Quinolone Antibacterials Containing the New 7-[3-(1-Aminoethyl)-1-pyrrolidinyl] Side Chain: The Effects of the 1-Aminoethyl Moiety and Its Stereochemical Configurations on Potency and in Vivo Efficacy

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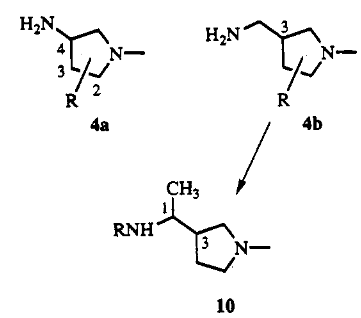
A series of stereochemically pure 7-[3-(1-aminoethyl)-1-pyrrolidinyl]-1,4-dihydro-4-oxoquinoline and 1,8-naphthyridine-3-carboxylic acids, with varied substituents at the 1-, 5-, and 8-positions, were synthesized to study the effects of the 7-[3-(1-aminoethyl)-1-pyrrolidinyl] moiety on potency and in vivo efficacy relative to the known 7-[3-(aminomethyl)-1-pyrrolidinyl] derivatives. The antibacterial efficacies of the target compounds and their relevant reference agents were determined in vitro using an assortment of Gram-negative and Gram-positive organisms and in vivo using *Escherichia coli* and *Streptococcus pyogenes* mouse infection models. The effects of the 7-[3-(1-aminoethyl)-1-pyrrolidinyl] moiety were also examined at the level of the target enzyme by employing a DNA-gyrase supercoiling inhibition assay. Selected compounds were further evaluated for potential phototoxic and clastogenic liabilities using a phototoxicity mouse model and an in vitro mammalian cell cytotoxicity assay. It was found that the differences in in vitro antibacterial activity between the stereoisomers were significantly greater than previously reported for other optically pure 3-substituted pyrrolidinyl side chains. Relative to their 7-[3-(aminomethyl)-1-pyrrolidinyl] analogs, the (3*R*,1*S*)-3-(1-aminoethyl)pyrrolidines generally conferred a 2–4-fold increase in Gram-positive in vitro activity and an average of 10-fold improvement in oral efficacy. The level of phototoxicity and cytotoxicity of the product quinolones was ultimately determined by the combined influence of the 7-[3-(1-aminoethyl)-1-pyrrolidinyl] side chains and the other quinolone substituents. From this study, several compounds were identified with outstanding antibacterial activity and low degrees of phototoxicity and mammalian cell cytotoxicity. One such agent, **34F**–*R*,*S* (PD 140248), showed the best overall blend of safety and efficacy.

The fluoroquinolones **1** (Table I) have emerged as one of the premier classes of antibacterials and have been the subject of many recent reviews.[1–9] Their broad-spectrum activity,[2,3,5,6] coupled with their oral and parenteral dosage options,[2] has enabled them to successfully challenge all of the conventional drugs employed for hospital and serious community infections.[1]

Structure–activity relationships for the quinolones have established that the 7-position is largely responsible for controlling the spectrum of antibacterial coverage[10–12] and the overall pharmacokinetics.[10] All of the fluoroquinolones currently approved for use in man contain a 1-piperazinyl moiety at the 7-position (R_7 in **1**), with ciprofloxacin (**2**) and ofloxacin (**3**) generally considered to be the most active of these agents.[4,5] Both have demonstrated good clinical success in widespread use.[7,9,13] These piperazinylquinolones are particularly effective for the treatment of infections caused by most Gram-negative organisms, including *Pseudomonas*, and for some infections caused by *Staphylococcus* and mycobacteria.[2–6] None of the current quinolones are considered front-line therapy for infections caused by the Gram-positive species *Streptococcus* or *Enterococcus*, or when anaerobes are among the causative pathogens.[5,6,14–16]

Gram-positive activity, especially against *Streptococcus* and *Enterococcus*, has been obtained for quinolones containing the 3-amino-substituted-1-pyrrolidines **4a** and **4b** (Figure 1) at the R_7-position.[3,10,11] Several of these pyrrolidinylquinolones, such as **5–9** in Table I, also retain good Gram-negative potency and therefore represent truly broad-spectrum agents.[3] Unfortunately, the pyrrolidinyl



F, R = H

G, R = Me

H, R = Et

3R,1S = RS; 3R,1R = RR; 3S,1S = SS; 3S,1R = SR

Figure 1. Substituted pyrrolidines.

side chains have less intrinsic oral absorption than the corresponding piperazines and require additional modifications of the quinolone structure to provide good oral efficacy.[17] Such changes include a halogen at C_8 (X = CF or CCl) as in **5**, **6**, and **8**,[18] an amino acid prodrug as in **7**,[19] or a difluorophenyl group at N_1 as in **9**.[12] The best in vivo efficacy was obtained with the 8-fluoro derivatives, but recently the fluorine at the 8-position has been shown to be associated with a number of undesirable effects such as increased phototoxicity[20] and cytotoxicity[21c–f] via inhibition of mammalian topoisomerase.[21a–c] Other methods for improving in vivo efficacy include the alkylation of the distal basic nitrogen of the side chain[10,11] or the alkylation of the heterocyclic ring.[22] Alkylations of the aminopyr-

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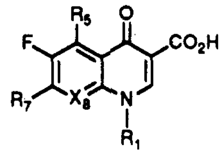
Table I. Fluoroquinolones



1

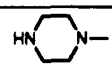
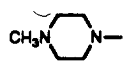
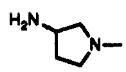
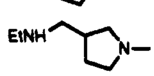
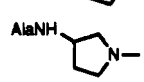
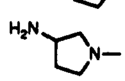
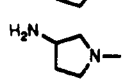
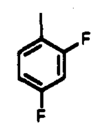
compound	R_7	X_8	R_1	R_5
ciprofloxacin (2)		CH		H
ofloxacin (3)		$-COCH_2C(CH_3)-$		H
Cl-938 (5)		CF		H
PD 117558 (6)		CF		H
Cl-990 (7)		N		H
CI-960 (8) (PD 127391)		CCl		H
tosufloxacin (9) (A-61827)		N		H

Table II. Quinolone and Naphthyridine Intermediates Used To Prepare Final Products

no.	substituents on 1				ref
	R_7	X	R_1	R_5	
17	Cl	N	c-C_3H_5	H	27
18	F	CH	c-C_3H_5	H	18, 31
19	F	CF	c-C_3H_5	NH_2	28
20	F	CF	c-C_3H_5	H	18
21	F	CH	c-C_3H_5	NH_2	28
22	F	CCF_3	c-C_3H_5	H	20
23	F	N	2,4-F_2Ph	H	29
24	F	$COCH_3$	c-C_3H_5	H	30
25	F	$COCH_3$	C_2H_5	H	30
26	F	CF	C_2H_5	H	31

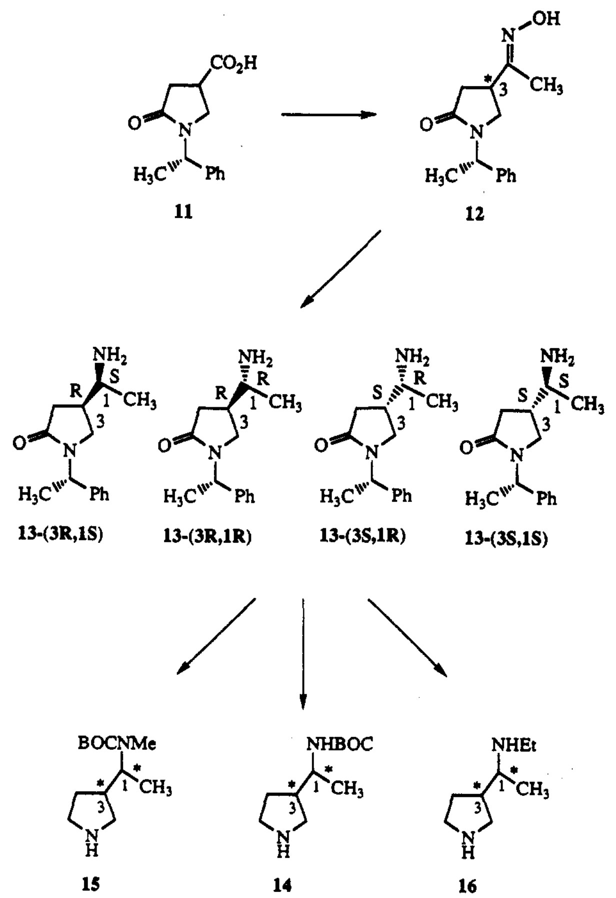
rolidines **4a,b** at positions 2,[23] 3,[24,25] and 4[24] have all been reported to improve oral efficacy.

