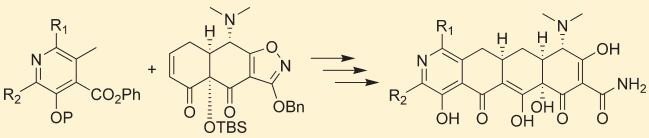# 8-Azatetracyclines: Synthesis and Evaluation of a Novel Class of Tetracycline Antibacterial Agents

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Ⓢ *Supporting Information*

ABSTRACT: A novel series of fully synthetic 8-azatetracyclines was prepared and evaluated for antibacterial activity. Compounds were identified that overcome both efflux (tet(K)) and ribosomal protection (tet(M)) tetracycline resistance mechanisms and are active against Gram-positive and Gram-negative organisms. Two compounds were identified that exhibit comparable efficacy to marketed tetracyclines in in vivo models of bacterial infection.

■ INTRODUCTION

The tetracycline class of antibacterial agents has seen widespread clinical use for over 50 years due to its broad spectrum antibacterial activity.[1] Tetracyclines inhibit bacterial growth by preventing protein biosynthesis through binding to the 30S ribosome, thereby blocking the binding of aminoacyl-tRNA to the acceptor site.[2] This class includes a number of naturally occurring compounds, such as chlorotetracycline (**1**)[3] and tetracycline (**2**),[4] as well as several semisynthetic analogues, such as doxycycline (**3**)[5] and minocycline (**4**) (Figure 1).[6] In addition to their use as antibiotics for serious infections, tetracyclines have also been prescribed for use in acne and rosacea.[7] As with many classes of antibiotics, widespread use of tetracyclines has led to resistance and has limited their use in more complicated bacterial infections.[8,9]

Two major bacterial resistance mechanisms have been identified for tetracyclines: (1) active transport from bacterial cells via efflux pumps[10] and (2) ribosomal protection.[11] The efflux pumps are comprised of inner membrane tetracycline efflux proteins that were first identified in Gram-negative organisms (tet(A-E))[10a,10b] and were later found in Gram-positive bacteria (tet(K-L)).[10c] The ribosomal protection mechanism (tet(M-O)) involves the expression of proteins that bind to the ribosome and allow protein synthesis to proceed even in the presence of tetracyclines. The exact mechanism by which this occurs remains unclear. Since these early discoveries, the number of efflux and ribosomal protection mechanisms identified has increased.[12]

The presence of the strongly activating 10-hydroxy group readily enables chemical modification of the tetracycline D-ring at the 7- and 9-positions. This strategy led to the introduction of the 7-dimethylamino group in compounds **4**−**6**, all of which have demonstrated improved activity in bacterial strains bearing efflux pump resistance mechanisms. More recently, the 9-substituted glycylcyclines, such as tigecycline (**5**),[13] and the aminomethylcyclines, such as amadacycline (**6**),[14] have shown improved activity for resistant organisms bearing the ribosomal protection mechanism.

In 2005, Myers and co-workers published a fully synthetic route to the tetracyclines, the key step of which involves the Michael−Dieckmann condensation of the AB precursor **8** with structurally diverse D-ring precursors.[15,16] This route provides potential access to a broad range of tetracyclines that are inaccessible via traditional semisynthesis.[17] One example of this is the introduction of a nitrogen atom into the D-ring, resulting in "azatetracyclines." One 7-azatetracycline and one 9-azatetracycline were disclosed in Myers original work, resulting in compounds with only modest antibacterial activity. Herein we demonstrate this powerful approach using pyridyl D-ring precursors **7** to generate novel 8-azatetracyclines, **10** (Scheme 1).

■ RESULTS AND DISCUSSION

Chemistry. To explore 7- and 9-substituted-8-azatetracyclines, we required intermediates that could be readily modified either prior to or after construction of the protected compound **9**. Thus, the halopyridines **15** and **16** were prepared according to Schemes 2 and 3. 3,5-Dichloroisonicotinic acid was treated with sodium benzylate to give compound **12**, which was esterified to the phenyl ester **13** by conversion to the acid chloride, followed by treatment with phenol and 4-(dimethylamino)pyridine (DMAP). Suzuki coupling[18] using methylboronic acid and $PdCl_2Cy_2$ catalyst[19] gave the parent D-ring precursor **14** in good yield. *N*-Oxide formation with hydrogen peroxide in acetic acid followed by treatment with either phosphorousoxychloride or phosphorousoxybromide gave **15a** and **15b**, respectively. For **15a**, the reaction gave a 4.5:1.0 ratio of the desired regioisomer, while a 25:1 ratio was observed for **15b**. Both desired regioisomers were isolated in pure form by recrystallization. The regiochemistry was assigned by conversion to the dimethylamino compound **15c** through a palladium-mediated amination.[20] ^{1}H NMR studies indicated an nOe between the methyl
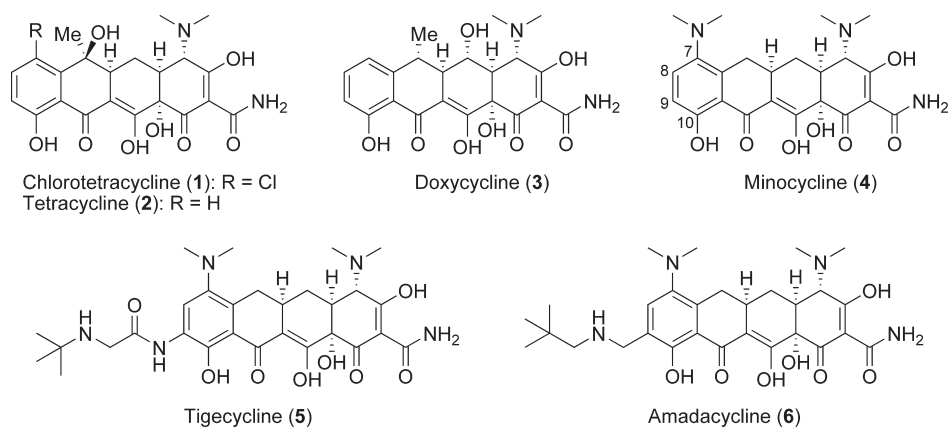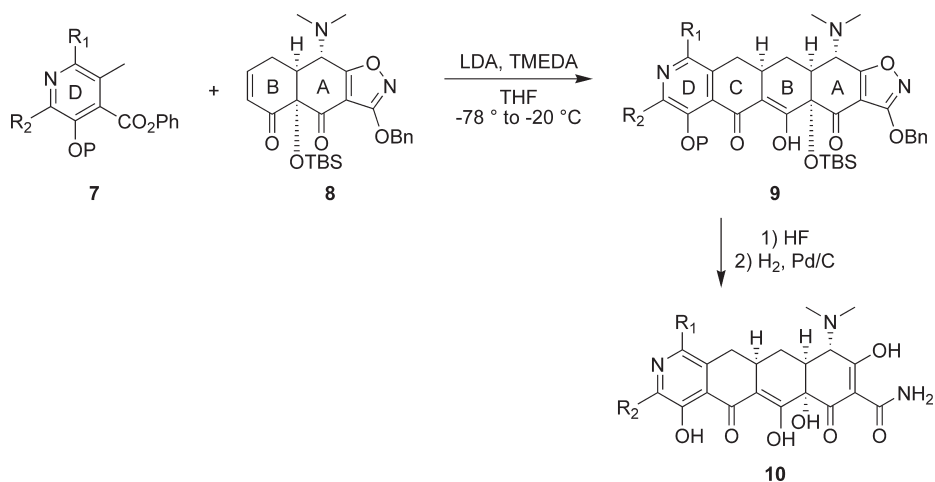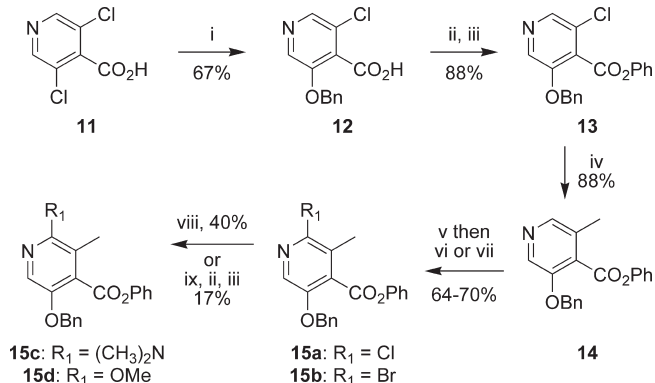
Figure 1. Examples of naturally occurring (**1** and **2**) and semisynthetic (**3**−**6**) tetracyclines.

Scheme 1. Fully Synthetic Approach to the 8-Azatetracyclines

**Scheme 2. Synthesis of D-Ring Precursors for 7-Substituted-8-azatetracyclines**[a]

[a] Reagents and conditions: (i) NaH (2 equiv), BnOH, NMP, 80 °C; (ii) (COCl)$_2$, cat. DMF, CH$_2$Cl$_2$; (iii) PhOH, Et$_3$N, DMAP, CH$_2$Cl$_2$; (iv) CH$_3$B(OH)$_2$, PdCl$_2$Cy$_2$, K$_3$PO$_4$, toluene, water, 100 °C; (v) H$_2$O$_2$, AcOH, 80 °C; (vi) POCl$_3$, toluene, 100 °C; (vii) POBr$_3$, toluene, 100 °C; (viii) dimethylamine in THF, Pd$_2$dba$_3$, Xantphos, K$_3$PO$_4$, 1,4-dioxane, 100 °C (sealed); (ix) NaOMe, NMP, 100−120 °C.

groups of the amine and the adjacent ring methyl group. The methoxy derivative **15d** was prepared from **15b** by treatment with sodium methoxide in 2-methylpyrrolidinone (NMP) at 120 °C. This gave the acid directly, which was re-esterified to the phenyl ester.

The dihalo compounds **16a** and **16b** were prepared by N-oxidation of **15a** and **15b** followed by treatment with POBr$_3$ (Scheme 3). Yields were typically moderate (45−50%), with reduction of the N-oxide to give the starting pyridines **15** observed as major side reactions in both cases. Palladium-mediated coupling of **16b** with t-butylcarbamate gave a ∼2.5:1 mixture of regioisomers, which were readily separated after conversion to the free amine **16c** upon treatment with HCl. Treatment of **16c** with sodium nitrite in the presence of HF-pyridine gave the fluoro derivative **16d** in good yield.[21] Similarly, a 2.4:1.0 ratio of regioisomers was observed in the palladium-mediated reaction of **16b** with dimethylamine to give **16e** and **16f**. Interestingly, the regioselectivity could be reversed by heating **16b** in the presence of dimethylamine without catalyst to give **16f** as the major isomer in a 3:1 ratio.
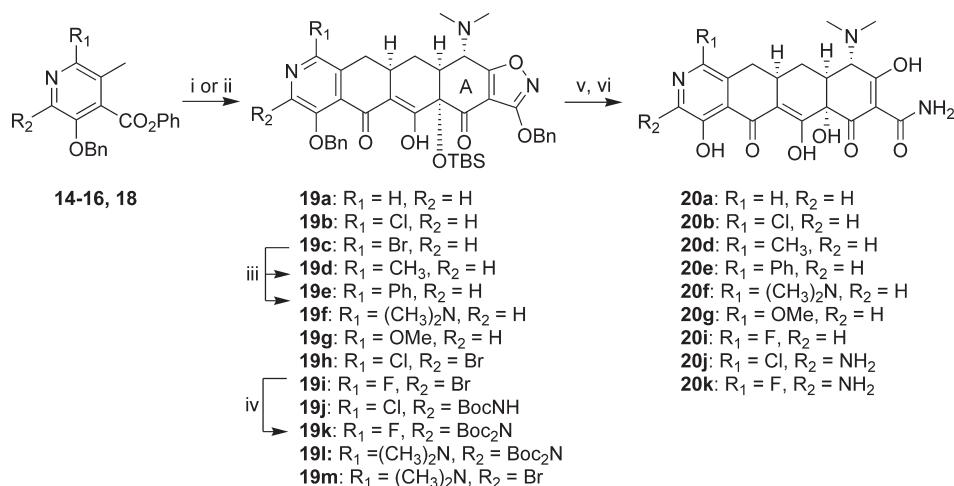
The 9-amino D-ring precursors **18a** and **18b** were prepared according to Scheme 4. Once again, palladium-mediated couplings using t-butylcarbamate proved to be highly effective for

Scheme 3. Synthesis of D-Ring Precursors for 7,9-Disubstituted-8-azatetracyclines[a]

15a: R$_1$ = Cl
15b: R$_1$ = Br

16a: R$_1$ = Cl
16b: R$_1$ = Br

16c

16d

16e: 58%

16f: 24%
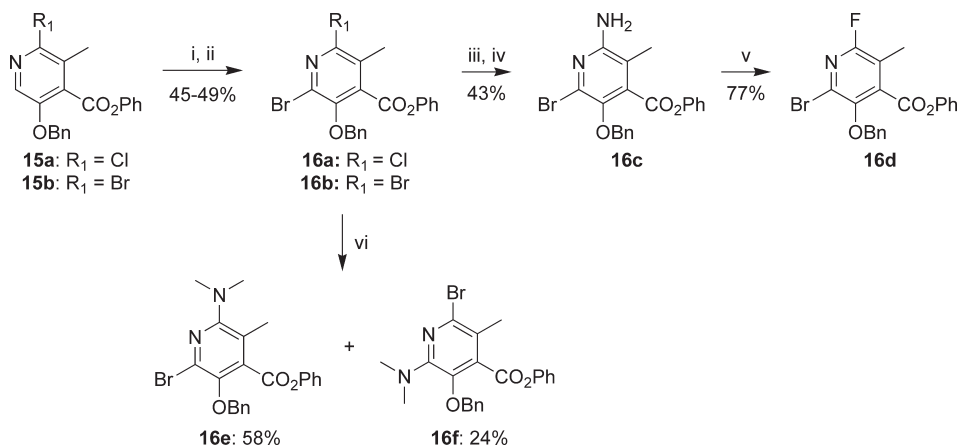
[a] Reagents and conditions: (i) for **15a**, mCPBA, CH$_2$Cl$_2$; for **15b**, H$_2$O$_2$, AcOH, 80 °C; (ii) POBr$_3$, toluene, 100 °C; (iii) BocNH$_2$, Pd$_2$dba$_3$, Xantphos, Cs$_2$CO$_3$, 1,4-dioxane, 80 °C; (iv) HCl, 1,4-dioxane; (v) HF-pyridine, NaNO$_2$, pyridine; (vi) dimethylamine in THF, Pd$_2$dba$_3$, Xantphos, Cs$_2$CO$_3$, 1,4-dioxane, 60 °C (sealed).

