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Novel DNA Gyrase Inhibiting Spiropyrimidinetriones with a Benzisoxazole Scaffold: SAR and in Vivo Characterization

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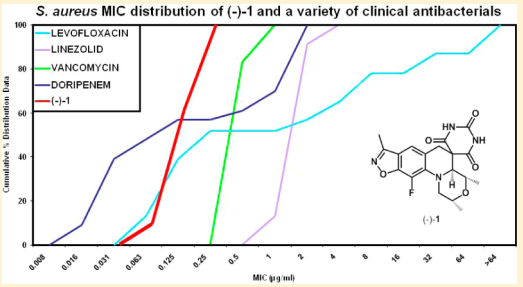
Supporting Information

ABSTRACT: The compounds described herein with a spirocyclic architecture fused to a benzisoxazole ring represent a new class of antibacterial agents that operate by inhibition of DNA gyrase as corroborated in an enzyme assay and by the inhibition of precursor thymidine into DNA during cell growth. Activity resided in the configurationally lowest energy (2*S*,4*R*,4a*R*) diastereomer. Highly active compounds against *Staphylococcus aureus* had sufficiently high solubility, high plasma protein free fraction, and favorable pharmacokinetics to suggest that in vivo efficacy could be demonstrated, which was realized with compound (−)-**1** in *S. aureus* mouse infection models. A high drug exposure NOEL on oral dosing in the rat suggested that a high therapeutic margin could be achieved. Importantly, (−)-**1** was not cross-resistant with other DNA gyrase inhibitors such as fluoroquinolone and aminocoumarin antibacterials. Hence, this class shows considerable promise for the treatment of infections caused by multidrug resistant bacteria, including *S. aureus*.



INTRODUCTION

The persistence and escalation of bacterial resistant to current drug regimens continues to prompt efforts toward identifying novel mode-of-action antibacterial agents.[1−4] Accordingly, targets essential for bacterial viability have been screened against compound libraries,[5,6] probed with substrate, product, or transition state mimetics,[7] or structurally enabled for computational based inhibitor design.[8−10] An alternative approach to addressing resistance would be to exploit an established target by introducing a novel binding mode and thereby a novel mode-of-inhibition. Here, we disclose initial work around compounds with a benzisoxazole scaffold fused with a piperidine ring that displays a spiropyrimidinetrione pharmacophore.[11] A key reaction in the synthesis of this class of compounds is the tertiary amino effect reaction (T-reaction) wherein the Knövenagel adduct of a precursor aromatic aldehyde undergoes a [1,5]-hydride shift with an *ortho*-dialkylamine substituent.[12−14] In this case, barbituric acid serves to form the Knövenagel adduct, and a Mannich cyclization following the hydride shift leads to a spiropyrimidinetrione (Figure 1). Previously, PNU-286607 (Figure 2), which shares the spiropyrimidinetrione pharmacophore, was shown to be effective against fluoroquinolone-resistant bacteria despite inhibition of the same bacterial targets, that is, the bacterial type II topoisomerases, DNA gyrase, and topoisomerase IV (Topo IV).[15,16] The course of substrate binding and conformational transformations during the catalytic process for type II topoisomerases leads to several possible modes-of-inhibition,[17−21] and indeed different classes of antibacterial agents are known to inhibit the two enzymes.[22−28] This new class of compounds, hereafter referred to as spiropyrimidinetrione (SPT) antibacterials, are structurally and biochemically differentiated from any class of DNA gyrase inhibitors.[29] We therefore set out to assess the potential for developing an efficacious drug by identifying analogues with potent antibacterial activity and sufficiently good DMPK attributes to evaluate their efficacy in mouse models of infection and to assess in vivo tolerability.

RESULTS AND DISCUSSION

Synthesis of Target Compounds. Three patent applications from Pfizer have disclosed PNU-286607 analogues, with most of them having two fluorine atoms and a heteroaryl substituent on the benzene scaffold as shown in Figure 2.[16,30,31] The benzisoxazole scaffold was designed to place an oxygen atom in the same position as the one fluorine atom, thereby either serving as an isosteric replacement or maintaining the capability to accept an admittedly weak hydrogen bond. The positioning of an oxygen atom in place of a fluorine atom through an isoxazole fusion is, to our knowledge, unprecedented in the literature. The distal substituent on the benzisoxazole scaffold enters into space nearby to, but offset from, the heteroaryl substituent of the compounds of Figure 2, thereby probing a differentiated binding environment. The synthesis of target spirocycles with a methyl substituent on the benzisoxazole (Table 1) utilized either *meso*-dimethylmorpholine to afford racemic product (Scheme 1) or chiral (*R*,*R*)-

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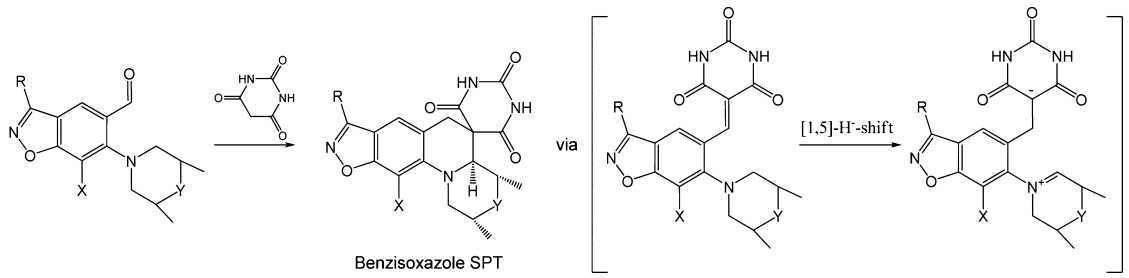


Figure 1. T-reaction for the synthesis of spiropyrimidinetriones (SPTs).

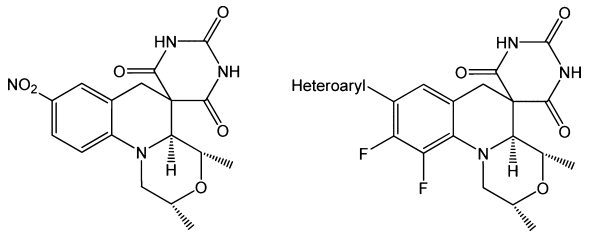
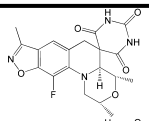
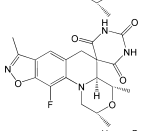
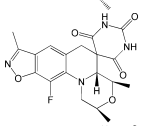
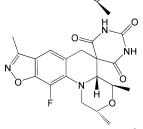
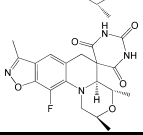


Figure 2. PNU-286607 and analogues.

dimethylmorpholine to afford chiral product (Scheme 2).[15] Along both routes, compounds **14a** and **14b** were made by regioselective displacement of the 2-position fluorine atom of **13**, followed by reduction to the alcohol and protection as TBDPS ethers. This set up a fluorine directed *ortho*-lithiation[32] followed by quenching with the Weinreb amide of acetic acid to afford methyl ketones **15a** and **15b**. The use of MTBE over other ethereal solvents proved important toward maximizing the yield, which was otherwise compromised by the return of starting material, presumably due to quenching by non-productive deprotonation of the Weinreb amide. Each fluorine atom *ortho* to the acetyl group of **15a** and **15b** was displaced by the anion of acetone oxime, and treatment with aqueous acid hydrolyzed both the oxime and silyl ether, enabling subsequent cyclization to the corresponding benzisoxazoles.[33] Oxidation with MnO_2 afforded aldehydes **16a** and **16b**. The final T-reaction was achieved by heating barbituric acid with the corresponding aldehyde at the appropriate temperature. In the case of **16a**, heating in 2-propanol at 80 °C effected clean conversion to racemic (±)-**1**, and the individual enantiomers (−)-**1** and (+)-**1** were separated by chiral supercritical-fluid chromatography (SFC). Temperatures above 80 °C resulted in conversion to other diastereomers and were therefore avoided. By contrast, the T-reaction with **16b** and barbituric acid was carried out at 120 °C to equilibrate diastereomers to give a 9:1 thermodynamic mixture. The major diastereomer was obtained by crystallization as the hydrate from aqueous methanol, matching the properties of (−)-**1** described in Scheme 1. The configuration was confirmed from a single-crystal X-ray structure of (−)-**1**, showing the morpholine ring in a lower energy chair conformation with the two pendant methyl substituents in equatorial orientations (Figure 3) and matching that of the (−)-enantiomer of PNU-286607.[15] The configuration and conformation were corroborated by NOESY NMR examination of (−)-**1** (see Supporting Information) wherein

Table 1. Influence of Stereochemistry on DNA Gyrase Inhibitory Potency, PPB, Solubility, and MIC

Cmpd	Structure	Eco IC_{50} (nM)	Human PPB (% f_u)	Solubility (μM)	MIC (μM)						
					Spn[a]	Spy[b]	MSSA[c]	MRQR[d] Sau	Hin[e]	Eco[f]	Eco[f] *tolC*
(±)-1		0.38	6.3	>1000	21	ND[g]	2.8	2.5	2.5	100	0.3
(-)-1		0.29	9.4	>1000	8.8	6.2	1.0	2.0	1.0	45	≤0.2
(+)-1		>83	3.5	>1000	>200	ND	>200	>200	>200	>200	50
(+)-2		13	8.2	190	100	200	25	50	25	>200	1.6
(-)-2		26	5.1	340	>200	>200	>200	>200	200	>200	12

[a]Spn: *S. pneumoniae*. [b]Spy: *S. pyogenes*. [c]Methicillin sensitive *S. aureus*. [d]Methicillin resistant, quinolone resistant *S. aureus*. [e]*H. influenzae*. [f]*E. coli*. [g]Not determined.

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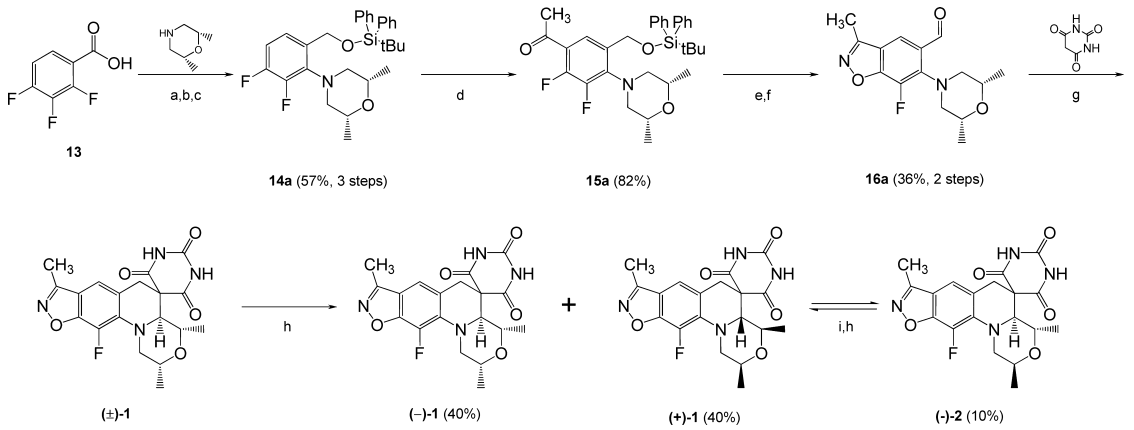
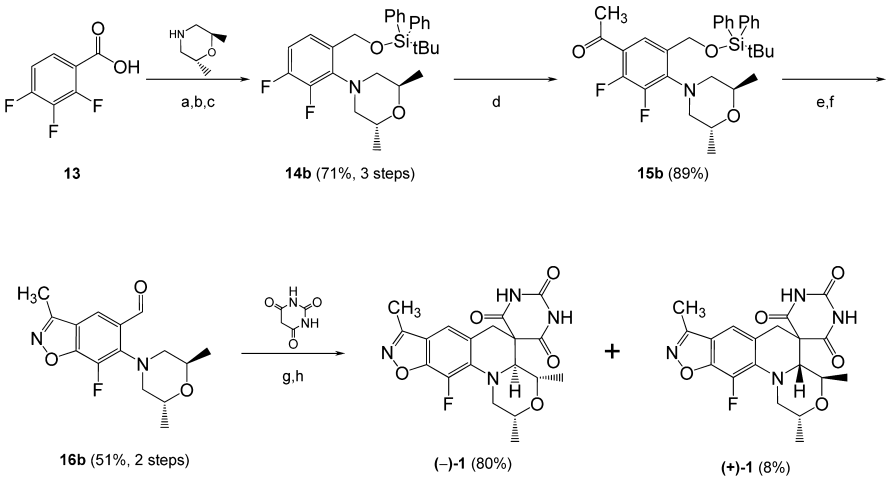
Scheme 1[a]



[a]Reagents and conditions: (a) LHMDS, THF, −78 °C → rt; (b) $NaBH_4$, I_2, EtOH; (c) *t*-Bu$(CH_3)_2$Si-Cl, imidazole; (d) *s*-BuLi, $CH_3C(=O)N(OMe)Me$, THF; (e) acetone−oxime, KO-*t*-Bu, THF, then 2.5% HCl, EtOH; (f) MnO_2, CH_2Cl_2, 3d; (g) *i*-PrOH, 80 °C, 16 h; (h) separate isomers, 120 °C; (i) 3:1 AcOH−water.

Scheme 2[a]



[a]Reagents and conditions: (a) LHMDS, THF, −78 °C → rt; (b) $NaBH_4$, I_2, EtOH; (c) *t*-Bu$(CH_3)_2$Si-Cl, imidazole; (d) *s*-BuLi, $CH_3C(=O)N(OMe)Me$, MTBE; (e) acetone−oxime, KO-*t*-Bu, THF, then 2.5% HCl, EtOH; (f) MnO_2, CH_2Cl_2, 3 d; (g) 3:1 AcOH−water, 120 °C; (h) separate isomers.

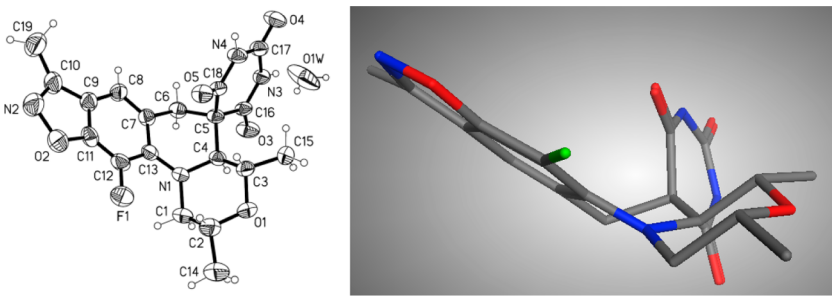


Figure 3. (left) X-ray structure of (−)-**1** (as water hydrate, Ortep representation). (right) Side view showing morpholine chair conformation of (−)-**1** with equatorial methyl groups (water and hydrogen atoms removed for clarity).

large coupling constants (12 and 9 Hz) were seen for the C2 and C3 morpholine hydrogen atoms (see Figure 3 for numbering) to the adjacent C1 and C4 hydrogen atoms indicative of *trans*-diaxial relationships, placing the methyl substituents in equatorial orientations. A through-space NOE was seen between the same two morpholine C2 and C3 hydrogen atoms, achieved by their axial orientations. A notable through-space NOE was seen between the C4 hydrogen atom and one of the C6 benzylic hydrogen atoms. Hence, solution and solid-state structures match closely. The configuration of (−)-**1** proved stable through prolonged (multiday) heating at 80 °C in solution and very much longer (greater than one year) at room temperature in the solid state. The minor diastereomer (+)-**2** was isolated by chromatography of the mother liquor

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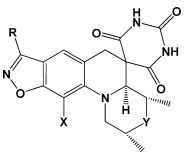
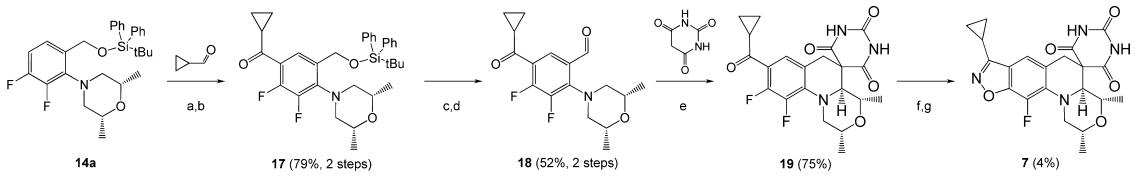
Table 2. Structure–Activity Relationships



							MIC (μM)							
compd	R	X	Y	human PPB (% f_u)	solubility (μM)	Eco FP IC_{50} (μM)	Spn[a]	Spy[b]	MSSA[c]	MSSA[c] + serum	MRQR[d] Sau	Hin[e]	Eco[f]	Eco[f] *tolC*$^-$
(−)-**1**	$-CH_3$	F	O	9.4	>1000	0.29	8.8	6.2	1.0	3.1	2.0	1.0	45	≤0.2
(±)-**3**	$-CH_3$	Cl	O	3	330	0.37	50	25	6.2	50	12	3.1	200	≤0.2
(−)-**4**	$-CF_3$	F	O	<1	53	0.61	12	3.1	0.78	100	1.6	1.6	25	≤0.2
(−)-**5**	$-CF_2H$	F	O	2.8	>1000	0.48	3.1	1.2	0.39	3.1	0.39	0.62	25	≤0.2
(±)-**6**	$-CH_2OCH_3$	F	O	9.6	180	1.6	12	6.2	3.1	ND[g]	6.2	12	200	≤0.2
(±)-**7**	cyclopropyl	F	O	5.2	29	0.34	25	6.2	1.6	ND	12	3.1	100	≤0.2
(±)-**8**	*t*-butyl	F	O	4.2	770	4.0	200	50	18	ND	18	200	>200	3.1
(±)-**9**	$-CH_3$	F	CH_2	<1	<1.0	0.34	100	200	25	50	25	>200	50	6.2
(−)-**10**	−Cl	F	O	<1	>1000	0.21	12	6.2	0.39	12	0.78	25	25	6.2
(−)-**11**	$-OCH_3$	F	O	9.0	510	0.97	25	6.2	3.1	ND	3.1	3.1	200	≤0.2
(−)-**12**	$-NH_2$	F	O	13	330	0.26	6.2	3.1	25	ND	50	1.6	100	≤0.2
linezolid				71	>1000	>32	3.1	3.1	6.2	12	6.2	23	>200	20
ciprofloxacin				74	240	0.27	1.5	1.5	0.91	ND	96	<0.1	0.06	<0.1

[a]Spn: *S. pneumoniae*. [b]Spy: *S. pyogenes*. [c]Methicillin-sensitive *S. aureus*. [d]Methicillin-resistant, quinolone-resistant *S. aureus*. [e]*H. influenzae*. [f]*E. coli*. [g]Not determined.

