n = 14)	MIC_{50}	0.016	0.016	8	0.031
	MIC_{90}	0.016	0.016	8	0.063
	MIC range	0.016−0.063	0.016−0.063	2−8	0.016−0.063
A. baumannii (n = 25)	MIC_{50}	0.13	0.13	NT	4
	MIC_{90}	1	1		32
	MIC range	0.016−2	0.016−2		0.13−>32

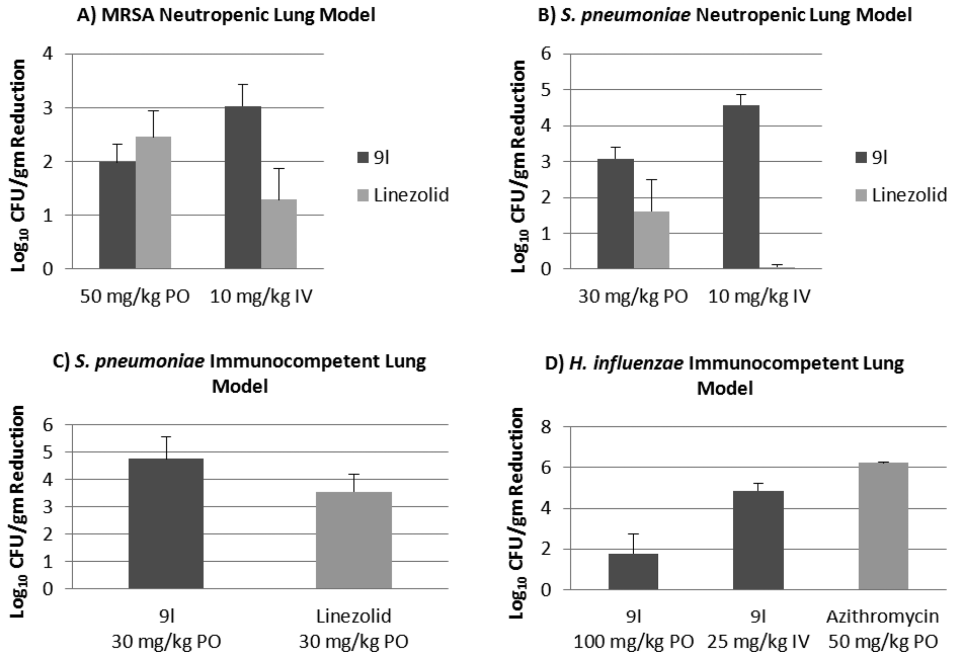

Figure 2. Efficacy of **9l** in rodent respiratory infection models.

tissue vs a 3.56 $\log_{10}$ reduction in CFU/g lung tissue for linezolid (MIC = 0.5 μg/mL). In an *H. influenzae* rat lung infection model, **9l** (MIC = 0.063 μg/mL) produced a 1.77 $\log_{10}$ reduction in CFU/g lung tissue when dosed orally at 100 mg/kg. Compound **9l** exhibited good efficacy when dosed at 25 mg/kg IV, with a 4.87 $\log_{10}$ reduction in CFU/g lung tissue. The bacterial count was below the level of quantitation (≥6 $\log_{10}$ reduction in CFU/g lung tissue) for azithromycin ((MIC = 1 μg/mL) when dosed orally at 50 mg/kg. In summary, compound **9l** exhibited significant oral activity in all four respiratory infection models, with efficacy comparable to or greater than linezolid.

CONCLUSIONS

Utilizing a fully synthetic chemistry platform to generate novel tetracyclines that in many cases are not readily accessed by traditional semisynthesis, we were able to significantly expand the chemical space at the C-9 position of the fluorocyclines. This approach demonstrates the ability to efficiently optimize compounds for both antibacterial activity, including overcoming

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both efflux and Tet(M)-mediated ribosomal protection tetracycline-resistance mechanisms, and pharmacokinetic properties. Specifically, two compounds (**9bb**, **9l**) were identified that showed superior potency versus standard of care therapies against panels of clinically important respiratory pathogens. Using a mouse sepsis model to screen for oral efficacy, the oral bioavailability of compound **9l** was initially demonstrated, enabling a more extensive evaluation of oral efficacy in several rodent lung infection models. While oral bioavailability in rat was found to be low, precedents set by other members of the tetracycline class suggest further examination of compound **9l** as an oral therapy in higher mammals is warranted.

EXPERIMENTAL PROCEDURES

General. Air and moisture sensitive liquids and reagents were transferred via syringe or cannula and were introduced into flame-dried glassware under a positive pressure of dry nitrogen through rubber septa. All reactions were stirred magnetically. Commercial reagents were used without further purification. Analytical thin-layer chromatography was performed on EM Science precoated glass-backed silica gel 60 Å F-254 250 μm plates. Visualization of the plates was effected by ultraviolet illumination and/or immersion of the plate in a basic solution of potassium permanganate in water followed by heating. Column chromatography was performed on a FlashMaster Personal system using ISOLUTE Flash Si II silica gel prepacked cartridges (available from Biotage). Preparative reversed-phase HPLC chromatography (HPLC) was accomplished using a Waters Auto-purification system with mass-directed fraction collection. All intermediate compounds were purified with a Waters Sunfire Prep C18 OBD column (5 μm, 19 mm × 50 mm; flow rate = 20 mL/min) using a mobile phase mixture of H_2O (A) and CH_3CN (B) containing 0.1% HCO_2H. The final tetracycline compounds were purified using a Phenomenex Polymerx 10 μ RP 100A column (10 μm, 30 mm × 21.2 mm; flow rate = 20 mL/min) using a mobile phase mixture of 0.05N HCl in H_2O (A) and CH_3CN (B). Unless otherwise described, all final tetracycline compounds were isolated as mono-, di-, or trihydrochloride salts following freeze-drying. ^{1}H NMR spectra were recorded on a JEOL ECX-400 (400 MHz) spectrometer and are reported in ppm using residual solvent as the internal standard ($CDCl_3$ at 7.24 ppm, DMSO-d_6 at 2.50 ppm, or CD_3OD at 3.31 ppm). High performance liquid chromatography–electrospray mass spectra (LC-MS) were obtained using an Waters Alliance HPLC system equipped with a binary pump, a diode array detector, a Waters Sunfire C18 (5 μm, 4.6 mm I.D. × 50 mm) column, and a Waters 3100 series mass spectrometer with electrospray ionization. Spectra were scanned from 100 to 1200 amu. The eluent was a mixture of H_2O (A) and CH_3CN (B) containing 0.1% HCO_2H at a flow rate of 1 mL/min. Purity of the final compounds was assessed by the following method: (a) time = 0, 100% A; (b) time = 0.5 min, 100% A; (c) time = 3.5 min, 100% B; (d) time = 5 min, 100% B; (e) time = 6 min, 100% A; (f) time = 7 min, 100% A. All final products were ≥95% purity as assessed by this method.