In this paper, we have studied the effects of adding a methyl group to the methylene spacer in **4b** to produce the 3-(1-aminoethyl)pyrrolidines **10F–H** (Figure 1).[26] In each case, all four possible stereoisomers have been prepared and coupled to a variety of quinolones and naphthyridines, which were tested for in vitro and in vivo activity. We wish to report that the 3-(1-aminoethyl)-1-pyrrolidinyl substituent and its stereochemical orientations profoundly influence both the in vitro and in vivo potency. Furthermore, this study has produced quinolones with enhanced Gram-positive activity and oral efficacy superior to those containing a halogen at the 8-position.

Chemistry

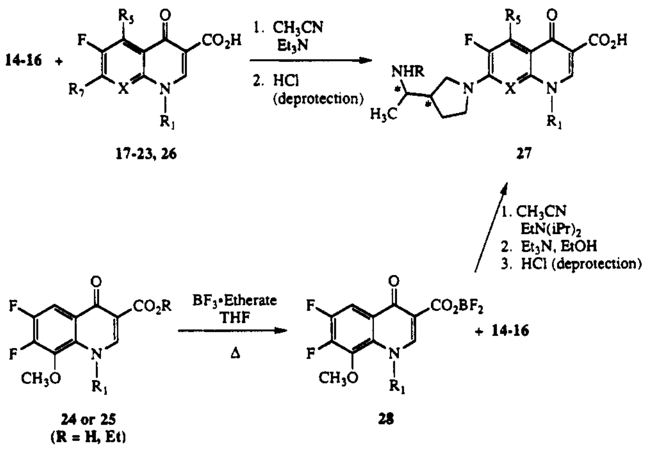
The quinolone nuclei used for the preparation of final products were prepared according to literature procedures as indicated in Table II. The synthesis of the pure diastereomers of the 3-(1-aminoethyl)pyrrolidines **10** is outlined in Scheme I. Specific experimental details have been published elsewhere.[32] The key step is the separation of the diastereomeric oximes **12** by column chromatography (≥99%). Reduction of **12**-*3R* produced **13**-*3R,1S* and **13**-*3R,1R*, and reduction of **12**-*3S* gave **13**-*3S,1R* and **13**-*3S,1S*. The individual diastereomers of **13** were separated and purified by chromatography to ≥20:1 diastereomeric purity as determined by HPLC and NMR (see Experimental Section). In addition, the absolute configuration of each diastereomer of **13** was determined by X-ray crystallography.[32] The pyrrolidinones **13** were then elab-

Scheme I[a]



[a] An asterisk denotes pure chiral centers.

Scheme II



orated into the BOC-NH, BOC-*N*-methyl, and *N*-ethylpyrrolidines **14**, **15**, and **16**, which were then coupled to the quinolone nuclei according to Scheme II. For the 7-haloquinolones **17–23** and **26**, the aromatic nucleophilic displacement by the pyrrolidine followed standard procedures,[18,20,28,29] to provide final quinolones of the generic form **27** (Scheme II). For the 8-methoxyquinolones **24** and **25**, the quinolone acid or ester was first converted to the borate ester **28**, which was then coupled using the pyrrolidines and Hunig's base. The borate ester products were hydrolyzed back to the quinolone acids using triethylamine in ethanol. Deprotection as necessary provided the final products **27**. All of the new and reference quinolones **30–39** prepared for this study are listed in Table III with physical constants and methods of preparation. In the numbering system chosen to identify compounds, the number refers to the quinolone nuclei and the letter, A–H, represents the side chain at the 7-position. These side chains are depicted in Figure 2. The *R*,*S* designations represent the stereochemistry at the 3- and 1-positions, respectively.

Biological Assays

In Vitro Antibacterial. All of the 7-[3-(1-aminoethyl)-1-pyrrolidinyl]quinolones (**30–39**) in Table III and selected reference agents were tested against 10 representative Gram-positive and Gram-negative organisms using standard microtitration techniques.[33] Their minimum inhibitory concentrations (MICs, μg/mL) were averaged from multiple experiments and recorded in Table IV. To determine the effects of the stereochemical orientations of the side chains at the target enzyme level, select compounds were tested for their inhibition of DNA gyrase (Table V), which was isolated and purified from *Escherichia coli* H560.[34] The initial cleavage method was employed, which measures the lowest concentration of drug (μg/mL) that will produce a detectable level of cleavage from relaxed bacterial Col E1 plasmid DNA, as visualized by agarose gel electrophoresis and staining with ethidium bromide.[34] The gyrase data is averaged from multiple experiments and is accurate to ±50%.

In Vivo Antibacterial. As previously reported,[35] the in vivo potency was determined in acute, lethal systemic infections in female Charles River CD-1 mice and expressed as the median protective dose (PD_{50}, mg/kg, Table IV). Sixteen mice per method of administration were employed. Single doses of compound were administered at the time of the bacterial challenge. The 95% confidence limits are generally ±30%.

Phototoxicity. Compounds with the best in vitro and in vivo efficacy were tested for oral phototoxicity in

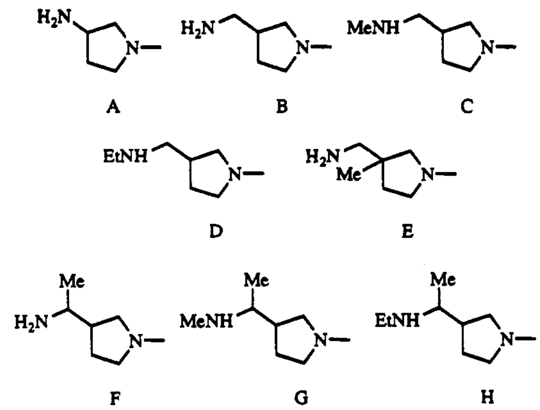


Figure 2. Pyrrolidinyl side-chain substituents (R_7) used in this study.

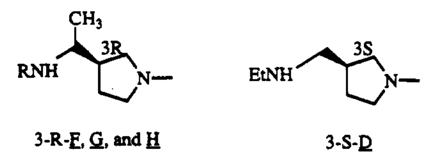


Figure 3. Stereochemical relationship between the most active stereoisomers of **F–H** vs **D**.

depilated female CD-1 mice.[20] Each day, five animals/dose and control were exposed to UV-A radiation (320–400 nm) for 3 h duration following oral administration. Dosing was continued until a definite positive response was elicited or for a maximum of 4 days. Any detectable redness or erythema (as observed 24 h postirradiation) relative to control animals was considered a positive photoresponse. The results are reported in Table VI as the highest dose producing no effect.

Mammalian Cell Cytotoxicity. Promising quinolones were also evaluated in a mammalian cell cytotoxicity assay[21d] to estimate their clastogenic potential.[21c–f] The clonogenic cytotoxicity was determined in Chinese hamster V-79 cells. The cells were grown overnight and treated with drug for 3 h at 37 °C, at which time the compound-containing media was replaced with fresh media. The cells were then incubated for 5 days and examined for colony formation. The concentration of drug-inhibiting colony formation by 50% (IC_{50}, μg/mL) relative to control was determined and recorded in Table VI.

Results and Discussion

For each parent quinolone **30–39**, Table IV shows the effects of the reference side chains A–E (see Figure 2) vs the stereoisomers of the 3-(1-aminoethyl)pyrrolidines F–H on antibacterial activity. In order to discern the effects derived from the new pyrrolidines F–H, it is important to first summarize the structure–activity relationships of the reference pyrrolidines A–E, and those of the quinolone nuclei themselves.