Scheme 3[a]



[a]Reagents and conditions: (a) *s*-BuLi, THF, −78 °C; (b) PCC, DCM, 0 °C; (c) 2.5% HCl, EtOH; (d) MnO_2, CH_2Cl_2, 3d; (e) *i*-PrOH, 80 °C, 16h; (f) $NH_2OH.HCl$, pyridine, 90 °C; (g) Cs_2CO_3, DMF, 100 °C.

from the crystallization, and the configuration was assigned by NMR. There was slow conversion of the higher energy (+)-**2** to (−)-**1** at room temperature in DMSO solution, so its characterization was best carried out soon after isolation. In the solid state, the configuration of (+)-**2** was maintained at room temperature for over a year. Finally, heating the inactive diastereomer (+)-**1** at 120 °C effected equilibration to a thermodynamic 9:1 mixture of (+)-**1** and (−)-**2**, which were separated by chromatography. With three stereocenters, only two of the four possible diastereomers for the chiral scaffold could be isolated although all would be sampled under thermodynamic equilibrating conditions (120 °C). Enantiomers (−)-**1** and (+)-**1** represent the lowest energy configuration, while enantiomers (−)-**2** and (+)-**2** are next lowest in energy. The morpholine of (+)-**2** primarily exists in a chair conformation, and the C14 methyl group (using the numbering in Figure 3) has an axial orientation as delineated by a diagnostic 1,3-axial NOE to the C3 ether C−H (see Supporting Information for NOESY and modeled conformation). A through-space NOE is seen between the axial C4 and C1 hydrogen atoms, and the two C1 hydrogen atoms show small 3.0 and 3.3 coupling constants to the C2 hydrogen atom in line with the latter being equatorial. Finally, the C3 and C2 hydrogen atoms showed a coupling constant (6.8 Hz) consistent with a *trans*-diaxial orientation. There is also minor (25%) conformation for (+)-**2** detected through an NOE between the C15 methyl group and the C2 hydrogen atom consistent with a ring flip to a boat conformation and accounting for an averaging of coupling constants overall. There are four diastereomers otherwise (two enantiomeric pairs) that are likely sampled in the course of the T-reactions depending on substrates and conditions. However, their propensity to convert to the more stable diastereomers via the retro-Mannich iminium intermediate (Figure 1) precluded their isolation. Neither of these two diastereomers would be able to orient the two morpholine methyl groups in lower energy equatorial positions without significant distortions to the overall molecular shape. Note that when the temperature was not too high (80 °C or below), the racemate (±)-**1** did not equilibrate to (±)-**2**. Shorter reaction times (1 h) for the conversion of **16a** to (±)-**1** afforded a third diastereomer (as seen by NMR) that could not be isolated from a mixture with (±)-**1** due to its facile conversion to (±)-**1** during all attempts at chromatographic separation. Compounds **3**, **4**, **5**, and **6** of Table 2 were constructed similarly via Scheme 1 or Scheme 2 with some modifications (for **5** and **6**) in the assembly of the benzisoxazole ring (see Experimental Section).

A variation from the sequence of Schemes 1 and 2 involved reaction of the lithium anion derived from **14a** with aldehydes followed by oxidation to the carbonyl as exemplified in Scheme 3 for the synthesis of (±)-**7**. In this particular sequence, the benzisoxazole was built after the assembly of the spiropyr-

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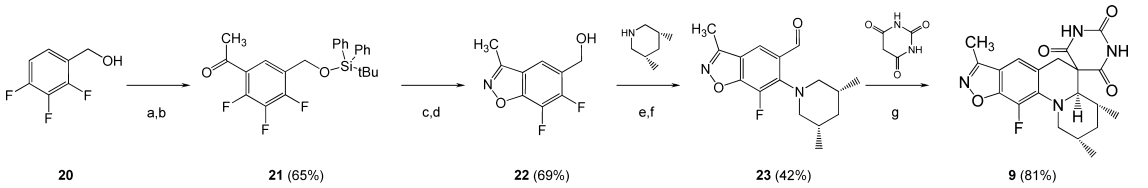
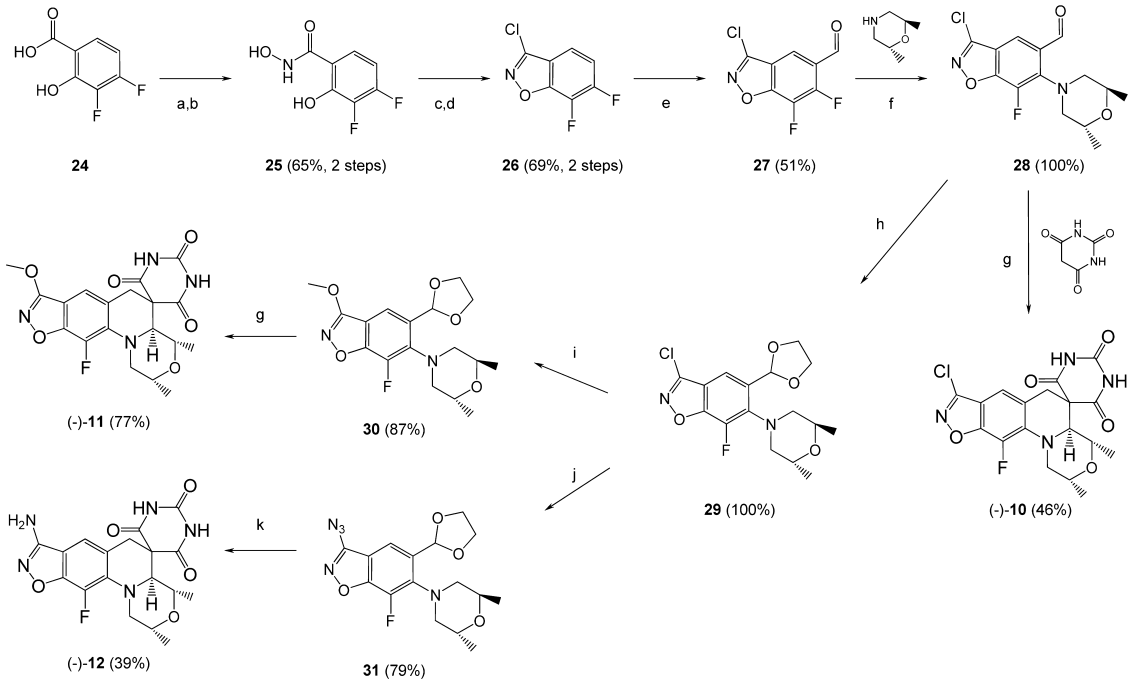
Scheme 4[a]



[a]Reagents and conditions: (a) TBDPS-Cl, imidazole; (b) *s*-BuLi, $CH_3C(=O)N(OMe)Me$, THF, −78 °C; (c) acetone−oxime, KO-*t*-Bu, THF, 0 °C; (d) 2.5% HCl, EtOH; (e) MnO_2, CH_2Cl_2, 3 d; (f) DIEA, CH_3CN, H_2O, reflux; (g) *i*-PrOH, 80 °C.

Scheme 5[a]



[a]Reagents and conditions: (a) HCl, MeOH, reflux; (b) NH_2OH, dioxane, rt; (c) CDI, THF, reflux; (d) $POCl_3$, 140 °C; (e) LTMP, DMF, −78 °C (f) DIEA, butyronitrile, H_2O, reflux; (g) AcOH, H_2O, 120 °C; (h) $HOCH_2CH_2OH$, *p*-TsOH, toluene, reflux; (i) NaOMe, MeOH, 100 °C; (j) NaN_3, DMSO, 100 °C; (k) Ph_3P, AcOH, H_2O, rt then barbituric acid, 120 °C.

imidinetrione, reflecting the flexibility of the reaction sequence order for the construction of target molecules, albeit the lower yield made the route less efficient. Compound (±)-**8** was also made in an analogous route to (±)-**7**. Scheme 4 exemplifies another sequence variation wherein the benzisoxazole ring was assembled before the introduction of an amine, enabling late stage variation of the amine portion of compounds (other analogues not included herein). The route complemented that of Schemes 1, 2, and 3, which were more amenable toward variation of benzisoxazole substituents. Protection of benzyl-alcohol **20** was followed by fluorine directed *ortho*-lithiation and Weinreb amide quenching to afford methylketone **21**. The fluorine atom adjacent to the acetyl group was selectively displaced by the anion of acetone oxime, and subsequent acid treatment led to alcohol **22**. Oxidation of **22** to the aldehyde followed by fluorine displacement with the dimethylpiperidine afforded **23**. The final T-reaction with barbituric acid was performed as described in Scheme 1 for (±)-**1**. In this case, a 9:1 ratio of diastereomers for (±)-**9** was obtained even at the lower 80 °C temperature, with the major diastereomer being isolated by fractional crystallization.

The synthesis of (−)-**10** with a chlorobenzisoxazole substituent and analogues derived from S_NAr displacement of the chlorine are outlined in Scheme 5. To this end, salicylic acid **24** was converted to **26** by procedures set out in the literature for the synthesis of chlorobenzisoxazoles.[33,34] An aldehyde was introduced by sequential fluorine directed lithiation and quenching with DMF, affording **27**. Displacement of the fluorine atom adjacent to the carboxaldehyde followed by the T-reaction with barbituric acid afforded (−)-**10** after separation from the analogous minor diastereomer described in Scheme 2. The aldehyde of **28** was protected as the ketal (**29**) for an S_NAr displacement of chlorine with methoxide and azide. The methyl ether **30** was transformed to (−)-**11** by in situ ketal hydrolysis and T-reaction. Azide **31** was converted to (−)-**12** by treatment with Ph_3P and barbituric acid, reducing the azide to the amine, hydrolyzing the ketal, and carrying out the T-reaction in a single reaction vessel.

Structure−Activity Relationships. There are eight possible diastereomers with the three chiral centers around the morpholine ring of (±)-**1**, and ultimately four of the diastereomers (two enantiomer pairs) were isolated and characterized biologically. As mentioned, the other diaster-

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eomers could not be isolated due to instability relative to configurational isomerization. Compound inhibitory potency against *Escherichia coli* DNA gyrase was determined in an assay where differential binding of a fluorescence tagged DNA chain with relaxed and supercoiled DNA was monitored.[35] The highest inhibitory potency resided in (−)-**1**, correlating with higher antibacterial activity against the Gram-positive *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* and the Gram-negative *Haemophilus influenzae* and *E. coli* (Table 1). The wild-type *E. coli* MIC was considerably higher compared to other bacteria, probably due to low membrane permeability, as indicated by the much lower MIC against the *tolC*$^{-}$ *E. coli* mutant in which the compound efflux machinery is disrupted. The lower activity against *E. coli* and other serious Gram-negative pathogens is therefore likely due to the limited capability for compounds to enter and remain in the bacteria. Because the net epimerization of the C14 methyl group (Figure 3) in going from (−)-**1** to (−)-**2** led to a reorientation from equatorial to axial positions and about a 25% population of the boat conformation, it is presumed the predominate conformations of the two compounds are quite similar and accessible at room temperature. The diminished activity for (−)-**2** would presumably result from a disfavored steric interaction with the target due to the axial methyl group. The 25% boat conformer was also presumably not well tolerated for target binding, perhaps in part due the reorientation of the C15 methyl group to the axial position. Also seen in Table 1, there was a measure of differential recognition for binding to human plasma proteins in that free fraction (fraction unbound or f_u) for (−)-**1** was 2−3-fold higher than that for opposite enantiomer (+)-**1**. This trend for the (−)-enantiomer, the active diastereomer, showing a higher f_u than the racemate, which in turn showed a higher f_u than the (+)-enantiomer, was seen consistently across many other SPT analogues (data not shown). A higher f_u would presumably correlate with improved in vivo activity as free drug is presumed necessary for expression of antibacterial activity. Conversely, a lower f_u can be beneficial for protection from clearance mechanisms and lead to more prolonged drug exposure. Hence, a balance must be achieved for antibacterial activity, PPB, and in vivo clearance for advancing a drug candidate.[36−38] Related to this, the extent of PPB can influence the level of free drug that would interact with secondary host targets including those involved in drug−drug interactions, which is a concern for drug safety in vivo.[37,39] Finally, consideration of solubility is important toward the viability of a drug candidate, especially for antibacterial agents where relatively high doses are administered.[40]

Table 2 shows the data for a series of modifications on the structure of **1** wherein enzyme inhibition potencies are often maintained but physical properties vary, influencing the microbiological profile. The table also includes MIC data for a methicillin sensitive *S. aureus* strain (MSSA) in the presence of 50% human serum as a measure of the influence of plasma protein on MIC; generally, more lipophilic compounds with a lower f_u show a larger shift to a higher MIC with serum.[41] As a dramatic example, replacement of the benzisoxazole CH_3 substituent of (−)-**1** with CF_3 made for highly protein bound compound (−)-**4** that showed a low MIC (0.78 μM) against MSSA under standard culture conditions but a notably higher MIC (100 μM) when 50% human serum was added. Replacement of the morpholine oxygen of (−)-**1** with carbon (compound (±)-**9**) maintained inhibitory potency but the increased lipophilicity contributed to notably low solubility and low f_u, perhaps leading to the higher MIC values. Similarly, replacement of the fluorine atom of (−)-**1** with a chlorine (compound (±)-**3**) maintained inhibitor potency, but the antibacterial activity was lower. The bulky *t*-butyl substituent of (±)-**8** was less well tolerated for binding potency, and therefore MIC values were higher. Having a CF_2H, Cl, or OCH_3 in place of CH_3 on the benzisoxazole (compounds (−)-**5**, (−)-**10**, and (−)-**11**) led to similar antibacterial activity across *S. aureus*, *S. pneumoniae*, *S. pyogenes*, and *H. influenzae*. The more polar NH_2 substituent of (−)-**12** led to decreased activity, in particular against *S. aureus*, despite high enzyme inhibitory potency, in line with general observations that higher polarity decreases permeability through lipophilic bacterial membranes.

In Vitro Microbiology. Analysis of incorporation of radioactive precursors in a *S. aureus* supported the topoisomerase mode-of-action for (−)-**1** (Figure 4).[38,42] The lowest IC_{50}

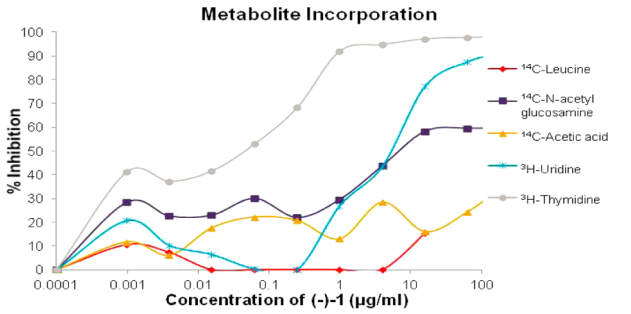

Figure 4. Inhibition of radioactive precursor incorporation by (−)-**1** in *S. aureus*.