Scheme 4. Synthesis of D-Ring Precursors for 9-Amino-8-azatetracyclines[a]

16d,e

17a: R$_1$ = F
17b: R$_1$ = (CH3)$_2$N

18a: R$_1$ = F
18b: R$_1$ = (CH3)$_2$N
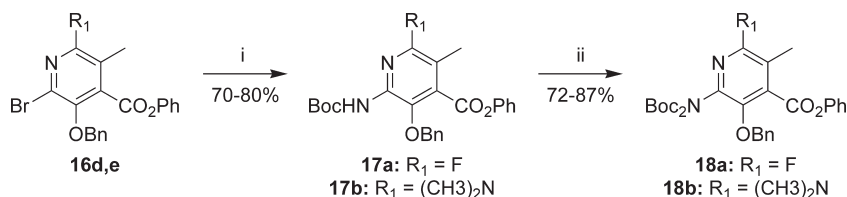
[a] Reagents and conditions: (i) BocNH$_2$, Pd$_2$dba$_3$, Xantphos, Cs$_2$CO$_3$, 1,4-dioxane, 80 °C; (ii) Boc$_2$O, DMAP, DMF.

Scheme 5. Synthesis of 8-Azatetracyclines[a]

14-16, 18

19a: R$_1$ = H, R$_2$ = H
19b: R$_1$ = Cl, R$_2$ = H
19c: R$_1$ = Br, R$_2$ = H
19d: R$_1$ = CH$_3$, R$_2$ = H
19e: R$_1$ = Ph, R$_2$ = H
19f: R$_1$ = (CH$_3$)$_2$N, R$_2$ = H
19g: R$_1$ = OMe, R$_2$ = H
19h: R$_1$ = Cl, R$_2$ = Br
19i: R$_1$ = F, R$_2$ = Br
19j: R$_1$ = Cl, R$_2$ = BocNH
19k: R$_1$ = F, R$_2$ = Boc$_2$N
19l: R$_1$ =(CH$_3$)$_2$N, R$_2$ = Boc$_2$N
19m: R$_1$ = (CH$_3$)$_2$N, R$_2$ = Br

20a: R$_1$ = H, R$_2$ = H
20b: R$_1$ = Cl, R$_2$ = H
20d: R$_1$ = CH$_3$, R$_2$ = H
20e: R$_1$ = Ph, R$_2$ = H
20f: R$_1$ = (CH$_3$)$_2$N, R$_2$ = H
20g: R$_1$ = OMe, R$_2$ = H
20i: R$_1$ = F, R$_2$ = H
20j: R$_1$ = Cl, R$_2$ = NH$_2$
20k: R$_1$ = F, R$_2$ = NH$_2$

[a] Reagents and conditions: (i) for **14**, **15b–d**, **16e**, and **18a**, LDA, TMEDA, −78 °C, THF then **8**, −78 °C to −20 °C; (ii) for **16a**, **16d**, and **18b**, LHMDS, **8**, THF, −78 °C to −20 °C; (iii) CH$_3$B(OH)$_2$ or PhB(OH)$_2$, PdCl$_2$dppf$_2$-CH$_2$Cl$_2$, K$_3$PO$_4$, toluene, 1,4-dioxane, water, 100 °C; (iv) BocNH$_2$, Pd$_2$dba$_3$, Xantphos, K$_3$PO$_4$, 1,4-dioxane, 100 °C; (v) aqueous HF, CH$_3$CN or 1,4-dioxane; (vi) 10% Pd/C, H$_2$, HCl, MeOH, 1,4-dioxane.

introduction of the amino group. Further protection of the mono-Boc intermediates **17** with Boc$_2$O and DMAP in DMF gave the di-Boc precursors **18**.

Synthesis of the 8-azatetracyclines is outlined in Scheme 5. Most of the D-ring precursors were lithiated with lithium diisopropylamide (LDA) at −78 °C, followed by treatment with the enone **8**. Warming to −20 °C yielded the fully protected tetracycline precursors **19**. For D-ring precursors **16a**, **16d**, and **18b**, a one-pot reaction was performed in which lithium bis(trimethylsilyl)amide (LHMDS) was added to a −78 °C

Scheme 6. Synthesis of 9-Glycylamido-8-azatetracyclines[a]

19j-l

21a: R₁ = Cl
21b: R₁ = F
21c: R₁ = (CH₃)₂N
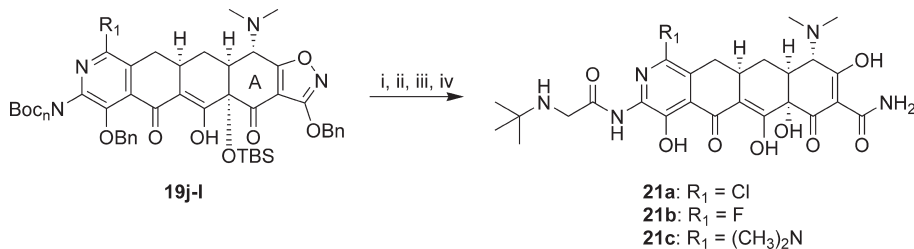
[a] Reagents and conditions: (i) HCl, 1,4-dioxane; (ii) bromoacetylchloride, THF then *t*-BuNH₂, 50 °C; (iii) aqueous HF, CH₃CN or 1,4-dioxane; (iv) 10% Pd/C, H₂, HCl, MeOH, 1,4-dioxane.

Scheme 7. Synthesis of 7,9-Disubstituted-8-azatetracyclines[a]

16a,d,e

22

23

19h,m

24

25

26
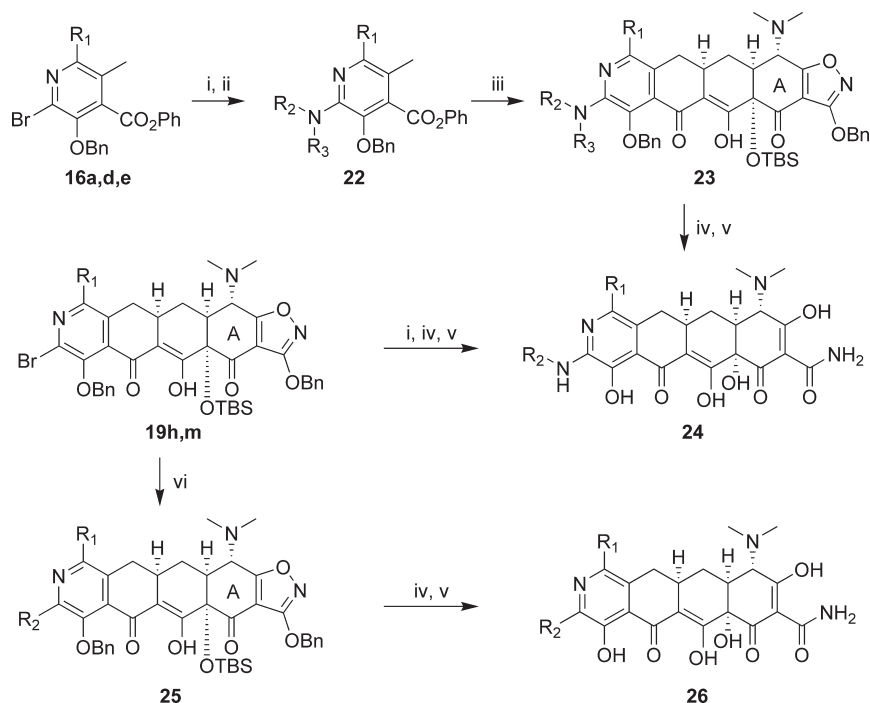
[a] Reagents and conditions: (i) R₂R₃NH2, Pd₂dba₃, Xantphos, K₃PO₄ or Cs₂CO₃, 1,4-dioxane, 100 °C or *n*-propylamine, CuI, 2-acetylcyclohexanone, K₃PO₄, DMF, 100 °C; (ii) LHMDS, THF then Boc₂O or CbzCl; (iii) LDA, TMEDA, −78 °C, THF then **8**, −78 °C to −20 °C or LHMDS, **8**, THF, −78 °C to −20 °C; (iv) aqueous HF, CH₃CN, or 1,4-dioxane; (v) 10% Pd/C, H₂, HCl, MeOH, 1,4-dioxane; (vi) R₂B(OH)₂, (Ph₃P)₄Pd, K₃PO₄, toluene, 1,4-dioxane, H₂O, 90 °C.
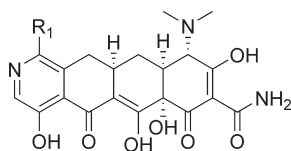
solution of the precursor and the enone **8** followed by warming to −20 °C. Yields varied from 20 to 84% depending on substrate, with larger reaction scales generally providing better results. The LHMDS procedure also typically gave higher yields but was only possible on the more highly electron deficient D-ring precursors. The 7-bromo intermediate **19c** was further derivatized at this stage via Suzuki couplings with methylboronic acid and phenylboronic acid to give **19d** and **19e**, respectively. Similarly, palladium-mediated amination with **19h** and *t*-butylcarbamate provided the 7-chloro-9-amino compound **19j**. Deprotection of the intermediates **19** with HF followed by hydrogenation in the presence of palladium on carbon gave the final 8-azatetracyclines **20a−k**.

To compare the 8-azatetracyclines directly to tigecycline, the 9-glycylamido-8-azatetracyclines **21a−c** were prepared (Scheme 6). The Boc groups of compounds **19j−l** were deprotected with HCl in 1,4-dioxane. Treatment with bromoacetylchloride in THF followed

by excess *t*-butylamine gave the acylated intermediates, which were deprotected under standard conditions to give the final analogues **21a−c**.

The bromopyridine intermediates proved to be useful for the introduction of alkylamines via palladium-mediated aminations and alkyl groups via Suzuki couplings, ultimately providing the 9-alkylamino-8-azatetracyclines **24** and 9-alkyl-8-azatetracyclines **26** (Scheme 7). Thus, **19h** was treated with various amines in the presence of Pd₂dba₃ and 9,9-dimethyl-4,5-bis-(diphenylphosphino)xanthenes (Xantphos) in 1,4-dioxane at 100 °C. Potassium phosphate was found to be the most effective base for this transformation, with cesium carbonate and sodium *t*-butoxide leading to rapid decomposition. Low yields were generally observed due to decomposition of both the starting material and products upon heating. Reaction times of 2−4 h were eventually settled on as a balance between product formation and decomposition. Copper-catalyzed aminations were also

Table 1. In Vitro Antibacterial Activity of 7-Substituted-8-azatetracyclines

| | | MIC (μg/mL) | | | | | | | |
| | | S. aureus | | | S. pneumoniae | | E. coli | | K. pneumoniae |
compd	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]
20a	H	1	32	16	0.25	32	0.5	>32	1
20b	Cl	1	32	32	1	32	2	>32	4
20d	CH$_3$	1	32	0.5	0.25	>32	1	>32	1
20e	Ph	0.063	32	1	0.031	4	0.5	>32	2
20f	(CH$_3$)$_2$N-	0.031	16	2	0.063	8	0.125	>32	0.25
20g	CH$_3$O-	0.125	8	4	0.063	16	0.25	>32	1
20i	F	0.063	8	8	0.016	8	0.25	>32	0.5
tetracycline		1	>32	32	0.25	32	2	>32	4
minocycline		0.125	16	0.25	<0.016	8	0.5	8	1

[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.

explored, but rapid decomposition of the starting material was observed. Hindered amines generally gave low yields as did reactions with the 7-dimethylamino compound **19m**. Alternatively, the alkylamines could be introduced prior to the Michael—Dieckmann reaction using either palladium or copper-mediated aminations[22] with **16a**, **16d**, and **16e**. Protection of the resulting aminopyridines upon treatment with LHMDS followed by Boc$_2$O or CbzCl gave the D-ring precursors **22**. Michael—Dieckmann reaction then gave the protected intermediates **23**. Suzuki reactions with **19h** or **19m** and various boronic acids were also carried out in moderate yields, providing the 9-alkyl intermediates **25**. Deprotection of the intermediates under standard conditions gave the final 8-azatetracyclines **24** and **26**.
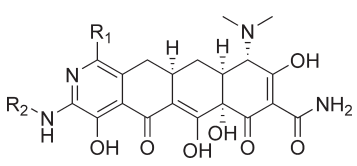
Biology. The in vitro antibacterial activities of all analogues were determined for a panel of Gram-positive (e.g., *Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram-negative (e.g., *Escherichia coli* and *Klebsiella pneumoniae*) bacteria. The activities of the 7-substituted-8-azatetracycline as well as several control tetracyclines for a representative set of these organisms are found in Table 1. The parent 8-azatetracycline **20a** showed comparable activity to tetracycline, with reasonable minimum inhibitory concentrations (MICs) of 0.25–1 μg/mL for the tetracycline-susceptible strains. Lacking a substituent at either C-7 or C-9, **20a** demonstrated poor activity for the strains bearing tet(M), tet(K), and tet(A). Overall, the 7-position appears to be very tolerant of substitution, allowing for bulky (**20e**), electron withdrawing (**20b** and **20i**), and electron donating (**20f** and **20g**) groups while maintaining activity against the tetracycline-susceptible strains. As with minocycline, introduction of the 7-dimethylamino group in **20f** led to improved activities for most strains tested. Modest (1–2 dilution) improvements in MICs were observed for the tet(M) strains, while a more significant improvement was seen for the strain bearing the tet(K) resistance mechanism. With the exception of the 7-chloro analogue, all of the 7-substituted-8-aza compounds exhibited improved MICs for the tet(K) strain, correlating well with literature reports on 7-substituted tetracyclines. Unfortunately, all of the 8-azatetracyclines appear to be substrates for the tet(A) efflux pump, exhibiting no antibacterial activity at the highest concentration tested (32 μg/mL). In general, substitution at the 7-position appears to be inefficient at overcoming the ribosomal protection resistance mechanism of the tet(M) strains. The only compound with two dilution improvements for both tet(M) strains was compound **20i**, the 7-fluoro analogue. This compound was also one of the more potent antibacterial compounds in this series.