Of the five reference pyrrolidines A–E, the data demonstrate that the 3-aminopyrrolidine (A) confers the best Gram-negative potency, including that against *Pseudomonas* (see **30A** vs **30B–E**, **31A** vs **31B–E**). This fact, coupled with the generally good Gram-positive activity provided by A, made the (3-aminopyrrolidinyl)quinolones the most balanced, broad-spectrum agents in the quinolone family[18] (see **30A**, **32A**, **34A**, and **38A**). Spacing the 3-amino group away from the ring by a single methylene to give B increased the Gram-positive potency of the quinolones by 2–8-fold, while decreasing the Gram-negative MICs by a similar margin (**30A** vs **30B**, **31A** vs **31B**, **35A** vs **35B**). Alkylation of the distal nitrogen of B to give pyrrolidines

Table III. Physical Properties and Method of Preparation for Quinolone Antibacterials Used in This Study

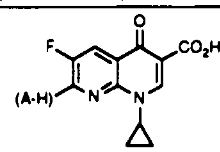
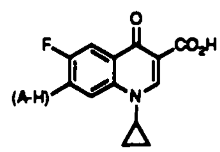
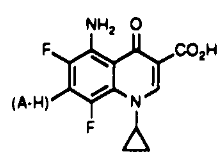
no.[f]	method of prep.[a] or ref	method of purif of final prod[b]	% yield[c]	mp (°C)	analysis formula (elements analyzed)[d]
				**30**	
30A	ref 18				
30B	ref 18				
30C	ref 18				
30D	ref 18				
30E	A	isoelect prec	47	257–259	$C_{18}H_{21}FN_4O_3\cdot H_2O$ (C,H,N)
30F-*R,S*	A	trit 2-PrOH	82	>300	$C_{18}H_{21}FN_4O_3\cdot HCl\cdot 0.22H_2O$ (C,H,N,F,Cl)
30F-*R,R*	A	trit 2-PrOH	62	>300	$C_{18}H_{21}FN_4O_3\cdot HCl$ (C,H,N,F,Cl)
30F-*S,S*	A	trit 2-PrOH	36	>300	$C_{18}H_{21}FN_4O_3\cdot HCl$ (C,H,N,F,Cl)
30F-*S,R*	A	trit 2-PrOH	65	>300	$C_{18}H_{21}FN_4O_3\cdot HCl$ (C,H,N,F,Cl)
30G-*R,S*	A	recryst MeOH/Et$_2$O	90	>300	$C_{19}H_{23}FN_4O_3\cdot 1.1HCl\cdot 0.25H_2O$ (C,H,N,Cl)
30G-*R,R*	A	trit 2-PrOH	76	>300	$C_{19}H_{23}FN_4O_3\cdot HCl\cdot 0.75H_2O$ (C,H,N,F,Cl)
30G-*S,S*	A	trit 2-PrOH	83	>300	$C_{19}H_{23}FN_4O_3\cdot HCl\cdot 0.5H_2O$ (C,H,N,Cl)
30G-*S,R*	A	recryst MeOH/Et$_2$O	31	>300	$C_{19}H_{23}FN_4O_3\cdot HCl\cdot 0.6H_2O$ (C,H,N,F,H$_2$O)
30H-*R,S*	B	isoelect prec	83	194–195	$C_{20}H_{25}FN_4O_3\cdot 0.5H_2O$ (C,H,N,F)
30H-*R,R*	B	isoelect prec	75	208–210	$C_{20}H_{25}FN_4O_3\cdot 0.05HF$ (C,H,N,F)
30H-*S,S*	B	isoelect prec	77	208–210	$C_{20}H_{25}FN_4O_3\cdot 0.5H_2O$ (C,H,N,F)
30H-*S,R*	B	isoelect prec	88	198–199	$C_{20}H_{25}FN_4O_3\cdot 0.45H_2O$ (C,H,N,F)
				**31**	
31A	ref 18				
31B	ref 18				
31C	ref 18				
31D	ref 18				
31E	A	isoelect prec	66	250–252	$C_{19}H_{22}FN_3O_3\cdot 0.5H_2O$ (C,H,N)
31F-*R,S*	A	trit 2-PrOH	63	>300	$C_{19}H_{22}FN_3O_3\cdot HCl$ (C,H,N,Cl,F)
31F-*R,R*	A	trit 2-PrOH	66	>300	$C_{19}H_{22}FN_3O_3\cdot HCl\cdot 2.6H_2O$ (C,H,N,Cl,H$_2$O)
31F-*S,S*	A	isoelect prec/dissolved HCl/conc/trit 2-PrOH	66	>300	$C_{19}H_{22}FN_3O_3\cdot 1.6HCl\cdot 1.1H_2O$ (C,H,N,F,Cl)
31F-*S,R*	A	isoelec prec/dissolved HCl/conc/trit 2-PrOH	75	>300	$C_{19}H_{22}FN_3O_3\cdot 1.4HCl\cdot 0.7H_2O$ (C,H,N,F,Cl,H$_2$O)
31G-*R,S*	A	trit 2-PrOH	32	229–230	$C_{20}H_{24}FN_3O_3\cdot 2HF\cdot 0.5H_2O$ (C,H,N,F)
31G-*R,R*	A	trit 2-PrOH	82	>300	$C_{20}H_{24}FN_3O_3\cdot HCl\cdot 0.5H_2O$ (C,H,N)
31G-*S,S*	A	trit 2-PrOH	80	>300	$C_{20}H_{24}FN_3O_3\cdot HCl\cdot 0.4H_2O$ (C,H,N,Cl)
31G-*S,R*	A	recryst MeOH/Et$_2$O	67	>300	$C_{20}H_{24}FN_3O_3\cdot HCl\cdot 0.8H_2O$ (C,H,N,Cl)
31H-*R,S*	B	none	86	211–213	$C_{21}H_{26}FN_3O_3\cdot 0.6HF$ (C,H,N,F)
31H-*R,R*	B	none	95	233–235	$C_{21}H_{26}FN_3O_3\cdot 0.33HF$ (C,H,N,F)
31H-*S,S*	B	none	94	233–234	$C_{21}H_{26}FN_3O_3\cdot 0.6HF$ (C,H,N,F)
31H-*S,R*	B	none	86	210–211	$C_{21}H_{26}FN_3O_3\cdot 0.75HF$ (C,H,N,F)
				**32**	
32A	ref 28,36				
32B	ref 36				
32C	ref 36				
32D	ref 28,36				
32E	A	isoelect prec	44	201–204	$C_{19}H_{22}F_2N_4O_3\cdot 0.5H_2O$ (C,H,N)
32F-*R,S*	A	trit Et$_2$O	72	251–254	$C_{19}H_{22}F_2N_4O_3\cdot HCl\cdot 0.35H_2O$ (C,H,N,Cl)
32F-*R,R*	A	trit EtOAc	77	273–275	$C_{19}H_{22}F_2N_4O_3\cdot 1.2HCl\cdot 1.7H_2O$ (C,H,N,Cl)
32F-*S,S*	A	trit EtOAc	46	252–254	$C_{19}H_{22}F_2N_4O_3\cdot 1.1HCl\cdot H_2O$ (C,H,N,Cl)
32F-*S,R*	A	trit EtOAc	56	233–235	$C_{19}H_{22}F_2N_4O_3\cdot 1.1HCl\cdot 0.8H_2O$ (C,H,N,Cl)
32G-*R,S*	A	none	46	203–205	$C_{20}H_{24}F_2N_4O_3\cdot 1.5HF\cdot 0.7H_2O$ (C,H,N,F)
32G-*R,R*	A	none	98	198–199	$C_{20}H_{24}F_2N_4O_3\cdot 1.5H_2O$ (C,H,N)
32G-*S,S*	A	none	77	>250	$C_{20}H_{24}F_2N_4O_3\cdot 2.0HCl\cdot 0.6H_2O$ (C,H,N)
32G-*S,R*	A	none	10	>250	$C_{20}H_{24}F_2N_4O_3\cdot 2HCl$ (C,H,N)
32H-*R,S*	B	isoelect prec	73	148–150	$C_{21}H_{26}F_2N_4O_3\cdot 0.4H_2O$ (C,H,N)
32H-*R,R*	B	isoelect prec	37	156–157	$C_{21}H_{26}F_2N_4O_3\cdot 2.6H_2O$ (C,H,N,H$_2$O)
32H-*S,S*	B	isoelect prec	50	156–157	$C_{21}H_{26}F_2N_4O_3\cdot 0.3H_2O$ (C,H,N)
32H-*S,R*	B	isoelect prec	50	136–138	$C_{21}H_{26}F_2N_4O_3\cdot 0.7H_2O$ (C,H,N)

Table III (Continued)