(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 Dihydrochloride (9a). 2-*t*-Butylaminoacetylchloride hydrochloride (4.2 mg, 0.022 mmol) was added to a solution of **8** (5 mg, 0.011 mmol) in DMF (0.1 mL). The reaction was stirred for 30 min. The reaction mixture was diluted with 0.05 N aqueous HCl (2 mL) and was purified directly by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0–100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 3.9 mg (62%) of the title compound as a yellow solid. ^{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-C-Benzene-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2,9-dicarboxamide Hydrochloride (9b). To a solution of compound **8** (20 mg, 0.045 mmol) in THF was added Na_2CO_3 (9.5 mg, 0.089 mmol) and 0.1 mL of a benzoyl chloride solution (54 μL in 1 mL THF, 0.047 mmol). The reaction mixture was stirred at room temperature for 1 h. HCl in MeOH (1 mL, 4 N) was added to the mixture at 0 °C. After 2 min, the mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0–100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 5.5 mg (21%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (d, J = 10.8 Hz, 1 H), 7.97 (d, J = 7.6 Hz, 2 H), 7.66–7.54 (m, 3 H), 4.11 (s, 1 H), 3.21–2.90 (m, 9 H), 2.37–2.24 (m, 2 H), 1.72–1.66 (m, 1 H). MS (ESI) m/z 552.1 (M + H).

The following compounds were prepared by similar methods to **9b**:

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-C-[3-(trifluoromethyl)benzene]-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2,9-dicarboxamide Hydrochloride (9c). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1 H), 8.21 (d, J = 8.0 Hz, 1 H), 8.14 (d, J = 10.4 Hz, 1 H), 7.92 (d, J = 8.0 Hz, 1 H), 7.76 (t, J = 8.0 Hz, 1 H), 4.08 (s, 1 H), 3.21–2.89 (m, 9 H), 2.35–2.22 (m, 2 H), 1.71–1.61 (m, 1 H). MS (ESI) m/z 620.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-9-C-(3-methoxybenzene)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2,9-dicarboxamide Hydrochloride (9d). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (d, J = 10.8 Hz, 1 H), 7.41–7.33 (m, 3 H), 7.09–7.07 (m, 1 H), 4.00 (s, 1 H), 3.78 (s, 3 H), 3.12–2.86 (m, 9 H), 2.23–2.13 (m, 2 H), 1.60–1.50 (m, 1 H). MS (ESI) m/z 582.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-C-[2-(dimethylamino)benzene]-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2,9-dicarboxamide Dihydrochloride (9e). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.19–8.14 (m, 2 H), 8.05 (d, J = 8.4 Hz, 1 H), 7.91–7.89 (m, 1 H), 7.76–7.74 (m, 1 H),.4.12 (s, 1 H), 3.32 (s, 6 H), 3.21–2.96 (m, 9 H), 2.41–1.98 (m, 2 H), 1.72–1.59 (m, 1 H). MS (ESI) m/z 595.0 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-C-[3-(dimethylamino)benzene]-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2,9-dicarboxamide Dihydrochloride (9f). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.13–8.06 (m, 2 H), 7.98 (d, J = 7.6 Hz, 1 H), 7.77 (d, J = 7.2 Hz, 1 H), 7.67 (t, J = 8.0 Hz, 1 H), 4.01 (s, 1 H), 3.26 (s, 6 H), 3.14–2.83 (m, 9 H), 2.27–2.13 (m, 2 H), 1.64–1.52 (m, 1 H). MS (ESI) m/z 595.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-C-[4-(dimethylamino)benzene]-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2,9-dicarboxamide Dihydrochloride (9g). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.08 (d, J = 10.8 Hz, 1 H), 7.98 (d, J = 8.4 Hz, 2 H), 7.49 (d, J = 8.4 Hz, 2 H), 4.02 (s, 1 H), 3.19 (s, 6 H), 3.12–2.88 (m, 9 H), 2.24–2.13 (m, 2 H), 1.60–1.51 (m, 1 H). MS (ESI) m/z 595.1 (M + H).

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]thiophene-2-carboxamide Hydrochloride (9h).** Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (d, J = 10.8 Hz, 1 H), 7.89 (d, J = 3.2 Hz, 1 H), 7.78 (d, J = 4.8 Hz, 1 H), 7.22 (t, J = 8.8 Hz, 1 H), 4.10 (s, 1 H), 3.20–2.98 (m, 9 H), 2.36–2.20 (m, 2 H), 1.68–1.61 (m, 1 H). MS (ESI) m/z 558.1 (M + H).

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]pyridine-3-carboxamide Dihydrochloride (9i).** Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.34 (s, 1 H), 9.04–9.00 (m, 2 H), 8.20–8.15 (m, 2 H), 4.07 (s, 1 H), 3.27–2.94 (m, 9 H), 2.34–2.18 (m, 2 H), 1.68–1.59 (m, 1 H). MS (ESI) m/z 553.1 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]pyrrolidine-2-carboxamide Dihydrochloride (9j). To a solution of compound

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8 (17.0 mg, 0.038 mmol) in DMF (200 μL) was added *N*-benzyloxycarbonyl-L-proline acid chloride (1.0 M in toluene, 57 μL). After 50 min, the reaction mixture was diluted to 3 mL with 0.05 N aqueous HCl and filtered to remove solids. The solution was purified directly by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 10−20% B gradient). Fractions with the desired MW were collected and freeze-dried. The material was dissolved in 1,4-dioxane:MeOH (1:3, 2.3 mL), and palladium on carbon (10%, 10 mg) was added. The flask was fitted with a septum and evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen for 1.7 h, was filtered through Celite, and was concentrated under reduced pressure. Half of the resulting residue was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−35% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 1.9 mg (30%) of compound the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (d, *J* = 11.0 Hz, 1 H), 4.59−4.56 (m, 1 H), 4.10 (s, 1 H), 3.48−3.33 (m, 2 H), 3.18−2.95 (m, 9 H), 2.59−2.50 (m, 1 H), 2.34−2.05 (m, 5 H), 1.70−1.60 (m, 1 H). MS (ESI) *m*/*z* 545.38 (M + H).

(2*R*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]pyrrolidine-2-carboxamide Dihydrochloride (9k). Prepared as described for **9j**. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (d, *J* = 11.0 Hz, 1 H), 4.59−4.53 (m, 1 H), 4.09 (s, 1 H), 3.48−3.37 (m, 2 H), 3.18−2.90 (m, 9 H), 2.59−2.50 (m, 1 H), 2.34−2.05 (m, 5 H), 1.70−1.59 (m, 1 H). MS (ESI) *m*/*z* 545.37 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-methylpyrrolidine-2-carboxamide Dihydrochloride (9l). Yellow solid. The second half of the intermediate described in **9j** (0.012 mmol) was dissolved in DMF (500 μL), and formaldehyde (37% aqueous solution, 5.3 μL, 0.072 mmol), triethylamine (5.0 μL, 0.036 mmol), and sodium triacetoxyborohydride (8.4 mg, 0.039 mmol) were added sequentially. After 2 h, the reaction mixture was diluted to 1.8 mL with 0.05 N aqueous HCl, and the solution was purified directly by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−30% B gradient). Fractions with the desired MW were collected and freeze-dried to provide a mixture of the desired compound and an overformylated product. The resulting compound mixture was dissolved in 4 N aqueous HCl solution (1.5 mL) and stirred for 50 h. The resulting solution was freeze-dried to provide 1.0 mg (15%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (d, *J* = 10.4 Hz, 1 H), 4.36 (t, *J* = 8.6 Hz, 1 H), 4.08 (s, 1 H), 3.82−3.73 (m, 1 H), 3.20−2.90 (m, 12 H), 2.73−2.68 (m, 1 H), 2.35−2.10 (m, 5 H), 1.70−1.60 (m, 1 H). MS (ESI) *m*/*z* 559.38 (M + H).