To make a direct comparison to tigecycline, the 9-glycylamido-8-azatetracyclines were prepared (Table 2). Whereas tigecycline is a very potent antibacterial agent against all of the organisms in our panel, including the four strains bearing tetracycline resistance mechanisms, the corresponding 8-azatetracycline analogues **21a**—**c** were found to have poor overall activity. The aniline precursors **20j** and **20k**, however, showed significant improvements in MICs for both tet(M) strains relative to the corresponding 9-unsubstituted compounds **20b** and **20i**. For the 7-Cl analogue **20j**, antibacterial activity also improved for the tet(K) and wild type strains. These compounds were also assessed for their ability to inhibit protein synthesis in a cell-free transcription/translation assay (Table 3). In this assay, compounds **20j** and **20k** were about 2-fold more potent than tetracycline, in agreement with their relative MICs for *E. coli*. Compounds **21a**—**c**, on the other hand, inhibited protein synthesis with IC$_{50}$s between 0.42 and 0.90 μg/mL, similar to tigecycline, despite having only modest antibacterial activity in the whole-cell assays. These data suggest that the compounds are active at the target, but that compounds **21a**—**c** are not capable of getting to the target in the whole-cell assay, possibly due to poor cell permeability.

On the basis of the results of compounds **20j** and **20k**, a series of 9-amino-8azatetracyclines was prepared (Table 4). Compounds bearing a small, unbranched alkylamino group at the 9-position (**24a**—**c**) proved to be very potent antibacterial agents against

Table 2. In Vitro Antibacterial Activity of 9-Glycylamido-8-azatetracyclines

MIC (μg/mL)

Cmpd	R$_1$	R$_2$	S. aureus Wild type[a]	tet(M)[b]	tet(K)[c]	S. pneumoniae Wild type[d]	tet(M)[c]	E. coli Wild type[e]	tet(A)[c]	K. pneumoniae Wild type[f]
20j	Cl	H	0.016	4	8	0.016	4	0.25	>32	0.5
20k	F	H	0.063	2	8	0.063	2	0.25	>32	1
21a	Cl	(side chain)	2	32	32	1	16	4	>32	8
21b	F	(side chain)	4	16	16	2	8	8	>32	16
21c	(CH$_3$)$_2$N	(side chain)	16	32	8	4	4	32	>32	>32
Tigecycline			0.063	0.125	0.063	0.016	0.016	0.125	0.5	0.125

[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. Transcription/Translation (TnT) Assay Data

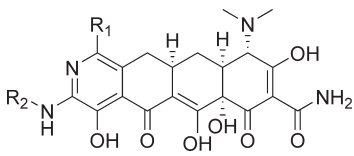| compd	E. coli[a] MIC (μg/mL)	TnT IC$_{50}$ (μg/mL)
20f	0.125	0.76
20j	0.25	1.1
20k	0.25	0.93
21a	4	0.78
21b	8	0.36
21c	32	0.71
24i	8	0.58
24l	0.5	0.70
24c	4	5.3
24n	2	2.8
24o	32	3.4
tetracycline	2	2.0
tigecycline	0.125	0.47

[a] ATCC 25922 is a tetracycline-susceptible strain.

the two tetracycline-susceptible Gram-positive strains, with MICs between 0.016 and 0.25 μg/mL. A 4-fold improvement in activity against the tet(M) S. aureus strain was observed for all of these compounds relative to **20j**, while MICs for the S. pneumoniae tet(M) strain were largely unchanged. For the tet(K) S. aureus strain, antibacterial activity improved with increasing size of the alkyl group. Among the tetracycline-susceptible Gram-negative strains, however, there was a clear loss in potency as the size of the alkyl group increased, a trend that continued for the larger branched alkyl compounds **24d−f**. This is not surprising, as it is known that Gram-negative antibacterial agents tend to be smaller and more polar.[23] These larger side chains also adversely affected the antibacterial activity for S. pneumoniae while having less effect on the MICs for the S. aureus strains. The 7-fluoro-9-n-propylamino

(**24n**) and 7-dimethylamino-9-n-propylamino (**24o**) analogues were prepared as comparisons to **24c**. While the 7-fluoro compound had MICs that tracked fairly closely to the 7-chloro analogue, the 7-dimethylamino compound exhibited no antibacterial activity for any of the strains at the highest concentration tested (32 μg/mL) with the exception of the tetracycline-susceptible S. pneumoniae strain. All three of the compounds had similar, albeit weak, activity in the transcription/translation assay, indicating the differences in MICs might be due to poor permeability of the 7-dimethylamino analogue. Introduction of a more polar heteroatom into the side chain (**24g−i,k**) resulted in improved antibacterial activity against the S. pneumoniae and Gram-negative strains but had minimal effect on activity toward either resistant S. aureus organisms. Interestingly, the oxygen bearing side chains (**24g** and **24h**) yielded significantly improved antibacterial activity toward tetracycline-susceptible Gram-positive strains relative to the all carbon side chains (**24e** and **24f**), while the nitrogen analogues (**24k** and **24i**) were less potent against S. aureus and showed more modest improvements against S. pneumoniae. The combination of a branched side chain with a distal amino group gave the most balanced antibacterial compounds in this series (**24l**, **24m**, and **24j**). These compounds combine good antibacterial activity toward both Gram-positive and Gram-negative organisms, with modest activity against tet(A) E. coli. Both compounds **24l** and **24m** are less potent than tigecycline but compare favorably to amadacycline.

The antibacterial activity of the 9-alkyl and phenyl substituted compounds were generally modest (Table 5). Here again, a larger substituent at C-9 yielded improved activity for tet(M) and tet(K) strains but resulted in loss of activity for wild type S. pneumoniae and Gram-negative strains. Introduction of a 7-Cl or 7-dimethylamino group also gave improved activity for most of

Table 4. In Vitro Antibacterial Activity of 9-Alkylamino-8-azatetracyclines

| | | | MIC (µg/mL) | | | | | | | |
| | | | S. aureus | | | S. pneumoniae | | E. coli | | K. pneumoniae |
Cmpd	R1	R2	Wild type[a]	tet(M)[b]	tet(K)[c]	Wild type[d]	tet(M)[c]	Wild type[e]	tet(A)[c]	Wild type[f]
20j	Cl	H	0.016	4	8	0.016	4	0.25	>32	0.5
24b	Cl	Me	0.016	1	2	0.016	4	0.125	32	0.5
24a	Cl	Et	0.031	1	0.5	0.031	4	0.5	16	2
24c	Cl	n-Pr	0.063	1	0.5	0.25	8	4	16	8
24d	Cl	i-Pr	1	4	2	2	16	32	>32	32
24e	Cl	(isobutyl structure)	0.5	1	0.5	1	8	16	>32	>32
24f	Cl	(branched alkyl structure)	0.25	2	0.25	8	32	>32	>32	>32
24g	Cl	(methoxyethyl structure)	0.031	4	1	0.063	4	1	32	4
24k	Cl	(dimethylaminoethyl structure)	2	4	1	0.5	2	4	32	8
24h	Cl	(isopropoxyethyl structure)	0.5	2	0.25	0.031	4	8	>32	16
24i	Cl	(dimethylaminopropyl structure)	2	8	2	1	4	8	>32	16
24l	Cl	(N,N-dimethyl branched structure)	0.5	2	0.125	0.016	0.125	0.5	8	2
24m	Cl	(pyrrolidinyl structure)	0.5	2	0.125	0.031	0.25	2	8	4
24j	F	(N,N-dimethyl branched structure)	1	2	0.5	0.5	1	1	8	2
24n	F	n-Pr	0.25	2	0.25	4	4	2	16	8
24o	(CH3)2N	n-Pr	>32	>32	>32	8	>32	>32	>32	>32
Amadacycline			0.5	2	0.25	0.031	0.125	2	16	2

[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.

the strains in the panel. When comparing the 7-Cl-9-alkyl compounds (26b) to the 7-Cl-9-aminoalkyl compounds (24c), it is clear that the nitrogen confers significant improvements in antibacterial activity. In the 7-dimethylamino case, however, the 7-dimethylamino-9-alkyl compound 26a is a balanced Gram-positive antibacterial agent while the 9-aminoalkyl compound 24o has no significant activity.

Compounds 20f and 24l were selected for further profiling. Both compounds inhibited protein synthesis in the transcription/translation assay with IC$_{50}$s comparable to tigecycline. Susceptibility testing was performed, and MIC$_{50}$ and MIC$_{90}$ values were calculated for recent clinical isolates of S. aureus, S. pneumoniae, Haemophilus influenzae, and E. coli (Table 6). The panels contained both tetracycline-susceptible and resistant strains, including 12 methicillin-resistant S. aureus (MRSA) strains.

As expected from the initial MIC data, compound 20f had very potent antibacterial activity for the tetracycline-susceptible strains but performed poorly against the resistant organisms. The compound did have MIC$_{90}$s that were significantly lower than tetracycline. Despite having higher MICs in the primary screen relative to tigecycline, compound 24l performed similarly in the S. aureus, S. pneumoniae, and H. influenzae panels, exhibiting clinically relevant MIC$_{90}$s in each case. These organisms are particularly relevant, as they are responsible for infections in a large portion of community-acquired bacterial pneumonia cases.

The pharmacokinetic profiles of the two 8-azatetracyclines were determined in Sprague—Dawley rats (Table 7). Compound 20f had very similar AUC, clearance, and volume of distribution profiles to both tetracycline and tigecycline, although the half-life

Table 5. In Vitro Antibacterial Activity of 9-Alkyl-8-azatetracyclines

			MIC (μg/mL)							
			S. aureus			S. pneumoniae		E. coli		K. pneumoniae
compd	R_1	R_2	wild type[a]	tet(M)[b]	tet(K)[c]	wild type[d]	tet(M)[c]	wild type[e]	tet(A)[c]	wild type[f]
26a	$(CH_3)_2N$	n-Pr	1	2	1	1	2	16	>32	16
26b	Cl	n-Pr	1	4	4	1	4	>32	>32	32
26c	Cl	CH_3	2	8	16	0.5	8	8	>32	8
26d	H	CH_3	4	32	32	1	16	8	>32	8
26e	Cl	Ph	1	2	1	8	8	>32	>32	>32
26f	H	Ph	4	8	16	2	8	16	>32	32

[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.
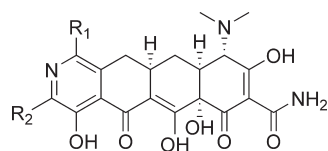
Table 6. MIC$_{90}$ Analysis

species	MIC (μg/mL)	20f	24l	tigecycline	tetracycline
S. aureus (n = 31)	MIC$_{50}$	≤0.016	0.125	0.063	0.125
	MIC$_{90}$	16	2	1	>64
	MIC range	≤0.016−32	0.063−4	≤0.016−1	0.063→64
S. pneumoniae (n = 19)	MIC$_{50}$	2	≤0.016	≤0.016	32
	MIC$_{90}$	4	0.063	≤0.016	>32
	MIC range	≤0.016−8	≤0.016−0.063	≤0.016-≤0.016	0.125→32
H. influenzae (n = 11)	MIC$_{50}$	0.5	1	0.5	4
	MIC$_{90}$	2	1	0.5	16
	MIC range	0.063−4	0.25−2	0.25−1	0.25−16
E. coli (n = 20)	MIC$_{50}$	0.25	1	0.25	2
	MIC$_{90}$	>32	4	0.5	>64
	MIC range	≤0.016−32	0.5−8	0.125−2	0.25→64

was somewhat shorter. Low oral bioavailability (%F) of 12.7% was observed but was comparable to tetracycline (12.1%). In humans, tetracycline is generally reported to have oral bioavailability of 50−70%.[24] We and others have found the oral bioavailability of several tetracycline derivatives to be significantly lower in rat than reported data in humans. Tigecycline, administered only intravenously in humans, had very low oral bioavailability. Compound 24l had a 4-fold higher AUC and 5-fold lower clearance than the other three compounds as well as a significantly lower volume of distribution. Unfortunately, oral bioavailability was comparable to tigecycline.

The compounds were also investigated in mouse S. aureus and E. coli septicemia models (Table 8). Both 8-azatetracyclines performed well in the S. aureus model following IV administration, with PD$_{50}$ values comparable to the two marketed control tetracyclines. 20f, the most potent of the four compounds, also provided the best protection, with 100% survival at the lowest dose at which it was administered (0.30 mg/kg). Despite having an 8-fold higher MIC, compound 24l provided equivalent

Table 7. Pharmacokinetic Properties of 20f and 24l in Sprague−Dawley Rats[a]

compd	AUC (h·ng/mL)	Cl$_{obs}$ (mL/h/kg)	Vz$_{obs}$ (mL/kg)	$t_{1/2}$ (hr)	%F
20f	1087	919	4177	3.1	12.7
24l	4872	202	1256	4.3	0.8
tetracycline	802	1230	3676	4.5	12.1
tigecycline	1052	929	6119	4.6	1.1

[a] Compounds were dosed at 1 mg/kg IV and 10 mg/kg PO with sterile water as vehicle.

protection relative to tigecycline, likely due to its higher AUC and lower clearance.[25] The E. coli model proved to be more challenging, requiring higher doses for protection. Compound 20f had a PD$_{50}$ of 4.3 mg/kg compared to 2.1 mg/kg for tigecycline. Compound 24l was 4-fold less potent than 20f and was comparable to tetracycline in this model.

Table 8. IV Efficacy in Mouse Septicemia Model[a]

compd	S. aureus			E. coli		
	MIC (μg/mL)	PD$_{50}$ (mg/kg)	95% C.I.	MIC (μg/mL)	PD$_{50}$ (mg/kg)	95% CI
20f	0.031	<0.30[b]		0.13	4.3	4.1−4.6
24l	0.5	0.36	0.36−0.56	0.5	17	4.1−30
tetracycline	0.25	0.35	0.34−0.37	1	17	7.3−27
tigecycline	0.063	0.35	0.24−0.47	0.13	2.1	1.8−2.4

[a] S. aureus ATCC 13709 (Smith) or E. coli ATCC 25922 was mixed with 5% mucin and inoculated by intraperitoneal injection at 2.1×10^6 cfu/mouse (S. aureus) or 2.0×10^7 cfu/mouse (E. coli). One hour postchallenge, mice received IV treatment (vehicle = sterile water) with the test article at concentrations ranging from 0.05−10 mg/kg (S. aureus) or 0.30−30 mg/kg (E. coli). The PD$_{50}$ in mg/kg was calculated as survival after 48 h. [b] Lowest dose was 0.30 mg/kg with 100% survival at all doses.

CONCLUSIONS

A novel, fully synthetic series of 8-azatetracycline analogues was prepared and optimized. Through modification of the 7- and 9-positions, compounds were found that overcome both *tet*(K) efflux and *tet*(M)-mediated ribosomal protection. The 7-chloro-9-alkylamino-8-azatetracycline **24l** exhibits clinically relevant MIC$_{90}$ profiles against both Gram-positive and Gram-negative organisms that include these resistance mechanisms. This compound also demonstrated in vivo efficacy in murine models of infection that is equivalent to currently marketed tetracycline antibacterial agents. The 7-dimethylamino-8-azatetracycline **20f** was efficacious when administered intravenously and had oral bioavailability in rat equivalent to tetracycline.