(0.06 μg/mL) was seen for thymidine incorporation indicating DNA biosynthesis inhibition. The IC_{50} for incorporation of uridine and *N*-acetylglucosamine, indicative of the disruption of RNA and cell wall biosynthesis, was much higher at approximately 5 and 8 μg/mL, respectively, and would be expected to follow the disruption of DNA biosynthesis. This data correlated well with similar studies carried out for PNU-286607, where DNA biosynthesis inhibition was also inhibited.[11] This profile was similar to other antibacterial drugs that inhibit type II topoisomerases.[24,27,43,44] Despite a similar inhibition profile, low MIC values were seen against a methicillin resistant, quinolone resistant *S. aureus* isolate (MRQR in Table 2), showing that the compounds were not cross-resistant to the fluoroquinolone class. To expand on this, the activity of (−)-**1** was surveyed against other bacterial strains resistant to DNA gyrase inhibitors including levofloxacin and novobiocin (Table 3). The MIC of (−)-**1** was not changed for the isolates, indicating that the mode of DNA gyrase inhibition of this class differs from the other two classes. Therefore, an SPT compound should not be compromised by pre-existing clinical resistance to the two drugs. Although the documentation of clinical strains of bacteria resistant to novobiocin is sparse, numerous laboratory spontaneous resistant mutants have been reported,[27,45] and all those tested including those of Table 3 were not cross-resistant to (−)-**1**. Hence, (−)-**1** does not share the mode-of-inhibition with novobiocin, namely that of binding to the ATP binding site of the topoisomerases. More broadly, there is no reduced susceptibility with (−)-**1** to other classes of antibacterials including linezolid, vancomycin, and doripenem (data not included). Finally, resistance frequencies for (−)-**1** in *S. aureus* (MSSA) cultures at concentrations of

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Table 3. Cross Resistance

strain	genotype		MIC (μg/mL)		
	GyrA−GyrB mutations	ParC−ParE mutations	levofloxacin	novobiocin	(−)-**1**
Sau516[a]	WT−WT	WT−WT	0.13	0.13	0.5
Sau2792	WT−R144A	WT−wild type	0.25	32	0.5
Sau2375	S84L, A457T−K65Q	S80Y, E84G, V693M−N141S	>16	4.3	0.50
Spn548[b]	WT−WT	WT−WT	1	1	4
Spn2800	WT−K143I, T172A	WT−WT	1	>16	4
Spn2485	S81F−D160E, T329A	S79F−WT	16	0.13	2

[a]Sau: *S. aureus*. [b]Spn: *S. pneumoniae*.

Table 4. PK Properties in Mouse and Rat

compd	mouse PPB (% f_u)	mouse Cl (mL/min/kg)	mouse *F* (%)	rat PPB (% f_u)	rat Cl (mL/min/kg)	rat *F* (%)
(−)-**1**	19	38	58	16	32	71
(−)-**4**	7.8	46	63	7.8	2.3	60
(−)-**5**	4.4	47	95	4.3	1.0	87

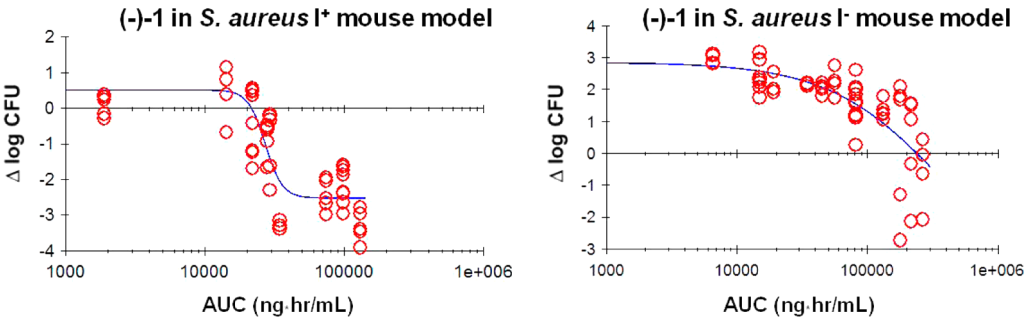

Figure 5. CFU response as to AUC for (−)-**1** in *S. aureus* (ARC516, MSSA) mouse thigh infection models: immunocompetent (left) and neutropenic (right).

two, four, and eight times the MIC were determined to be 1.7×10^{-8}, $\leq 1 \times 10^{-9}$, and $\leq 1 \times 10^{-9}$, respectively. The resistance frequency was lower than those recorded for ciprofloxacin and other fluoroquinolones against *S. aureus*.[46] At two times the MIC, an isolate was characterized and showed only a 2-fold elevation in MIC and no changes in any of the four genes (*gyrA*, *gyrB*, *parC*, or *parE*) encoding for type II topoisomerases. An inoculum of the isolate was plated in the presence of four times the MIC of (−)-**1** to afford two spontaneous mutants at a frequency of 1.1×10^{-8}, each 8-fold elevated in MIC compared to wild-type. Genomic analysis of the topoisomerase genes of the resultant second step mutants showed changes in only *gyrB* Trp592Leu in one strain and Ala439Ser in the other. These residues are located near the DNA binding region of GyrB that abuts the DNA cleavage domain of GyrA and did not confer cross-resistance to ciprofloxacin. This data also strongly supports the novel mode-of-inhibition of (−)-**1** relative to ciprofloxacin.

In Vivo Characterization. The in vivo PK properties of (−)-**1**, (−)-**4**, and (−)-**5** were determined toward selecting a compound for evaluation for efficacy in a *S. aureus* infection study in the mouse and tolerability studies in the rat (Table 4). A correlation was seen in the rat data in that the more highly protein bound (−)-**4** and (−)-**5** were protected from clearance relative to (−)-**1** and, indeed, the free drug exposure on IV dosing of (−)-**4** and (−)-**5** in the rat calculate to about 8-fold higher than that of (−)-**1**. However, despite the lower f_u for (−)-**4** and (−)-**5** relative to (−)-**1**, clearances of the three compounds were comparable in the mouse. This, combined with the smaller MIC serum shift relative to MIC for (−)-**1** and the assumption that the shift with human serum translates to what might be seen in the mouse, made it the better candidate for profiling for in vivo efficacy. The compound otherwise showed promising oral bioavailability of 58 and 71% in the mouse and rat, respectively, supporting the capability for PO administration.

A mouse *S. aureus* (MSSA) thigh infection model was carried out in both immunocompetent and immunosuppressed mouse models via IP administration of 10−400 mg/kg single doses of (−)-**1** (Figure 5). Compound (−)-**1** was highly efficacious in a dose dependent fashion for both mouse models, offering a measure of in vivo validation of the scaffold series. An exposure (AUC or area under the curve) of AUC = 20 μg·h/mL resulted in no net change in CFU at the end of treatment when compared to the bacterial load at the start of treatment. A maximal response of 3-log relative to the untreated control was achieved at 70 μg·h/mL in the immunocompetent model. In a neutropenic (immunosuppressed) mouse model, a 3-log drop in CFU was seen at about 120 μg·h/mL, although higher doses to record a maximum response were not investigated. Accounting for free drug concentrations relative to PPB, the 3-log decreases in CFU correspond to 13 μmol·h/L for the immunocompetent mouse and 23 μg·h/L for the neutropenic mouse.

An oral tolerability study was conducted in Wistar Hannover rats with (−)-**1** as a single daily dose and multiple doses over 14 days. In the acute single dose phase, 1000 mg/kg was not tolerated, but doses up to 750 mg/kg were well tolerated. Dosing for 14 days was carried out at 250, 500, and 750 mg/kg. One animal at 750 mg/kg was prematurely sacrificed due to

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signs of toxicity (subdued behavior, ataxia), and no significant histopathogical findings were seen in two of three rats dosed at the 750 mg/kg and any of the 500 or 250 mg/kg dosed rats. In the one rat that was sacrificed, the most significant pathologic effects seen were marked apoptosis in the rapidly dividing tissue such as the gastrointestinal and hemopoietic cells. At high doses, fluoroquinolones are known to induce cell death via cell cycle arrest and mitochondrial membrane breakdown, an effect that may be related to the topoisomerase mode-of-inhibition of the class.[47] As seen with fluoroquinolones,[48] (−)-**1** inhibited human topoisomerase IIα with an IC_{50} = 50 μM, 170-fold higher than that for *E. coli* DNA gyrase. A NOEL of 500 mg/kg/day for (−)-**1** was established. Table 5 summarizes the toxicokinetic exposures of the drug in blood sample at day 14 from four treated rats at the three doses. Increasing the dose from 500 to 750 mg/kg did not proportionately increase drug exposure, likely due to solubility and/or absorption limit. On the basis of the NOEL, the C_{max} and AUC equaled 440 μmol/L and 4400 μmol·h/L, respectively, and taking f_u into account, fC_{max} and fAUC equaled 70 μmol/L and 700 μmol·h/L, respectively. The fAUC represents a 50-fold and 30-fold therapeutic index relative to the 3-log drop in CFU for the mouse immunocompetent and neutropenic models, respectively.

Table 5. Toxicokinetic Results (Day 14 of 14 Day Repeat Dosing) for (−)-1

dose (mg/kg)	no. of animals	C_{max} (μmol/L)	AUC (μmol·h/L)
250	4 (2 M, 2 F)	290 ± 150	2300 ± 970
500	4 (2 M, 2 F)	440 ± 82	4400 ± 890
750	4 (2 M, 2 F)	487 ± 180	4600 ± 1500

CONCLUSIONS

In summary, the therapeutic margin based on in vivo correlations from rodent models bodes well for this novel SPT chemotype and the continuation of optimization work toward identifying a drug candidate. Efficient synthetic procedures were devised to enable scale-up of large compound quantities (ultimately over 500 g of (−)-**1** were prepared for the biological studies) as well as to enable variation of substituents for SAR work. A novel mode-of-inhibition for DNA gyrase was demonstrated by no loss of susceptibility to bacteria resistant to fluoroquinolones and aminocoumarins, and the generation of spontaneous resistant mutants to (−)-**1** with GyrB mutations map to the DNA binding site and do not confer cross-resistance to fluoroquinolones. This is clearly important as it mitigates issues with DNA gyrase based pre-existing resistance. Activity against the Gram-positive bacteria *S. aureus* (including methicillin and fluoroquinolone resistant strains) and *S. pyogenes* bolstered the prospects for addressing skin and skin structure infections as did the demonstration of in vivo efficacy against *S. aureus*. Activity against *S. pneumoniae* and *H. influenzae* brings in the additional possibility for the treatment of respiratory tract infections such as community acquired bacterial pneumonia. A next-generation SPT compound would need improvements in the antibacterial activity and spectrum relative to (−)-**1**, in particular improving the activity against the Streptococci while maintaining favorable physicochemical, PK, and tolerability properties.

EXPERIMENTAL SECTION

General Considerations. All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions at 300 K using a Bruker Ultrashield 300 MHz instrument or a Bruker Ultrashield 400 MHz instrument. ^{13}C NMR spectra were recorded in DMSO-d_6 solutions at 300 K and 126 MHz using a Bruker DRX-500 500 MHz instrument with a QNP cryoprobe or at 101 MHz using a Bruker Ultrashield 400 MHz instrument or at 75.5 MHz using a Bruker Ultrashield 300 MHz instrument. ^{19}F NMR spectra were recorded at 282 MHz in $CDCl_3$ or DMSO-d_6 solutions at 300 K using a Bruker Ultrashield 300 MHz instrument. Chemical shifts are reported as parts per million relative to TMS (0.00) for ^{1}H and ^{13}C NMR and $CFCl_3$ for ^{19}F NMR. High-resolution mass spectra (HRMS) were obtained using a hybrid quadrupole time-of-flight mass spectrometer (microTOFq II, Bruker Daltonics) in ESI^+ mode. Silica gel chromatographies were performed on an ISCO Combiflash Companion instruments using ISCO RediSep flash cartridges (particle size: 35−70 μm) or Silicycle SiliaSep flash cartridges (particle size: 40−63 μm). Preparative reverse phase HPLC was carried out using YMC Pack ODS-AQ (100 mm × 20 mm ID, S-5 μ particle size, 12 nm pore size) on Agilent instruments. When not indicated, compound intermediates and reagents were purchased from chemical supply houses. All final compounds (compounds **1**−**12**) were determined to be greater than 95% pure via analysis by reversed phase UPLC-MS (retention times, RT, in minutes) was used with a Waters Acquity UPLC instrument with DAD and ELSD and a UPLC HSS T3, 2.1 mm × 30 mm, 1.8 μm column and a gradient of 2−98% acetonitrile in water with 0.1% formic acid over 2.0 min at 1 mL/min. Injection volume was 1 μL and the column temperature was 30 °C. Detection was based on electrospray ionization (ESI) in positive and negative polarity using a Waters ZQ mass spectrometer (Milford, MA, USA), diode-array UV detector from 210 to 400 nm, and evaporative light scattering detector (Sedex 75, Sedere, Alfortville Cedex, France).

rel-2-((2R,6S)-2,6-Dimethyl-morpholin-4-yl)-3,4-difluoro-benzoic Acid. LHMDS (1 M in THF, 156 mL, 156 mmol) was added to a stirred solution of 2,3,4-trifluorobenzoic acid (25.0 g, 142 mmol) in 250 mL of THF at −78 °C under N_2 and the solution stirred for 45 min. In a separate reaction flask, lithium bis(trimethylsilyl)amide (1 M in THF, 156 mL, 156 mmol) was added to a solution of *cis*-2,6-dimethylmorpholine (17.4 mL, 142 mmol) in 200 mL of THF and stirred for 45 min at −78 °C. This latter solution was added to the first solution, and stirring was continued for 1 h at −78 °C. Stirring was continued for an additional 12 h with warming to room temperature. Solvents were evaporated, and the residue was dissolved in EtOAc, which was washed with 1 N HCl, water, and brine. The organic layer was dried (Na_2SO_4) and concentrated to give the title compound as a semisolid. Yield: 27.5 g, (72%). UPLC (ESI) m/z $(M + H)^+$: 271.2 for $C_{13}H_{15}F_2NO_3$. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.2 (s, 6H), 2.9 (d, 2H), 3.1 (d, 2H), 3.9 (m, 2H), 7.2 (s, 1H), 7.3 (t, 1H), 8.1 (m, 1H).

rel-[2-[(2R,6S)-2,6-Dimethylmorpholin-4-yl]-3,4-difluoro-phenyl]-methanol. $NaBH_4$ (12.6 g, 359 mmol) was added portionwise to a stirred and ice-bath cooled solution of the previous compound in 250 mL of THF. Afterward, a solution of iodine (32.5 g, 139 mmol) in 250 mL of THF was added at a rate to maintain the internal temperature 10 °C. After the addition was complete, the mixture was brought to room temperature and heated at reflux for 12 h. It was cooled to room temperature and quenched with MeOH (250 mL). Solvents were evaporated and the residue treated with 2 M NaOH (500 mL) for 2 h. The mixture was extracted with EtOAc (3 × 150 mL), and the combined organic phases were washed with water and brine before being dried (Na_2SO_4) and concentrated to give the title compound as a gummy solid. UPLC (ESI) m/z $(M + H)^+$: 257.2 for $C_{13}H_{17}F_2NO_2$. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (s, 6H), 3.0 (d, 3H), 3.1 (d, 2H), 3.9 (m, 2H), 4.78 (s, 2H), 6.9 (d, 1H), 7.0 (t, 1H).

*rel-(2R,6S)-4-[6-({[tert-Butyl(diphenyl)silyl]oxy}methyl)-2,3-difluorophenyl]-2,6-dimethylmorpholine (**14a**).* Imidazole (8.5 g, 126 mmol) followed by *t*-butyl chlorodiphenylsilane (30 mL, 115 mmol) were added over a period of 15 min to an ice-cooled solution of the

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previous compound (27.0 g, 105 mmol) in CH_2Cl_2. The mixture was brought to room temperature and stirred for 12 h. The reaction mixture diluted with CH_2Cl_2 and washed successively with 1 N HCl (1 × 250 mL), water, and brine. The organic layer dried (Na_2SO_4), filtered, and concentrated. The residue was purified over a silica gel flash column using a gradient of EtOAc in petroleum ether to give the title compound as a white solid. Yield: 45 g (94%). UPLC (ES) MH^+: 495.6 for $C_{29}H_{35}F_2NO_2Si$. ^1H NMR (400 MHz, $CDCl_3$) δ 1.1 (s, 15 H), 2.6 (d, 2 H), 2.8 (m, 2H), 3.5 (t, 2H), 4.7 (s, 2H), 7.0 (q, 1H); 7.3 (t, 1H), 7.4 (m, 10H).