(2*R*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-methylpyrrolidine-2-carboxamide Dihydrochloride (9m). Prepared as described for **9l**. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (d, *J* = 10.4 Hz, 1 H), 4.45−4.34 (m, 1 H), 4.08 (s, 1 H), 3.84−3.74 (m, 1 H), 3.20−2.90 (m, 12 H), 2.79−2.65 (m, 1 H), 2.33−2.05 (m, 5 H), 1.70−1.58 (m, 1 H). MS (ESI) *m*/*z* 559.40 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-ethylpyrrolidine-2-carboxamide Dihydrochloride (9n). Prepared as described for **9l** using acetaldehyde instead of formaldehyde. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (d, *J* = 11.0 Hz, 1 H), 4.48−4.40 (m, 1 H), 4.10 (s, 1 H), 3.85−3.76 (m, 1 H), 3.50−3.30 (m, 2 H), 3.25−2.90 (m, 10 H), 2.75−2.62 (m, 1 H), 2.36−2.02 (m, 5 H), 1.70−1.58 (m, 1 H), 1.35 (t, *J* = 6.9 Hz, 3 H). MS (ESI) *m*/*z* 573.33 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-(propan-2-yl)-pyrrolidine-2-carboxamide Dihydrochloride (9o). Prepared as described for **9l** using acetone instead of formaldehyde. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (d, *J* = 10.5 Hz, 1 H), 4.61−4.53 (m, 1 H), 4.09 (s, 1 H), 3.78−3.60 (m, 2 H), 3.42−3.30 (m, 2 H), 3.20−2.95 (m, 8 H), 2.70−2.58 (m, 1 H), 2.38−2.00 (m, 5 H), 1.70−1.60 (m, 1 H), 1.44−1.36 (m, 6 H). MS (ESI) *m*/*z* 587.36 (M + H).

(2*S*,4*R*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-4-fluoro-1-methylpyrrolidine-2-carboxamide Dihydrochloride (9p). To a solution of compound **8** (20 mg, 0.045 mmol) in THF was added Na_2CO_3 (9.5 mg, 0.089 mmol), (4*R*)-4-fluoro-1-methyl-L-proline (9.8 mg, 0.067 mmol), and HATU (34.6 mg, 0.047 mmol). The reaction mixture was stirred at room temperature for 20 h. HCl in MeOH (1 mL, 4 N) was added to the mixture at 0 °C. After 2 min, the mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 6.0 mg (21%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (d, *J* = 10.8 Hz, 1 H), 5.51 (d, *J* = 51.6 Hz, 1 H), 4.76−4.72 (m, 1 H), 4.22−4.16 (m, 1 H), 4.10 (s, 1 H), 3.74−3.63 (m, 1 H), 3.21−2.97 (m, 14 H), 2.35−2.21 (m, 2 H), 1.69−1.60 (m, 1 H). MS (ESI) *m*/*z* 577.1 (M + H).

The following compounds were prepared by similar methods to **9p**:

(2*S*,4*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-4-fluoro-1-methylpyrrolidine-2-carboxamide Dihydrochloride (9q). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (d, *J* = 10.8 Hz, 1 H), 5.48 (d, *J* = 51.2 Hz,1 H), 4.60−4.56 (m, 1 H), 4.11 (s, 1 H), 4.05−3.98 (m, 1 H), 3.67−3.54 (m, 1 H), 3.24−2.96 (m, 13 H), 2.55−2.44 (m, 1 H), 2.34−2.22 (m, 2 H), 1.70−1.66 (m, 1 H). MS (ESI) *m*/*z* 577.1 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-4,4-difluoro-1-methylpyrrolidine-2-carboxamide Dihydrochloride (9r). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (d, *J* = 10.8 Hz, 1 H), 4.76−4.71 (m, 1 H), 4.17−4.12 (m, 1 H), 4.09 (s, 1 H), 3.96−3.86 (m, 1 H), 3.67−3.53 (m, 1 H), 3.55−3.53 (m, 1 H), 3.25−2.73 (m, 12 H), 2.33−2.19 (m, 2 H), 1.68−1.59 (m, 1 H). MS (ESI) *m*/*z* 595.3 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]piperidine-2-carboxamide Dihydrochloride (9s). To a solution of (*S*)-(−)-1-Cbz-piperidinecarboxylic acid (34.2 mg, 0.13 mmol), and HATU (50.0 mg, 0.13 mmol) in DMF (200 μL) was added triethylamine (18 μL, 0.13 mmol). After 30 min, aniline **8** (17.5 mg, 0.039 mmol) was added. After 16 h, the reaction mixture was diluted to 3 mL with 0.05 N aqueous HCl and the solution was purified directly by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 15−70% B gradient). Fractions with the desired MW were collected and freeze-dried. The material was dissolved in 1,4-dioxane:MeOH (1:3, 1.2 mL), and palladium on carbon (10%, 4 mg) was added. The flask was fitted with a septum and was evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen gas for 1.5 h, was filtered through Celite, and was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−35% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 0.75 mg (4%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (d, *J* = 11.0 Hz, 1 H), 4.12−4.06 (m, 2 H), 3.48−3.40 (m, 2 H), 3.20−2.90 (m, 9 H), 2.36−2.18 (m, 3 H), 2.02−1.90 (m, 2 H), 1.82−1.60 (m, 4 H). MS (ESI) *m*/*z* 559.37 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-methylpiperidine-2-carboxamide Dihydrochloride (9t). To a solution of compound **9s** (8.7 mg, 0.0138 mmol) in DMF (750 μL) were added sequentially formaldehyde (37% aqueous solution, 6.2 μL, 0.083 mmol), triethylamine (5.8 μL, 0.041 mmol), and sodium triacetoxyborohydride (11 mg, 0.051 mmol). After 17 h, the reaction mixture was

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concentrated to remove amine and 6 N aqueous HCl (500 μL) was added. The solution was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 15−50% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 2.4 mg (31%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (d, J = 11.0 Hz, 1 H), 4.08−4.04 (m, 1 H), 3.59−3.53 (m, 1 H), 3.20−3.10 (m, 5 H), 3.06−2.96 (m, 5 H), 2.90 m (s, 3 H), 2.36−2.25 (m, 2 H), 2.11−2.05 (m, 1 H), 2.02−1.94 (m, 2 H), 1.90−1.74 (m, 2 H), 1.71−1.58 (m, 2 H). MS (ESI) m/z 573.33 (M + H).