The current work demonstrates the ability of this chemistry platform to generate antibacterial compounds that are inaccessible through traditional semisynthesis, providing the medicinal chemist a broader array of tools through which essential antibacterial properties can be modulated. Importantly, this includes the ability to overcome ever evolving resistance mechanisms and to identify structure−activity relationships to deliver compounds with desired pharmacokinetic properties.

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 Autopurification 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$_2$O (A) and CH$_3$CN (B) containing 0.1% HCO$_2$H. The final 8-azatetracycline 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$_2$O (A) and CH$_3$CN (B). All final 8-azatetracycline 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$_3$OD at 3.31 ppm). High performance liquid chromatography−electrospray mass spectra (LC-MS) were obtained using a 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$_2$O (A) and CH$_3$CN (B) containing 0.1% HCO$_2$H 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.

3-(Benzyloxy)-5-chloroisonicotinic Acid (12). Sodium hydride (60% dispersion in mineral oil, 4.37 g, 109 mmol) was added portionwise to a solution of 3,5-dichloroisonicotinic acid (10.2 g, 53.3 mmol) in NMP (100 mL). After gas evolution ceased, benzyl alcohol (5.52 mL, 53.3 mmol) was added dropwise. After gas evolution ceased, the reaction mixture was heated to 80 °C. After 1 h, the reaction was complete and was allowed to cool to room temperature and stand overnight. The reaction mixture was diluted with water (300 mL) and was washed with Et$_2$O (2 × 100 mL, discarded). The aqueous layer was brought to pH ∼2 with conc HCl, causing a precipitate to form. The mixture was diluted with brine (100 mL) and was allowed to stand for 30 min. The solid was collected by filtration, was washed with water (3×), and was dried in a 45 °C vacuum oven overnight. This gave 9.36 g (67%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.30−14.10 (bs, 1 H), 8.52 (s, 1H), 8.34 (s, 1 H), 7.50−7.30 (m, 5 H), 5.33 (s, 2 H). MS (ESI) *m/z* 264.20 (M + H).

Phenyl 3-(Benzyloxy)-5-chloroisonicotinate (13). Oxalyl chloride (4.9 mL, 56 mmol) was added to a suspension of **12** (3.71 g, 14.1 mmol) in CH$_2$Cl$_2$ (50 mL) over ∼2 min. DMF was added dropwise in ∼20 μL portions every 5 min until complete solution was achieved. After stirring for an additional 30 min, the reaction mixture was concentrated under reduced pressure. The resulting solid was dissolved in CH$_2$Cl$_2$ (50 mL), and phenol (2.65 g, 28.1 mmol), DMAP (0.172 g, 1.41 mmol), and Et$_3$N (9.76 mL, 70.4 mmol) were added. After 1 h, the reaction mixture was diluted with CH$_2$Cl$_2$ (50 mL) and was washed with water (2 × 100 mL) and NaHCO$_3$ (saturated, aqueous, 100 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−25% EtOAc in hexanes gradient), yielding 4.20 g (88%) of the product as an off-white solid. R_f = 0.43 in 30% EtOAc/hexanes. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.48 (s, 1 H), 7.52−7.30 (m, 8 H), 7.15− 7.08 (m, 2 H), 5.44 (s, 2 H). MS (ESI) *m/z* 340.25 (M + H).

Phenyl 3-(Benzyloxy)-5-methylisonicotinate (14). Compound **13** (2.01 g, 5.92 mmol), methyl boronic acid (1.06 g, 17.7 mmol), dichlorobis(tricyclohexylphosphine)palladium(II) (102 mg, 0.296 mmol), and K$_3$PO$_4$ (3.76 g, 17.7 mmol) were heated to 100 °C in toluene (20 mL) and water (2 mL). After 16 h, the reaction mixture was allowed to cool to room temperature and was diluted with EtOAc (20 mL). This was washed

with water (20 mL) and brine (20 mL), dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−40% EtOAc in hexanes gradient), yielding 1.66 g (88%) of the product as a white solid. R_f = 0.18 in 30% EtOAc/hexanes. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (s, 1H), 8.26 (s, 1 H), 7.52−7.30 (m, 8 H), 7.20−7.10 (m, 2 H), 5.37 (s, 2 H), 2.38 (s, 3 H). MS (ESI) m/z 320.27 (M + H).

Phenyl 5-(Benzyloxy)-2-chloro-3-methylisonicotinate (15a). Compound 14 (9.66 g, 30.3 mmol) was heated to 80 °C in acetic acid (50 mL) and hydrogen peroxide (30% aqueous solution, 17 mL). After 6 h, LC/MS indicated that the starting material was consumed and the N-oxide present. Most of the acetic acid and water were removed under reduced pressure. The material was then diluted with EtOAc (200 mL), and the mixture was washed with NaHCO$_3$ (saturated aqueous solution, 3 × 50 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure, yielding 9.37 g (92%) of the crude N-oxide. The material was dissolved in toluene (60 mL), phosphorousoxychloride (7.69 g, 50.1 mmol) was added, and the reaction mixture was heated to 100 °C. After 2 h, LC/MS indicated that the reaction was complete. Upon cooling to room temperature, the reaction mixture was poured into a solution of K$_2$CO$_3$ (40 g) in water (200 mL). This mixture was extracted with EtOAc (3 × 150 mL), and the combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. ^{1}H NMR indicated a mixture of regioisomers (4.5:1). The material was redissolved in EtOAc (30 mL) with heating and was then allowed to cool to room temperature and stand overnight. A white crystalline solid was obtained (4.99 g, 51%). Another 1.92 g (19%) of the white crystalline solid was obtained from the mother liquor after standing in a refrigerator overnight. Both were the desired pure regioisomer 15a by ^{1}H NMR. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.05 (s, 1H), 7.43−7.30 (m, 7 H), 7.29−7.20 (m, 1 H), 7.07 (d, J = 8.2 Hz, 2 H), 5.21 (s, 2 H), 2.46 (s, 3 H). MS (ESI) m/z 398.22, 400.22 (M + H).

Phenyl 5-(Benzyloxy)-2-bromo-3-methylisonicotinate (15b). Compound 14 (25.9 g, 81.1 mmol) was heated to 80 °C in acetic acid (160 mL) and hydrogen peroxide (30% aqueous solution, 40 mL). After stirring overnight, LC/MS indicated that the starting material was consumed and the N-oxide present. The reaction mixture was concentrated under reduced pressure, was diluted with EtOAc, and was washed with NaHCO$_3$ (saturated aqueous solution, 3×). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure, yielding 25.4 g (93% crude) of the N-oxide as a tan solid. The material was suspended in toluene (200 mL) and was heated to 80 °C, at which point most of the solid had dissolved. Phosphorousoxybromide (25 g, 87 mmol) was added in one portion. After 30 min, LC/MS indicated the reaction was complete. Upon cooling to room temperature, the reaction mixture was poured into a solution of K$_2$CO$_3$ (83 g) in water (300 mL). This was stirred for 30 min at room temperature. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined extracts were dried over MgSO$_4$, filtered through Celite, and concentrated under reduced pressure. The material was recrystallized from EtOAc/hexanes (1:1), yielding 20.6 g (64%) of the product as an off-white solid. Single isomer by ^{1}H NMR. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.05 (s, 1H), 7.43−7.30 (m, 7 H), 7.29−7.20 (m, 1 H), 7.07 (d, J = 8.2 Hz, 2 H), 5.21 (s, 2 H), 2.46 (s, 3 H). MS (ESI) m/z 398.22, 400.22 (M + H).

Phenyl 5-(Benzyloxy)-2-(dimethylamino)-3-methylisonicotinate (15c). Compound 15b (1.06 g, 2.66 mmol), dimethylamine (2.0 M solution in THF, 4.0 mL, 8.0 mmol), K$_3$PO$_4$ (1.7 g, 8.0 mmol), tris(dibenzylideneacetone)dipalladium (120 mg, 0.13 mmol), and Xantphos (220 mg, 0.40 mmol) were heated to 100 °C in 1,4-dioxane (10 mL) in a sealed pressure vessel. After heating overnight, the reaction mixture was cooled to room temperature, was diluted with EtOAc (100 mL), and was washed with water (3 × 50 mL) and brine (50 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−15% EtOAc in hexanes gradient), yielding

388 mg (40%) of the title compound as a white solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.96 (s, 1H), 7.45−7.20 (m, 8 H), 7.08 (d, J = 8.2 Hz, 2 H), 5.16 (s, 2 H), 2.76 (s, 6 H), 2.37 (s, 3 H). MS (ESI) m/z 363.45 (M + H).

Phenyl 5-(Benzyloxy)-2-methoxy-3-methylisonicotinate (15d). Sodium methoxide (38 mg, 0.70 mmol) was added to a solution of compound 15b (118 mg, 0.351 mmol) in NMP (2 mL), and the reaction mixture was heated to 100 °C. After heating overnight, additional sodium methoxide (57 mg, 1.1 mmol) was added and heating was continued at 100 °C. After 1 h, the reaction mixture was heated to 120 °C. After an additional 1 h, the reaction mixture was cooled to room temperature, water (20 mL) was added, and the pH was adjusted to 2 with 6 N aqueous HCl. This was extracted with EtOAc (3 × 20 mL), and the combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude acid intermediate was dissolved in CH$_2$Cl$_2$ (10 mL), and oxalyl chloride (0.123 mL, 1.40 mmol) was added. After 1 h, the reaction mixture was concentrated under reduced pressure. The material was dissolved in CH$_2$Cl$_2$ (10 mL), and phenol (66 mg, 0.70 mmol), DMAP (4 mg, 0.04 mmol), and Et$_3$N (0.245 mL, 1.76 mmol) were added. After 1 h, the reaction mixture was diluted with EtOAc (50 mL) and was washed with water (2 × 30 mL) and brine (30 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 10 g column, 0−12% EtOAc in hexanes gradient), yielding 20.3 mg (17%) of clean product. An additional 29.1 mg of product contaminated with some residual phenol was also isolated. R_f = 0.39 in 20% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.75 (s, 1H), 7.45−7.24 (m, 9 H), 7.10 (d, J = 7.3 Hz, 2 H), 5.13 (s, 2 H), 3.91 (s, 3 H), 2.67 (s, 3 H). MS (ESI) m/z 336.1, 338.1 (M + H).

Phenyl 3-(Benzyloxy)-2-bromo-6-chloro-5-methylisonicotinate (16a). Compound 15a (4.99 g, 14.1 mmol) was dissolved in CH$_2$Cl$_2$ (30 mL), and 3-chloroperbenzoic acid (77%, 6.33 g, 28.2 mmol) was added in one portion. After 6 h, an additional portion of 3-chloroperbenzoic acid (3.15 g, 14.1 mmol) was added, and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with CH$_2$Cl$_2$ (200 mL) and was washed with Na$_2$CO$_3$ (saturated aqueous solution, 100 mL) and brine. The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 70 g column, 5−50% EtOAc in hexanes gradient), yielding 3.90 g (75%) of the pure N-oxide as an off-white solid (R_f = 0.33 in 66% EtOAc/hexanes). The N-oxide was dissolved in toluene (30 mL), phosphorousoxybromide (6.36 g, 22.1 mmol) was added, and the reaction mixture was heated to 80 °C. After 1 h, the reaction mixture was allowed to cool to room temperature and was poured into a solution of K$_2$CO$_3$ (10 g) in water (50 mL). This mixture was extracted with EtOAc (3 × 100 mL), and the combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−10% EtOAc in hexanes gradient), yielding 2.06 g (45%) of the desired product 16a as an oil which slowly solidified on standing overnight. In addition, 1.01 g (20%) of compound 15a was recovered. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.05 (s, 1H), 7.43−7.30 (m, 7 H), 7.29−7.20 (m, 1 H), 7.07 (d, J = 8.2 Hz, 2 H), 5.21 (s, 2 H), 2.46 (s, 3 H). MS (ESI) m/z 398.22, 400.22 (M + H).

Phenyl 3-(Benzyloxy)-2,6-dibromo-5-methylisonicotinate (16b). Compound 15b (8.54 g, 21.4 mmol) was heated to 80 °C in acetic acid (120 mL) and hydrogen peroxide (30% aqueous solution, 30 mL). After stirring overnight, LC/MS indicated that the starting material was mostly consumed and the N-oxide present. The reaction mixture was cooled to room temperature and was diluted with brine (150 mL). The mixture was cooled in a −20 °C freezer for 30 min. The resulting precipitate was collected by filtration and was washed with water/brine (1:1, 2×). The solid was dissolved in CH$_2$Cl$_2$ and was dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. This gave 7.83 g (88% crude) of the

N-oxide as a tan solid. The material was suspended in toluene (200 mL) and was heated to 100 °C, at which point most of the solid had dissolved. A solution of phosphorousoxybromide (10.8 g, 37.8 mmol) in toluene (10 mL) was added rapidly. After 45 min, the reaction mixture was allowed to cool to room temperature and was poured into a solution of K_2CO_3 (40 g) in water (150 mL). After stirring for 30 min at room temperature, the layers were separated and the aqueous layer was extracted with EtOAc. The combined extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−8% EtOAc in hexanes gradient to isolate the product then 15% EtOAc in hexanes to recover **15b**), yielding 5.02 g (49%) of the product as an off-white solid. Compound **15b** was also recovered. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49−7.32 (m, 8 H), 7.10 (d, J = 7.3 Hz, 2 H), 5.15 (s, 2 H), 2.41 (s, 3 H). MS (ESI) m/z 476.18, 478.16, 480.16 (M + H).

Phenyl 3-(Benzyloxy)-2-bromo-6-[(*t*-butoxycarbonyl)-amino]-5-methylisonicotinate. Compound **16b** (2.15 g, 4.50 mmol), *t*-butylcarbamate (633 mg, 5.40 mmol), Cs_2CO_3 (2.93 g, 9.00 mmol), tris-(dibenzylideneacetone)dipalladium (206 mg, 0.225 mmol), and Xantphos (380 mg, 0.673 mmol) were weighed into a flask. This was evacuated and backflushed with nitrogen (3×), and 1,4-dioxane (15 mL) was added. The reaction mixture was heated to 80 °C. After 3 h, the reaction mixture was cooled to room temperature, was diluted with EtOAc (100 mL), and was washed with water (2 × 50 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−12% EtOAc in hexanes gradient), yielding 1.43 g (62%) of a 4:1 mixture of the two regioisomeric compounds. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.51−7.46 (m, 2 H), 7.42−7.32 (m, 7 H), 7.30−7.24 (m, 1 H), 7.12 (d, J = 8.2 Hz, 0.5 H), 7.03 (d, J = 8.2 Hz, 2 H), 6.88 (s, 0.25 H), 6.71 (s, 1 H), 5.12 (s, 2 H), 5.02 (s, 0.5 H), 2.43 (s, 0.75 H), 2.34 (s, 3 H), 1.50 (s, 9 H), 1.47 (s, 2.25 H). MS (ESI) m/z 535.10, 537.10 (M + Na).