*rel-1-{5-({[tert-Butyl(diphenyl)silyl]oxy}methyl)-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluorophenyl}ethanone (**15a**).* *sec*-Butyllithium (1.4 M in cyclohexane, 66.4 mL, 93 mmol) was added to a stirred solution of **14a** (15.0 g, 30.0 mmol) in THF (150 mL) at −78 °C under N_2. After stirring for 1 h, *N*-methoxy-*N*-methyl-acetamide (3.4 mL, 45 mmol) was added and, after stirring for 30 min at −78 °C, the solution was allowed reach room temperature with stirring continuing for 12 h. The reaction mixture treated with saturated aqueous NH_4Cl solution and the aqueous layer extracted with EtOAc (2 × 100 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated. The residue was purified over a silica gel flash column using a gradient of EtOAc in petroleum ether to give the title compound as yellow solid. Yield: 13.2 g (82%). UPLC (ES) MH^+: 538.6 for $C_{31}H_{37}F_2NO_3Si$. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.1 (s, 15H), 2.8 (m, 4H), 3.4 (m, 2H), 4.6 (s, 2H), 7.3 (t, 4H), 7.4 (t, 2H), 7.6 (d, 4H), 7.8 (s, 1H), 10.2 (s, 1H).

rel-1-(5-((tert-Butyldiphenylsilyloxy)methyl)-4-((2R,6S)-2,6-dimethylmorpholino)-3-fluoro-2-(propan-2-ylideneaminooxy)phenyl)-ethanone. Propan-2-one oxime (913 mg, 12.50 mmol) in 15 mL of THF was treated with KOtBu (935 mg, 8.33 mmol) at room temperature for 45 min. A solution of **15a** (2.24 g, 4.17 mmol) in 10 mL of THF was added, and the mixture was stirred at rt for 3 h. After quenching with satd aqueous NH_4Cl, the mixture was extracted with EtOAc. The EtOAc was washed with water and brine, dried (Na_2SO_4), and concentrated to give the title compound (2.5 g, 100%). UPLC (ES) MH^+: 591.3 for $C_{34}H_{43}FN_2O_4Si$; used in the next step without any further purification.

rel-1-(5-((tert-Butyldiphenylsilyloxy)methyl)-4-((2R,6S)-2,6-dimethylmorpholino)-3-fluoro-2-(propan-2-ylideneaminooxy)phenyl)-ethanone. A solution of the preceding compound (2.4 g, 4.06 mmol) in 30 mL of EtOH was treated with 10 mL of 10% aq HCl at 75 °C for 2 h. After cooling to rt, the mixture was diluted with EtOAc and washed wtih 10% aqueous $NaHCO_3$. The organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (35−55% EtOAc gradient in hexanes) to afford 0.65 g (42%) of the title compound. UPLC (ES) MH^+: 295 for $C_{15}H_{19}FN_2O_3$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, J = 6.4 Hz, 6H) 2.55 (s, 3H) 2.9−3.2 (m, 4H) 3.7−4.0 (m, 2H) 4.82 (s, 2H) 7.31 (s, 1H).

*rel-6-((2R,6S)-2,6-Dimethylmorpholino)-7-fluoro-3-methylbenzo-[d]isoxazole-5-carbaldehyde (**16a**).* A mixture of the preceding compound (0.65 g, 2.21 mmol) and MnO_2 (3.84 g, 44.2 mmol) in 30 mL of CH_2Cl_2 was stirred at rt for 3 d. The MnO_2 was filtered and rinsed through with CH_2Cl_2. The filtrate was concentrated and dried in vacuo to give the title compound (0.547 g, 85%) that was used in the next step without further purification. UPLC (ES) MH^+: 293 for $C_{15}H_{17}FN_2O_3$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, J = 6.2 Hz, 6H) 2.58 (s, 3H) 2.9−3.25 (m, 4H) 3.7−4.0 (m, 2H) 7.94 (s, 1H) 10.38 (s, 1H).

*rel-(2R,4S,4aS)-11-Fluoro-2,4,8-trimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (±)-**1**.* A solution of **16a** (543 mg, 1.86 mmol) and barbituric acid (238 mg, 1.86 mmol) in 50 mL of *i*-PrOH was heated at 80 °C for 16 h. Solvent was removed, and the residue was chromatographed on silica gel (EtOAc eluent) to afford 528 mg (71%) of the title compound as a white solid. UPLC RT = 0.90 min. (ES) MH^+: 403.0 for $C_{19}H_{19}FN_4O_5$. ^1H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H), 11.44 (s, 1H), 7.17 (s, 1H), 2.90−4.2 (m, 7H), 2.41 (s, 3H), 1.13 (d, J = 6.1 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H).

The enantiomers of 528 mg of (±)-**1** were separated by SFC on a Chiralpak AD column (21 mm × 250 mm, 5 μ), 80% CO_2, 20% MeOH, 0.1% dimethylethylamine, 40 °C, 100 bar. The first eluting compound (+)-**1** (202 mg, 40%) was consistent with (2*S*,4*R*,4a*R*)-11-fluoro-2,4,8-trimethyl-2,4,4a,6-tetrahydro-1*H*,1′*H*-spiro[isoxazolo[4,5-*g*][1,4]oxazino[4,3-*a*]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′*H*)-trione (+)-**1**. UPLC RT = 0.90 min. (ES) MH^+: 403.0 for $C_{19}H_{19}FN_4O_5$. ^1H NMR (300 MHz, DMSO-d_6) δ 11.9 (s, 1H), 11.5 (s, 1H), 7.18 (s, 1H), 4.09 (d, J = 13.0 Hz, 1H), 3.94 (d, J = 8.9 Hz, 1H), 3.74−3.87 (m, 1H), 3.61−3.73 (m, 1H), 3.5−3.6 (m, 1H), 3.09 (t, J = 12.6 Hz, 1H), 2.96 (d, J = 14.0 Hz, 1H), 2.42 (s, 3H), 1.14 (d, J = 6.2 Hz, 3H), 0.89 (d, J = 6.4 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.1. ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.6, 154.6, 151.5 (d, J_{CF} = 12.1 Hz), 149.4, 134.5 (d, J_{CF} = 2.7 Hz), 133.8 (d, J_{CF} = 240 Hz), 122.6 (d, J_{CF} = 1.6 Hz), 114.9 (d, J_{CF} = 2.7 Hz), 114.3, 72.0, 71.7, 64.4, 56.4 (d, J_{CF} = 9.3 Hz), 53.1, 38.7, 18.2, 18.1, 9.3. HRMS (ES) MH^+ calcd for $C_{19}H_{20}FN_4O_5$ 403.1412, found 403.1421; [α] = +296 (c = 0.1 in MeOH); >98% ee by SFC chiral analysis.

The second eluting compound (199 mg, 39%) was consistent with (2*R*,4*S*,4a*S*)-11-fluoro-2,4,8-trimethyl-2,4,4a,6-tetrahydro-1*H*,1′*H*-spiro[isoxazolo[4,5-*g*][1,4]oxazino[4,3-*a*]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′*H*)-trione (−)-**1**. UPLC RT = 0.90 min. (ES) MH^+: 403.0 for $C_{19}H_{19}FN_4O_5$. ^1H NMR (300 MHz, DMSO-d_6) δ 11.8 (s, 1H), 11.4 (s, 1H), 7.17 (s, 1H), 4.09 (d, J = 13.0 Hz, 1H), 3.94 (d, J = 8.9 Hz, 1H), 3.74−3.87 (m, 1H), 3.61−3.73 (m, 1H), 3.49−3.58 (m, 1H), 3.09 (t, J = 12.6 Hz, 1H), 2.96 (d, J = 14.0 Hz, 1H), 2.42 (s, 3H), 1.14 (d, J = 6.2 Hz, 3H), 0.89 (d, J = 6.4 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.1. ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.6, 154.6, 151.5 (d, J_{CF} = 11.7 Hz), 149.4, 134.5 (d, J_{CF} = 2.2 Hz), 133.8 (d, J_{CF} = 240 Hz), 122.6, 114.9, 114.3, 72.0, 71.7, 64.4, 56.4 (d, J_{CF} = 8.8 Hz), 53.1, 38.8, 18.2, 18.1, 9.3. HRMS (ES) MH^+ calcd for $C_{19}H_{20}FN_4O_5$ 403.1412, found 403.1393; [α] = −291 (c = 0.1 in MeOH), >98% ee by SFC chiral analysis.

*(2S,4S,4aS)-11-Fluoro-2,4,8-trimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**2**.* A mixture of (+)-**1** (320 mg, 0.80 mmol) in 1.2 mL of EtOH and 0.8 mL of water was heated at 150 °C in a microwave reactor for 5 min. After cooling to rt and then to 4 °C overnight, precipitated white solids were collected, rinsed with cold 60% EtOH, and dried in vacuo at 80 °C to afford 175 mg of material identical to starting (+)-**1**. The mother liquor was concentrated and purified by reverse phase HPLC (Waters Xbridge C18 column, 45−60% gradient of CH_3CN/10 mM aqueous NH_4OAc) to afford two materials. The first eluting material afforded additional (+)-**1** and the second eluting material afforded 14 mg (11%) of the title compound as a white solid. UPLC RT = 0.87 min. MH^+: 403.0 for $C_{19}H_{19}FN_4O_5$. ^1H NMR (300 MHz, DMSO-d_6) δ 10.80 (br s, 2 H), 7.23 (s, 1 H), 3.93−4.19 (m, 2H), 3.75 (d, J = 13.2 Hz, 1 H), 3.67 (d, J = 6.8 Hz, 1 H), 3.42−3.62 (m, 2 H), 3.16 (d, J = 15.3 Hz, 1 H), 2.44 (s, 3 H), 1.27 (d, J = 6.0 Hz, 3 H), 1.00 (d, J = 6.2 Hz, 3 H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −152.5. ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.2, 169.2, 154.7, 150.7 (d, J_{CF} = 12.4 Hz), 149.8, 135.4 (d, J_{CF} = 2.2 Hz), 134.6 (d, J_{CF} = 242.2 Hz), 122.7, 115.2 (d, J_{CF} = 5.1 Hz), 66.2, 65.3, 64.8, 52.9 (d, J_{CF} = 9.5 Hz), 50.9, 37.4, 18.7, 16.9, 9.3. HRMS (ES) MH^+ calcd for $C_{19}H_{20}FN_4O_5$ 403.1412, found 403.1411; [α] = −164 (c = 0.1 in MeOH).

2-((2R,6R)-2,6-Dimethylmorpholino)-3,4-difluorobenzoic Acid. LHMDS (1M, 622 mL, 0.622 mol) in THF was added dropwise to a solution of 2,3,4-trifluorobenzoic acid (100 g, 0.56 mol) in anhydrous THF (500 mL) cooled to −78 °C, maintaining the temperature below −65 °C, and the mixture was stirred at −78 °C for 45 min. In a separate flask, LHMDS (1M, 622 mL, 0.622 mol) in THF was added dropwise to a solution of (2*R*,6*R*)-2,6-dimethylmorpholine (67.0 g, 0.56 mol) in THF (210 mL) cooled to −78 °C, maintaining the temperature below −65 °C, and the mixture was stirred at −78 °C for 45 min. The contents of the second flask were then transferred via cannula to the first flask over 30 min, stirring was continued for 1 h at −78 °C, and the mixture was warmed to rt with stirring for 18 h. The solvent was removed, and the residue was poured into 1 N HCl and extracted with EtOAc (3 × 750 mL). The combined organic extracts were washed with water (1 L) and brine (1 L), dried (Na_2SO_4), and concentrated to give the title compound (131.4 g, 85%) as a solid.

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UPLC (ES) MH^+: 272 for $C_{13}H_{15}F_2NO_3$. ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (d, J = 6.4 Hz, 6 H) 2.84 (dd, J = 11.5, 5.5 Hz, 2 H) 3.20 (d, J = 11.3 Hz, 2 H) 3.86−4.19 (m, 2 H) 7.14−7.40 (m, 1 H) 7.44−7.61 (m, 1 H) 14.14 (s, 1 H).

(2-((2R,6R)-2,6-Dimethylmorpholino)-3,4-difluorophenyl)-methanol. $NaBH_4$ (73.1 g, 1.93 mol) was added portionwise to a solution of the previous compound (131.0 g, 0.483 mol) in 400 mL of THF at 0 °C. A solution of iodine (183 g, 0.724 mol) in 1 L of THF was added dropwise, keeping the temperature below 10 °C. The reaction mixture was then heated at reflux for 18 h. After cooling to rt, the reaction was quenched with MeOH (370 mL), the solvent was removed, and the residue was suspended in 2N NaOH (1.5 L) with stirring at rt for 1.5 h. The mixture was extracted with EtOAc (3 × 1 L). The combined organic extracts were washed with brine (1.5 L), dried over anhydrous Na_2SO_4, filtered, and evaporated to yield the title compound (107 g, 86%) as colorless liquid. UPLC (ES) MH^+: 258 for $C_{13}H_{17}F_2NO_2$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (d, J = 5.8 Hz, 6 H) 2.73−3.41 (m, 4 H) 4.00−4.29 (m, 2 H) 4.60−4.89 (m, 2 H) 6.78−7.19 (m, 2 H).

*(2R,6R)-4-(6-((tert-Butyldiphenylsilyloxy)methyl)-2,3-difluorophenyl)-2,6-dimethylmorpholine (**14b**).* A solution of *tert*-butylchlorodiphenylsilane (125.3 g, 0.457 mol) in 100 mL of CH_2Cl_2 was added dropwise to a solution of the preceding compound (107 g, 0.416 mol) and imidazole (33.95 g, 0.499 mol) in 1 L of CH_2Cl_2 at 0 °C before stirring at rt for 16 h. The reaction mixture was poured into 1 N HCl (1 L), the layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (500 mL), water (500 mL), and brine (500 mL) before being dried (Na_2SO_4). Removal of solvent was followed by column chromatography on silica gel (3:97 EtOAc: petroleum ether) to afford the title compound (158 g, 83%) as a yellow oil. UPLC (ES) MH^+: 496 for $C_{29}H_{35}F_2NO_2Si$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.57−7.76 (m, 4H), 7.3−7.5 (m, 7H), 6.9−7.1 (m, 1H), 4.6−4.9 (m, 2H), 3.7−4.0 (m, 2H), 2.8−3.15 (m, 2H), 2.44−2.77 (m, 2H), 1.09 (s, 9H), 1.04 (d, J = 6.4 Hz, 6H).

*1-(5-((tert-Butyldiphenylsilyloxy)methyl)-4-((2R,6R)-2,6-dimethylmorpholino)-2,3-difluorophenyl)ethanone (**15b**).* Under N_2, *sec*-butyllithium (1.3 M in cyclohexane/hexane, 69.8 mL, 90.89 mmol) in 20 mL of MBTE was added via cannula to a solution of **14b** (18 g, 36.31 mmol) in 80 mL of MTBE cooled to −75 °C at a rate to keep the temperature below −70 °C. The resultant slurry was stirred for 1 h before *N*-methoxy-*N*-methylacetamide (9.74 g, 94.4 mmol) was added dropwise, keeping the internal reaction temperature above −60 °C. After stirring cold for 1 h, the reaction slurry was quenched with 200 mL of saturated aqueous NH_4Cl. The mixture was warmed to rt and extracted with EtOAc. The EtOAc was washed with brine, dried (Na_2SO_4), and concentrated to give a an oil that was chromatographed on silica gel (5% EtOAc in hexanes) to afford the title compound as an oil (17.4 g, 89%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, J = 7.7 Hz, 1H), 7.53−7.64 (m, 4H), 7.26−7.39 (m, 6H), 4.7 (d, J = 13.9 Hz, 1H), 4.62 (d, J = 13.9 Hz, 1H), 3.74−3.9 (m, 2H), 2.98 (d, J = 11.3 Hz, 2H), 2.61 (dd, J = 5.4, 11.4 Hz, 2H), 2.55 and 2.53 (2s, 3H), 1.03 (s, 9H), 0.98 (d, J = 6.4 Hz, 6H).

(6-((2R,6R)-2,6-Dimethylmorpholino)-7-fluoro-3-methylbenzo[d]-isoxazol-5-yl)methanol. Potassium *t*-butoxide (1.0 M in THF, 61.3 mL, 61.3 mmol) was added under N_2 to a solution of acetone oxime (4.48 g, 61.3 mmol) in 40 mL of THF at 0 °C. The resulting slurry was warmed to rt and stirred for 30 min. After cooling to 0 °C, a solution of the **15b** (23.6 g, 43.8 mmol) in 10 mL of THF was added, and the resulting yellow slurry was warmed to rt with stirring over 3.5 h. The reaction mixture was quenched with aqueous NH_4Cl and extracted with EtOAc (2×). The EtOAc was washed with brine, dried (Na_2SO_4), and concentrated to give dark-orange oil. The oil was dissolved 180 mL of EtOH, and 65.7 mL of 1 M HCl was added. The mixture was heated at 70 °C for 3 h, and after cooling to rt, EtOH solvent was removed. The aqueous residue neutralized with saturated aqueous $NaHCO_3$ and extracted twice with EtOAc. The combined organic extract were washed with brine, dried (Na_2SO_4), and concentrated to give an oil that was chromatographed on silica gel (5−25% gradient of EtOAc in hexanes) to afford the title compound as yellow solid (7.7 g, 60%). UPLC (ES) MH^+: 295.03 for $C_{15}H_{19}FN_2O_3$. ^1H NMR (300 MHz, DMSO-d_6) δ 7.65 (s, 1H), 5.36 (t, J = 5.3 Hz, 1H), 4.71 (t, J = 4.5 Hz, 2H), 4.04 (dd, J = 5.8, 3.2 Hz, 2H), 3.18 (d, J = 0.75 Hz, 1H), 3.14 (br s, 1H), 2.80 (dd, J = 11.3, 5.65 Hz, 2H), 2.55 (m, 4H), 1.23 (d, J = 5.8 Hz, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −145.1.

*6-((2R,6R)-2,6-Dimethylmorpholino)-7-fluoro-3-methylbenzo[d]-isoxazole-5-carbaldehyde (**16b**).* A mixture of the preceding compound (13.0 g, 44.2 mmol) and MnO_2 (26.9 g, 309 mmol) in 400 mL of CH_2Cl_2 was stirred for 3 days at rt. The reaction was incomplete by TLC, additional MnO_2 (11.5 g) was added, and the mixture was stirred at rt overnight. The reaction mixture was filtered through Celite, rinsing through with CH_2Cl_2 and with 15% *i*-PrOH in dichloromethane. The combined filtrate was concentrated to afford the title compound as a yellow oil (11g, 85%). ^1H NMR (300 MHz, $CDCl_3$) δ 10.51 (s, 1H), 7.96 (s, 1H), 5.31 (s, 1H), 4.23 (dt, J = 3.2, 6.1 Hz, 2H), 3.41 (d, J = 11.68 Hz, 2H), 3.04 (dd, J = 5.65, 11.7 Hz, 2H), 1.34 (d, J = 6.4 Hz, 6H).