(2*R*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]piperidine-2-carboxamide Hydrochloride (9u). Prepared as described for **9t**. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (d, J = 11.0 Hz, 1 H), 4.13−4.06 (m, 2 H), 3.50−3.43 (m, 2 H), 3.20−2.90 (m, 9 H), 2.38−2.18 (m, 3 H), 2.04−1.88 (m, 2 H), 1.83−1.60 (m, 4 H). MS (ESI) m/z 559.38 (M + H).

Phenyl 3-Amino-2-(benzyloxy)-5-fluoro-6-methylbenzoate (13). To a 250 mL round-bottom flask was added phenyl 3-fluoro-6-hydroxy-2-methylbenzoate (14.47 g, 56.30 mmol), tetrabutylammonium bromide (0.90 g, 2.80 mmol), 1,2-dichloroethane (60 mL), and water (60 mL). The clear bilayer was cooled in a 20 °C water bath. Nitric acid (7.2 mL, 70 wt %, 113 mmol) was added. The reaction was stirred at room temperature overnight. The organic layer was separated, washed with water (60 mL × 2) and brine, and dried over Na_2SO_4. The solvent was removed to give 17.7 g (100%) of the nitrated intermediate as a brown oil. The material (17.7 g, 56.30 mmol) was dissolved in acetone (177 mL), and anhydrous potassium carbonate (15.6 g, 113.00 mmol), potassium iodide (0.47 g, 2.80 mmol), and benzyl bromide (7.03 mL, 59.10 mmol) were added. The suspension was then heated to 56 °C for 4 h. The solid was removed by filtration and washed with acetone (30 mL). The filtrate was concentrated to give a paste which was partitioned between methyl t-butyl ether (120 mL) and water (80 mL). The organic layer was washed with water (80 mL) and brine, dried over anhydrous Na_2SO_4, and concentrated to give 21.1 g (98%) of the benzylated intermediate as a brown oil. This material (21.1 g, 55.4 mmol) was dissolved in THF (230 mL) in a 1 L round-bottom flask. The solution was cooled in a cold water bath to 10 °C. A solution of sodium hydrosulfite (56.7 g, 276.8 mmol) was added. The temperature quickly rose from 10 to 20.4 °C after the addition. The yellow suspension was stirred while the cold water bath slowly warmed to room temperature overnight to give an orange cloudy solution. The reaction mixture was diluted with EtOAc (460 mL). The organic layer was washed with water (150 mL × 2) and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The material was purified by silica gel chromatography (9:1 heptane/EtOAc) to yield 15.8 g (80%, 3 steps) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45−7.32 (m, 7 H), 7.28−7.23 (m, 1 H), 7.16−7.12 (m, 2 H), 7.57 (d, J = 11.0 Hz, 1 H), 5.00 (s, 2 H), 3.99 (br s, 2 H), 2.26 (d, J = 1.8 Hz, 3 H). MS (ESI) m/z 352.31 (M + H).

Phenyl 2-(Benzyloxy)-3-[bis(prop-2-en-1-yl)amino]-5-fluoro-6-methylbenzoate (14). To an solution of **13** (10.0 g, 28.5 mmol) in NMP (50 mL) was added allylbromide (7.65 mL, 85.5 mmol) and potassium carbonate (11.8 g, 85.5 mmol). Potassium iodide (1 g, 6 mmol) was added, and the reaction mixture was heated to 100 °C. After 16 h, the reaction was cooled, diluted with water (60 mL), and extracted with EtOAc (75 mL, then 2 × 50 mL). The combined organic extracts were washed with water (2 × 35 mL) and were dried (Na_2SO_4), filtered, and concentrated. Purification via flash column chromatography on silica gel (RediSep, 125 g, 1−6% EtOAc in hexanes gradient) gave 10.97 g (89%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.30 (m, 7 H), 7.42−7.20 (m, 1 H), 7.00 (d, J = 8.5 Hz, 2 H), 6.72 (d, J = 11.0 HZ, 1 H), 5.77−5.70 (m, 2 H), 5.20−5.12 (m, 6 H), 3.81 (d, J = 6.1 Hz, 4 H), 2.26 (s, 3 H). MS (ESI) m/z 432.34 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-13-(Dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-8-(di-2-propen-1-ylamino)-10-fluoro-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-naphthaceno[2,3-*d*]isoxazole-4,6(4a*H*,11*H*)-dione (15). A solution of **14** (875 mg, 2.03 mmol) in THF (6.5 mL) was added to a solution of LDA in THF (0.051 M, 40 mL, 2.03 mmol) and TMEDA (304 μL, 2.03 mmol) at −78 °C. The reaction was stirred at −78 °C for 15 min. A solution of enone **6** (784 mg, 1.62 mmol) in THF (6.5 mL) was added dropwise, followed by addition of LHMDS (1.0 M in THF, 2.03 mL, 2.03 mmol). The reaction mixture was slowly warmed to −10 °C over 1 h, was quenched with NH_4Cl (saturated, aqueous solution, 6 mL), and was warmed to 25 °C. The solution was poured into NH_4Cl (saturated, aqueous solution, 20 mL) and was extracted with EtOAc (2 × 75 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated. The material was purified by preparative HPLC (Sunfire Prep C18 column, 88−100% B gradient). Fractions with the desired MW were collected and freeze-dried to give 552 mg (41%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 16.22 (s, 1 H), 7.49−7.47 (m, 4 H), 7.37−7.31 (m, 6 H), 6.80 (d, J = 11.0 Hz, 1 H), 5.76−5.64 (m, 2 H), 5.35 (s, 2 H), 5.17−5.11 (m, 4 H), 4.98 (d, J = 9.2, 1 H), 4.87 (d, J = 9.8 Hz, 1 H), 3.96 (m, J = 10.4 Hz, 1 H), 3.83−3.71 (m, 4 H), 3.14 (dd, J = 14.7, 4.3 Hz, 1 H), 3.0−2.87 (m, 1 H), 2.55−2.35 (m, 9 H), 2.11 (d, J = 14.7 Hz, 1 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) m/z 820.55 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-8-Amino-13-(dimethylamino)-9-[(dimethylamino)methyl]-4a-[[(1,1-dimethylethyl)-dimethylsilyl]oxy]-10-fluoro-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-naphthaceno[2,3-*d*]isoxazole-4,6(4a*H*,11*H*)-dione (16). A solution of **15** (550 mg, 0.671 mmol) in degassed CH_2Cl_2 (5 mL) was added under nitrogen via syringe to a flame-dried flask containing *N*,*N*-dimethylbarbituric acid (324 mg, 2.07 mmol) and tetrakis(triphenylphosphine)palladium(0) (56.9 mg, 0.0492 mmol). The resulting solution was heated to 35 °C for 4 h and was concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient). Fractions with the desired MW were collected and freeze-dried to give 352 mg (71%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 16.10 (s, 1 H), 7.51−7.43 (m, 4 H), 7.9−7.29 (m, 6 H), 6.61 (d, J = 9.8 Hz, 1 H), 5.35 (s, 2 H), 4.87 (dd, J = 22.6, 10.4 Hz, 2 H), 3.96 (d, J = 10.4 Hz, 1 H), 3.91 (s, 2 H), 3.12 (dd, J = 15.3, 10.1 Hz, 1 H), 3.04−2.92 (m, 1 H), 2.55−2.31 (m, 9 H), 2.11 (d, J = 14.7 Hz, 1 H), 0.82 (s, 9 H), 0.27 (s, 3 H), 0.12 (s, 3 H). MS (ESI) m/z 740.44 (M + H).