Phenyl 2-Amino-5-(benzyloxy)-6-bromo-3-methylisonicotinate (16c). The above regioisomeric mixture (1.43 g, 2.78 mmol) was stirred in 4 M HCl in 1,4-dioxane (20 mL) and 1,4-dioxane (5 mL) overnight. The reaction mixture was diluted with EtOAc (100 mL) and was washed with NaHCO$_3$ (saturated aqueous solution, 2 × 100 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 20 g column, 0−30% EtOAc in hexanes gradient), yielding 805 mg (70%) of the major regioisomer **16c** and 270 mg (24%) of the minor regioisomer. Data for **16c**: R_f = 0.39 in 40% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.50−7.42 (m, 2 H), 7.40−7.30 (m, 5 H), 7.30−7.24 (m, 1 H), 7.04 (d, J = 8.2 Hz, 2 H), 5.06 (s, 2 H), 4.56 (s, 2 H), 2.16 (s, 3 H). MS (ESI) m/z 413.11, 415.01 (M + H). Data for phenyl 2-amino-3-(benzyloxy)-6-bromo-5-methylisonicotinate: R_f = 0.72 in 40% EtOAc/hexanes; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42−7.34 (m, 8 H), 7.13−7.10 (m, 2 H), 5.01 (s, 2 H), 4.64 (s, 2 H), 2.35 (s, 3 H); MS (ESI) m/z 413.09, 415.09 (M + H).

Phenyl 3-(Benzyloxy)-2-bromo-6-fluoro-5-methylisonicotinate (16d). HF-Pyridine solution (~70% HF, 2 mL) was added to a 0 °C solution of compound **16c** (884 mg, 2.14 mmol) in pyridine (1 mL). Sodium nitrite (177 mg, 2.57 mmol) was added (bubbling observed), and the reaction mixture was allowed to slowly warm to room temperature. After 3 days, the reaction mixture was poured into Na$_2$CO$_3$ (saturated aqueous solution, 30 mL) and was extracted with EtOAc (3 × 30 mL). The combined extracts were washed with HCl (1 N aqueous solution, 2 × 50 mL) and were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 20 g column, 0−6% EtOAc in hexanes gradient), yielding 687 mg (77%) of the product as a colorless oil that slowly solidified on standing. R_f = 0.39 in 10% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.50−7.42 (m, 2 H), 7.40−7.30 (m, 5 H), 7.30−7.24 (m, 1 H), 7.08−7.02 (m, 2 H), 5.14 (s, 2 H), 2.33 (s, 3 H). MS (ESI) m/z 416.17, 418.15 (M + H).

Phenyl 3-(Benzyloxy)-2-bromo-6-(dimethylamino)-5-methylisonicotinate (16e) and Phenyl 3-(Benzyloxy)-6-bromo-2-(dimethylamino)-5-methylisonicotinate (16f). Compound **16b** (1.51 g, 3.16 mmol), Cs_2CO_3 (3.1 g, 9.5 mmol), tris(dibenzylideneacetone)dipalladium (145 mg, 0.158 mmol), and Xantphos (267 mg, 0.474 mmol) were weighed into a vial equipped with a septum. This was evacuated and flushed with nitrogen (3×), and 1,4-dioxane (7 mL) and dimethylamine (2.0 M solution in THF, 4.75 mL, 9.50 mmol) were added. The reaction mixture was heated to 60 °C and was stirred for 4 h. The reaction mixture was allowed to cool to room temperature, was filtered through Celite, and was concentrated under reduced pressure. The material was purified by column chromatography (Biotage 50 g column, 0−10% EtOAc in hexanes gradient), yielding 868 mg (58%) of the major regioisomer **16e** and 354 mg (24%) of the minor regioisomer **16f**. Data for **16e**: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.50−7.46 (m, 2 H), 7.41−7.30 (m, 5 H), 7.30−7.24 (m, 1 H), 7.08−7.02 (m, 2 H), 5.08 (s, 2 H), 2.85 (s, 6 H), 2.32 (s, 3 H); MS (ESI) m/z 441.19, 443.18 (M + H); R_f = 0.29 in 10% EtOAc/hexanes. The regiochemistry was confirmed by an NOE between the methyl protons and the *N*,*N*-dimethylamino protons (0.61%). Data for **16f**: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.41−7.31 (m, 7 H), 7.30−7.20 (m, 1 H), 7.01 (d, J = 7.3 Hz, 2 H), 4.94 (s, 2 H), 3.04 (s, 6 H), 2.33 (s, 3 H); MS (ESI) m/z 441.19, 443.18 (M + H); R_f = 0.40 in 10% EtOAc/hexanes. The regiochemistry was confirmed by NOE between the benzylic protons of the *O*-benzyl group and the *N*,*N*-dimethylamino protons (0.44%).

Phenyl 5-Benzyloxy-6-[(*t*-butoxycarbonyl)amino]-2-fluoro-3-methylisonicotinate (17a). Compound **16d** (538 mg, 1.29 mmol), *t*-butylcarbamate (302 mg, 2.58 mmol), Cs_2CO_3 (840 mg, 2.58 mmol), tris(dibenzylideneacetone)dipalladium (59 mg, 0.065 mmol), and Xantphos (109 mg, 0.190 mmol) were weighed into a flask. This was evacuated and backflushed with nitrogen (3×), and 1,4-dioxane (3 mL) was added. The reaction mixture was heated to 80 °C. After 4 h, the reaction mixture was cooled to room temperature, was diluted with EtOAc (50 mL), and was washed with water (2 × 25 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 20 g column, 0−12% EtOAc in hexanes gradient), yielding 451 mg (77%) of the product. R_f = 0.35 in 20% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42−7.24 (m, 8 H), 7.04−6.99 (m, 2 H), 4.99 (s, 2 H), 2.38 (s, 3 H), 1.40 (s, 18 H). MS (ESI) m/z 453.14 (M + H).

Phenyl 3-Benzyloxy-2-[bis(*t*-butoxycarbonyl)amino]-6-fluoro-5-methylisonicotinate (18a). Compound **17a** (451 mg, 1.00 mmol) was treated with di-*t*-butyldicarbonate (1.09 g, 5.00 mmol) and DMAP (24 mg, 0.20 mmol) in DMF (15 mL). After 30 min, the reaction mixture was diluted with EtOAc (100 mL) and was washed with water (3 × 50 mL) and brine (50 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 10 g column, 0−8% EtOAc in hexanes gradient), yielding 481 mg (87%) of the title compound. R_f = 0.16 in 10% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42−7.24 (m, 8 H), 7.04−6.99 (m, 2 H), 4.99 (s, 2 H), 2.38 (s, 3 H), 1.40 (s, 18 H). MS (ESI) m/z 575.25 (M + Na).

Phenyl 5-Benzyloxy-6-[(*t*-butoxycarbonyl)amino]-2-(dimethylamino)-3-methylisonicotinate (17b). Compound **16e** (467 mg, 1.06 mmol), *t*-butylcarbamate (372 mg, 3.17 mmol), Cs_2CO_3 (1.04 g, 3.17 mmol), tris(dibenzylideneacetone)dipalladium (48 mg, 0.053 mmol), and Xantphos (89.3 mg, 0.159 mmol) were weighed into a vial. This was evacuated and backflushed with nitrogen (3×), and 1,4-dioxane (3 mL) was added. The reaction mixture was heated to 100 °C. After 4 h, the reaction mixture was cooled to room temperature and was filtered through Celite. The filtrate was concentrated under reduced pressure, and the material was purified by column chromatography (Biotage 20 g column, 0−16% EtOAc in hexanes gradient), yielding 493 mg (98%) of the product containing

~20% of an unidentified impurity. R_f = 0.45 in 30% EtOAc/hexanes. ¹H NMR (400 MHz, CDCl₃) δ 7.43−7.32 (m, 8 H), 7.30−7.24 (m, 1 H), 7.15−7.10 (m, 2 H), 6.80 (s, 1 H), 4.94 (s, 2 H), 2.85 (s, 6 H), 2.31 (s, 3 H), 1.48 (s, 9 H). MS (ESI) *m/z* 478.27 (M + H).

Phenyl 3-Benzyloxy-2-[bis(*t*-butoxycarbonyl)amino]-6-(dimethylamino)-5-methylisonicotinate (18b). Compound **17b** (490 mg, 1.03 mmol) was treated with di-*t*-butyldicarbonate (672 mg, 3.08 mmol) and DMAP (12.5 mg, 0.103 mmol) in DMF (5 mL). After stirring overnight, the reaction mixture was diluted with EtOAc (25 mL) and was washed with water (3 × 20 mL) and brine (20 mL). The organics were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 10 g column, 0−10% EtOAc in hexanes gradient), yielding 424 mg (72%) of the product. R_f = 0.22 in 15% EtOAc/hexanes. ¹H NMR (400 MHz, CDCl₃) δ 7.42−7.24 (m, 8 H), 7.06−7.00 (m, 2 H), 4.92 (s, 2 H), 2.79 (s, 6 H), 2.36 (s, 3 H), 1.39 (s, 18 H). MS (ESI) *m/z* 578.33 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-13-(Dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]-isoquinoline-4,6(4a*H*,11*H*)-dione (19a). LDA was prepared at −78 °C from *n*-butyllithium (1.6 M solution in hexane, 0.495 mL, 0.792 mmol) and diisopropylamine (0.112 mL, 0.792 mmol) in THF (5 mL). TMEDA (0.318 mL, 2.11 mmol) was added, followed by dropwise addition of a solution of compound **14** (169 mg, 0.529 mmol) in THF (2 mL). This resulted in a deep-red colored solution. After 5 min, a solution of **8** (128 mg, 0.264 mmol) in THF (2 mL) was added. After complete addition, the reaction mixture was allowed to warm to −20 °C over 1 h. The reaction was quenched by the addition of ammonium chloride (saturated, aqueous solution, 20 mL) and was extracted with EtOAc (2 × 20 mL). The combined extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient), yielding 65.5 mg (35%) of the title compound as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.77 (s, 1 H), 8.36 (s, 1 H), 8.16 (s, 1 H), 7.55−7.24 (m, 10 H), 5.40−5.25 (m, 4 H), 3.93 (d, J = 11.0 Hz, 1 H), 3.16−3.04 (m, 1 H), 2.98−2.90 (m, 1 H), 2.76−2.64 (m, 1 H), 2.60−2.40 (m, 8 H), 2.12 (d, J = 14 Hz, 1 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 708.72 (M + H).

The following compounds were synthesized by methods similar to **19a**.

(4a*S*,11a*R*,12a*S*,13*S*)-10-Chloro-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19b). Prepared from **15a** in 38% yield, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.6 (s, 1 H), 8.12 (s, 1 H), 7.56−7.49 (m, 4 H), 7.42−7.32 (m, 6 H), 5.37 (s, 2 H), 5.07 (s, 2 H), 3.91 (d, J = 11.0 Hz, 1 H), 3.13−3.02 (m, 1 H), 2.97 (dd, J = 15.6 4.9 Hz, 1 H), 2.65−2.45 (m, 3 H), 2.50 (s, 6 H), 2.14 (d, J = 15.6 Hz, 1 H), 0.82 (s, 9 H), 0.27 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 742.56 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-10-Bromo-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19c). Prepared from **15b** in 71% yield, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.55 (s, 1 H), 8.12 (s, 1 H), 7.55−7.24 (m, 10 H), 5.40−5.22 (m, 4 H), 3.90 (d, J = 11.0 Hz, 1 H), 3.25 (dd, J = 16.5 Hz, J = 4.88 Hz, 1 H), 3.12−3.02 (m, 1 H), 2.62−2.42 (m, 9 H), 2.14 (d, J = 14.0 Hz, 1 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 786.63, 788.63 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-10,13-Bis(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19f). Prepared from **15c** in 50% yield, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.65 (s, 1 H), 8.01 (s, 1 H), 7.52−7.22 (m, 10 H), 5.36 (s, 2 H),

5.17 (q, J = 11.0 Hz, 2 H), 4.02 (d, J = 11.0 Hz, 1 H), 3.05 (dd, J = 17.2 Hz, J = 11.6 Hz, 1 H), 2.98−2.86 (m, 1 H), 2.73 (s, 6 H), 2.62−2.38 (m, 9 H), 2.12 (d, J = 13.4 Hz, 1 H), 0.81 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 751.79 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-13-(Dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-10-methoxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19g). Prepared from **15d** in 27% yield, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.70 (s, 1 H), 7.83 (s, 1 H), 7.55−7.24 (m, 10 H), 5.36 (s, 2 H), 5.17 (q, J = 13.4 Hz, 2 H), 3.98 (d, J = 11.0 Hz, 1 H), 3.72 (s, 3 H), 3.18 (d, J = 16.5 Hz, 1 H), 3.04 −2.96 (m, 1 H), 2.60−2.30 (m, 9 H), 2.15 (d, J = 14 Hz, 1 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 738.66 (M + H).

2-[(4a*S*,11a*R*,12a*S*,13*S*)-13-(Dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-10-fluoro-4,4a,6,11,11a,12,12a,13-octahydro-5-hydroxy-4,6-dioxo-3,7-bis(phenylmethoxy)isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinolin-8-yl]-imidodicarbonic Acid, 1,3-Bis(1,1-dimethylethyl) Ester (19k). Prepared from **18a** in 56% yield, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.59 (s, 1 H), 7.56−7.24 (m, 10 H), 5.36 (s, 2 H), 4.92 (q, J = 67.8 Hz, J = 9.16 Hz, 2 H), 3.91 (d, J = 11.0 Hz, 1 H), 3.20−3.02 (m, 2 H), 2.62−2.45 (m, 9 H), 2.19−2.12 (m, 1 H), 1.37, (s, 18 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 941.59 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-8-Bromo-10,13-bis(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19m). Prepared from **16e** in 54% yield, yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 15.65 (s, 1 H), 7.52−7.22 (m, 10 H), 5.36 (s, 2 H), 5.17 (q, J = 11.0 Hz, 2 H), 4.01 (d, J = 11.0 Hz, 1 H), 3.05 (dd, J = 17.2 Hz, J = 11.6 Hz, 1 H), 2.98−2.86 (m, 1 H), 2.73 (s, 6 H), 2.62−2.38 (m, 9 H), 2.12 (d, J = 13.4 Hz, 1 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.13 (s, 3 H). MS (ESI) *m/z* 829.49, 831.48 (M + H).