*(2R,4S,4aS)-11-Fluoro-2,4,8-trimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**1**.* A mixture of **16b** (655 mg, 2.24 mmol) and barbituric acid (287 mg, 2.24 mmol) in 3 mL of EtOH and 1 mL of water was heated at 120 °C for 1 h in a microwave reactor. UPLC analysis of the reaction mixture showed and 9:1 mixture of materials. The resultant solution was chilled in a freezer at −10 °C overnight, during which solids precipitated. The solids were filtered, rinsed with cold 3:1 EtOH−water, and dried under vacuum at 50 °C overnight to afford 647 mg (72%) of the title compound. The filtrate was concentrated, and the residue was purified by SFC on an (S,S) Whelk-O1 column (21 mm × 250 mm, 5 μ), 85% CO_2, 15% MeOH, 40 °C, 150 bar to separate two diastereomers. The analytical data for the major diastereomer (74 mg, 8%) and the precipitated solids was consistent with (−)-**1** isolated via the racemic route. UPLC RT = 0.91 min. (ES) MH^+: 403.0 for $C_{19}H_{19}FN_4O_5$. ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.1. ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.6, 154.6, 151.5 (d, J_{CF} = 12.1 Hz), 149.4, 134.5 (d, J_{CF} = 2.2 Hz), 133.8 (d, J_{CF} = 239.9 Hz), 122.6 (d, J_{CF} = 1.1 Hz), 122.4, 114.9 (d, J_{CF} = 3.3 Hz), 114.3, 72.0, 71.7, 64.4, 56.4 (d, J_{CF} = 9.3 Hz), 53.1, 38.7, 18.2, 18.1, 9.3. HRMS (ES) MH^+ calcd for $C_{19}H_{20}FN_4O_5$ 403.1412, found 403.1422; [α] = −297 (c = 0.1 in MeOH); >98% ee by SFC chiral analysis. The minor diastereomer (51 mg, 6%) was consistent with (2*R*,4*R*,4a*R*)-11-fluoro-2,4,8-trimethyl-2,4,4a,6-tetrahydro-1*H*,1′*H*-spiro[isoxazolo[4,5-*g*][1,4]oxazino[4,3-*a*]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′*H*)-trione (+)-**2**. UPLC RT = 0.88 min. (ES) MH^+: 403.0 for $C_{19}H_{19}FN_4O_5$. ^1H NMR (400 MHz, DMSO-d_6) δ 11.4 (br s, 2H), 7.23 (s, 1H), 4.05−4.15 (m, 1H), 4.00 (quin, J = 6.4 Hz, 1H), 3.74 (dd, J = 3.9, 13.2 Hz, 1H), 3.66 (d, J = 6.8 Hz, 1H), 3.45−3.6 (m, 2H), 3.17 (d, J = 14.8 Hz, 1H), 2.44 (s, 3H), 1.26 (d, J = 5.5 Hz, 3H), 1.01 (d, J = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.4, 169.3, 154.7, 150.7 (d, J_{CF} = 11.7 Hz), 150.0, 135.4 (d, J_{CF} = 2.2 Hz), 134.5 (d, J_{CF} = 242.2 Hz), 122.7, 115.2, 115.1 (d, J_{CF} = 3.7 Hz), 66.2, 65.3, 64.8, 52.9 (d, J_{CF} = 9.5 Hz), 50.8, 37.5, 18.7, 16.9, 9.3. ^{19}F NMR (282 MHz, DMSO-d_6) δ −152.3. HRMS (ES) MH^+ calcd for $C_{19}H_{20}FN_4O_5$ 403.1412, found 403.1411; [α] = +148 (c = 0.1 in MeOH).

rel-3-Chloro-2-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-4-fluorobenzoic Acid. The title compound was prepared as described for 2-((2*R*,6*S*)-2,6-dimethyl-morpholin-4-yl)-3,4-difluoro-benzoic acid using 4.4 g (22.8 mmol) of 3-chloro-2,4-difluorobenzoic acid and 2.8 mL (22.8 mmol) of *cis*-2,6-dimethylmorpholine and 2 × 25 mL (25.1 mmol) LHMDS to afford 5.2 g (80%) of product. MS (ES) MH^+: 288.0, 289.8 for $C_{13}H_{15}ClFNO_3$. ^1H NMR (300 MHz, DMSO-d_6) δ 13.7 (br s, 1H), 7.60 (t, J = 7.54 Hz, 1H), 7.23 (t, J = 8.57 Hz, 1H), 3.60−3.93 (m, 2H), 2.74−3.03 (m, 4H), 1.08 (d, J = 6.22 Hz, 6H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −107.7.

rel-Methyl 3-Chloro-2-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-4-fluorobenzoate. A solution of the preceding compound (10.0 g, 34.8 mmol) and 1 mL of conc H_2SO_4 in 50 mL of methanol was heated at reflux for 12 h. The reaction mixture was concentrated and diluted with EtOAc. The organic layer was washed with H_2O (2 × 20 mL), dried (Na_2SO_4), and concentrated. The residue purified by silica gel chromatography (EtOAc−petroleum ether gradient) to obtain the

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title compound as a white solid. Yield: 9.0 g (86%). MS (ES) MH^+: 301.9, 302.8 for $C_{14}H_{17}ClFNO_3$. ^1H NMR (300 MHz, CD_2Cl_2) δ 7.36 (dd, J = 6.2, 8.7 Hz, 1H), 6.85 (t, J = 8.4 Hz, 1H), 3.81 (s, 3H), 3.73 (ddd, J = 2.5, 6.4, 9.3 Hz, 2H), 2.81−2.96 (m, 2H), 2.62−2.80 (m, 2H), 1.04 (d, J = 6.2 Hz, 6H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −107.8.

rel-Methyl 5-Acetyl-3-chloro-2-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-4-fluorobenzoate. A solution of the preceding compound (1.5 g, 4.96 mmol) in 10 mL of anhydrous THF was added dropwise to a stirred solution of LDA (3.1 equiv) at −50 °C in THF, and the solution was stirred for 1 h at −50 °C. *N*-Methoxy-*N*-methylacetamide (1.66 g, 14.9 mmol) in THF (5 mL) was added dropwise, and stirring was continued for 1 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4). Solvent was removed, and the residue was purified by silica gel chromatography (EtOAc−petroleum ether gradient) to obtain 800 mg (47%) of the title compound. MS (ES) MH^+: 344 for $C_{16}H_{19}ClFNO_4$.

rel-Methyl-3-chloro-2-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-4-fluoro-5-[N-hydroxyethanimidoyl]benzoate. Hydroxylamine hydrochloride (223 mg, 3.48 mmol) was added to a solution of the preceding compound (800 mg, 2.32 mmol) in 6 mL of methanol:pyridine (1:1), and the mixture was stirred at rt for 12 h. Solvents were removed under vacuum, and the residue was chromatographed on silica gel (EtOAc−petroleum ether gradient) to give 700 mg (87%) of the title compound. MS (ES) MH^+: 359 for $C_{16}H_{20}ClFN_2O_4$.

rel-7-Chloro-6-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-3-methyl-1,2-benzoxazole-5-carboxylic Acid. A solution of the preceding compound (700 mg, 1.94 mmol) and Cs_2CO_3 (1.9 g, 5.84 mmol) in DMF (4 mL) was heated at 130 °C for 14 h. The reaction mixture was cooled to rt and filtered through Celite rinsing through with EtOAc. The solvent was removed under vacuum, and the residue was purified by silica gel chromatography (EtOAc−petroleum ether gradient) to afford the title compound. Yield: 250 mg, (41%). MS (ES) MH^+: 335 for $C_{15}H_{17}ClN_2O_4$.

rel-[7-Chloro-6-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-3-methyl-1,2-benzoxazol-5-yl]methanol. BF_3 (1.6 mL, 2.59 mmol) was added to a stirred solution of $NaBH_4$ (78 mg, 2.43 mmol) in 1 mL of diglyme, and the generated diborane gas was purged into solution of the preceding compound (250 mg, 0.8 mmol) in THF (1 mL). The mixture was stirred at rt for 30 min before being quenched with methanol (1 mL) and concentrated. The residue was purified by silica gel chromatography (ethyl acetate−petroleum ether gradient) to give the title compound as a solid. Yield: 130 mg (52%). MS (ES) MH^+: 311 for $C_{15}H_{19}ClN_2O_3$.

rel-7-Chloro-6-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-3-methyl-1,2-benzoxazole-5-carbaldehyde. NMO (103 mg, 8.8 mmol) and TPAP (31 mg, 0.88 mmol) were added to a solution of the preceding compound (130 mg, 4.4 mmol) in 5 mL of anhydrous CH_2Cl_2 was added at 0 °C, and the mixture was warmed to rt with stirring for 1 h. The reaction mixture was filtered, the solvents were removed under vacuum, and the residue was purified by silica gel chromatography (EtOAc−petroleum ether gradient) to give the title compound as a yellow solid. Yield: 35 mg, (27%). MS (ES) MH^+: 309 for $C_{15}H_{17}ClN_2O_3$.

*rel-(2R,4S,4aS)-11-Chloro-2,4,8-trimethyl-1,2,4,4a-tetrahydro-2′H,6H-spiro[1,4-oxazino[4,3-a][1,2]oxazolo[4,5-g]quinoline-5,5′-pyrimidine]-2′,4′,6′(1′H,3′H)-trione (±)-**3**.* A solution of the preceding compound (35 mg, 0.12 mmol) and barbituric acid (16 mg, 0.113 mmol) in *i*-PrOH was heated at reflux for 16 h. Solvents were evaporated, and the residue was chromatographed on silica gel (gradient of MeOH in $CHCl_3$) to give the title compound as a solid. Yield: 10 mg (25%). UPLC RT = 0.93 min. (ES) MH^+: 419.3, 421.3 for $C_{19}H_{19}ClN_4O_5$. ^1H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 11.45 (s, 1H), 7.30 (s, 1H), 4.49 (d, J = 12.80 Hz, 1H), 3.9−4.05 (m, 2H), 3.6−3.7 (m, 1H), 3.54 (d, J = 14.3 Hz, 1H), 3.0−3.10 (m, 2H), 2.42 (s, 3H), 1.17 (d, J = 6.02 Hz, 3H), 0.90 (d, J = 6.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.7, 167.6, 160.8, 155.2, 149.5, 144.1, 122.4, 118.0, 113.0, 97.7, 72.6, 72.5, 65.8, 56.4, 52.8, 40.1, 18.2, 18.1, 9.5. HRMS (ES) MH^+ calcd for $C_{19}H_{20}ClN_4O_5$ 419.1117, found 419.1130.

rel-1-(5-((tert-Butyldiphenylsilyloxy)methyl)-4-((2R,6S)-2,6-dimethylmorpholino)-2,3-difluorophenyl)-2,2,2-trifluoroethanone. *sec*-Butyllithium (1.3 M in hexane, 11.5 mL, 15.0 mmol) was added to a stirred solution of **14a** (2.478 g, 5 mmol) in 10 mL of THF at −78 °C under N_2. After stirring for 1 h at −78 °C, 2,2,2-trifluoro-*N*-methoxy-*N*-methylacetamide (3.14 g, 20.0 mmol) was added. After stirring for 30 min at −78 °C, the solution was warmed to rt. The reaction was quenched with satd aqueous NH_4Cl and extracted with EtOAc. The EtOAc was dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (30−70% CH_2Cl_2 in hexanes gradient) to afford the title compound. MS (ES) MH^+: 610 for $C_{31}H_{34}F_5NO_3Si{\cdot}H_2O$ (hydrate form). ^1H NMR (300 MHz, $CDCl_3$) δ 7.64−7.70 (m, 4H), 7.4−7.5 (m, 7H), 4.65 (s, 2H), 3.4−3.6 (m, 2H), 2.8−2.9 (m, 4H), 1.12 (s, 9H), 1.10 (s, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −73.7, −134.0, −146.1.

1-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-2,2,2-trifluoro-ethanone Oxime. A mixture of the preceding compound (1.9 g, 3.2 mmol), pyridine (2.6 mL, 32 mmol), and hydroxylamine hydrochloride (223 mg, 3.21 mmol) in 50 mL of ethanol was heated at 80 °C for 40 h. The mixture was concentrated, diluted with water, and extracted with EtOAc. The organic phases were dried (Na_2SO_4) and concentrated to give the title compound (1.95 g, 98%), which was used in the next step without further purification. MS (ES) MH^+: 607 for $C_{31}H_{35}F_5N_2O_3Si$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.03−1.15 (m, 15H), 2.56−2.91 (m, 4H), 3.33−3.59 (m, 2H), 4.58−4.80 (m, 2H), 7.27−7.51 (m, 7H), 7.57−7.74 (m, 4H).

rel-6-((2R,6S)-2,6-Dimethylmorpholino)-7-fluoro-3-(trifluoromethyl)benzo[d]isoxazol-5-yl)methanol. A mixture of the preceding compound (1.9 g, 3.13 mmol) and $CsCO_3$ (5.10 g, 15.66 mmol) in 20 mL of DMF was heated at 60 °C for 4 h. Solids were filtered off and rinsed through with EtOAc. The filtrate was diluted with water, and the organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by chromatography on silica gel (20−35% EtOAc in hexanes) to afford the title compound (0.460 g, 42.2%). MS (ES) MH^+: 349 for $C_{15}H_{16}F_4N_2O_3$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 4.86 (s, 2H), 3.73−3.96 (m, 3H), 3.04−3.14 (m, 2H), 2.92−2.99 (m, 2H), 1.26 (d, J = 6.2 Hz, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −62.3, −140.8.

rel-6-((2R,6S)-2,6-Dimethylmorpholino)-7-fluoro-3-(trifluoromethyl)benzo[d]isoxazole-5-carbaldehyde. A mixture of the preceding compound (460 mg, 1.32 mmol) and MnO_2 (4.59 g, 52.8 mmol) in 50 mL of CH_2Cl_2 was stirred at rt for 3 d. The MnO_2 was filtered off and rinsed through with CH_2Cl_2. The filtrate was concentrated and chromatographed on silica gel (20% EtOAc in hexanes) to give the title compound (260 mg, 56.9%). MS (ES) MH^+: 347 for $C_{15}H_{14}F_4N_2O_3$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.3 (s, 1H), 8.06 (s, 1H), 3.7−4.0 (m, 2H), 3.0−3.3 (m, 4H), 1.23 (d, J = 6.2 Hz, 6H).

*(2R,4S,4aS)-11-Fluoro-2,4-dimethyl-8-(trifluoromethyl)-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]-quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (±)-**4**.* A mixture of the preceding compound (255 mg, 0.74 mmol) and barbituric acid (104 mg, 0.81 mmol) in 10 mL of *i*-PrOH was heated at reflux for 3 days. Solvent was removed, and the solid residue was triturated with MeOH. The solids were collected by filtration to afford give 220 mg of the title compound. The mother liquor concentrated and purified on silica gel (35−50% EtOAc gradient in CH_2Cl_2) to get afford additional (122 mg) material. Total yield: 322 mg, 96%. MS (ES) MH^+: 457 for $C_{19}H_{16}F_4N_4O_5$. ^1H NMR (300 MHz, DMSO-d_6) δ 11.9 (s, 1H), 11.6 (s, 1H), 7.35 (s, 1H), 3.54−4.28 (m, 5H), 2.86−3.24 (m, 2H), 1.14 (d, J = 6.0 Hz, 3H), 0.89 (d, J = 6.4 Hz, 3H)

The (2*S*,4*R*,4a*R*) and (2*R*,4*S*,4a*S*) enantiomers were separated by SFC using a Chiralpak AD, 21 mm × 250 mm, 5 μ column (elution with 25% MeOH, 75% CO_2 at 60 mL/min, 40 °C, and 100 bar with detection at 220 nm), providing (+)-**4** and (−)-**4**:

(2S,4R,4aR)-11-Fluoro-2,4-dimethyl-8-(trifluoromethyl)-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]-

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*quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (+)-**4***. First eluting compound, 129 mg (38%). UPLC RT = 1.12 min. (ES) MH^+: 457.0 for $C_{19}H_{16}F_4N_4O_5$. ^1H NMR (300 MHz, DMSO-d_6) δ 0.89 (d, J = 5.5 Hz, 3H), 1.14 (d, J = 5.1 Hz, 3H), 2.83−3.21 (m, 2H), 3.57−4.23 (m, 5H), 7.34 (s, 1H), 11.72 (s, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −61.6, −155.9; >98% ee by chiral HPLC; [α] = +285 (c = 0.1 in MeOH).