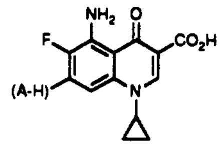
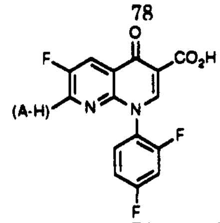
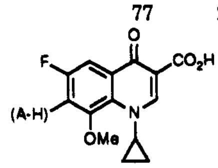
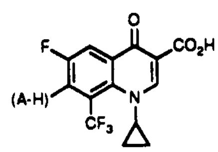
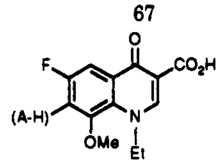
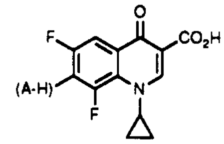
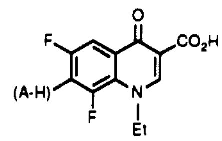
no.[f]	method of prep.[a] or ref	method of purif of final prod[b]	% yield[c]	mp (°C)	analysis formula (elements analyzed)[d]
			33		
33A	ref 28				
33D	ref 28				
33F-*R,S*	A	trit 2-PrOH/Et_2O	75	>300	$C_{19}H_{23}FN_4O_3$·1.75HCl·2.5H_2O (C,H,N,Cl)
33G-*R,S*	A	recryst EtOH/H_2O	44	>280	$C_{20}H_{25}FN_4O_3$·HCl·0.5H_2O (C,H,N,Cl)
33H-*R,S*	A	dissolved HCl/conc/trit 2-PrOH	78	>300	$C_{21}H_{27}FN_4O_3$·1.8HCl·1.5H_2O (C,H,N,Cl)
			34		
34A (9[g])	ref 29				
34B	A	trit 2-PrOH/Et_2O	74	272–275	$C_{20}H_{17}F_3N_4O_3$·HCl·2.3H_2O (C,H,N,Cl)
34D	B	none	100	189–191	$C_{22}H_{21}F_3N_4O_3$·1.5H_2O (C,H,N)
34F-*R,S*	A	none	86	263–265	$C_{21}H_{19}F_3N_4O_3$·1.1HCl·0.9H_2O (C,H,N,Cl)
34F-*R,R*	A	none	95	265–268	$C_{21}H_{19}F_3N_4O_3$·1.1HCl·1.2H_2O (C,H,N,Cl)
34G-*R,S*	A	recryst EtOH/Et_2O	82	278–280	$C_{22}H_{21}F_3N_4O_3$·HCl (C,H,N,Cl)
34G-*R,S*	B	isoelect prec	71	136–138	$C_{23}H_{23}F_3N_4O_3$·2.0H_2O (C,H,N)
34H-*R,R*	B	isoelect prec	77	203–205	$C_{23}H_{23}F_3N_4O_3$·1.7H_2O (C,H,N)
			35		
35A	C	isoelec prec/dissolved in HCl/conc/	89	200–202	$C_{18}H_{20}FN_3O_4$·HCl·1.5H_2O (C,H,N)
35B	C	trit 2-PrOH/ether	54	>300	$C_{19}H_{22}FN_3O_4$·2.0HCl·3.0H_2O (C,H,N,Cl,H_2O)
35C	C	isoelect prec	42	207–208	$C_{21}H_{26}FN_3O_4$·1.1H_2O (C,H,N)
35E	C	isoelect prec	75	179–182	$C_{20}H_{24}FN_3O_4$·0.5H_2O
35F-*R,S*	C	trit 2-PrOH	89	236–238	$C_{20}H_{24}FN_3O_4$·1.25HCl·2.5H_2O (C,H,N,Cl)
35F-*R,R*	C	trit 2-PrOH	77	215–218	$C_{20}H_{24}FN_3O_4$·1.1HCl·2.4H_2O (C,H,N,Cl)
35F-*S,R*	C	recryst 2-PrOH	62	188–190	$C_{20}H_{24}FN_3O_4$·HCl·0.2HF·2.2H_2O (C,H,N,Cl)
35G-*R,S*	C	trit EtOAc	73	166–167	$C_{21}H_{26}FN_3O_4$·HCl·1.4H_2O (C,H,N,Cl)
35G-*R,R*	C	isoelect prec/dissolved HCl/conc	38	212–213	$C_{21}H_{26}FN_3O_4$·HCl·2.0H_2O (C,H,N,Cl)
35H-*R,S*	C	chromatographed	53		$C_{22}H_{28}FN_3O_3$·1.22CH_2Cl_2 (C,H,N)[e]
35H-*R,R*	C	chromatographed	66	97–99	$C_{22}H_{28}FN_3O_3$ (C,H,N)
			36		
36A	ref 20				
36D	ref 20				
36F-*R,S*	A	none	89	>300	$C_{20}H_{21}F_4N_3O_3$·1.8HCl·1.3H_2O (C,H,N,Cl)
36F-*R,R*	A	trit 2-PrOH	47	>300	$C_{20}H_{21}F_4N_3O_3$·1.6HCl·1.2HF·2.4H_2O (C,H,N,Cl)
36G-*R,S*	A	trit 2-PrOH	79	210–212	$C_{21}H_{23}F_4N_3O_3$·1.1HCl·1.4H_2O (C,H,N,F,Cl)
36G-*R,R*	A	trit 2-PrOH	69	261–262	$C_{21}H_{23}F_4N_3O_3$·HCl·1.1H_2O (C,H,N,F,Cl)
36H-*R,S*	B	trit 2-PrOH	72	175–177	$C_{22}H_{25}F_4N_3O_3$·0.2HF·1.1H_2O (C,H,N,H_2O)
36H-*R,R*	B	none	67	146–147	$C_{22}H_{25}F_4N_3O_3$·0.5H_2O (C,H,N)
			37		
37A	C	trit Et_2O	73	229–231	$C_{17}H_{20}FN_3O_4$·1.1HCl·0.8H_2O (C,H,N,Cl)
37F-*R,S*	C	trit 2-PrOH	72	>280	$C_{19}H_{24}FN_3O_4$·1.6HCl·1.8H_2O (C,H,N,Cl)
37G-*R,S*	C	trit Et_2O	70	>280	$C_{20}H_{26}FN_3O_4$·1.1HCl·1.5H_2O (C,H,N,Cl)

Table III (Continued)

no.[f]	method of prep.[a] or ref	method of purif of final prod[b]	% yield[c]	mp (°C)	analysis formula (elements analyzed)[d]
		 **38**			
38A (5)	ref 18				
38B (6)	ref 18				
38D	ref 18				
38F-*R,S*	A	none	72	>300	$C_{19}H_{21}F_2N_3O_3 \cdot HCl \cdot 0.8H_2O$ (C,H,N,Cl)
		 **39**			
39A	A	isoelect prec	48	240–242	$C_{16}H_{17}F_2N_3O_3 \cdot 0.5H_2O$ (C,H,N)
39B	ref 31				
39D-S	ref 37				
39F-*R,S*	A	washed 2-PrOH	72	289–290	$C_{18}H_{21}F_2N_3O_3 \cdot HCl$ (C,H,N,F,Cl)
39F-*R,R*	A	washed 2-PrOH	68	285–286	$C_{18}H_{21}F_2N_3O_3 \cdot HCl \cdot H_2O$ (C,H,N,F,Cl)
2[h]	ref 18				

[a] See Experimental Section for general methods. [b] Trituration (trit) refers to grinding the solids under solvent to produce a fine powder. Isoelectric precipitation (isoelec prec) refers to dissolving the compound in NaOH to pH 10.5–11.0 and then slowly adjusting to pH to 7.0–7.2 and filtering the resultant solids. [c] Yields are those from coupling to final products and include the deprotection and hydrolysis steps. [d] Symbols refer to those elements analyzed for. Analyses were ±0.4% of the theoretical values. [e] Solvent was present in analytical sample and was observed in ^{1}H NMR spectra. Chemical and optical purities were >98% by HPLC. [f] See Figure 2 for structures of side chains A–H. [g] Tosufloxacin. [h] Ciprofloxacin.

C and D, or alkylation of the 3-position of the pyrrolidine ring to give E, produced quinolones with reduced Gram-negative and Gram-positive effectiveness (see any series of C, D, or E quinolones vs B).

In vivo, the (3-aminopyrrolidinyl)quinolones (A) displayed the best oral efficacy against systemic *E. coli* infections in mice, but were less effective vs the *Streptococcus pyogenes* infection model. Most of this variance was likely due to the 2–4-fold MIC differential between *E. coli* and *S. pyogenes*. The increased in vitro Gram-positive activity conferred by pyrrolidine B did not translate into in vivo effectiveness (see **30B**, **31B**, **32B**, and **38B**). Alkylating the distal nitrogen or the pyrrolidine ring (C–E), even though in vitro activity was reduced, did improve in vivo efficacy vs *S. pyogenes* by 2–10-fold (**32D** vs **32A** and **32B**; **36D** vs **36A**; **38D** vs **38A** and **38B**; and **30E** vs **30B**).

The quinolone nuclei can be ranked on the basis of combined in vitro and in vivo antibacterial effectiveness. This exercise, using all the data in Table IV, produces the following descending rank order: 6,8-difluoroquinolones **38** > N_1-(difluorophenyl)naphthyridines **34** ≥ 5-amino-6,8-difluoroquinolones **32** > 8-methoxyquinolones **35** > 8-(trifluoromethyl)quinolones **36** ≥ 1-cyclopropylnaphthyridines **30** ≥ 5-aminoquinolones **33** ≥ quinolones **31**.