(4a*S*,11a*R*,12a*S*,13*S*)-8-Bromo-10-chloro-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19h). Compound **16a** (793 mg, 1.84 mmol) and compound **8** (885 mg, 1.84 mmol) were dissolved in THF (16 mL), and the solution was cooled to −78 °C. LHMDS (1.0 M solution in THF, 5.5 mL, 5.5 mmol) was added slowly via syringe. After 10 min, the reaction mixture was allowed to slowly warm to 0 °C over 1 h. A phosphate buffer solution (pH 7.0, 20 mL) was added, followed by the addition of ammonium chloride (saturated, aqueous solution, 50 mL). The resulting mixture was extracted with CH₂Cl₂ (3 × 50 mL), and the combined extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The resulting brown solid was washed with cold methanol (3 × 5 mL) to afford 1.11 g (74%) of the title compound as a yellow−brown powder. The organics were concentrated under reduced pressure and purified by column chromatography (Biotage 20 g column, 5−20% EtOAc in hexanes gradient), yielding an additional 150 mg (10%) of the product. ¹H NMR (400 MHz, CDCl₃) δ 15.45 (br, 1 H), 7.54−7.48 (m, 4 H), 7.40−7.30 (m, 6 H), 5.36 (s, 2 H), 5.03 (abq, J = 10.4 Hz, 2 H), 3.87 (d, J = 11.0 Hz, 1 H), 3.27−3.23 (m, 1 H), 3.10−3.00 (m, 1 H), 2.65−2.57 (m, 1 H), 2.57−2.43 (m, 8 H), 2.16 (d, J = 11.0 Hz, 1 H), 0.81 (s, 9 H), 0.26 (s, 3 H), 0.12 (s, 3 H). MS (ESI) *m/z* 820.37, 822.37 (M + H).

The following compounds were prepared by methods similar to **19h**.

(4a*S*,11a*R*,12a*S*,13*S*)-8-Bromo-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-10-fluoro-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4a*H*,11*H*)-dione (19i). Prepared from **16d** in 45% yield, yellow solid. ¹H NMR

(400 MHz, CDCl$_3$) δ 15.55 (s, 1 H), 7.56−7.26 (m, 10 H), 5.36 (s, 2 H), 5.02 (s, 2 H), 3.88 (d, J = 10.4 Hz, 1 H), 3.18−3.04 (m, 2 H), 2.62−2.58 (m, 1 H), 2.58−2.40 (m, 8 H), 2.17 (d, J = 14.6 Hz, 1 H), 0.81 (s, 9 H), 0.25 (s, 3 H), 0.12 (s, 3 H). MS (ESI) m/z 804.34, 806.34 (M + H).

2-[(4aS,11aR,12aS,13S)-10,13-Bis(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4,4a,6,11,11a,12,12a,13-octahydro-5-hydroxy-4,6-dioxo-3,7-bis(phenylmethoxy)isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinolin-8-yl]-imidodicarbonic Acid, 1,3-Bis(1,1-dimethylethyl) Ester (19l). Prepared from **18b** in 53% yield, yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.50 (s, 1 H), 7.52−7.24 (m, 10 H), 5.36 (s, 2 H), 4.85 (q, J = 86.1 Hz, J = 9.76 Hz, 2 H), 4.02 (d, J = 10.4 Hz, 1 H), 3.08−3.00 (m, 1 H), 3.00−2.82 (m, 1 H), 2.77 (s, 6 H), 2.62−2.38 (m, 9 H), 2.20−2.12 (m, 1 H), 1.35, (br s, 18 H), 0.79 (s, 9 H), 0.26 (s, 3 H), 0.12 (s, 3 H). MS (ESI) m/z 966.59 (M + H).

(4aS,11aR,12aS,13S)-13-(Dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-10-methyl-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinoline-4,6(4aH,11H)-dione (19d). A solution of compound **19c** (52 mg, 0.067 mmol), methylboronic acid (40 mg, 0.67 mmol), dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane adduct (3 mg, 0.003 mmol), and K$_3$PO$_4$ (142 mg, 0.667 mmol) in toluene (1 mL), 1,4-dioxane (1 mL), and water (0.2 mL) was heated to 70 °C. After 2 h, the reaction mixture was heated to 100 °C. After an additional 2 h, the reaction mixture was diluted with EtOAc (20 mL) and was washed with water (15 mL) and brine (15 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient), yielding 18.3 mg (38%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.71 (s, 1 H), 8.24 (s, 1 H), 7.55−7.24 (m, 10 H), 5.36 (s, 2 H), 5.30−5.20 (m, 2 H), 3.95 (d, J = 10.4 Hz, 1 H), 3.10−2.92 (m, 2 H), 2.62−2.42 (m, 12 H), 2.12 (d, J = 14.0 Hz, 1 H), 0.82 (s, 9 H), 0.26 (s, 3 H), 0.14 (s, 3 H). MS (ESI) m/z 722.72 (M + H).

(4aS,11aR,12aS,13S)-13-(Dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-10-phenyl-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinoline-4,6(4aH,11H)-dione (19e). A solution of compound **19c** (31 mg, 0.040 mmol), phenylboronic acid (24.5 mg, 0.201 mmol), dichloro[1,1′-bis(diphenylphosphino)-ferrocene]palladium(II) dichloromethane adduct (1.6 mg, 0.002 mmol), and sodium carbonate (21.3 mg, 0.201 mmol) in toluene (1 mL), 1,4-dioxane (1 mL), and water (0.2 mL) was heated to 100 °C via microwave reactor for 10 min. The reaction mixture was diluted with EtOAc (10 mL) and was washed with water (5 mL) and brine (5 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient), yielding 16.9 mg (54%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.66 (s, 1 H), 8.48 (s, 1 H), 7.52−7.24 (m, 15 H), 5.42−5.30 (m, 4 H), 3.97 (d, J = 10.4 Hz, 1 H), 3.00−2.86 (m, 2 H), 2.78−2.62 (m, 1 H), 2.58−2.30 (m, 8 H), 2.00 (d, J = 14.0 Hz, 1 H), 0.80 (s, 9 H), 0.25 (s, 3 H), 0.14 (s, 3 H). MS (ESI) m/z 784.75 (M + H).

N-[(4aS,11aR,12aS,13S)-10-Chloro-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4,4a,6,11,11a,12,12a,13-octahydro-5-hydroxy-4,6-dioxo-3,7-bis(phenylmethoxy)isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinolin-8-yl]-carbamic Acid, 1,1-Dimethylethyl Ester (19j). Compound **19h** (100 mg, 0.122 mmol), t-butylcarbamate (42.9 mg, 0.366 mmol), K$_3$PO$_4$ (77.7 mg, 0.366 mmol), tris(dibenzylideneacetone)dipalladium (5.6 mg, 0.006 mmol), and Xantphos (10.3 mg, 0.018 mmol) were weighed into a vial equipped with a septum. The vial was evacuated and flushed with nitrogen (3×), and 1,4-dioxane (0.5 mL) was added. The reaction mixture was heated to 100 °C and stirred for 2 h. The reaction mixture was allowed

to cool to room temperature, was filtered through Celite, and was concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 80−100% B gradient), yielding 41.7 mg (40%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.65−15.45 (br s, 1 H), 7.56−7.50 (m, 2 H), 7.43−7.34 (m, 7 H), 7.29−7.20 (m, 1 H), 5.38 (s, 2 H), 4.94 (dd, J = 29.3 Hz, J = 11.0 Hz, 2 H), 3.90 (d, J = 11.0 Hz, 1 H), 3.27 (dd, J = 16.4 Hz, J = 11.6 Hz, 1 H), 3.12−3.03 (m, 1 H), 2.68−2.60 (m, 1 H), 2.60−2.45 (m, 8 H), 2.18 (d, J = 14.6 Hz, 1 H), 1.47 (s, 9 H), 0.85 (s, 9 H), 0.29 (s, 3 H), 0.15 (s, 3 H). MS (ESI) m/z 857.67 (M + H).

6-Demethyl-6-deoxy-8-azatetracycline Dihydrochloride (20a). Aqueous HF (48%, 0.4 mL) was added to a solution of **19a** (65.5 mg, 0.093 mmol) in CH$_3$CN (0.6 mL) in a plastic vial. After 18 h, the reaction mixture was poured into a solution of K$_2$HPO$_4$ (4.8 g) in water (20 mL). The mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was dissolved in MeOH (1 mL) and 1,4-dioxane (1 mL), and palladium on carbon (Degussa, 10 wt %, ∼5 mg) was added. An atmosphere of hydrogen was introduced, and the reaction mixture was stirred for 2 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx column, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 18.4 mg (41%, 2 steps) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 8.55 (s, 1 H), 8.36 (s, 1 H), 4.19 (s, 1 H), 3.26−2.98 (m, 9 H), 2.71 (t, J = 14.2 Hz, 1 H), 2.39−2.32 (m, 1 H), 1.75−1.63 (m, 1 H). MS (ESI) m/z 416.44 (M + H).

The following compounds were synthesized by similar methods to **20a**.

7-Chloro-6-demethyl-6-deoxy-8-azatetracycline Hydrochloride (20b). Prepared from **19b** in 24% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.08 (d, J = 6.0 Hz, 1 H), 4.09 (s, 1 H), 3.08−2.92 (m, 9 H), 2.30−2.15 (m, 2 H), 1.70 −1.58 (m, 1 H). MS (ESI) m/z 450.18 (M + H).

6-Demethyl-6-deoxy-7-methyl-8-azatetracycline Dihydrochloride (20d). Prepared from **19d** in 34% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 8.35 (s, 1 H), 4.19 (s, 1 H), 3.25−2.95 (m, 9 H), 2.80−2.25 (m, 5 H), 1.80−1.63 (m, 1 H). MS (ESI) m/z 430.46 (M + H).

6-Demethyl-6-deoxy-7-phenyl-8-azatetracycline Dihydrochloride (20e). Prepared from **19e** in 24% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 8.53 (s, 1 H), 7.62 (s, 5 H), 4.18 (s, 1 H), 3.14−2.82 (m, 9 H), 2.81−2.66 (m, 1 H), 2.24−2.14 (m, 1 H), 1.66−1.52 (m, 1 H). MS (ESI) m/z 492.48 (M + H).

6-Demethyl-6-deoxy-7-(dimethylamino)-8-azatetracycline Dihydrochloride (20f). Prepared from **19f** in 96% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 8.22 (s, 1 H), 4.19 (s, 1 H), 3.38−3.30 (m, 2 H), 3.24 (s, 6 H), 3.12−2.95 (m, 7 H), 2.60 (t, J = 14.2 Hz, 1 H), 2.42−2.34 (m, 1 H), 1.75−1.63 (m, 1 H). MS (ESI) m/z 459.50 (M + H).

6-Demethyl-6-deoxy-7-methoxy-8-azatetracycline Dihydrochloride (20g). Prepared from **19g** in 51% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 7.80 (s, 1 H), 4.15 (s, 1 H), 3.97 (s, 3 H), 3.40−2.98 (m, 9 H), 2.32−2.20 (m, 2 H), 1.72−1.56 (m, 1 H). MS (ESI) m/z 446.39 (M + H).

6-Demethyl-6-deoxy-7-fluoro-8-azatetracycline Hydrochloride (20i). Prepared from **19i** in 68% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 7.81 (s, 1 H), 4.17 (s, 1 H), 3.26−2.96 (m, 9 H), 2.42−2.25 (m, 2 H), 1.70−1.58 (m, 1 H). MS (ESI) m/z 434.27 (M + H).

9-Amino-7-chloro-6-demethyl-6-deoxy-8-azatetracycline (20j). Prepared from **19j** in 68% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.19 (s, 1 H), 3.20−2.98 (m,

8 H), 2.35−2.22 (m, 2 H), 1.68−1.56 (m, 1 H). MS (ESI) m/z 465.32 (M + H).

9-Amino-6-demethyl-6-deoxy-7-fluoro-8-azatetracycline Dihydrochloride (20k). Prepared from **19k** in 49% yield, 2 steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.20 (s, 1 H), 3.40−2.90 (m, 9 H), 2.42−2.22 (m, 2 H), 1.70−1.54 (m, 1 H). MS (ESI) m/z 449.27 (M + H).

N-[(4aS,11aR,12aS,13S)-10-Chloro-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4,4a,6,11,11a,12,12a,13-octahydro-5-hydroxy-4,6-dioxo-3,7-bis(phenylmethoxy)isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinolin-8-yl]-2-[(1,1-dimethylethyl)amino]-acetamide. Compound **19j** (68.3 mg, 0.080 mmol) was stirred in 4 M HCl in 1,4-dioxane for 2 h. The reaction mixture was concentrated under reduced pressure. The material was dissolved in THF (2 mL), and bromoacetylchloride (12.5 mg, 0.080 mmol) was added. After stirring overnight, an additional portion of bromoacetylchloride (0.020 mL) was added. After 4 h, the reaction mixture was concentrated under reduced pressure. MS (ESI) m/z 879.42 (M + H). t-Butylamine (0.063 mL, 0.60 mmol) was added to a solution of the intermediate (35 mg, 0.040 mmol) in THF (1 mL), and the reaction mixture was heated to 50 °C. After stirring overnight, the reaction mixture was concentrated under reduced pressure and was purified by preparative HPLC (Sunfire Prep C18 column, 50−100% B gradient). This gave 16.8 mg (49%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.20 (br s, 1 H), 7.52−7.46 (m, 2 H), 7.43−7.30 (m, 8 H), 5.36 (s, 2 H), 4.95 (dd, J = 40.6 Hz, J = 11.0 Hz, 2 H), 3.88 (d, J = 11.0 Hz, 1 H), 3.32−3.24 (m, 1 H), 3.12−3.03 (m, 1 H), 2.66−2.59 (m, 1 H), 2.58−2.42 (m, 8 H), 2.18 (d, J = 14.6 Hz, 1 H), 1.30−1.10 (br s, 9 H), 0.82 (s, 9 H), 0.27 (s, 3 H), 0.12 (s, 3 H). MS (ESI) m/z 870.60 (M + H).