*(2R,4S,4aS)-11-Fluoro-2,4-dimethyl-8-(trifluoromethyl)-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]-quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**4***. Second eluting compound, 124 mg (37%). UPLC RT = 1.12 min. (ES) MH^+: 457.0 for $C_{19}H_{16}F_4N_4O_5$. ^1H NMR (300 MHz, DMSO-d_6) δ 0.89 (d, J = 6.2 Hz, 3H), 1.14 (d, J = 6.0 Hz, 3H), 2.89−3.25 (m, 2H), 3.56−4.30 (m, 5H), 7.34 (s, 1H), 11.7 (s, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −61.6, −155.9. ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.7, 167.7, 153.6 (d, J_{CF} = 12.4 Hz), 149.4, 148.5 (q, J_{CF} = 37.6 Hz), 136.2, 133.1 (d, J_{CF} = 241.5 Hz), 126.3 (d, J_{CF} = 2.2 Hz), 119.9 (q, J_{CF} = 272.5 Hz), 113.8, 108.0, 72.1, 71.7, 64.5, 56.3 (d, J_{CF} = 9.5 Hz), 52.7, 38.1, 18.1, 18.1; >98% ee by chiral HPLC; [α] = −305 (c = 0.1 in MeOH). HRMS (ES) MH^+ calcd for $C_{19}H_{17}F_4N_4O_5$ 457.1130, found 457.1152.

1-(5-((tert-Butyldiphenylsilyloxy)methyl)-4-((2R,6R)-2,6-dimethylmorpholino)-2,3-difluorophenyl)-2,2-difluoroethanone. *sec*-Butyllithium (1.3 M in hexane, 4.7 mL, 6.1 mmol) was added to a stirred solution of **14b** (1.5 g, 3.03 mmol) in 15 mL of THF at −70 °C under N_2. After stirring for 1.5 h at this temperature, 2,2-difluoro-*N*-methoxy-*N*-methylacetamide (1.26 g, 9.08 mmol) was added. After stirring for 30 min at −78 °C, the reaction was quenched with satd aqueous NH_4Cl and warmed to rt. The mixture was extracted with EtOAc, which was dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (10% EtOAc in hexanes) to afford 1.54 g (89%) of the title compound. UPLC (ES) MH^+: 574 for $C_{31}H_{35}F_4NO_3Si$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.60−7.74 (m, 4H), 7.36−7.52 (m, 6H), 6.38 (td, J = 54,3 Hz 1H), 4.66 (s, 2H), 3.4−3.6 (m, 2H), 2.8−2.9 (m, 4H), 1.08−1.14 (m, 15H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −127.0, −134.8, −146.6.

1-(5-((tert-Butyldiphenylsilyloxy)methyl)-4-((2R,6R)-2,6-dimethylmorpholino)-2,3-difluorophenyl)-2,2-difluoroethanone Oxime. A mixture of the preceding compound (8.85 g, 15.4 mmol), pyridine (12.5 mL, 154 mmol), and hydroxylamine hydrochloride (1.07 g, 15.4 mmol) in 100 mL of EtOH was heated at 90 °C for 8 h. The reaction mixture was concentrated, diluted with water, and extracted with EtOAc. The EtOAc was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel chromatography (15% EtOAc in hexanes) to give the title compound (8.45 g, 93%). UPLC (ES) MH^+: 589.3 for $C_{31}H_{36}F_4N_2O_3Si$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.65−7.7 (m, 4H), 7.3−7.5 (m, 7H), 7.13 (t, J = 57 Hz, 1H), 4.7−4.9 (m, 2H), 3.8−4.0 (m, 2H), 3.0 (m, 2H), 2.7 (m, 2H), 1.1 (s, 7H), 1.07 (d, J = 6.8 Hz, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −117.1, −124.4, −135.4, −138.9.

(3-(Difluoromethyl)-6-((2R,6R)-2,6-dimethylmorpholino)-7-fluorobenzo[d]isoxazol-5-yl)methanol. A mixture of the preceding compound (8.4 g, 14.3 mmol) and Cs_2CO_3 (18.6 g, 57.1 mmol) in 60 mL of DMF heated at 60 °C for 2 h. The mixture was filtered and solids rinsed through with EtOAc. The EtOAc was washed with water, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (20−30% EtOAc gradient in hexanes) to afford 2.59 g (55%) of the title compound. UPLC (ES) MH^+: 331.0 for $C_{15}H_{17}F_3N_2O_3$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.0 (t, J = 53 Hz, 1H), 4.7−5.1 (m, 2H), 4.0−4.3 (m, 2H), 2.79−3.41 (m, 4H), 1.34 (d, J = 6.0 Hz, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −115.8, −140.4.

3-(Difluoromethyl)-6-((2R,6R)-2,6-dimethylmorpholino)-7-fluorobenzo[d]isoxazole-5-carbaldehyde. A mixture of the preceding compound (2.56 g, 7.75 mmol) and MnO_2 (13.5 g, 155 mmol) in 150 mL was stirred at rt for 4 d. MnO_2 was filtered off by rinsing through with CH_2Cl_2. The filtrate was concentrated to afford the title compound (2.44 g, 96%). MS (ES) MH^+: 329.2 for $C_{15}H_{15}F_3N_2O_3$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.4 (s, 1H), 8.16 (s, 1H), 7.01 (t, J = 53 Hz, 1H), 4.1−4.4 (m, 2H), 2.9−3.5 (m, 4H), 1.32 (d, J = 6.6 Hz, 6 H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −115.9, −142.8.

*(2R,4S,4aS)-8-(Difluoromethyl)-11-fluoro-2,4-dimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]-quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**5***. A solution of the previous compound (2.42 g, 7.36 mmol) and barbituric acid (0.94 g, 7.36 mmol) in 100 mL of ethanol was heated at 90 °C for 4 days. Solvent was removed and the residue was purified by reverse phase HPLC (CH_3CN/water gradient) to isolate the title compound as a solid (4.66 g, 63%). UPLC RT = 1.02 min. MS (ES) MH^+: 439.1 for $C_{19}H_{17}F_3N_4O_5$. ^1H NMR (300 MHz, $CDCl_3$) δ 1H NMR (300 MHz, DMSO-d_6) δ 11.8 (br s, 1H), 11.5 (br s, 1H), 7.3−7.7 (t, J = 36 Hz, 1H), 7.24 (s, 1H), 4.05 (d, J = 13.6 Hz, 1H), 3.9 (d, J = 8.85 Hz, 1H), 3.55−3.8 (m, 3H), 3.0−3.14 (m, 1H), 2.87 (d, J = 14.3 Hz, 1H), 1.08 (d, J = 6.0 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −117.1, −155.9. ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.8, 167.6, 152.9 (d, J_{CF} = 12.4 Hz), 152.5 (t, J_{CF} = 29.3 Hz), 149.4, 135.6, 133.4 (d, J_{CF} = 241.5 Hz), 125.1, 114.6, 110.1 (t, J_{CF} = 237 Hz), 109.1, 72.1, 71.7, 64.5, 56.3 (d, J_{CF} = 9.5 Hz), 52.9 (s), 38.2 (s), 18.2 (s), 18.1 (s); [α] = −285 (c = 0.1 in MeOH). HRMS (ES) MH^+ calcd for $C_{19}H_{18}F_3N_4O_5$ 439.1224, found 439.1241.

rel-1-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-2-methoxy-ethanone. Prepared from **14b** and *N*,2-dimethoxy-*N*-methyl-2-methylacetamide as described for **15b**. MS (ES) MH^+: 568.8 for $C_{32}H_{39}F_2NO_4Si$.

rel-1-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-2-methoxy-ethanone Oxime. A mixture of the preceding compound (8.85 g, 15.4 mmol), pyridine (12.5 mL, 154 mmol), and hydroxylamine hydrochloride (1.07 g, 15.4 mmol) in 100 mL of EtOH was heated at 90 °C for 8 h. The reaction mixture was concentrated, diluted with water, and extracted with EtOAc. The EtOAc was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel chromatography (15% EtOAc in hexanes) to give the title compound (8.45 g, 93%). MS (ES) MH^+: 583.8 for $C_{32}H_{40}F_2N_2O_4Si$.

rel-[6-[(2R,6S)-2,6-Dimethylmorpholin-4-yl]-7-fluoro-3-(methoxymethyl)-1,2-benzoxazol-5-yl]methanol. A mixture of the preceding compound (8.4 g, 14.3 mmol) and $CsCO_3$ (18.6 g, 57.1 mmol) in 60 mL of DMF heated at 60 °C for 2 h. The mixture was filtered and solids rinsed through with EtOAc. The EtOAc was washed with water, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (20−30% EtOAc gradient in hexanes) to afford 2.59 g (55%) of the title compound. MS (ES) MH^+: 325.4 for $C_{16}H_{21}FN_2O_4$.

rel-6-[(2R,6S)-2,6-Dimethylmorpholin-4-yl]-7-fluoro-3-(methoxymethyl)-1,2-benzoxazole-5-carbaldehyde. A mixture of the preceding compound (2.56 g, 7.75 mmol) and MnO_2 (13.5 g, 155 mmol) in 150 mL was stirred at rt for 4 d. MnO_2 was filtered off by rinsing through with CH_2Cl_2. The filtrate was concentrated to afford the title compound (2.44 g, 96%). MS (ES) MH^+: 323.4 for $C_{16}H_{19}FN_2O_4$.

*rel-(2R,4S,4aS)-11-Fluoro-8-(methoxymethyl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2′H,6H-spiro[1,4-oxazino[4,3-a][1,2]oxazolo-[4,5-g]quinoline-5,5′-pyrimidine]-2′,4′,6′(1′H,3′H)-trione (±)-**6***. A solution of the previous compound (2.42 g, 7.36 mmol) and barbituric acid (0.94 g, 7.36 mmol) in 100 mL of ethanol was heated at 90 °C for 4 days. Solvent removed and the residue was purified by reverse phase HPLC (CH_3CN/water gradient) to isolate the title compound as a solid (4.66 g, 63%). UPLC RT = 0.92 min. (ES) MH^+: 433.3 for $C_{20}H_{21}FN_4O_6$. ^1H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H), 11.48 (s, 1H), 7.23 (s, 1H), 4.72 (s, 2H), 4.10 (d, J = 12.8 Hz, 1H), 3.95 (d, J = 8.8 Hz, 1H), 3.74−3.86 (m, 1H), 3.66−3.73 (m, 1H), 3.63 (d, J = 14.0 Hz, 1H), 3.34 (s, 3H), 3.03−3.18 (m, 1H), 2.94 (d, J = 14.0 Hz, 1H), 1.15 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.1. ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.9, 167.6, 155.5, 152.0 (d, J_{CF} = 12.4 Hz), 149.4, 134.7 (d, J_{CF} = 2.0 Hz), 133.7 (d, J_{CF} = 240.0 Hz), 123.3, 115.3 (d, J_{CF} = 3.0 Hz), 112.8, 72.1, 71.7, 64.5, 64.4, 58.2, 56.4 (d, J_{CF} = 8.8 Hz), 53.0, 38.5, 18.2, 18.1. HRMS (ES) MH^+ calcd for $C_{20}H_{22}FN_4O_6$ 433.1518, found 433.1519.

rel-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-cyclopropyl-methanol. *sec*-Butyllithium (4.5 mL, 1.4 M in cyclohexane, 6.3 mmol) was added to a stirred solution of **14a** (1 g, 2.0 mmol) in 100 mL of THF at −78 °C, and stirring was continued for 15 min. Cyclopropane carboxaldehyde (735 mg, 7.0 mmol) in 5 mL of THF was added dropwise, and the

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solution was stirred for an additional 1 h. The reaction mixture was quenched with satd aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4), and solvent was removed. The residue was chromatographed over silica gel (EtOAc–petroleum ether gradient), providing the title compound. Yield: 1.04 g, (92%). MS (ES) MH^+: 566.2 for $C_{33}H_{41}F_2NO_3Si$.

rel-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-cyclopropyl-methanone. PCC (1.07 g, 5 mmol) was added to a solution of the preceding compound (1.0 g, 1.77 mmol) in 15 mL of CH_2Cl_2 at 0 °C, and the mixture was allowed to stir for 1h at rt. The reaction mixture was filtered through Celite, and solvents were removed from the filtrate. The residue was purified by silica gel chromatography (EtOAc–petroleum ether gradient), providing the title compound. Yield: 828 mg (83%). MS (ES) MH^+: 564.2 for $C_{33}H_{39}F_2NO_3Si$.

rel-Cyclopropyl-[4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-5-(hydroxymethyl)phenyl]methanone. TBAF (181 mg, 1.46 mmol) was added in small portions to a stirred solution of the preceding compound (820 mg, 1.46 mmol) in 10 mL of THF at 0 °C, and stirring was continued for 1 h with warming to rt. The solvent was removed and the residue was purified by chromatography on silica gel (EtOAc–petroleum ether gradient), providing the title compound. Yield: 395 mg, (75%). MS (ES) MH^+: 362.2 for $C_{20}H_{21}F_2NO_3$.

rel-Cyclopropyl-[4-[(2S,6R)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-5-(hydroxymethyl)phenyl]methanone. PCC (234 mg, 1.08 mmol) was added to a solution of the previous compound (390 mg, 1.08 mmol) in 5 mL of CH_2Cl_2 at 0 °C, and the mixture was stirred with warming to room temperature for 1 h. The reaction mixture was filtered through Celite rinsing through with CH_2Cl_2. The filtrate was concentrated, and the residue was purified by silica gel chromatography column (EtOAc–petroleum ether gradient), providing the title compound. Yield: 244 mg, (70%). MS (ES) MH^+: 324.2 for $C_{17}H_{19}F_2NO_3$.

rel-(2R,4S,4aS)-8-(Cyclopropanecarbonyl)-9,10-difluoro-2,4-dimethyl-spiro[2,4,4a,6-tetrahydro-1H-[1,4]oxazino[4,3-a]quinoline-5,5′-hexahydropyrimidine]-2′,4′,6′-trione. A solution of the preceding compound (244 mg, 0.76 mmol) and barbituric acid (106 mg, 0.83 mmol) in *i*-PrOH was heated at reflux for 12 h. The solvent was removed, and the residue was chromatographed on neutral alumina (MeOH gradient in CH_2Cl_2) to give 245 mg (75%) of the title compound as an off-white solid. MS (ES) MH^+: 434.1 for $C_{21}H_{21}F_2N_3O_5$.

rel-(2R,4S,4aS)-8-Cyclopropyl-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2′H,6H-spiro[1,4-oxazino[4,3-a][1,2]oxazolo[4,5-g]-quinoline-5,5′-pyrimidine]-2′,4′,6′(1′H,3′H)-trione (±)-7. A solution of the previous compound (212 mg, 0.49 mmol) and hydroxylamine hydrochloride (51 mg, 0.73 mmol) in 3 mL of MeOH:pyridine was heated to 90 °C for 12 h. Solvents were removed, and the residue was chromatographed on silica gel (EtOAc gradient in petroleum ether) to give 92 mg (42%) of *rel*-(2*R*,4*S*,4a*S*)-8-[*C*-cyclopropyl-*N*-hydroxycarbonimidoyl]-9,10-difluoro-2,4-dimethyl-spiro[2,4,4a,6-tetrahydro-1*H*-[1,4]oxazino[4,3-*a*]quinoline-5,5′-hexahydropyrimidine]-2′,4′,6′-trione as a solid. UPLC RT = 1.00 min. (ES) MH^+: 429.2 for $C_{21}H_{22}F_2N_4O_5$. ^1H NMR (400 MHz, DMSO-d_6) δ: 11.1 (br s, 3H), 4.0 (d, 1H), 3.8 (d, 1H), 3.7 (m, 1H), 3.6 (m, 1H), 3.3 (m, 1H), 3.0 (m, 1H), 2.9 (d, 1H), 2.3 (m, 1H), 1.1 (d, 3H), 0.85 (d, 3H), 0.8 (d, 2H), 0.3 (m, 1H). The material was dissolved in 1.5 mL of DMF along with Cs_2CO_3 (82 mg, 0.41 mmol), and the mixture was heated to 100 °C for 14 h. The reaction mixture was cooled to rt and filtered through Celite. The filtrate was concentrated, and the residue was purified by reverse phase HPLC (CH_3CN gradient in water with 0.1% TFA). UPLC RT = 1.00 min. MS (ES) MH: $C_{21}H_{21}FN_4O_5$. ^1H NMR (400 MHz, DMSO-d_6) δ 11.8 (br s, 1H), 11.5 (br s, 1H), 7.20 (s, 1H), 4.07 (d, J = 13.0 Hz, 1H), 3.93 (d, J = 8.8 Hz, 1H), 3.72–3.83 (m, 1H), 3.61–3.72 (m, 1H), 3.55 (d, J = 14.3 Hz, 1H), 3.08 (t, J = 12.4 Hz, 1H), 2.94 (d, J = 14.3 Hz, 1H), 2.12–2.27 (m, 1H), 1.13 (d, J = 6.3 Hz, 3H), 0.96–1.11 (m, 4H), 0.88 (d, J = 6.3 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.1. ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.4, 168.2, 160.3, 152.4 (d, J_{CF} = 11.9 Hz), 150.0, 135.0 (d, J_{CF} = 1.8 Hz), 134.3 (d, J_{CF} = 240.1 Hz), 123.2, 115.2 (d, J_{CF} = 2.7 Hz), 113.7, 72.5, 72.2, 64.8, 56.8 (d, J_{CF} = 9.2 Hz), 53.6, 39.2, 18.7, 18.6, 8.2, 7.8, 6.8. HRMS (ES) MH^+ calcd for $C_{21}H_{22}FN_4O_5$ 429.1569, found 429.1580.