When analyzing the effects of adding a single methyl group to the methylene spacer of B, C, and D, giving the side chains F, G, and H, one notices that these side chains, like their precursors, confer much greater activity vs the Gram-positive organisms. It is also immediately clear that the stereochemistry of the 3- and 1-centers has a significant and consistent effect on in vitro and in vivo activity. For all the quinolones tested, the in vitro and in vivo potencies conferred by the side chains F–H tend to vary with stereochemical configuration in the descending rank order of 3*R*,1*S* ≥ 3*R*,1*R* ≥ 3*S*,1*R* ≥ 3*S*,1*S*. While the differences in activity between close pairs are small, usually 1–4-fold, the differences between the most active (3*R*,1*S*)-quinolones and the least active 3*S*,1*S* derivatives are consistently 2–8-fold, with certain organisms approaching differences in potency of >30-fold (see *S. pneumonia* for **30G**-*R,S* vs **30G**-*S,S* and **32G**-*R,S* vs **32G**-*S,S*). These in vitro differences are reflected at the target enzyme level. The DNA gyrase inhibition data from Table V demonstrates that the *R,S* and *R,R* isomers of **30F** and **31F** are 3–15 times superior to the *S,R* and *S,S* isomers at inducing initial DNA cleavage. In vivo, the differences in oral efficacy between stereoisomers parallels the in vitro results with the *R,S* isomers, displaying an average of 6–10-fold superiority over the *S,S* and *S,R* derivatives. It appears that the center of asymmetry at the pyrrolidinyl 3-position has a greater overall influence on activity than the adjacent 1-position. The most active isomers (3*R*,1*S* and 3*R*,1*R*) bear the same configuration at C_3 as the most favorable enantiomer of the 3-[(ethylamino)methyl]pyrrolidine D (Figure 3).[37] In contrast to the large differences in quinolone activity conferred by the various stereoisomers of F–H, the quinolones containing the enantiomers of D showed only a small 1–3-fold variance. This is the first case where stereochemical configuration spatially distant from the quinolone nucleus has created such a large biological difference between stereoisomers.[12]

If one compares the 3*R*,1*S* isomers of F–H to the reference side chains B–D, the effect of methylating the methylene spacer atom can be discerned. Examining all the quinolones tested shows that the methylated spacer has little effect on Gram-negative potency. (See **30F**-*R,S* vs **30B**, **30G**-*R,S* vs **30C**, **30H**-*R,S* vs **30D**, etc.) However, against Gram-positive organisms, the compounds containing pyrrolidines F–H confer an average 2–4-fold superiority relative to the quinolones with side chains B–D. (Compare **30–32G**-*R,S* and **30–32H**-*R,S* vs **30–32C** and **30–32D**.) Compared to the 3-aminopyrrolidine A, the *R,S* and *R,R* isomers of F–H always provide quinolones with improved Gram-positive potency. The quinolones bearing the primary amino side chain **F**-*R,S* also approach the Gram-negative potency of the reference compounds containing side chain A.

Comparison of the oral efficacy for the quinolones containing side chains F–H vs the reference agents with side chains A–E immediately reveals that the 3-(1-aminoethyl)pyrrolidines confer 4–10 times more activity in the *S. pyogenes* infection model with the average closer to 10 (see the *R,S* isomers of **30–32** vs the derivatives **30–32B–D**). This in vivo oral superiority reaches 100-fold when comparing **39F**-*R,S* to **39B**. Against *E. coli*, quinolones with side chains F-*R,S*, G-*R,S*, or H-*R,S* show more modest advantages of 2–5-fold over comparable analogs of B, C, or D. They do not compete with the 3-aminopyrrolidine A for conferring oral efficacy vs *E. coli*. As with the reference agents, alkylation of the distal nitrogen further improves the in vivo performance (see corresponding stereoisomers of **31F** relative to **31G** and **31H**, and **33F**-*R,S* relative to **33H**-*R,S*).

The in vivo enhancement of the 3-(1-aminoethyl) moiety is so great that even the quinolones with the *S,R* and *R,R* side chains, which are the less active of the stereoisomers in vitro, are often more active orally than the reference quinolones, especially vs *S. pyogenes* (see **30F**-*R,R*, **30F**-*S,R* vs **30B**, **31H**-*R,R* vs **31D**, and **39F**-*R,R* vs **39B**). The magnitude of the observed in vivo superiority of these quinolones can be partly explained by their generally better MICs for *S. pyogenes* vs the reference agents, and the fact that the reference quinolones are racemic mixtures, which has been shown to account for a 2-fold reduction in oral efficacy vs Gram-positive infections in mice.[37] Nevertheless, even with correction for these variables, the *R,S* isomers of the [3-(1-aminoethyl)-1-pyrrolidinyl]quinolones still have a baseline edge in oral efficacy of 3-fold over the reference quinolones. Other workers have shown that alkylation of the R_7 heterocyclic substituent of the quinolones tends to improve their water solubility[23,40] and increase lipophilicity.[23] Such physical chemical properties were associated with improved in vivo efficacy and this phenomena could help explain the in vivo enhancement described in this series.

The outstanding in vitro and in vivo Gram-positive potency conferred by the *R,S* isomers of F–H made it possible for several of the substituted quinolones to now compete with the 6,8-difluoroquinolones **38**. Relative to the reference agents **5** (**38A**) and **6** (**38D**), several of the new quinolones meet or exceed these standards for combined in vitro and in vivo activity. These include 1-cyclopropylnaphthyridines **30F**-*R,S*, **30G**-*R,S* and **30G**-*R,R*, and **30H**-*R,S* and **30H**-*R,R*; 5-amino-6,8-difluoroquinolones **32F**-*R,S* and **32F**-*R,R*, **32G**-*R,S* and **32G**-*R,R*, **32H**-*R,S* and **32H**-*R,R*; 1-(2,4-difluorophenyl)naphthyridine **34F**-*R,S*; 8-methoxyquinolones **35F**-*R,S* and **35F**-*R,R*, **35G**-*R,S*, and **35H**-*R,S*; 8-(trifluoromethyl)quinolones **36F**-*R,S* and **36G**-*R,S*; and 1-ethyl-6,8-difluoroquinolone **39F**-*R,S*.

As described above, the 8-fluoroquinolones have been reported to increase phototoxicity in animal models,[20] and also cytotoxicity in in vitro mammalian cell assays.[21a,b,d] Therefore, the compounds with activity competitive with **38A** and **38D** were tested for both phototoxicity and cytotoxicity (Table VI).

We have previously reported that most of the quinolones in widespread clinical use display oral no-effect doses (NEDs) of ≥30 mg/kg in the mouse phototoxicity model.[20,39] The reference 6,8-difluoroquinolones **38A**, **38D**, and **39D** and the agents **38F**-*R,S* and **39F**-*R,S* represent the high extremes of induced phototoxicity with NEDs ranging from 1.5 to 10 mg/kg. The [3-(1-ethylamino)-1-pyrrolidinyl]naphthyridines **30** and the 5-amino-6,8-difluoroquinolone analogs **32** show a fair degree of phototoxicity relative to their reference quinolones (see **30A** vs **30F**-*R,S*, **30G**-*R,S*, and **30H**-*R,S*). Clearly, the improved in vivo efficacy might account for the increased phototoxicity. However, several quinolones containing the F–H side chains show NEDs of ≥100 mg/kg.

Quinolone cytotoxicity in mammalian cells has become an important aspect of their overall safety profile. Several workers have previously demonstrated a strong correlation between mammalian topoisomerase II inhibition and cytotoxicity to mammalian cells.[21a,c,g,f] Recently, these correlations were extended to include in vitro clastogenic endpoints.[21c,e,f] Over a wide variety of quinolones, the concentration causing mammalian cell cytotoxicity was well-correlated to that concentration inducing micronuclei formation, a clastogenic endpoint.[21,c,e] From this and related work,[21d] it has been shown that most of the quinolones in clinical use display a 50% cytotoxicity concentration vs mammalian cells of ≥100 μg/mL.[21c,d] Of the new quinolones described in this study, only a small percentage have IC_{50} values ≥100 μg/mL, and many, especially those with an 8-fluoro group, are cytotoxic at concentrations ≤10 μg/mL. Only **34F**-*R,S*, **35H**-*R,S*, and **36G**-*R,S* meet the phototoxicity and cytotoxicity criteria, while possessing overall antibacterial activity comparable to the reference standards **5** (**38A**) and **6** (**38D**).