9-[2-(tert-Butylamino)acetamido]-7-chloro-6-demethyl-6-deoxy-8-azatetracycline Dihydrochloride (21a). Aqueous HF (48%, 0.4 mL) was added to a solution of the preceding intermediate (16.8 mg, 0.019 mmol) in CH$_3$CN (0.6 mL) in a plastic vial. After 18 h, the reaction mixture was poured into a solution of K$_2$HPO$_4$ (7.8 g) in water (15 mL). The mixture was extracted with EtOAc (3×). Sodium chloride (10 g) was added to the aqueous layer, and this was extracted with EtOAc (2×). The combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was dissolved in MeOH (1 mL) and 1,4-dioxane (1 mL), and palladium on carbon (Degussa, 10 wt %, ~5 mg) was added. An atmosphere of hydrogen was introduced, and the reaction mixture was stirred for 1 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. 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 1.2 mg (10%, 2 steps) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.30−4.20 (s, 2 H), 4.19 (s, 1 H), 3.25−2.96 (m, 9 H), 2.48−2.28 (m, 2 H), 1.72−1.58 (m, 1 H), 1.43 (s, 9 H). MS (ESI) m/z 578.48 (M + H).

The following compounds were prepared according to methods similar to **21a**:

9-[2-(tert-Butylamino)acetamido]-6-demethyl-6-deoxy-7-fluoro-8-azatetracycline Dihydrochloride (21b). Prepared from **19k** in 68% yield for the acylation/amine displacement and 58% yield for the deprotections, yellow solid. ^{1}H NMR (400 MHz, CD3OD with 1 drop DCl) δ 4.24−4.65 (m, 3 H), 3.26−2.97 (m, 9 H), 2.38−2.28 (m, 2 H), 1.69−1.56 (m, 1 H), 1.44 (s, 9 H). MS (ESI) m/z 562.37 (M + H).

9-[2-(tert-Butylamino)acetamido]-6-demethyl-6-deoxy-7-(dimethylamino)-8-azatetracycline Trihydrochloride (21c). Prepared from **19l** in 43% yield for the acylation/amine displacement and 55% yield for the deprotections, yellow solid. ^{1}H NMR (400 MHz,

CD3OD with 1 drop DCl) δ 4.30−4.40 (m, 3 H), 3.40−3.25 (m, 8 H), 3.12−3.04 (m, 4 H), 2.99 (s, 3 H), 2.62−2.52 (m, 1 H), 2.41−2.35 (m, 1 H), 1.73−1.60 (m, 1 H), 1.45 (s, 9 H). MS (ESI) m/z 587.43 (M + H).

(4aS,11aR,12aS,13S)-10-Chloro-13-(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-8-(ethylamino)-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinoline-4,6(4aH,11H)-dione. Compound **19h** (50 mg, 0.061 mmol), K$_3$PO$_4$ (39 mg, 0.18 mmol), tris(dibenzylideneacetone)dipalladium (2.8 mg, 0.003 mmol), and Xantphos (5.1 mg, 0.009 mmol) were added to a small vial equipped with a septum. After the flask was evacuated and flushed with nitrogen (3×), 1,4-dioxane (0.5 mL) and ethylamine (2.0 M solution in THF, 0.091 mL, 0.18 mmol) were added. The reaction mixture was heated to 100 °C and stirred for 3 h. The reaction mixture was allowed to cool to room temperature and was filtered through Celite. The material was purified by preparative HPLC (Sunfire Prep C18 column, 90−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 11 mg (23%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.6 (br, 1 H), 7.56−7.49 (m, 4 H), 7.42−7.30 (m, 6 H), 5.37 (s, 2 H), 5.03 (s, 2 H), 3.89 (d, J = 11.0 Hz, 1 H), 3.24−3.04 (m, 3 H), 2.97 (dd, J = 15.6, 4.9 Hz, 1 H), 2.64−2.43 (m, 3 H), 2.49 (s, 6 H), 2.14 (d, J = 15.6 Hz, 1 H), 1.25 (t, J = 6.8 Hz, 3 H), 0.81 (s, 9 H), 0.25 (s, 3 H), 0.11 (s, 3 H). MS (ESI) m/z 785.57 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-(ethylamino)-8-azatetracycline Dihydrochloride (24a). Aqueous HF (48%, 0.3 mL) was added to a solution of the preceding intermediate (11 mg, 0.014 mmol) in CH$_3$CN (7 mL) in a plastic vial. After 18 h, the reaction mixture was poured into a solution of K$_2$HPO$_4$ (2 g) in water (10 mL). The mixture was extracted with EtOAc (3×). The combined EtOAc extracts were dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The material was dissolved in MeOH (2 mL) and 1,4-dioxane (2 mL), and palladium on carbon (Degussa, 10 wt %, 5.6 mg) was added. An atmosphere of hydrogen was introduced, and the reaction mixture was stirred for 1 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. 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 3.2 mg (46%, 2 steps) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.08 (s, 1 H), 3.43 (q, J = 7.4 Hz, 2 H), 3.08−2.92 (m, 9 H), 2.30−2.15 (m, 2 H), 1.67−1.55 (m, 1 H), 1.22 (t, J = 7.4 Hz, 3H). MS (ESI) m/z 493.24 (M + H).

The following compounds were prepared by similar methods to **24a**:

7-Chloro-6-demethyl-6-deoxy-9-(methylamino)-8-azatetracycline Dihydrochloride (24b). Prepared from **19h** and methylamine (1.0 M solution in THF) in 21% yield for the amination step, 19% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.08 (s, 1 H), 3.41 (s, 3 H), 3.08−2.92 (m, 9 H), 2.30−2.15 (m, 2 H), 1.67−1.55 (m, 1 H). MS (ESI) m/z 479.22 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-(propylamino)-8-azatetracycline Dihydrochloride (24c). Prepared from **19h** and n-propylamine in 24% yield for the amination step, 40% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.16 (s, 1H), 3.47 (t, J = 7.3 Hz, 2 H), 3.15−2.96 (m, 9 H), 2.32−2.20 (m, 2 H), 1.78−1.55 (m, 3 H), 1.01 (t, J = 7.4 Hz, 3 H). MS (ESI) m/z 507.29 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-(propan-2-ylamino)-8-azatetracycline Dihydrochloride (24d). Prepared from **19h** and 2-propylamine in 11% yield for the amination step, 16% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.21−4.13 (m, 1 H), 4.08 (s, 1 H), 3.14−2.92 (m, 9 H), 2.31−2.15 (m, 2 H), 1.67−1.56 (m, 1 H), 1.23 (dd, J = 6.4, 1.8 Hz, 6 H). MS (ESI) m/z 507.24 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[(2-methylpropyl)amino]-8-azatetracycline Dihydrochloride (24e). Prepared from **19h** and 2-methylpropan-1-amine in 15% yield for the amination step, 57% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.18 (s, 1H), 3.38 (d, J = 7.3 Hz, 2 H), 3.20−2.96 (m, 9 H), 2.34−2.20 (m, 2 H), 2.10−1.98 (m, 1H), 1.68−1.54 (m, 1 H), 1.00 (d, J = 6.4 Hz, 6 H). MS (ESI) m/z 521.40 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[(3-methylbutyl)amino]-8-azatetracycline Dihydrochloride (24f). Prepared from **19h** and 3-methyl-butan-1-amine in 25% yield for the amination step, 66% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.09 (s, 1 H), 3.45−3.40 (m, 2 H), 3.20 (s, 1 H), 3.08−2.93 (m, 9 H), 2.30−2.16 (m, 2 H), 1.72−1.58 (m, 2 H), 1.55−1.48 (m, 2 H), 0.96 (d, J = 6.4 Hz, 6 H). MS (ESI) m/z 535.30 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[(2-methoxyethyl)amino]-8-azatetracycline Dihydrochloride (24g). Prepared from **19h** and 2-methoxyethylamine in 19% yield for the amination step, 44% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.13 (s, 1H), 3.72−3.60 (m, 4H), 3.40 (s, 3 H), 3.12−2.95 (m, 9 H), 2.32−2.21 (m, 2 H), 1.69−1.56 (m, 1 H). MS (ESI) m/z 523.32 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[[2-(propan-2-yloxy)ethyl]-amino]-8-azatetracycline Dihydrochloride (24h). Prepared from **19h** and 2-(propan-2-yloxy)ethan-1-amine in 9% yield for the amination step, 30% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.19 (s, 1H), 3.82−3.69 (m, 5H), 3.20−2.98 (m, 9 H), 2.35−2.22 (m, 2 H), 1.68−1.56 (m, 1 H), 1.18 (d, J = 6.4 Hz, 6H). MS (ESI) m/z 551.41 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[[3-(dimethylamino)propyl]-amino]-8-azatetracycline Trihydrochloride (24i). Prepared from **19h** and (3-aminopropyl)dimethylamine in 9% yield for the amination step, 21% for the 2 deprotection steps, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.09 (s, 1 H), 3.70−3.46 (m, 4 H), 3.10−2.90 (m, 15 H), 2.31−2.17 (m, 2 H), 2.10−2.00 (m, 2 H), 1.68−1.56 (m, 1 H). MS (ESI) m/z 550.34 (M + H).

Phenyl 3-(Benzyloxy)-2-[[(benzyloxy)carbonyl][3-(dimethyl-amino)-2,2-dimethylpropyl]amino]-6-fluoro-5-methylpyr-idine-4-carboxylate (22a). Compound **16d** (100 mg, 0.24 mmol), Cs$_2$CO$_3$ (235 mg, 0.720 mmol), tris(dibenzylideneacetone)dipalladium (11 mg, 0.012 mmol), and Xantphos (20 mg, 0.036 mmol) were weighed into a flask. This was evacuated and backflushed with nitrogen (3×), and 1,4-dioxane (0.5 mL) and N,N-dimethylneopentanediamine (0.057 mL, 0.36 mmol) were added. The reaction mixture was heated to 70 °C. After 1.5 h, the reaction mixture was cooled to room temperature and was filtered through Celite. The filtrate was concentrated under reduced pressure and was purified by column chromatography (Biotage 10 g column, 0−3% MeOH in CH$_2$Cl$_2$ gradient), yielding 76.9 mg (69%) of the intermediate. R_f = 0.25 in 5% MeOH/CH$_2$Cl$_2$. MS (ESI) m/z 466.28 (M + H). LHMDS (1.0 M in THF, 0.22 mL, 0.22 mmol) was added dropwise to a −78 °C solution of the above intermediate (93 mg, 0.20 mmol) in THF (5 mL). After 5 min, benzyl chloroformate (0.084 mL, 0.60 mmol) was added. After 15 min, the reaction mixture was quenched by the addition of NH$_4$Cl (saturated, aqueous solution, 15 mL) and was extracted with EtOAc. The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 10 g column, 0−7% MeOH in CH$_2$Cl$_2$ gradient), yielding 111 mg (93%) of the product. R_f = 0.42 in 10% MeOH/CH$_2$Cl$_2$. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.45−7.20 (m, 13 H), 7.06−6.98 (m, 2 H), 5.16 (s, 2 H), 4.87 (s, 2 H), 3.78 (br s, 2 H), 2.38 (s, 3 H), 2.18 (br s, 6 H), 1.62 (br s, 2 H), 0.90 (br s, 6 H). MS (ESI) m/z 600.28 (M + H).

N-[(4aS,11aR,12aS,13S)-13-(Dimethylamino)-4a-[[(1,1-di-methylethyl)dimethylsilyl]oxy]-10-fluoro-4,4a,6,11,11a,12, **12a,13-octahydro-5-hydroxy-4,6-dioxo-3,7-bis(phenyl-methoxy)isoxazolo[5′,4′:6,7]naphth[2,3-g]isoquinolin-8-yl]-N-[3-(dimethylamino)-2,2-dimethylpropyl]-carbamic Acid, Phenylmethyl Ester (23a).** A solution of compound **22a** (54 mg, 0.090 mmol) in THF (0.5 mL) was added dropwise to a −78 °C solution of LDA (2.0 M solution in THF, 0.112 mL, 0.224 mmol) and TMEDA (0.081 mL, 0.54 mmol) in THF (2 mL), resulting in an orange-colored solution. After 10 min, a solution of **8** (43 mg, 0.090 mmol) in THF (0.5 mL) was added dropwise over ∼30 s. After complete addition, the reaction mixture was allowed to warm to −10 °C over 1 h. The reaction was quenched by the addition of ammonium chloride (saturated, aqueous solution), was diluted with water, and was extracted with EtOAc (2×). The combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 20−100% B gradient), yielding 22.9 mg (26%) of the desired product as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.55 (br s, 1 H), 7.52−7.20 (m, 15 H), 5.36 (s, 2 H), 5.20−5.00 (m, 2 H), 4.80 (s, 2 H), 3.90 (d, J = 11.0 Hz, 1 H), 3.19−3.05 (m, 2 H), 2.62−2.57 (m, 1 H), 2.56−2.12 (m, 19 H), 0.94−0.74 (m, 15 H), 0.26 (s, 3 H), 0.12 (s, 3 H). MS (ESI) m/z 988.59 (M + H).

6-Demethyl-6-deoxy-9-[[3-(dimethylamino)-2,2-dimethyl-propyl]amino]-7-fluoro-8-azatetracycline Trihydrochloride (24j). Aqueous HF (48%, 0.4 mL) was added to a solution of **23a** (22.9 mg, 0.0232 mmol) in CH$_3$CN (0.8 mL) in a plastic vial. After 18 h, the reaction mixture was poured into a solution of K$_2$HPO$_4$ (4.8 g) in water. The mixture was extracted with EtOAc, and the combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was dissolved in MeOH (1 mL), 0.5 M HCl in MeOH (0.2 mL), and 1,4-dioxane (1 mL), and palladium on carbon (Degussa, 10 wt %, ∼2 mg) was added. An atmosphere of hydrogen was introduced, and the reaction mixture was stirred for 3 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx column, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 9.9 mg (67%, 2 steps) of the desired product as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.16 (s, 1 H), 3.43 (s, 2 H), 3.15−2.90 (m, 17 H), 2.30−2.22 (m, 1 H), 2.22−2.12 (m, 1 H), 1.65−1.54 (m, 1 H), 1.17, (s, 6 H). MS (ESI) m/z 562.43 (M + H).