rel-1-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-2,2-dimethyl-propan-1-ol. sec-Butyllithium (4.5 mL, 1.4 M in cyclohexane, 6.3 mmol) was added to a stirred solution of **14a** (1 g, 2.0 mmol) in 100 mL of THF at −78 °C, and stirring was continued for 15 min. Pivalaldehyde (0.76 mL, 7.0 mmol) in 5 mL of THF was added dropwise, and the solution was stirred for an additional 1 h. The reaction mixture was quenched with satd aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4), and solvent was removed. The residue was chromatographed over silica gel (EtOAc–petroleum ether gradient), providing the title compound. Yield: 1.1 g, (94%). MS (ES) MH^+: 582.8 for $C_{34}H_{45}F_2NO_3Si$.

rel-1-[5-[[tert-Butyl(diphenyl)silyl]oxymethyl]-4-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2,3-difluoro-phenyl]-2,2-dimethyl-propan-1-one. NMO (439 mg, 3.8 mmol) and TPAP (61 mg, 0.19 mmol) were added to a stirred solution of the preceding compound (1.1 g, 1.9 mmol) in CH_2Cl_2:CH_3CN (1:1, 10 mL) 0 °C. The mixture was allowed to warm to room temperature with stirring for 12 h. The solvents were removed, and the residue was purified by silica gel chromatography (EtOAc–petroleum ether gradient) to provide 727 mg (66%) of the title compound. MS (ES) MH^+: 580.8 for $C_{34}H_{43}F_2NO_3Si$.

rel-1-[4-[(2R,6S)-2,6-Dimethylmorpholin-4-yl]-2,3-difluoro-5-(hydroxymethyl)phenyl]-2,2-dimethyl-propan-1-one. TBAF (3265 mg, 1.25 mmol) was added in small portions to a stirred solution of the preceding compound (725 mg, 1.25 mmol) in 10 mL of THF at 0 °C and stirring was continued for 1h with warming to rt. The solvent was removed and the residue was purified by silica gel chromatography (EtOAc–petroleum ether gradient), providing the title compound. Yield: 320 mg, (75%). MS (ES) MH^+: 342.4 for $C_{18}H_{25}F_2NO_3$.

rel-2-[(2R,6S)-2,6-Dimethylmorpholin-4-yl]-5-(2,2-dimethylpropanoyl)-3,4-difluorobenzaldehyde. NMO (213 mg, 1.84 mmol) and TPAP (40 mg, 0.09 mmol) were added to a stirred solution of the preceding compound (320 mg, 0.94 mmol) in 10 mL of CH_2Cl_2:CH_3CN (1:1) at 0 °C, and the mixture was stirred for 12 h with warming to rt. The solvents were removed, and the residue was purified by silica gel chromatography (EtOAc–petroleum ether gradient) to give title compound. Yield: 242 mg, (76%). MS (ES) MH^+: 340.4 for $C_{18}H_{23}F_2NO_3$.

rel-(2R,4S,4aS)-8-(2,2-Dimethylpropanoyl)-9,10-difluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2′H,6H-spiro[1,4-oxazino[4,3-a]-quinoline-5,5′-pyrimidine]-2′,4′,6′(1′H,3′H)-trione. A solution of the preceding compound (240 mg, 0.71 mmol) and barbituric acid (100 mg, 0.78 mmol) in *i*-PrOH was heated at reflux for 12 h. The solvent was removed, and the residue was chromatographed on neutral alumina (MeOH gradient in CH_2Cl_2) to give 258 mg (81%) of the title compound as a white solid. MS (ES) MH^+: 450.4 for $C_{22}H_{25}F_2N_3O_5$.

rel-(2R,4S,4aS)-9,10-Difluoro-8-[N-hydroxy-2,2-dimethylpropanimidoyl]-2,4-dimethyl-1,2,4,4a-tetrahydro-2′H,6H-spiro[1,4-oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(1′H,3′H)-trione. A solution of preceding compound (250 mg, 0.57 mmol) and hydroxylamine hydrochloride (60 mg, 0.86 mmol) in 3 mL 1:1 MeOH:pyridine was heated to 90 °C for 12 h. Solvents were removed, and the residue was chromatographed on silica gel (EtOAc–petroleum ether gradient) to give title compound. Yield: 0.225 mg (85%). MS (ES) MH^+: 465.5 for $C_{22}H_{26}F_2N_4O_5$.

rel-(2R,4S,4aS)-8-tert-Butyl-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2′H,6H-spiro[1,4-oxazino[4,3-a][1,2]oxazolo[4,5-g]quinoline-5,5′-pyrimidine]-2′,4′,6′(1′H,3′H)-trione (±)-8. A mixture of the preceding compound (220 mg, 0.47 mmol) and Cs_2CO_3 (155 mg, 0.47 mmol) in 1.5 mL of DMF was heated at 100 °C for 14 h. The reaction mixture was filtered through Celite rinsing through with EtOAc. The solvent was removed, and the residue was purified by reverse phase HPLC (10 mM ammonium acetate in water, CH_3CN gradient), providing 45 mg (22%) of the title compound as a white solid. UPLC RT = 1.10 min, MS (ES) MH^+: 445.2 for $C_{22}H_{25}FN_4O_5$. ^1H NMR (400 MHz, DMSO-d_6) δ 0.9 (d, 3H), 1.1 (d, 3H), 1.4 (s, 9H), 2.9 (d, 1H), 3.1 (t, 1H), 3.6 (m, 1H), 3.7 (m, 2H), 3.9 (d, 1H),

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4.1 (d, 1H), 7.4 (s, 1H), 11.3 (bs, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.3. ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.4, 169.0, 164.7, 152.3 (d, J_{CF} = 11.7 Hz), 151.4, 134.1, 133.7 (d, J_{CF} = 238.6 Hz), 122.9, 115.9, 111.9, 72.3, 71.6, 64.4, 56.4, 56.3, 52.7, 33.0, 28.7, 18.2, 18.1. HRMS (ES) MH$^+$ calcd for $C_{22}H_{26}FN_4O_5$ 445.1882, found 445.1893.

*1-(5-((tert-Butyldiphenylsilyloxy)methyl)-2,3,4-trifluorophenyl)-ethanone (**21**).* A solution of *s*-butyllithium (1.4 M in cyclohexane, 6.42 mL, 9.0 mmol) was added slowly to a solution of the *tert*-butyldiphenyl(2,3,4-trifluorobenzyloxy)silane[29,49] (3 g, 7.49 mmol) in 30 mL of THF cooled in a dry ice−acetone bath to maintain a temperature below −60 °C. After stirring in for 20 min, *N*-methoxy-*N*-methylacetamide (1.035 mL, 9.74 mmol) was added dropwise, maintaining a temperature below −60 °C. The mixture was stirred cold for 40 min before being quenched with NH_4Cl (aqueous) and warmed to room temperature. The solution was partitioned between EtOAc and water. The EtOAc was separated and washed with brine. The combined aqueous layers were extracted with EtOAc, which was washed with brine. The combined EtOAc layers were dried ($MgSO_4$) and concentrated to give an oil that was chromatographed on silica gel (10% CH_2Cl_2 in hexanes followed by gradient elution to 70% CH_2Cl_2 in hexanes) to give two materials. The first eluting component (0.76 g) was consistent with starting material, and the second eluting component (2.05 g) was consistent with the title compound. MS (ES) MH$^+$: 443.0 for $C_{25}H_{25}F_3O_2Si$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.1 (s, 9H), 2.6 (d, 3H), 4.8 (s, 2H), 7.3−7.5 (m, 6H) 7.6−7.7 (m, 4H), 7.8−7.95 (m, 1H. ^{19}F NMR (282 MHz, DMSO-d_6) δ −131.4, −132.9, −159.4.

*(6,7-Difluoro-3-methyl-1,2-benzisoxazol-5-yl)methanol (**22**).* Potassium *t*-butoxide (1 M solution in THF, 23.8 mL) was added to a stirred solution of propan-2-one oxime (1.73 g, 23.7 mmol) in THF (25 mL) at 0 °C under N_2, and the mixture was stirred with warming to rt for 1 h. The mixture was cooled to −78 °C and a solution of the preceding compound (3.0 g, 6.8 mmol) in THF (15 mL) was added, and stirring was continued for 20 min at −78 °C before warming to −20 °C for 2 h. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution and extracted with EtOAc. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in 130 mL of ethanol and treated with 5 mL of 5% aqueous HCl with heating at 45 °C for 8 h. Addition of 10% aqueous $NaHCO_3$ was followed by extraction with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), and concentrated. The residue was purified over silica gel (15−20% EtOAc in hexanes gradient) to give the title compound as yellow solid. Yield: 600 mg, (46%). MS (ES) MH$^+$: 200 for $C_9H_7F_2NO_2$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.6(s, 3H), 4.6 (d, 2H), 5.55 (s, 1H), 7.7 (d, 1H).

6,7-Difluoro-3-methylbenzo[d]isoxazole-5-carbaldehyde. A mixture of the preceding compound (0.7 g, 3.51 mmol) and MnO_2 (6.11 g, 70.30 mmol) in 70 mL of CH_2Cl_2 was stirred at rt for 3 days. TLC shows conversion to a higher R_f spot with starting material. Additional MnO_2 (5 g) was added, and the mixture was heated at reflux for 24 h. The mixture was filtered through Celite rinsing through with CH_2Cl_2. Solvent was removed from the filtrate to give 640 mg of the title compound as a tan solid. MS (ES) MH$^+$: 198 for $C_9H_5F_2NO_2$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.6 (s, 3H), 8.0 (d, 1H), 10.4 (s, 1H. ^{19}F NMR (282 MHz, DMSO-d_6) δ −145.2, −157.1.

*rel-6-((3S,5R)-3,5-Dimethylpiperidin-1-yl)-7-fluoro-3-methylbenzo[d]isoxazole-5-carbaldehyde (**23**).* A solution of the previous compound (110 mg, 0.56 mmol), (3*S*,5*R*)-*rel*-3,5-dimethylpiperidine (HCl salt) (167 mg, 1.12 mmol), and DIEA (0.292 mL, 1.67 mmol) in 7 mL of CH_3CN was heated at reflux overnight. The mixture was diluted with EtOAc and washed with water and brine. The combined aqueous layers were extracted with EtOAc, which was washed with brine. The combined EtOAc layers were dried ($MgSO_4$) and concentrated to give an oil that was chromatographed on silica gel (50% hexanes in CH_2Cl_2 followed by gradient elution to 100% CH_2Cl_2) to give the title compound as yellow solid. MS (ES) MH$^+$: 291 for $C_{16}H_{19}FN_2O_2$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.7−0.9 (q, 1H), 0.94 (d, 6H), 1.9 (m, 4H), 2.6 (s, 3H), 2.8−3.0 (m, 2H), 3.2−3.3 (m, 2H), 7.9 (s, 1H), 10.3 (s, 1H. ^{19}F NMR (282 MHz, DMSO-d_6) δ −144.2.

*rel-(2S,4R,4aR)-11-Fluoro-2,4,8-trimethyl-1,2,3,4,4a,6-hexahydro-1′H-spiro[isoxazolo[4,5-g]pyrido[1,2-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (±)-**9**.* A solution of 72 mg (0.25 mmol) of the preceding compound and 32 mg (0.25 mmol) barbituric acid in 3 mL of *i*-PrOH was heated at refflux overnight. Solvent was removed, and the title compound (80 mg, 81%) was isolated as a white solid by crystallization from 3:1 ethanol−water. UPLC RT = 1.09 min, .MS (ES) MH$^+$: 401.1 for $C_{20}H_{21}FN_4O_4$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.6 (d, 3H), 0.8−1.1 (m, 1H), 0.9 (d, 3H), 1.8 (m, 3H), 2.4 (s, 3H), 2.6−3.1 (m, 2H) 3.5 (d,1H), 3.8 (d, 1H) 3.9−4.0 (m, 1H) 7.1 (s, 1H) 11.4 (br s, 2H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −160.0. ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.4, 167.8, 154.5, 151.6 (d, J_{CF} = 12.4 Hz), 149.6, 135.7 (d, J_{CF} = 2.9 Hz), 133.9 (d, J_{CF} = 240.0 Hz), 123.1 (d, J = 1.5 Hz), 114.5, 113.8, 66.2, 58.1(d, J_{CF} = 8.8 Hz), 54.1, 43.0, 38.8, 32.4, 31.3, 18.6, 18.4, 9.3. HRMS (ES) MH$^+$ calcd for $C_{10}H_{22}FN_4O_4$ 401.1620, found 401.1626.

Methyl 3,4-Difluoro-2-hydroxybenzoate. A solution of 3,4-difluoro-2-hydroxybenzoic acid (6.45 g, 37.05 mmol) and sulfuric acid (6 mL, 113 mmol) in MeOH (25 mL) was heated at reflux overnight. The mixture was diluted with water and extracted with ether. The ether was washed with aqueous $NaHCO_3$, water, and brine. The combined aqueous layers were twice more extracted with ether, which was washed with $NaHCO_3$, water, and brine. The combined ether extracts were dried ($MgSO_4$) and concentrated to give 6.4 g of product as a white solid. MS (ES) MH$^-$: 187.1 for $C_8H_6F_2O_3$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.8 (s, 1H), 7.65 (ddd, J = 9.0, 6.3, 2.3 Hz 1H), 6.9−7.1 (m, 1H), 3.9 (s, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −128.7, −160.5.

*3,4-Difluoro-N,2-dihydroxybenzamide (**25**).* A solution of hydroxylamine (50% in water) (50 mL, 816 mmol) was added to a solution of the preceding compound (7.75 g, 41.2 mmol) in dioxane (200 mL), and the mixture was stirred at room temperature for 3 days. The mixture was partitioned between water and EtOAc. The aqueous layer was acidified with concentrated HCl and extracted with EtOAc twice more. The EtOAc layers were washed with brine, combined, and concentrated to give 7.9 g of a solid. LC-MS ES MH$^+$: 190.0 for $C_7H_5F_2NO_3$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.14 (br s, 1H), 11.7 (s, 1H), 9.54 (br s, 1H), 7.37−7.72 (m, 1H), 6.96 (ddd, J = 7.4, 9.2, 10.0 Hz, 1H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −131.6, −161.6.

6,7-Difluoro-1,2-benzisoxazol-3(2H)-one. A mixture of **25** (7.91 g, 41.8 mmol) and carbonyl diimidazole (13.6 g, 83.7 mmol) in 200 mL of THF was heated at reflux for 90 min.The mixture was partitioned between EtOAc and water and acidified with conc HCl. The solution was extracted three times with EtOAc, each extract being washed with water and brine. Drying ($MgSO_4$) of the combined extracts and removal of solvent gave 6.88 g of product as an off-white solid. MS (ES) (M − H)$^-$: 170 for $C_7H_3F_2NO_2$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.3−7.5 (m, 1H), 7.6 (m, 1H), 12.9 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −137.6, −162.4.

*3-Chloro-6,7-difluorobenzo[d]isoxazole (**26**).* TEA (5.6 mL, 40.2 mmol) was added to an ice bath cooled mixture of the preceding compound (6.88 g, 40.2 mmol) and $POCl_3$ (13.1 mL, 141 mmol) in a microwave reactor vessel (exothermic), and the mixture was heated at 140 °C for 6 h in a microwave reactor. Solvent was removed, and the mixture was taken up in ether and washed with Na_2CO_3 (2×) and brine. The combined aqueous layers were twice more extracted with ether, which was washed with aqueous Na_2CO_3 and brine. The combined ether layers were dried ($MgSO_4$) and concentrated to give an oil that slowly solidified. The material was chromatographed on silica gel (hexanes followed by gradient elution to 50% CH_2Cl_2 in hexanes) to afford 5.64 g (71%) of product as an off-white solid. ^{1}H NMR ($CDCl_3$) δ 7.2−7.35 (m, 1H), 7.4−7.5 (m, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −133.4, −157.9.

*3-Chloro-6,7-difluorobenzo[d]isoxazole-5-carbaldehyde (**27**).* A solution of *n*-butyllithium (2.5 M in hexanes) (16.7 mL, 42 mmol) was added slowly to a solution of 2,2,6,6-tetramethylpiperidine (7.6 mL, 45 mmol) in THF (50 mL) cooled in a dry ice−acetone bath. The solution was warmed to 0 °C and recooled in a dry ice−acetone bath

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before transferring via syringe to a solution of **26** (5.64 g, 30 mmol) in THF (50 mL). After 1 h stirring, DMF (1.0 mL, 13.2 mmol) was added all at once and the mixture was stirred with cooling in a dry ice−acetone bath for 45 min. The mixture was transferred via cannula to a solution of acetic acid (6.8 mL, 119 mmol) in 100 mL of Et_2O, and the mixture was warmed to rt. The mixture was diluted with EtOAc and washed with brine. The combined aqueous layers were extracted again with EtOAc, which was washed with brine. Drying ($MgSO_4$) of the combined extracts and removal of solvent gave a brown oil that was chromatographed on silica gel (10% CH_2Cl_2 in hexanes followed by gradient elution to 100% CH_2Cl_2) to give 3.32 g (51%) of product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.4 (s, 1H), 8.1 (dd, J = 5.3, 1.7 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −142.3, 155.9.