In summary, we have shown that the 3-(1-aminoethyl)-pyrrolidines confer excellent Gram-positive potency both in vitro and in vivo. Unlike their 3-(aminomethyl)-pyrrolidinyl analogs, the stereoisomers of F–H, display significant differences in MICs and in oral efficacy ranging from 6- to 30-fold between the most active 3*R*,1*S* and the least active 3*S*,1*S* isomers. Relative to the reference quinolones containing the pyrrolidinyl side chains A–E, the *R,S* isomers of F–H confer a 2–4-fold improvement in in vitro Gram-positive activity and an average 10-fold improvement in oral efficacy. In particular, the quinolones **34F**-*R,S*, **35H**-*R,S*, and **36G**-*R,S* display extremely high levels of antibacterial efficacy with low levels of phototoxicity and mammalian cell cytotoxicity. These agents are ideal candidates for further advancement with **34F**-*R,S* (PD 140248) showing the best overall blend of safety and efficacy.

Experimental Section

All melting points were determined on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined in KBr on a Mattson Cygnus 100 or a Nicolet MX1 instrument. Proton magnetic resonance (NMR) were recorded on either a Varian XL-200 or a Bruker AM250 spectrometer; shifts are reported in δ units relative to internal tetramethylsilane. All mass spectra were obtained on a Finnigan 4500 GCMS or a VG analytical 7070 E/F spectrometer. Elemental analyses were performed on a CEC Model 240 elemental analyzer and all compounds prepared had analytical results ±0.4% of the theoretical values. Column chromatography was accomplished using E. Merck silica gel, 230–400 mesh, and concentrations were performed in vacuo at 10–30 mmHg. Final compounds were assayed for purity by using a Waters high-performance liquid chromatography (HPLC) system equipped with a 5-μM Alltech CN column and a mobile phase consisting of 20% THF/80% 0.5 M $NH_4H_2PO_4$ (adjusted to pH 3 with H_3PO_4); in all cases, the purity exceeded 97%.

General Method. Procedures for the Determination of Isomeric Purity of 13-*R,S*, 13-*R,R*, 13-*S,R*, and 13-*S,S*. The purity of pyrrolidinones **13** was determined via the two following methods which gave comparable results.

High-Pressure Liquid Chromatography. Small samples (<10 mg) of each of the chromatographed pyrrolidines **13** were

Table IV. In Vitro and in Vivo Antibacterial Activity of the [3-(1-Aminoethyl)-1-pyrrolidinyl]quinolones and the Reference Agents from Table III

	in vitro antibacterial activity (MICs[a] μg/mL)										in vivo antibacterial activity; mouse protection (PD_{50}, mg/kg)[b]			
	Gram-negative organisms[c]					Gram-positive organisms[c]					*E. c.*		*S. py.*	
no.	*E. cl.*	*E. c.*	*K. pn.*	*P. r.*	*P. ae.*	*S. au.* H	*S. au.* U	*E. f.*	*S. pn.*	*S. py.*	po[d]	sc[e]	po[d]	sc[e]
30A	0.025	0.013	0.025	0.05	0.1	0.2	0.013	0.2	0.2	0.2	2	0.6	32	14
30B	0.1	0.05	0.1	0.4	0.2	0.1	0.003	0.025	0.1	0.025	17	1	39	4
30C	0.2	0.1	0.2	0.4	0.8	0.2	0.025	0.2	0.025	0.05	3	1	21	7
30D	0.2	0.1	0.2	0.8	0.8	0.1	0.05	0.1	0.1	0.2	7	1	17	8
30E	0.1	0.2	0.1	0.4	0.8	0.025	0.006	0.05	0.025	0.05	10	2	6	2
30F-*R,S*	0.05	0.025	0.05	0.1	0.4	0.025	0.003	0.013	0.003	0.013	2	0.4	3	1
30F-*R,R*	0.05	0.05	0.1	0.4	0.8	0.05	0.006	0.05	0.006	0.013	6	1	14	2
30F-*S,S*	0.05	0.05	0.2	0.4	1.6	0.2	0.025	0.1	0.05	0.05	8	1	28	10
30F-*S,R*	0.05	0.05	0.1	0.2	0.8	0.2	0.025	0.1	0.05	0.05	4	1	15	5
30G-*R,S*	0.1	0.1	0.2	0.4	1.6	0.1	0.013	0.1	0.013	0.025	1	0.5	2	2
30G-*R,R*	0.1	0.1	0.2	0.4	1.6	0.1	0.05	0.1	0.025	0.05	5	1	8	5
30G-*S,S*	0.4	0.2	0.4	0.8	3.1	0.8	0.2	0.8	0.4	0.4			23	15
30G-*S,R*	0.1	0.1	0.2	0.8	1.6	0.2	0.05	0.2	0.1	0.1			11	5
30H-*R,S*	0.1	0.1	0.2	0.8	1.6	0.1	0.025	0.05	0.025	0.05	2	1	2	2
30H-*R,R*	0.4	0.1	0.2	1.6	1.6	0.05	0.025	0.05	0.025	0.05	7	6	6	4
30H-*S,S*	0.8	0.4	0.8	3.1	6.3	0.8	0.4	0.8	0.2	0.4			12	12
30H-*S,R*	0.2	0.2	0.4	1.6	3.1	0.2	0.1	0.2	0.1	0.4	3	1	15	9
31A	0.025	0.025	0.05	0.1	0.2	0.2	0.025	0.1	0.1	0.1	3	0.5	97	11
31B	0.1	0.05	0.1	0.2	0.8	0.05	0.006	0.05	0.013	0.013	>100	2	>100	4
31C	0.2	0.1	0.2	0.4	0.8	0.2	0.025	0.1	0.05	0.05	35	2	>50	5
31D	0.4	0.2	0.2	0.8	1.6	0.4	0.013	0.2	0.2	0.05	35	3	46	5
31E	0.8	0.2	0.4	0.8	0.8	0.1	0.013	0.1	0.025	0.05	43	3	31	3
31F-*R,S*	0.05	0.05	0.1	0.2	0.4	0.05	0.025	0.025	0.013	0.003	47	1	11	1
31F-*R,R*	0.2	0.1	0.4	0.8	1.6	0.2	0.05	0.1	0.025	0.025	72	2	>100	2
31F-*S,S*	0.2	0.2	0.4	0.8	1.6	0.8	0.1	0.4	0.2	0.1	100	4	>100	12
31F-*S,R*	0.2	0.2	0.4	0.8	1.6	0.4	0.05	0.2	0.1	0.1	35	2	>100	3
31G-*R,S*	0.2	0.2	0.4	0.8	1.6	0.2	0.05	0.1	0.05	0.05	7	1	7	1
31G-*R,R*	0.2	0.1	0.4	0.8	1.6	0.2	0.05	0.2	0.05	0.05			36	3
31G-*S,S*	0.4	0.4	0.8	3.1	6.3	1.6	0.2	0.8	0.4	0.4				
31G-*S,R*	0.2	0.2	0.4	0.8	3.1	0.4	0.05	0.2	0.2	0.1			82	7
31H-*R,S*	0.2	0.2	0.4	0.8	6.3	0.1	0.025	0.1	0.05	0.05	13	2	8	1
31H-*R,R*	0.2	0.2	0.4	0.8	6.3	0.1	0.025	0.1	0.05	0.05			12	4
31H-*S,S*	0.4	0.4	0.8	1.6	12.5	0.8	0.4	0.8	0.4	0.4				
31H-*S,R*	0.4	0.4	0.8	1.6	6.3	0.4	0.2	0.4	0.2	0.2				
32A	0.013	0.006	0.006	0.025	0.05	0.013	0.006	0.025	0.006	0.025	1	0.3	28	8
32B	0.025	0.013	0.025	0.05	0.1	0.006	0.003	0.013	0.003	0.003	12	1	11	0.6
32C	0.05	0.025	0.05	0.2	0.4	0.013	0.006	0.025	0.003	0.003	8	1		
32D	0.05	0.05	0.05	0.1	0.2	0.013	0.003	0.025	0.003	0.013	16	2	5	3
32E	0.1	0.2	0.1	0.2	0.8	0.013	0.003	0.025	0.003	0.013	9	1	8	1
32F-*R,S*	0.013	0.013	0.013	0.025	0.4	0.003	0.003	0.003	0.003	0.003	2	0.3	0.6	0.1
32F-*R,R*	0.025	0.025	0.025	0.05	0.4	0.003	0.003	0.006	0.006	0.006	4	0.7	2	0.2
32F-*S,S*	0.05	0.05	0.1	0.4	0.4	0.025	0.013	0.05	0.025	0.05			>25	7
32F-*S,R*	0.1	0.05	0.1	0.2	0.8	0.025	0.006	0.05	0.013	0.013			14	4
32G-*R,S*	0.025	0.025	0.05	0.1	0.8	0.003	0.003	0.013	0.003	0.003	3	0.4	0.6	0.3
32G-*R,R*	0.05	0.05	0.1	0.2	0.8	0.006	0.003	0.013	0.006	0.006	3	2	2	0.7
32G-*S,S*	0.4	0.4	0.8	1.6	3.1	0.8	0.4	1.6	0.8	1.6				
32G-*S,R*	0.2	0.2	0.8	0.8	3.1	0.1	0.05	0.2	0.2	0.2			13	13
32H-*R,S*	0.025	0.025	0.05	0.1	0.8	0.003	0.003	0.013	0.003	0.003	2	1	0.3	0.3
32H-*R,R*	0.1	0.05	0.1	0.2	1.6	0.006	0.003	0.025	0.003	0.003			1	0.5
32H-*S,S*	0.4	0.2	0.4	1.6	3.1	0.1	0.05	0.2	0.05	0.2			13	9
32H-*S,R*	0.1	0.1	0.1	0.4	0.8	0.013	0.003	0.025	0.013	0.013			6	3
33A	0.013	0.013	0.025	0.1	0.4	0.1	0.025	0.1	0.025	0.05	8	1	110	6
33D	0.1	0.1	0.4	0.8	1.6	0.2	0.025	0.05	0.05	0.1	90	3	>100	11
33F-*R,S*	0.025	0.025	0.05	0.1	0.4	0.05	0.003	0.013	0.003	0.003			18	4
33G-*R,S*	0.1	0.1	0.2	0.4	1.6	0.05	0.006	0.025	0.013	0.025			8	1
33H-*R,S*	0.1	0.1	0.2	0.8	1.6	0.05	0.025	0.05	0.013	0.013			5	1
34A	0.025	0.025	0.1	0.1	0.4	0.05	0.013	0.1	0.05	0.05	0.5	0.2	5	3
34B	0.05	0.05	0.1	0.2	0.4	0.05	0.013	0.025	0.025	0.025			10	1
34D	0.2	0.2	0.4	0.8	1.6	0.1	0.025	0.2	0.1	0.1	6	4	8	3
34F-*R,S*	0.2	0.1	0.2	0.4	0.8	0.05	0.006	0.025	0.006	0.006	3	1	1	1
34F-*R,R*	0.1	0.1	0.2	0.4	1.6	0.1	0.025	0.1	0.1	0.05			9	3
34G-*R,S*	0.8	0.4	0.8	1.6	3.1	0.1	0.025	0.2	0.025	0.05	6	2	4	1
34H-*R,S*	0.8	0.4	0.8	1.6	3.1	0.05	0.013	0.2	0.1	0.1	25	13	2	2
34H-*R,R*	0.8	0.8	1.6	3.1	3.1	0.2	0.05	0.4	0.2	0.2			10	6
35A	0.025	0.025	0.05	0.1	0.2	0.05	0.013	0.05	0.025	0.025	3	0.4	10	3
35B	0.05	0.05	0.1	0.1	0.8	0.025	0.003	0.013	0.003	0.003			11	0.6
35D	0.1	0.1	0.2	0.4	1.6	0.025	0.006	0.05	0.013	0.013			4	0.4
35E	0.1	0.1	0.2	0.4	1.6	0.025	0.006	0.025	0.013	0.013	18	3	5	1
35F-*R,S*	0.05	0.025	0.05	0.05	0.4	0.006	0.003	0.006	0.003	0.003	4	1	1	0.3
35F-*R,R*	0.05	0.05	0.1	0.2	0.8	0.025	0.003	0.025	0.013	0.013			6	1
35F-*S,R*	0.8	0.4	0.4	0.4	1.6	0.05	0.013	0.05	0.025	0.025			16	6
35G-*R,S*	0.05	0.05	0.1	0.2	0.8	0.013	0.003	0.025	0.003	0.003	5	1	1	0.1