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]pyridine-2-carboxamide Dihydrochloride (9v).** To a solution of compound **16** (21.1 mg, 0.028 mmol) was added picolinoyl chloride hydrochloride (15.8 mg, 0.088) and triethylamine (11.7 μL, 0.084 mmol). Upon completion, the reaction mixture was filtered through Celite and was concentrated under reduced pressure. The material was dissolved in 1,4-dioxane (1 mL) and HF (50% aqueous solution, 200 μL) was added. After stirring overnight, the reaction mixture was poured into a solution of K_2HPO_4 (2.6 g) in water (30 mL) and was extracted with EtOAc (2 × 25 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was dissolved in 1,4-dioxane:MeOH (1:1, 1 mL), and palladium on carbon (10%, 10 mg) was added. The flask was fitted with a septum and was evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen gas for 2 h. The reaction mixture was filtered through Celite and was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 10−60% B gradient). Fractions with the desired MW were collected and freeze-dried to provide 5.8 mg (37%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.73−8.69 (m, 1 H), 8.58−8.52 (m, 1 H), 8.27−8.21 (m, 1 H), 8.08−8.00 (m, 1 H), 7.66−7.60 (m, 1 H), 4.09 (s, 1 H), 3.29−2.92 (m, 9 H), 2.38−2.18 (m, 2 H), 1.72−1.60 (m, 1 H). MS (ESI) m/z 553.27 (M + H).

The following compounds were prepared by similar methods to **9v**:

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-methyl-1*H*-pyrrole-2-carboxamide Hydrochloride (9w).** Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (d, J = 11.6 Hz, 1 H), 6.98−6.86

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(m, 2 H), 6.17−6.10 (m, 1 H), 4.08 (s, 1 H), 3.94 (s, 3 H), 3.19−2.90 (m, 9 H), 2.33−2.18 (m, 2 H), 1.80−1.56 (m, 1 H). MS (ESI) *m/z* 555.32 (M + H).

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-5-methyl-1,2-oxazole-3-carboxamide Hydrochloride (9x).** Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (d, J = 11.0 Hz, 1 H), 6.59 (s, 1 H), 4.09 (s, 1 H), 3.19−2.90 (m, 9 H), 2.52 (s, 3 H), 2.34−2.18 (m, 2 H), 1.71−1.58 (m, 1 H). MS (ESI) *m/z* 557.26 (M + H).

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-methyl-1*H*-pyrazole-3-carboxamide Hydrochloride (9y).** Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (d, J = 11.0 Hz, 1 H), 7.68 (s, 1 H), 6.82−6.76 (m, 1 H), 4.09 (s, 1 H), 3.99 (s, 3 H), 3.16−2.90 (m, 9 H), 2.31−2.16 (m, 2 H), 1.70−1.56 (m, 1 H). MS (ESI) *m/z* 556.31 (M + H).

***N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1,3-thiazole-2-carboxamide Hydrochloride (9z).** Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (d, J = 11.0 Hz, 1 H), 8.02 (d, J = 3.0 Hz, 1 H), 7.95 (d, J = 2.4 Hz, 1 H), 4.09 (s, 1 H), 3.20−2.90 (m, 9 H), 2.34−2.17 (m, 2 H), 1.70−1.56 (m, 1 H). MS (ESI) *m/z* 559.23 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]azetidine-2-carboxamide Dihydrochloride (9aa). To a suspension of 1-Fmoc-L-azetidine-2-carboxylic acid (135 mg, 0.42 mmol) and HATU (164 mg, 0.43 mmol) in THF (1.5 mL) was added triethylamine (60 μL, 0.43 mmol). After 30 min, compound **16** (106 mg, 0.14 mmol) was added. After 18 h, the reaction mixture was concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient). Fractions with the desired MW were collected and freeze-dried to provide 131 mg of the intermediate **17aa** as a yellow powder. The material was dissolved in CH_2Cl_2 (2 mL), and piperidine (500 μL) was added. After 30 min, the reaction solution was poured into aqueous pH 7 phosphate buffer and was extracted with EtOAc (3 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography on silica gel (Silicycle, 5 g, 0−50% EtOAc in hexane gradient) provided 47.6 mg of the intermediate. Half of the intermediate (24 mg) was dissolved in acetonitrile (1 mL), and HF (50% aqueous solution, 200 μL) was added. After 18.5 h, the reaction mixture was poured into a solution of K_2HPO_4 (2.5 g) in water (20 mL) and was extracted with EtOAc (2 × 25 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Palladium on carbon (10%, 12.5 mg) was added to a solution of the intermediate in 1,4-dioxane:MeOH (1:1, 1 mL). The flask was fitted with a septum and was evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen gas for 4.5 h, was filtered through Celite, and was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 15−50% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 2.0 mg (5%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 11.0 Hz, 1 H), 5.29−5.24 (m, 1 H), 4.20−4.11 (m, 1 H), 4.09 (s, 1 H), 3.19−2.89 (m, 10 H), 2.69−2.56 (m, 1 H), 2.33−2.19 (m, 2 H), 1.68−1.56 (m, 1 H). MS (ESI) *m/z* 531.30 (M + H).

(2*S*)-*N*-[(5a*R*,6a*S*,7*S*,10a*S*)-9-Carbamoyl-7-(dimethylamino)-4-fluoro-1,8,10a,11-tetrahydroxy-10,12-dioxo-5,5a,6,6a,7,10,10a,12-octahydrotetracen-2-yl]-1-methylazetidine-2-carboxamide Dimesylate (9bb). To a suspension of compound **16** (302 mg, 0.408 mmol) and *N*-methyl-L-azetidine-2-carboxylic acid (148 mg, 1.28 mmol) in CH_2Cl_2 (6 mL) was added *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (395 mg, 1.23 mmol) and DIEA (285 μL, 1.64 mmol). After 16.5 h, the resulting orange solution was concentrated under reduced pressure and was purified by preparative HPLC (Sunfire Prep C18 column, 50−90% B gradient). Fractions with the desired MW were collected and freeze-dried to provide 147 mg (43%) of **17bb**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 16.04 (s, 1 H), 10.10 (s, 1 H), 8.48 (d, J = 11.0 Hz, 1 H), 7.54−7.48 (m, 4 H), 7.40−7.32 (m, 5 H), 5.36 (s, 2 H), 4.99 (d, J = 9.8 Hz, 1 H), 4.90 (d, J = 9.8 Hz, 1 H), 3.96 (d, J = 10.4 Hz, 1 H), 3.54 (t, J = 7.9 Hz, 1 H), 3.39−3.34 (m, 1 H), 3.25−3.19 (m, 1 H), 3.05−2.92 (m, 2 H), 2.58−2.36 (m, 10 H), 2.23−2.06 (m, 4 H), 0.81 (s, 9 H), 0.28 (s, 3 H), 0.11 (s, 3 H). MS (ESI) *m/z* 837.37 (M + H).