*3-Chloro-6-((2R,6R)-2,6-dimethylmorpholino)-7-fluorobenzo[d]-isoxazole-5-carbaldehyde (**28**).* A solution of **27** (10 g, 46 mmol), (2*R*,6*R*)-2,6-dimethylmorpholine (6.35 g, 55 mmol), and K_2CO_3 (12.7 g, 92 mmol) in butyronitrile (100 mL) and water (10 mL) was heated at reflux for 2 h. The mixture was cooled to room temperature before being partitioned between EtOAc and water. The EtOAc was separated and washed with brine. The combined aqueous layers were extracted with EtOAc, which was washed with brine. The combined EtOAc was dried ($MgSO_4$) and concentrated to give an oil that slowly solidified (15.3 g, 100% yield) and was sufficiently clean to be used in subsequent steps. For characterization purposes, material was chromatographed on silica gel (100% CH_2Cl_2 followed by gradient elution to 5% EtOAc in CH_2Cl_2) to give the title compound. MS (ES) MH$^+$: 312.9, 314.7 for $C_{14}H_{14}ClFN_2O_3$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.45 (s, 1H), 8.0 (d, J = 1.1 Hz, 1H), 4.24 (m, 2H), 3.43 (dm, J = 11.9 Hz, 2H), 3.0−3.1 (m, 2H), 1.33 (d, J = 6.4 Hz, 6H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −143.4.

*3-Chloro-6-[(2R,6R)-2,6-dimethylmorpholin-4-yl]-5-(1,3-dioxolan-2-yl)-7-fluoro-1,2-benzoxazole (**29**).* A suspension of **28** (37 g, 118 mmol), *p*-TsOH acid (1.12 g, 5.9 mmol), and ethylene glycol (26.4 mL, 473 mmol) in 100 mL toluene was heated at reflux with azeotropic removal of water via a Dean−Stark trap for 3 h. After cooling to rt, solvent was removed and the residue was partitioned between Et_2O and aqueous Na_2CO_3. The Et_2O was separated and washed with water and brine. The Et_2O was dried ($MgSO_4$) and concentrated to give an oil that slowly solidified under vacuum (42.5 g, 100%). LC-MS ES MH$^+$ 357.0, 359.0 for $C_{16}H_{18}ClFN_2O_4$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71 (s, 1H), 6.20 (s, 1 H), 3.9−4.2 (m, 6H), 3.2−3.3 (m, 2H), 2.90 (dd, J = 11.1, 5.1 Hz, 2H), 1.22 (d, J = 6.2 Hz, 6 H).

*(2R,4S,4aS)-8-Chloro-11-fluoro-2,4-dimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**10**.* A mixture of **28** (500 mg, 1.4 mmol) and barbituric acid (180 mg, 1.4 mmol) in 2 mL of EtOH and 2 mL of water was heated at 120 °C for 1 h. Solvent was removed, and the residue was recrystallized from methanol, affording a methanol solvate. The solids were dissolved in 1:1 CH_3CN−water and lyophilized to afford a white solid that was heated at 60 °C under vacuum for 2 d to afford 422 mg (71%) of product consistent with the title compound. UPLC RT = 1.03 min. MS (ES) MH$^+$: 423.2, 425.3 for $C_{18}H_{17}ClFN_4O_5$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.9 (s, 1H), 11.5 (s, 1H), 7.23 (s, 1H), 4.11 (d, J = 13.4 Hz, 1H), 3.98 (d, J = 8.85 Hz, 1H), 3.78 (d, J = 6.2 Hz, 1H), 3.6−3.7 (m, 2H), 3.13 (t, J = 12.0 Hz, 1H), 2.95 (d, J = 14.3 Hz, 1H), 1.15 (d, J = 5.8 Hz, 3H), 0.89 (d, J = 6.2 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −156.8. ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 167.6, 152.8 (d, J_{CF} = 13.2 Hz), 149.4, 148.5 (d, J_{CF} = 2.7 Hz), 136.0 (d, J_{CF} = 2.2 Hz), 133.2 (d, J_{CF} = 241.5 Hz), 124.8 (d, J_{CF} = 2.2 Hz), 113.7 (d, J_{CF} = 2.7 Hz), 110.7, 72.1, 71.6, 64.5, 56.3 (d, J_{CF} = 9.3 Hz), 52.8, 38.3, 18.2, 18.1. HRMS (ES) MH$^+$ calcd for $C_{18}H_{17}ClFN_4O_5$ 423.0866, found 423.0858; [α] = −273 (c = 0.1 in MeOH).

*6-((2R,6R)-2,6-Dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluoro-3-methoxybenzo[d]isoxazole (**30**).* A mixture of **29** (0.5 g, 1.40 mmol) and sodium methoxide (0.5 M in MeOH, 2.8 mL, 1.4 mmol) was heated at 100 °C for 1.5 h in a microwave reactor. The mixture was diluted with EtOAc, which was washed with water and brine. The combined aqueous layers were extracted with EtOAc, which was washed with brine. The combined EtOAc layers were dried (MgSO4) and concentrated to give an the title compound (430 mg, 87%) as an oil that slowly solidified. LC-MS ES MH$^+$ 353.0 for $C_{17}H_{21}ClFN_2O_5$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 6.22 (s, 1H), 4.05−4.11 (s and m, total of 7H), 3.9−4.00 (m, 2H), 3.20 (br s, 2H), 2.88 (br s, 2H), 1.26 (d, J = 6.5 Hz, 6H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −143.5.

*(2R,4S,4aS)-11-Fluoro-8-methoxy-2,4-dimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**11**.* A mixture of **30** (430 mg, 1.22 mmol) and barbituric acid (156 mg, 1.22 mmol) in MeOH (4 mL) and water (0.5 mL) were heated at 120 °C for 1 h in a microwave reactor. Solvent was removed, and the residue was purified by chiral SFC (21 mm × 250 mm, 5 μ (S,S) Whelk-O1 column, 80% CO_2, 20% MeOH) to give 391 mg (77%) of the title compound was a white solid. UPLC RT = 0.94 min. MS (ES) MH$^+$: 419.2 for $C_{19}H_{19}FN_4O_6$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.76 (br s, 1H), 11.49 (br s, 1H), 7.08 (s, 1H), 4.09 (m., 1H), 4.04 (s, 3H), 3.93 (d, J = 8.6 Hz, 1H), 3.77 (m, 1H), 3.62−3.72 (m, 1H), 3.57 (d, J = 14.1 Hz, 1H), 3.17 (d, J = 4.7 Hz, 1H), 3.09 (t, J = 12.2, 1H), 2.92 (d, J = 14.3 Hz, 1H), 1.14 (d, J = 6.0 Hz, 3H), 0.88 (d, J = 6.2 Hz, 3H). ^{19}F NMR (282 MHz, DMSO-d_6) δ −157.6. ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 167.6, 166.4 (d, J_{CF} = 2.7 Hz), 152.8 (d, J_{CF} = 12.6 Hz), 149.5, 135.1, 133.8 (d, J_{CF} = 239.9 Hz), 122.6, 113.8 (d, J_{CF} = 2.7 Hz), 105.2, 72.0, 71.7, 64.4, 57.6, 56.3 (d, J_{CF} = 9.3 Hz), 53.1, 48.6, 38.5, 18.1; [α] = −303 (c = 0.1 in MeOH). HRMS (ES) MH$^+$ calcd for $C_{19}H_{20}FN_4O_6$ 419.1361, found 419.1360.

*3-Azido-6-((2R,6R)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazole (**31**).* A mixture of **29** (0.1 g, 0.28 mmol) and NaN_3 (0.055 g, 0.84 mmol) in DMSO (3 mL) was heated at 100 °C for 6 h in a microwave reactor. The mixture was diluted with Et_2O and water. The Et_2O was separated and washed with water three more times. The combined aqueous layers were twice more extracted with Et_2O, and each extract was washed with water three more times. The combined Et_2O layers were dried ($MgSO_4$) and concentrated to give an oil that slowly solidified. The material was purified by chromatography on silica gel (100% CH_2Cl_2 with gradient elution to 40% EtOAc in CH_2Cl_2) to afford 80 mg (79%) of the title compound as a white solid. LC-MS ES MH$^+$ 363.0 for $C_{16}H_{18}FN_5O_4$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 6.28 (s, 1H), 4.14−4.28 (m, 4H), 3.91−4.11 (m, 2H), 3.32 (br s, 2H), 2.98 (br s, 2H), 1.34 (br s, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ −142.5.

*(2R,4S,4aS)-8-Amino-11-fluoro-2,4-dimethyl-2,4,4a,6-tetrahydro-1H,1′H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5′-pyrimidine]-2′,4′,6′(3′H)-trione (−)-**12**.* Ph_3P (57.7 mg, 0.22 mmol) was added to a suspension of 3-azido-6-((2*R*,6*R*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[*d*]isoxazole (80 mg, 0.22 mmol) in 2 mL of acetic acid and 200 μL of water, during which gas evolution was observed and the mixture became homogeneous. After stirring for 10 min, barbituric acid (28.2 mg, 0.22 mmol) was added and the mixture was heated at 120 °C for 1 h. Solvent was removed, and the residue was purified by chiral SFC (21 mm × 250 mm, 5 μ (S,S) Chiralpak A1 column, 70% CO_2, 30% MeOH) to give 37 mg (42%) of the title compound was a white solid. UPLC RT = 0.75 min. MS (ES) MH$^+$: 404.3 for $C_{18}H_{18}FN_5O_5$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (br s, 1H), 11.44 (br s, 1H), 7.09 (s, 1H), 6.23 (s, 2H), 4.05 (d, J = 12.6 Hz, 1H), 3.90 (d, J = 8.8 Hz, 1H), 3.72−3.83 (m, 1H), 3.59−3.71 (m, 1H), 3.45 (d, 14.3 Hz, 1H), 3.29 (s, 1H), 3.01−3.11 (m, 1H), 2.94 (d, J = 14.0 Hz, 1H), 1.13 (d, J = 6.0 Hz, 3H), 0.88 (d, J_{CF} = 6.37 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.1, 168.7, 159.0, 151.7 (d, J_{CF} = 12.1 Hz), 150.8, 134.70, 134.67 (d, J_{CF} = 238.8 Hz), 121.3, 115.2, 109.6, 72.6, 72.2, 65.1, 56.9 (d, J = 8.8 Hz), 53.6, 40.9, 18.67, 18.66. ^{19}F NMR (282 MHz, $CDCl_3$) δ −157.3; [α] = −158.9 (c = 0.1 in DMSO). HRMS (ES) MH$^+$ calcd for $C_{18}H_{18}FN_5O_5$ 404.1370, found 433.1365.

Colorless crystals of a monohydrate form of (−)-**1** were prepared by recrystallization from ethanol/water (4:1). The diffraction data were collected at 180 K on a Nonius KappaCCD diffractometer. The crystal structure was solved and refined with the SHELXTL package. All the hydrogen atoms attached to the C atoms and N atoms were

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calculated. Absolute configuration was established by using anomalous dispersion method, and the Flack parameter is found to be 0.1(3). The diffraction data was deposited into the Cambridge Crystallographic Data Centre (CCDC 1013311).

Biological Experimental Procedures. Compounds for oral, iv, and ip dosing in mice and rats were formulated in 0.2 M meglumine/20% hydroxypropyl-β-cyclodextrin in rats and DMA:TEG:saline 40/40/20% in mice. Wistar Hannover rats used for pharmacokinetic and toxicology studies were obtained from Charles River Laboratories (Raleigh, NC). CD-1 mice were obtained from Charles River Laboratories (Kingston, NY) and used for pharmacokinetic and efficacy studies. All animals were housed and acclimated in the animal facility on site before each study. All experimental procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Inhibition of of DNA Gyrase Activity. IC_{50} values against *E. coli* DNA gyrase and human topoisomerase IIα were determined using literature procedures.[35,48]

Plasma Protein Binding Determination. Human, rat, and mouse plasma protein binding was determined from a 10 μM compound solution in a Dianorm plasma well incubating at 37 °C for 16 h. Free fractions were calculated from ratios of drug concentration in buffer and plasma wells determined by LC-MS/MS.

Pharmacokinetic Studies. Pharmacokinetic properties of selected compounds were studied in male rats and male mice. Plasma pharmacokinetics were determined from 0 to 24 h following 15 min iv infusions at 3 mg/kg or oral administration at 10 mg/kg. Serial 200 μL samples of whole blood were taken from the jugular vein of each animal at time intervals. Concentration of compound in plasma was determined by LC-MS/MS, and pharmacokinetic parameters were estimated using a noncompartmental model in WinNonLin (Pharsight). Mean results were determined for each experiment with three mice or three rats.

Minimum Inhibitory Concentration (MIC). MIC values were determined by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines,[50,51] as described previously.[27] For more potent compounds with MIC values <12.5 μM, the reported data represents the averages of three or more replications.

DNA Biosynthesis Inhibition. Incorporation of radiolabeled precursors (^{3}H-thymidine, ^{3}H-uridine, ^{14}C-leucine, ^{14}C-acetic acid, and ^{14}C–*N*-acetyl glucosamine) of biological macromolecules was measured in *S. aureus* (MSSA) following previously described methods.[27,44]

Isolation and Characterization Resistant Bacteria. Suspensions of cultures of methicillin-sensitive *S. aureus* (ARC516) were transferred onto plates of Mueller–Hinton agar containing (−)-**1** at four concentrations: 0, 1.56, 3.12, and 6.25 μM. The frequency of spontaneous resistance was determined in two independent studies performed in triplicate on each occasion and was calculated from the number of colonies on the compound-containing plates divided by the number of colonies on compound-free plates after incubation for 48 h. Values across studies were averaged. The incubations were extended to 4 d for isolation and analysis of colonies. When bacteria were plated on agar plates containing a 2.12 and 6.25 μM of (−)-**1**, no resistant colonies were recovered. One colony was selected from the 1.56 μM plate, recovered on drug-free plates, and frozen in glycerol stocks. Genomic DNA was prepared from this isolated resistant variant (designated SAA4419), as well as from the original susceptible parent strain, and the genes for the topoisomerase subunits (*gyrA*, *gyrB*, *parC*, and *parE*) were amplified by PCR and sequenced using standard protocols described previously.[52] Gene sequences from the variant strains were compared to those from the parent strain ARC516 and found to be identical, Suspensions of cultures of SAA4419 were transferred onto plates of Mueller–Hinton agar with containing (−)-**1** at four concentrations: 0, 3.12, 6.25, and 12.5 μM as described above for ARC516. Two colonies were selected from the 3.12 μM plate and recovered on drug-free plates frozen in glycerol stocks. Genomic DNA was prepared these isolated resistant variants and found to be identical to ARC516 except for two different point mutations found in the *gyrB* gene: Trp519Leu for the first strain (designated SAA4419) and Ala438Ser for the second strain (designated SAB4419). All MIC values were determined using CLSI methodology.[50,51]

In Vivo Efficacy. Mouse thighs were infected with a mid-log culture of *S. aureus* (MSSA, ARC516 from the AstraZeneca Research Collection) to achieve a target inoculum of 5×10^5 CFU. Groups of five animals each received an intraperitoneal injection of test compound at doses of 3–100 mg/kg/day on a q24h or q6h regimen starting 2 h after infection. A group of 10 mice were sacrificed at 2 h after infection. Two control groups of five mice received linezolid at 160 mg/kg q24 (positive control group) or vehicle alone (negative control group). Efficacy and control groups were sacrificed 24 h after the start of treatment. Thighs were removed, weighed, and homogenized and aliquots plated onto tryptic soy agar plates and incubated at 37 °C overnight for CFU/g thigh determination. The experiment was repeated using mice that were rendered neutropenic by injecting cyclophosphamide intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before experimental infection.

In Vivo Safety. In the single dose phase, groups of three male rats were given doses up to 1000 mg/kg/day (−)-**1** once daily. The animals were then observed for up to seven days before being killed for scheduled necropsy. In the repeat dose phase, groups of three male and three female rats were dosed once daily for 14 days at 0, 250, 500, and 750 mg/kg/day and killed for scheduled necropsy on day 15. Additional groups of two males and two females were dosed at 250, 500, and 750 mg/kg/day for 14 days for toxicokinetics. These animals were killed without necropsy following completion of blood sampling. The following were assessed in this study: clinical observations, body weight, food consumption, body temperature, hematology, plasma chemistry, toxicokinetics, and gross and microscopic pathology.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Included are NMR NOESY spectra for (−)-**1** and (+)-**2** and a model for (+)-**2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): The authors are or have been previously employees of AstraZeneca.

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■ ABBREVIATIONS USED

CDI, carbonyl dimidazole; CFU, colony-forming unit; Cl, clearance; *F*, oral bioavailability; f_u, fraction unbound; DIEA, diisopropylaminoethylamine; GyrA, A-subunit of DNA gyrase; GyrB, B-subunit of DNA gyrase; MRQR, methicillin resistant, quinolone resistant *S. aureus*; MSSA, methicillin sensitive *S. aureus*; NMM, *N*-methylmorpholine; ParC, C-subunit of topoisomerase IV; ParE, E-subunit of topoisomerase IV; PPB, plasma protein binding; S_NAr, nucleophilic aromatic substitution; *tolC*, gene encoding outer membrane transport channel; TBDPS, *tert*-butyldimphenylsilyl; TK, toxicokinetics

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