Table IV (Continued)

	in vitro antibacterial activity (MICs[a] μg/mL)										in vivo antibacterial activity; mouse protection (PD_{50}, mg/kg)[b]			
	Gram-negative organisms[c]					Gram-positive organisms[c]					*E. c.*		*S. py.*	
no.	*E. cl.*	*E. c.*	*K. pn.*	*P. r.*	*P. ae.*	*S. au.* H	*S. au.* U	*E. f.*	*S. pn.*	*S. py.*	po[d]	sc[e]	po[d]	sc[e]
35G-*R,R*	0.1	0.1	0.2	0.4	3.1	0.05	0.013	0.05	0.013	0.025	12	3	4	1
35H-*R,S*	0.1	0.1	0.2	0.4	3.1	0.013	0.003	0.025	0.006	0.006	5	2	0.5	0.2
35H-*R,R*	0.2	0.2	0.4	0.8	3.1	0.013	0.003	0.013	0.003	0.003	14	4	3	0.6
36A	0.1	0.1	0.2	0.4	1.6	0.2	0.1	0.4	0.4	0.4	3	1	32	22
36D	0.4	0.4	0.8	1.6	6.3	0.05	0.025	0.1	0.05	0.05	5	2	2	1
36F-*R,S*	0.2	0.2	0.2	0.4	1.6	0.013	0.006	0.025	0.006	0.006			0.7	0.3
36F-*R,R*	0.4	0.8	0.8	0.8	6.3	0.1	0.025	0.2	0.1	0.1			3	2
36G-*R,S*	0.4	0.4	0.8	0.8	3.1	0.025	0.013	0.1	0.013	0.013	4	2	0.8	0.3
36G-*R,R*	0.4	0.2	1.6	3.1	6.3	0.05	0.025	0.1	0.05	0.05			3	2
36H-*R,S*	0.4	0.8	1.6	1.6	6.3	0.05	0.013	0.1	0.013	0.025	5	3	0.7	0.5
36H-*R,R*	1.6	0.8	1.6	3.1	6.3	0.05	0.05	0.1	0.05	0.1			2	0.8
37A	0.2	0.2	0.2	0.4	0.8	0.2	0.05	0.4	0.4	0.2	13	7	74	25
37F-*R,S*	0.1	0.05	0.1	0.1	0.8	0.006	0.003	0.006	0.003	0.003	9	3	3	0.2
37G-*R,S*	0.8	0.2	0.2	0.4	1.6	0.025	0.003	0.025	0.013	0.013	22	4	2	0.3
38A(5)	0.025	0.013	0.025	0.05	0.1	0.05	0.025	0.05	0.05	0.05	1	0.2	8	2
38B	0.013	0.025	0.05	0.1	0.2	0.013	0.003	0.013	0.006	0.003	6	1	4	0.4
38D(6)	0.1	0.05	0.1	0.2	0.4	0.025	0.006	0.025	0.025	0.025	4	1	1	0.8
38F-*R,S*	0.013	0.013	0.013	0.025	0.4	0.013	0.003	0.006	0.006	0.006	2	0.3	0.4	0.1
39A	0.1	0.05	0.1	0.1	0.2	0.2	0.05	0.4	0.4	0.2	3	1	21	7
39B	0.2	0.1	0.4	0.4	0.4	0.1	0.1	0.1	0.1	0.05	90	1	100	5
39D-*S*	0.2	0.1	0.1	0.2	1.6	0.05	0.05	0.1	0.1	0.1	10	3	10	4
39F-*R,S*	0.05	0.05	0.1	0.2	0.8	0.025	0.006	0.013	0.003	0.003	3	1	1	0.4
39F-*R,R*	0.1	0.1	0.2	0.4	1.6	0.1	0.025	0.1	0.05	0.05	15	2	19	2
2	0.05	0.05	0.05	0.1	0.4	3.1	0.2	0.8	1.6	0.8	1	0.3	180	19

[a] Minimum inhibitory concentrations. [b] Dose required to prevent death in 50% of the animals. Dose administered at time of lethal infection. [c] Definitions of organism abbreviations: *E. cl.* = *Enterobacter cloacae* MA 2646, *E. c.* = *E. coli* Vogel, *K. pn.* = *Klebsiella pneumoniae* MGH2, *P. r.* = *Providencia rettgeri* M1771, *P. ae.* = *Pseudomonas aeruginosa* UI-18, *S. au.* H = *Staphylococcus aureus* H 228, *S. au.* U = *S. aureus* UC 76, *E. f.* = *Enterococcus faecalis* MGH-2, *S. pn.* = *Streptococcus pneumoniae* SV-1, *S. py.* = *S. pyogenes* C 203. [d] Oral administration. [e] Subcutaneous administration.