To a solution of **17bb** (147 mg, 0.175 mmol) in 1,4-dioxane (3.5 mL) was added HF (50% aqueous solution, 750 μL). After 4 h, the reaction solution was poured into a solution of K_2HPO_4 (9 g) in water (90 mL) and was extracted with EtOAc (2 × 50 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was dissolved in 1,4-dioxane:MeOH (1:1, 4 mL), and palladium on carbon (10%, 43.5 mg) was added. The flask was fitted with a septum and was evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen gas for 3.25 h. The reaction mixture was filtered through Celite and was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 10−35% B gradient). Fractions with the desired MW were combined. The solution was cooled to 0 °C, and the pH was adjusted to 7.4 via dropwise addition of 0.5 M aqueous NaOH solution (approximately 7.8 mL). The aqueous solution was extracted with CH_2Cl_2 (3 × 60 mL), and the combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to provide 79.7 mg (0.146 mmol, 73%) of the free base. This yellow solid was dissolved in MeOH (3 mL), and $MeSO_3H$ (19 μL, 0.292 mmol) was added. The solution was concentrated under reduced pressure, dried under vacuum, and freeze-dried from water to provide 105 mg of the title compound as a yellow salt. ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (d, J = 11.0 Hz, 1 H), 5.16 (t, J = 8.6 Hz, 1 H), 4.21−4.12 (m, 1 H), 4.09−4.02 (m, 2 H), 3.17−2.85 (m, 10 H), 2.68 (s, 6 H, mesylate H), 2.64−2.59 (m, 1 H), 2.34−2.15 (m, 2 H), 1.70−1.58 (m, 1 H). MS (ESI) *m/z* 545.18 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-(thiophene-2-sulfonamido)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Hydrochloride (10b). To a solution of compound **8** (20 mg, 0.045 mmol) in THF was added DIEA (11.5 mg, 0.089 mmol) and 2-thiophenesulfonyl chloride (12.2 mg, 0.067 mmol). The reaction mixture was stirred for 20 h. HCl in MeOH (1 mL, 4 N) was added to the mixture at 0 °C. After 2 min, the mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 2.0 mg (7%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (dd, J = 5.2, 1.2 Hz, 1 H), 7.59 (d, J = 2.8 Hz, 1 H), 7.52 (d, J = 10.4 Hz, 1 H), 7.09 (t, J = 4.4 Hz, 1 H), 4.07 (s, 1 H), 3.11−2.92 (m, 9 H), 2.30−2.18 (m, 2 H), 1.68−1.58 (m, 1 H). MS (ESI) *m/z* 593.9 (M + H).

The following compounds were prepared by similar methods to **10b**:

(4*S*,4a*S*,5a*R*,12a*S*)-9-(Benzenesulfonamido)-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Hydrochloride (10a). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, J = 7.6 Hz, 2 H), 7.58−7.46 (m, 4 H), 4.07 (s, 1 H), 3.10−2.92 (m, 9 H), 2.35−2.25 (m, 2 H), 1.65−1.55 (m, 1 H). MS (ESI) *m/z* 552.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-(methanesulfonamido)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Hydrochloride (10c). Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.44 (d, J = 10.0 Hz, 1 H), 4.10 (s, 1 H), 3.21−2.90 (m, 12 H), 2.34−2.22 (m, 2 H), 1.67−1.61 (m, 1 H). MS (ESI) *m/z* 526.1 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-[(methylcarbamoyl)amino]-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Hydrochloride (11). To a suspension of compound **8** (0.260 g, 0.50 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (0.139 mL, 1.00 mmol). The reaction was stirred until a clear solution was formed.

Methylisocyanate (89.4 μL, 1.50 mmol) was added dropwise, and the reaction mixture was stirred for 1 h. Additional methylisocyanate (45 μL, 0.75 mmol) was added, and the reaction mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 15−65% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 80 mg (32%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (d, J = 11.4 Hz, 1 H), 4.07 (s, 1 H), 3.04 (s, 3 H), 2.96 (s, 3 H), 3.13−2.93 (m, 3 H), 2.77 (s, 3 H), 2.27−2.15 (m, 2 H), 1.69−1.57 (m, 1 H). MS (ESI) m/z 505.41 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-[(pyrimidin-4-yl)amino]-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Trihydrochloride (18). A vessel containing aniline **16** (18.2 mg, 0.024 mmol), Pd_2dba_3 (3.0 mg, 0.0033 mmol), Xantphos (3.4 mg, 0.0059 mmol), K_3PO_4 (40 mg, 0.188 mmol), and 4,6-dichloropyrimidine (6.5 mg, 0.044 mmol) was evacuated and backfilled with nitrogen gas three times. 1,4-Dioxane (500 μL) was added, and the reaction mixture was heated at 80 °C for 4.5 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient). Fractions with the desired MW were collected and freeze-dried to provide 7.5 mg (37%) of the intermediate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.97 (s, 1 H), 8.48 (s, 1 H), 8.33 (d, J = 5.5 Hz, 1 H), 7.52−7.46 (m, 2 H), 7.40−7.28 (m, 8 H), 7.07 (s, 1 H), 6.11 (s, 1 H), 5.34 (s, 2 H), 4.97 (d, J = 11.6 Hz, 1 H0, 4.88 (d, J = 11.0 Hz, 1 H), 3.95 (d, J = 10.4 Hz, 1 H), 3.28−3.19 (m, 1 H), 3.09−2.98 (m, 1 H), 2.61−2.54 (m, 1 H), 2.54−2.39 (m, 8 H), 2.16 (d, J = 14.6 Hz, 1 H), 0.83 (s, 9 H), 0.28 (s, 3 H), 0.14 (s, 3 H). MS (ESI) m/z 852.57 (M + H).

To a solution of the intermediate (7.5 mg, 0.0088 mmol) in 1,4-dioxane (1.4 mL) was added HF (50% aqueous solution, 200 μL). After 15.5 h, the reaction solution was poured into a solution of K_2HPO_4 (2.4 g) in water (20 mL) and was extracted with EtOAc (2 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was dissolved in 1,4-dioxane:MeOH (1:1, 1 mL), and palladium on carbon (10%, 10 mg) was added. The flask was fitted with a septum and was evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen gas for 2.5 h. The reaction mixture was filtered through Celite and was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 10−50% B gradient). Fractions with the desired MW were collected and freeze-dried to provide 2.2 mg (48%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.83 (s, 1 H), 8.37−8.25 (m, 1 H), 8.18−8.05 (m, 1 H), 7.30−7.20 (m, 1 H), 4.10 (s, 1 H), 3.20−2.90 (m, 9 H), 2.40−2.29 (m, 1 H), 2.27−2.19 (m, 1 H), 1.72−1.58 (m, 1 H). MS (ESI) m/z 526.31 (M + H).