The following compounds were prepared according to methods similar to **24j**:

7-Chloro-6-demethyl-6-deoxy-9-[[2-(dimethylamino)ethyl]-amino]-8-azatetracycline Trihydrochloride (24k). Prepared from **16a** and N,N-dimethylethylenediamine. Yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.13 (s, 1 H), 3.84−3.77 (m, 2 H), 3.44−3.38 (m, 2 H), 3.10−2.92 (m, 15 H), 2.32−2.20 (m, 2 H), 1.68−1.55 (m, 1 H). MS (ESI) m/z 536.25 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[[3-(dimethylamino)-2,2-dimethylpropyl]amino]-8-azatetracycline Trihydrochloride (24l). Prepared from **16a** and N,N-dimethylneopentanediamine. Yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.10 (s, 1 H), 3.51−3.36 (m, 4 H), 3.10−2.92 (m, 15 H), 2.33−2.18 (m, 2 H), 1.68−1.57 (m, 1 H), 1.174 (s, 3 H), 1.169 (s, 3 H). MS (ESI) m/z 578.35 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-[[2-methyl-2-(pyrrolidin-1-ylmethyl)propyl]amino]-8-azatetracycline Trihydrochloride (24m). Prepared from **16a** and 2,2-dimethyl-3-(pyrrolidin-1-yl)-propan-1-amine. Yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.09 (s, 1 H), 3.88−3.78 (m, 2 H), 3.50−3.39 (m, 2 H), 3.18 −2.92 (m, 9 H), 2.32−2.08 (m, 6 H), 1.67−1.56 (m, 1 H), 1.15 (s, 6 H). MS (ESI) m/z 604.29 (M + H).

6-Demethyl-6-deoxy-7-fluoro-9-(propylamino)-8-azate-tracycline Dihydrochloride (24n). Prepared from **16d** and

n-propylamine. Yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.09 (s, 1 H), 3.33 (t, *J* = 6.9 Hz, 2 H) 3.42−3.38 (m, 2 H), 3.20 (s, 1 H), 3.07−2.94 (m, 8 H), 2.91 (dd, *J* = 14.6, 4.6 Hz, 1 H), 2.23−2.12 (m, 2 H), 1.68−1.55 (m, 3 H), 0.96 (t, *J* = 7.3 Hz, 3 H). MS (ESI) *m/z* 491.23 (M + H).

Phenyl 5-Benzyloxy-2-(dimethylamino)-3-methyl-6-(propylamino)isonicotinate. Copper(I) iodide (4.7 mg, 0.025 mmol) and K$_3$PO$_4$ (209 mg, 0.984 mmol) were added to a vial equipped with a septum. After the vial was evacuated and flushed with nitrogen (3×), a solution of compound **16e** (217 mg, 0.492 mmol) in DMF (2 mL), 1-propylamine (0.162 mL, 1.97 mmol), and 2-acetylcyclohexanone (0.013 mL, 0.098 mmol) were added. The reaction mixture was heated to 100 °C for 3 h. The reaction mixture was allowed to cool to room temperature, was diluted with EtOAc (50 mL), and was washed with water (3 × 40 mL). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 10 g column, 0−12% EtOAc in hexanes gradient), yielding 74.6 mg (36%) of the title compound as a thick oil. R_f = 0.32 in 10% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.50−7.46 (m, 2 H), 7.41−7.30 (m, 5 H), 7.30−7.24 (m, 1 H), 7.08−7.02 (m, 2 H), 5.08 (s, 2 H), 2.85 (s, 6 H), 2.32 (s, 3 H). MS (ESI) *m/z* 420.34 (M + H).

Phenyl 5-Benzyloxy-6-[(*t*-butoxycarbonyl)(propyl)amino]-2-(dimethylamino)-3-methylisonicotinate (22b). LHMDS (1.0 M solution in THF, 0.167 mL, 0.167 mmol) was added to a solution of phenyl 5-benzyloxy-2-(dimethylamino)-3-methyl-6-(propylamino)isonicotinate (63.5 mg, 0.152 mmol) in THF (2 mL), resulting in a bright-yellow solution. A solution of di-*t*-butyldicarbonate (99.5 mg, 0.456 mmol) in THF (1 mL) was added. After 10 min, the reaction was quenched by the addition of ammonium chloride (saturated, aqueous solution) and was extracted with EtOAc (2×). The organics were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by column chromatography (Biotage 10 g column, 0−12% EtOAc in hexanes gradient), yielding 13.6 mg (17%) of the product. R_f = 0.22 in 10% EtOAc/hexanes. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40−7.28 (m, 7 H), 7.28−7.22 (m, 1 H), 7.05−7.00 (m, 2 H), 4.90 (s, 2 H), 2.79 (s, 6 H), 2.34 (s, 3 H), 1.76−1.66 (m, 2 H), 1.56−1.50 (m, 2 H), 0.91 (t, *J* = 7.56 Hz, 3 H). MS (ESI) *m/z* 520.38 (M + H).

N-[(4aS,11aR,12aS,13S)-10,13-Bis(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4,4a,6,11,11a,12,12a,13-octahydro-5-hydroxy-4,6-dioxo-3,7-bis(phenylmethoxy)isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinolin-8-yl]-N-propyl-carbamic Acid, 1,1-Dimethylethyl Ester (23b). A solution of **22b** (24 mg, 0.047 mmol) in THF (0.5 mL) was added dropwise to a −78 °C solution of LDA (0.5 M solution in THF, 0.102 mL, 0.051 mmol) and TMEDA (0.041 mL, 0.27 mmol) in THF (2 mL), resulting in a yellowish−orange colored solution. After 10 min, a solution of **8** (16.4 mg, 0.034 mmol) in THF (0.5 mL) was added dropwise. After complete addition, the reaction mixture was allowed to warm to −20 °C over ~45 min. The reaction was quenched by the addition of ammonium chloride (saturated, aqueous solution) and was extracted with EtOAc (2×). The combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 90−100% B gradient), yielding 6.1 mg (20%) of the desired product as a yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 15.56 (s, 1 H), 7.52−7.24 (m, 10 H), 5.36 (s, 2 H), 4.99−4.64 (m, 2 H), 4.02 (d, *J* = 11.0 Hz, 1 H), 3.10−2.81 (m, 2 H), 2.78 (s, 6 H), 2.62−2.40 (m, 10 H), 2.20−2.14 (m, 1 H), 1.80−1.45 (m, 2 H), 1.44−1.15 (m, 11 H), 1.00−0.70 (m, 12 H), 0.25 (s, 3 H), 0.12 (s, 3 H). MS (ESI) *m/z* 908.65 (M + H).

6-Demethyl-6-deoxy-7-(dimethylamino)-9-(propylamino)-8-azatetracycline Dihydrochloride (24o). Aqueous HF (48%, 0.4 mL) was added to a solution of **20o** (6.1 mg, 0.007 mmol) in CH$_3$CN

(0.6 mL) in a plastic vial. After 18 h, the reaction mixture was poured into a solution of K$_2$HPO$_4$ (4.8 g) in water (15 mL). The mixture was extracted with EtOAc (3×). The combined EtOAc extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was dissolved in MeOH (1 mL) and 1,4-dioxane (1 mL), and palladium on carbon (Degussa, 10 wt %, ~2 mg) was added. An atmosphere of hydrogen was introduced, and the reaction mixture was stirred for 4 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx, 0−100% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 2.8 mg (70%, 2 steps) of the desired product as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.19 (s, 1 H), 3.44 (t, *J* = 7.6 Hz, 2 H), 3.24−3.12 (m, 7 H), 3.12−2.97 (m, 8 H), 2.39−2.26 (m, 2 H), 1.73−1.56 (m, 3 H), 0.98 (t, *J* = 8.4 Hz, 3 H); MS (ESI) *m/z* 516.42 (M + H).

(4aS,11aR,12aS,13S)-10,13-Bis(dimethylamino)-4a-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-11a,12,12a,13-tetrahydro-5-hydroxy-3,7-bis(phenylmethoxy)-8-(2-propen-1-yl)-isoxazolo[5′,4′:6,7]naphth[2,3-*g*]isoquinoline-4,6(4aH,11H)-dione (25a). Compound **19m** (105 mg, 0.126 mmol), tetrakis(triphenylphosphine)palladium(0) (15 mg, 0.013 mmol), and K$_3$PO$_4$ (80 mg, 0.38 mmol) were weighed into an 8 mL vial. This was sealed with a septum and was evacuated and backflushed with nitrogen (3×). Toluene (1 mL), 1,4-dioxane (1 mL), and water (0.5 mL) were added, followed by allylboronic acid pinacol ester (63.7 mg, 0.379 mmol). The reaction mixture was heated to 90 °C. After 2.5 h, the reaction mixture was cooled to room temperature and was concentrated under reduced pressure. The material was purified by preparative HPLC (Sunfire Prep C18 column, 90−100% B gradient), yielding 28.6 mg (29%) of the title compound as a yellow solid. MS (ESI) *m/z* 791.64 (M + H).

6-Demethyl-6-deoxy-7-(dimethylamino)-9-propyl-8-azatetracycline Dihydrochloride (26a). Aqueous HF (48%, 0.4 mL) was added to a solution of **25a** (28.6 mg, 0.036 mmol) in 1,4-dioxane (1 mL) in a plastic vial. After 18 h, the reaction mixture was poured into a solution of K$_2$HPO$_4$ (4.8 g) in water. The mixture was extracted with EtOAc, and the combined extracts were dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The material was dissolved in MeOH (1 mL), 0.5 M HCl in MeOH (0.5 mL), and 1,4-dioxane (1 mL), and palladium on carbon (Degussa, 10 wt %, ~5 mg) was added. An atmosphere of hydrogen was introduced, and the reaction mixture was stirred for 1 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The material was purified by preparative HPLC (Phenomenex Polymerx column, 0−70% B gradient). Fractions with the desired MW were collected and freeze-dried to yield 14.3 mg (69%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD with 1 drop DCl) δ 4.20 (s, 1 H), 3.38−3.15 (m, 7 H), 3.14−2.94 (m, 8 H), 2.91−2.82 (m, 2 H), 2.56 (t, *J* = 13.8 Hz, 1 H), 2.40−2.32 (m, 1 H), 1.86−1.76 (m, 2 H), 1.74−1.62 (m, 1 H), 1.01 (t, *J* = 7.34 Hz, 3 H). MS (ESI) *m/z* 501.35 (M + H).

The following compounds were prepared according to methods similar to **26a**:

7-Chloro-6-demethyl-6-deoxy-9-propyl-8-azatetracycline Hydrochloride (26b). Prepared from **19h** and allylboronic acid pinacol ester, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.10 (s, 1 H), 3.22−2.92 (m, 9 H), 2.81−2.74 (m, 2 H), 2.49−2.38 (m, 1 H), 2.26−2.19 (m, 1 H), 1.77−1.60 (m, 3 H), 0.97 (t, *J* = 7.3 Hz, 3 H). MS (ESI) *m/z* 492.27 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-methyl-8-azatetracycline Hydrochloride (26c). Prepared from **19h** and methylboronic acid, yellow solid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 4.09 (s, 1 H), 3.22−2.92 (m, 9 H), 2.85 (s, 3 H), 2.49−2.38 (m, 1 H), 2.26−2.19 (m, 1 H), 1.77−1.60 (m, 1H). MS (ESI) *m/z* 464.30 (M + H).

6-Demethyl-6-deoxy-9-methyl-8-azatetracycline Dihydrochloride (26d). Prepared from **19h** as an over-reduced side product

in the synthesis of **26c**. ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.78 (s, 1 H), 4.12 (s, 1 H), 3.22−2.91 (m, 9 H), 2.78 (s, 3 H), 2.49−2.21 (m, 1 H), 1.77−1.62 (m, 1H). MS (ESI) *m/z* 430.46 (M + H).

7-Chloro-6-demethyl-6-deoxy-9-phenyl-8-azatetracycline Hydrochloride (26e). Prepared from **19h** and phenylboronic acid. ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.04−7.98 (m, 2 H), 7.47−7.39 (m, 3 H), 4.08 (s, 1 H), 3.14−2.92 (m, 9 H), 2.57−2.47 (m, 1 H), 2.28−2.07 (m, 1 H), 1.73−1.65 (m, 1H). MS (ESI) *m/z* 526.26 (M + H).

6-Demethyl-6-deoxy-9-phenyl-8-azatetracycline Dihydrochloride (26f). Prepared from **19h** as an over-reduced side product in the synthesis of **26e**. ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.04−7.98 (m, 2 H), 7.47−7.39 (m, 3 H), 4.08 (s, 1 H), 3.14−2.92 (m, 9 H), 2.57−2.47 (m, 1 H), 2.28−2.07 (m, 1 H), 1.73−1.65 (m, 1H). MS (ESI) *m/z* 526.26 (M + H).

Susceptibility Testing. Compound stocks were prepared 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)), *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).^{26} 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.

In vitro Transcription/Translation Assay. Compound stocks prepared and diluted in sterile deionized water were assayed for inhibition of coupled in vitro transcription/translation using an *E. coli* S30 extract system with a firefly luciferase readout from Promega (cat. no. L1020, Madison, WI). Briefly, compounds were diluted into water and added to reaction mix aliquoted to black-walled 96-well microtiter plates (cat. no. 3650, Costar, Corning, NY). An appropriate three-point titration was used for each compound, and reactions were run in duplicate. The final total reaction volume was 20 μL. Plates were incubated at 37 °C for one hour and then placed on ice for 5 min to arrest transcription/translation. Luciferase substrate (25 μL, Promega cat. no. E1500) was added to each well and luminescence was detected on a BMG LabTech LUMIstar-OPTIMA instrument. Positive assay control values, from reactions without inhibitor, were averaged per plate to determine percent inhibition of luciferase production. Results were plotted using Microsoft Excel and 50% inhibition values (IC$_{50}$) were determined.

ASSOCIATED CONTENT

ⓢ Supporting Information. MIC data for the complete panel of bacteria for all compounds as well as individual MIC data for the MIC$_{90}$ calculations for compounds **20f**, **24l**, tigecycline, and tetracycline. 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; AUC, area under the curve; Boc, *t*-butoxycarbonyl; Cbz, benzyloxycarbonyl; Cl, clearance; Cy, cyclohexyl; dba, dibenzylideneacetone; DMAP, 4-(dimethylamino)pyridine; dppf, (diphenylphosphino)ferrocene; %*F*, percent oral bioavailability; IV, intravenous; LDA, lithium diisopropylamide; LHMDS, lithium bis(trimethylsilyl)amide; mCPBA, 3-chloroperbenzoic acid; 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*; NMP, 2-methylpyrrolidinone; nOe, nuclear Overhauser effect; PD$_{50}$, dose at which 50% protection is observed; PO, oral; TMEDA, *N*,*N*,*N'*,*N'*-tetramethylethylenediamine; Vz, volume of distribution; Xantphos, 9,9-dimethyl-4,5-bis-(diphenylphosphino)-xanthenes

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