General Procedure for Reductive Amination Products 20a−e. Compound **16** (1.0 equiv) was dissolved in 1,2-dichloroethane (~0.1 M). HOAc (5 equiv) and the aldehyde (1.5 equiv) were added. The mixture was stirred for 1 h. $Na(OAc)_3BH$ (3.0 equiv) was added, and the resulting mixture was stirred for an additional hour. The mixture was washed with H_2O (10 mL) and concentrated to give the crude intermediates **19a−e**. The crude material was dissolved in 1,4-dioxane (1.4 mL), and HF (50% aqueous solution, 200 μL) was added. After 15.5 h, the reaction solution was poured into a solution of K_2HPO_4 (2.4 g) in water (20 mL) and was extracted with EtOAc (2 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was dissolved in 1,4-dioxane:MeOH (1:1, 1 mL), and palladium on carbon (10%, 10 mg) was added. The flask was fitted with a septum and was evacuated and backfilled three times with hydrogen gas. The reaction mixture was stirred under an atmosphere of hydrogen gas for 2.5 h. The reaction mixture was filtered through Celite and was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 10−100% B gradient). Fractions with the desired MW were collected and freeze-dried to provide the title compounds.

The following compounds were prepared by the general procedure described above:

(4*S*,4a*S*,5a*R*,12a*S*)-9-{[2-(*tert*-Butylamino)ethyl]amino}-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Trihydrochloride (20a). Prepared from *t*-BuN(Cbz)CH_2CHO. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 6.72 (d, J = 11.0 Hz, 1 H), 4.07 (s, 1 H), 3.54−3.46 (m, 2 H), 3.26−3.19 (m, 2 H), 3.03 (s, 3 H), 2.95 (s, 3 H), 3.14−2.92 (m, 3 H), 2.23−2.14 (m, 2 H), 1.67−1.55 (m, 1 H), 1.38 (s, 9 H). MS (ESI) m/z 547.51 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4,9-Bis(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (20b). Prepared from aqueous formaldehyde. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.46−7.42 (m, 1 H), 4.15 (s, 1 H), 3.33 (s, 6 H), 3.04 (s, 3 H), 2.96 (s, 3 H), 3.17 −2.95 (m, 3 H), 2.44−2.34 (m, 1 H), 2.29−2.22 (m, 1 H), 1.71−1.60 (m, 1 H). MS (ESI) m/z 476.29 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-(propylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (20c). Prepared from *n*-propanal. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.39 (d, J = 9.2 Hz, 1 H), 4.10 (s, 1 H), 3.34 (t, J = 7.8 Hz, 2 H), 3.04 (s, 3 H), 2.96 (s, 3 H), 3.21 −2.95 (m, 3 H), 2.35 (t, J = 13.7 Hz, 1 H), 2.27−2.20 (m, 1 H), 1.82−1.72 (m, 2 H), 1.71−1.60 (m, 1 H), 1.05 (t, J = 7.4 Hz, 3 H). MS (ESI) m/z 490.32 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-7-fluoro-9-[(3-methylbutyl)amino]-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (20d). Prepared from isovaleraldehyde. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.44 (d, J = 9.2 Hz, 1 H), 4.12 (s, 1 H), 3.42−3.37 (m, 2 H), 3.05 (s, 3 H), 2.97 (s, 3 H), 3.21−2.97 (m, 3 H), 2.39−2.30 (m, 1 H), 2.29−2.22 (m, 1 H), 1.79−1.59 (m, 4 H), 0.98 (d, J = 6.4 Hz, 6 H). MS (ESI) m/z 518.43 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-[(2,2-dimethylpropyl)amino]-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (20e). Prepared from 2,2-dimethyl-1-propanal. Yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 6.83 (d, J = 10.5 Hz, 1 H), 4.06 (s, 1 H), 3.34 (t, J = 7.8 Hz, 2 H), 3.03 (s, 3 H), 2.95 (s, 3 H), 3.11−2.93 (m, 5 H), 2.27−2.14 (m, 2 H), 1.67−1.57 (m, 1 H), 1.04 (s, 9 H). MS (ESI) m/z 518.48 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-9-(Aminomethyl)-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (21). Benzyl *N*-(hydroxymethyl)carbamate (92 mg, 0.51 mmol) was added to a TFA/CH_3SO_3H (1 mL/1 mL) solution of compound **7a** (110 mg, 0.25 mmol) and was stirred for 30 min. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−30% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 23 mg (17%) of the title compound. ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, J = 9.2 Hz, 1 H), 4.16 (s, 2 H), 4.13 (s, 1 H), 3.21−2.94 (m, 3 H), 3.06 (s, 3 H), 2.97 (s, 3), 2.37−2.22 (m, 2 H), 1.70−1.58 (m, 1 H). MS (ESI) m/z 462.26 (M + H).

(4*S*,4a*S*,5a*R*,12a*S*)-4-(Dimethylamino)-9-{[(2,2-dimethylpropyl)amino]methyl}-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (22). Triethylamine (2 μL, 0.014 mmol) was added to a mixture of **21** (3 mg, 0.0065 mmol) and pivaldehyde (0.8 μL, 0.0072 mmol) in DMF (0.1 mL). The reaction was stirred for 15 min, and $NaBH(OAc)_3$ (3 mg, 0.013 mmol) and HOAc (2 μL) were added. The reaction was stirred for 1 h. The material was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 1 mg (26%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (d, J = 9.1 Hz, 1 H), 4.30 (s, 2 H), 4.09 (s, 1 H), 3.23−2.93 (m, 5 H), 3.04 (s, 3 H), 2.95 (s, 3 H), 2.40−2.19 (m, 2 H), 1.71−1.60 (m, 1 H), 1.05 (s, 9 H). MS (ESI) m/z 532.27 (M + H).

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(4*S*,4a*S*,5a*R*,12a*S*)-9-{[2-(*tert*-Butylamino)acetamido]-methyl}-4-(dimethylamino)-7-fluoro-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide Dihydrochloride (23). 2-*t*-Butylaminoacetylchloride hydrochloride (5.8 mg, 0.031 mmol) was added to a solution of **21** (12 mg, 0.026 mmol) in DMF (0.2 mL). The reaction mixture was stirred for 30 min and was diluted with 0.05 N HCl (2 mL). The solution was purified by preparative HPLC (Phenomenex Polymerx 10 μ RP 100A column, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 3.0 mg (18%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (d, J = 9.6 Hz, 1 H), 4.46 (s, 2 H), 4.08 (s, 1 H), 3.81 (s, 2 H), 3.18−2.92 (m, 3 H), 3.03 (s, 3 H), 2.96 (s, 3 H), 2.32−2.18 (m, 2 H), 1.69−1.60 (m, 1 H), 1.38 (s, 9 H). MS (ESI) m/z 575.30 (M + H).

Susceptibility Testing. Compound stocks were prepared and serially diluted in sterile deionized water. Tetracycline-susceptible isolates SA100 (*S. aureus* ATCC 13709, Smith), SA101 (*S. aureus* ATCC 29213), SP106 (*S. pneumoniae* ATCC 49619), EF103 (*E. faecalis* ATCC 29212), EC107 (*E. coli* ATCC 25922), KP109 (*K. pneumoniae* ATCC 13883), AB110 (*Acinetobacter baumannii* ATCC 19606), EC108 (*Enterobacter cloacae* ATCC 13047), and PA111 (*Pseudomonas aeruginosa* ATCC 27853) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Tetracycline-resistant isolates *S. aureus* SA158 (*tet*(K)), *S. pneumoniae* SP160 (*tet*(M)), *E. faecalis* EF159 (*tet*(M)), *E. coli* EC155 (*tet*(A)), and *K. pneumoniae* KP153 (*tet*(A)) were obtained from Marilyn Roberts' lab at the University of Washington. *S. aureus* SA161 (*tet*(M)) was obtained from Micromyx (Kalamazoo, MI). 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).[24] 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.

Animal Efficacy Models. All animal efficacy models were performed at Vivisource, Waltham, MA.

Mouse Systemic Infection Model. *S. aureus* ATCC 13709 was grown to log phase in a liquid culture. Bacteria were diluted in 5% mucin to a concentration to achieve 90% mortality within 48 h after infection. The final inoculum concentration of bacteria was 1.98×10^6 CFU/mouse. CD-1 female mice (18−20 g) were infected with 0.5 mL of bacterial suspension via intraperitoneal injection, 6 per dose concentration. For the screening model, mice were treated with the test article as either a single 30 mg/kg dose via oral gavage or a 3 mg/kg intravenous dose one hour post infection. Infection control mice were dosed with vehicle (sterile water). After 48 h, survival was recorded. For PD_{50} determinations, mice received oral treatment with the test article at concentrations ranging from 0.30 to 30 mg/kg one hour post infection. The PD_{50} in mg/kg was calculated as survival after 48 h.

MRSA Neutropenic Lung Model. Female Balb/C mice weighing 18−20 g were rendered neutropenic through two consecutive IP cyclophosphamide injections of 150 and 100 mg/kg on days −4 and −1, respectively. Mice were infected with *S. aureus* VL-137 (MRSA) via intranasal inoculation under light anesthesia. At 2 and 12 h post infection, mice were treated with compound **9l** or linezolid either orally at 50 mg/kg/dose or intravenously at 10 mg/kg/dose. Six mice were treated with each drug concentration. Twenty-four hours post treatment, mice were euthanized by CO_2 inhalation. The lungs were aseptically removed, weighed, homogenized, serially diluted, and plated on TSA media. The plates were incubated overnight at 37 °C in 5% CO_2. CFU per gram of lung tissue was calculated by enumerating the plated colonies then adjusting for serial dilutions and the weight of the lung.

***S. pneumoniae* Neutropenic Lung Model.** Female Balb/c mice weighing 18−20 g were rendered neutropenic through two consecutive IP cyclophosphamide injections of 150 and 100 mg/kg on days −4 and −1, respectively. Mice were infected with *S. pneumoniae* SPN160 via intranasal administration of 0.05 mL under light anesthesia. At 2 and 12 h post infection, mice were treated with compound **9l** or linezolid at either 30 mg/kg/dose via oral gavage or 10 mg/kg/dose intravenously. Six mice were treated with each drug concentration. Twenty-four hours post initiation of treatment, mice were euthanized by CO_2 inhalation. The lungs of the mice were aseptically removed, weighed, homogenized, serially diluted, and plated on TSA-II medium. The plates were incubated overnight at 37 °C in 5% CO_2. CFU per gram of lung tissue was calculated by enumerating the plated colonies then adjusting for serial dilutions and the weight of the lung.

***S. pneumoniae* Immunocompetent Lung Model.** Female CD-1 mice weighing 18−20 g were infected with *S. pneumoniae* 1629 (VL-172) via intranasal administration of 0.05 mL under light anesthesia. At 5, 24, and 36 h post infection, mice were treated with compound **9l** or linezolid at 30 mg/kg/dose via oral gavage. Six mice were treated with each drug concentration. Forty-eight hours post initiation of treatment, mice were euthanized by CO_2 inhalation. The lungs of the mice were aseptically removed, weighed, homogenized, serially diluted, and plated on TSA-II medium. The plates were incubated overnight at 37 °C in 5% CO_2. CFU per gram of lung tissue was calculated by enumerating the plated colonies then adjusting for serial dilutions and the weight of the lung.

***H. influenzae* Immunocompetent Lung Model.** Male Sprague−Dawley rats weighing 175−200 g were infected with *H. influenzae* 551 via intratracheal administration of 0.05 mL under light anesthesia. At 5, 24, and 48 h post infection, rats were treated with compound **9l** at either 100 mg/kg/dose orally or 25 mg/kg/dose intravenously, or with azithromycin at 50 mg/kg/dose orally. Six rats were treated with each drug concentration. Seventy-two hours post initiation of treatment, rats were euthanized by CO_2 inhalation. The lungs of the rats were aseptically removed, weighed, homogenized, serially diluted, and plated on TSA-II medium. The plates were incubated overnight at 37 °C in 5% CO_2. CFU per gram of lung tissue was calculated by enumerating the plated colonies then adjusting for serial dilutions and the weight of the lung.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MIC data for the complete panel of bacteria for all compounds and MIC data for the lung infection model strains. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

ATCC, American Type Culture Collection; BLQ, below limit of quantitation; Boc, *t*-butoxycarbonyl; Cbz, benzyloxycarbonyl; CFU, colony forming units; dba, dibenzylideneacetone; DCE, 1,2-dichloroethane; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; *F*, oral bioavailability; HATU, 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; IV, intravenous; LDA, lithium diisopropylamide; LHMDS, lithium bis(trimethylsilyl)amide; MIC, minimum inhibitory concentration; MIC_{50}, minimum inhibitory concentration required to inhibit growth of 50% of organisms; MIC_{90}, minimum inhibitory concentration required to inhibit growth

of 90% of organisms; MRSA, methicillin-resistant *Staphylococcus aureus*; MW, molecular weight; NMP, 2-methylpyrrolidinone; NT, not tested; PD_{50}, dose at which 50% protection (survival) is observed; PK, pharmacokinetic; PO, oral; SAR, structure activity relationships; TMEDA, *N,N,N′,N′*-tetramethylethylenediamine; TFA, trifluoroacetic acid; Xantphos, 9,9-dimethyl-4,5-bis-(diphenylphosphino)xanthenes

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