Table V. DNA-Gyrase Inhibition[a] by Select Quinolones

no.	initial inhibn concn (μg/mL)	no.	initial inhibn concn (μg/mL)
30A	0.75	**31E**	1.0
30B	0.75	**31F**-*R,S*	0.50
30E	5.00	**31F**-*R,R*	0.75
30F-*R,S*	0.75	**31F**-*S,S*	7.5
30F-*R,R*	0.75	**31F**-*S,R*	7.5
30F-*S,S*	2.5	**34F**-*R,S*	0.5
30F-*S,R*	2.5	**35G**-*R,S*	<0.5
31A	0.25	**36H**-*R,S*	0.88
31B	0.50		

[a] Initial inhibition as measured by the concentration of drug that first induces cleavage of DNA-Gyrase complex. See ref 34.

dissolved in CH_2Cl_2, treated with 3 drops of Et_3N and a small amount of di-*tert*-butyl dicarbonate, and stirred for 20 min. These BOC derivatives were then assayed on a Waters HPLC equipped with an Alltech silica gel column and monitored at a wavelength of 214 nm. The mobile phase consisted of 4:1 octane/2-propanol at a flow rate of 1.5 mL/min.

NMR Spectroscopy. The doublet in the NMR spectrum of **13** occurring at approximately δ 1.0 corresponds to the methyl group adjacent to the exocyclic amine. The exact position of this doublet was characteristic of the specific diastereomer, and the presence of extraneous doublets in the region was diagnostic of isomeric contamination. These diagnostic doublets appeared at following resonances: δ 0.82 for **13**-*R,R*, δ 1.01 for **13**-*R,S*, δ 1.09 for **13**-*S,R*, and δ 1.19 for **13**-*S,S*.

The purities obtained via method 1 correlated well with those obtained via method 2 and indicated isomeric purities of ≥20:1.

General Method A. Preparation of (3*R*,1*S*)-7-[3-(1-Aminoethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid (30F-*R,S*). A mixture of 0.68 g (2.4 mmol) of 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**17**), 0.60 g (2.8 mmol) of **14**, 0.74 (7.3 mmol) of Et_3N, and 25 mL of CH_3CN was refluxed for 2 h. The mixture was cooled to 5 °C, diluted with Et_2O, and filtered. The solids were washed with H_2O and Et_2O and were dried to give 1.02 g (92%) of a white powder. This material was suspended in 20 mL of absolute EtOH, treated with 5 mL of 1 N HCl, and refluxed for 2 h. The homogeneous solution was cooled to room temperature and stirred for 18 h. The solvent was evaporated; the residue was triturated with 2-PrOH, and the solids which formed were filtered, washed with 2-PrOH and Et_2O, and dried to give 0.64 g (89%) of **30F**-*R,S*: mp >300 °C; IR 1703, 1631 cm^{-1}; NMR (DMSO-d_6) δ 1.05 (m, 2 H, cyclopropyl), 1.18–1.50 (m, 5 H, 2 cyclopropyl plus CH_3), 1.65–1.95 (m, 1 H, pyrrolidine), 2.15 (m, 1 H, pyrrolidine), 2.48 (m, 1 H, pyrrolidine), 3.30 (m, 1 H, C*H*NH_2), 3.47–3.87 (m, 3 H, pyrrolidine), 3.97 (m, 1 H, pyrrolidine), 4.10 (m, 1 H, cyclopropyl), 8.00 (d, *J* = 13 Hz, 1 H, C_5H), 8.53 (s, 1 H, C_2H).

Table VI. Results of Select Quinolones in the Mouse Phototoxicity and Hamster V-79 Cell Cytotoxicity Assays

no.	phototoxicity no-effect dose (mg/kg)	clonogenic cytotoxicity 50% inhibn (μg/mL)	no.	phototoxicity no-effect dose (mg/kg)	clonogenic cytotoxicity 50% inhibn (μg/mL)
2	>100	>200	**32H**-*R,S*		42
5 (38A)	3	30	**32H**-*R,R*		15
6 (38D)	10	11	**33A**	>300	12
38F-*R,S*	<3	<7.8	**33G**-*R,S*	>100	290
30A	>100	98	**34A**	>100	120
30F-*R,S*	30	33	**34F**-*R,S*	>100	280
30F-*S,R*	>100	84	**34G**-*R,S*	>100	310
30G-*R,S*	18	130	**35A**	>100	45
30G-*R,R*	30	88	**35F**-*R,S*	>100	<7.8
30H-*R,S*	<30	130	**35F**-*R,R*		41
30H-*R,R*		87	**35G**-*R,S*	>100	33
31G-*R,S*	100	180	**35H**-*R,S*	>100	110
32A	55	15	**36A**	>100	250
32C	>100	7.8	**36F**-*R,S*	>100	19
32F-*R,S*	<30	<7.8	**36G**-*R,S*	>100	130
32F-*R,R*		<7.8	**37F**-*R,S*		19
32G-*R,S*	<30	17	**39D**	1.5	190
32G-*R,R*	<30	24	**39F**-*R,S*	<10	9.8

Compounds **30F**-*R,R*, **30F**-*S,R*, and **30F**-*S,S* were prepared in an identical fashion, giving rise to the following physical data.

30F-*R,R*: NMR (DMSO-d_6) δ 1.00–1.47 (m, 7 H), 1.68–2.00 (m, 1 H), 2.11–2.35 (m, 1 H), 2.52 (m, 1 H), 3.17–3.87 (m, 4 H), 3.95 (m, 1 H), 4.17 (m, 1 H), 8.00 (d, J = 13 Hz, 1 H), 8.58 (s, 1 H).

30F-*S,R*: NMR (DMSO-d_6) δ 0.93–1.17 (m, 2 H), 1.17–1.47 (m, 5 H), 1.63–1.95 (m, 1 H), 2.00–2.37 (m, 1 H), 2.47 (m, 1 H), 3.35 (m, 1 H), 3.50–3.87 (m, 3 H), 3.93 (m, 1 H), 4.25 (m, 1 H), 8.00 (d, J = 13 Hz, 1 H), 8.58 (s, 1 H).

30F-*S,S*: NMR (DMSO-d_6) δ 1.00–1.50 (m, 7 H), 1.65–2.00 (m, 1 H), 2.22 (m, 1 H), 2.52 (m, 1 H), 3.15–3.85 (m, 4 H), 3.95 (m, 1 H), 4.17 (m, 1 H), 8.00 (d, J = 13 Hz, 1 H), 8.58 (s, 1 H).

Compounds **30G**-*S,S*, **31F**-*R,S*, **31F**-*R,R*, **31G**-*R,S*, **31G**-*R,R*, **31G**-*S,S*, **36G**-*R,S*, **30E**, **31E**, **32E**, **39F**-*R,S*, and **39F**-*R,R* were prepared in identical fashion to **30F**-*R,S*. Compounds **31F**-*S,R* and **31F**-*S,S* were prepared similarly except that TFA in CH_2Cl_2 was used to remove the *tert*-butoxycarbonyl protecting group instead of 1 N HCl in EtOH. Alternatively, the BOC-protected intermediate could be dissolved in 25 mL of CH_2Cl_2, cooled to 5 °C, and treated with gaseous HCl for 10 min. After warming to room temperature, the solution was concentrated and the residue triturated with 2-PrOH as before. This modified procedure was used to synthesize compounds **30G**-*S,R*, **31G**-*S,R*, **32F**-*R,S*, **32F**-*S,R*, **32F**-*R,R*, **32G**-*S,R*, **32G**-*S,S*, **32G**-*R,R*, **32G**-*R,S*, **34F**-*R,R*, **34G**-*R,S*, **39F**-*R,R*, **39F**-*R,S*, **38F**-*R,S*, **34B**, and **39A**.

At times, it was necessary to purify the *tert*-butoxycarbonyl intermediate prior to deprotection; chromatography on E. Merck silica gel, using 10:1 CH_2Cl_2/MeOH as eluent, proved to be the method of choice. Deprotection was then effected with gaseous HCl in CH_2Cl_2 as outlined previously. Compounds **36F**-*R,R*, **36F**-*R,S*, **30G**-*R,S*, **34F**-*R,S*, **33F**-*R,S*, and **33G**-*R,S* (eluting with 20:1 CH_2Cl_2/MeOH) were prepared in this manner, while compounds **30G**-*R,R* and **36G**-*R,R* (eluting with 40:1 CH_2Cl_2/MeOH) were deprotected with 1 N HCl in EtOH at reflux.

General Method B. Preparation of (3*R*,1*S*)-5-Amino-1-cyclopropyl-7-[3-[1-(ethylamino)ethyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (32H-*R,S*). A solution of 0.60 g (2.0 mmol) of 5-amino-1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (**19**), 0.31 g (2.2 mmol) of pyrrolidine **16**, 0.61 g (6.0 mmol) of Et_3N, and 20 mL of CH_3CN was refluxed for 5 h and then stirred at room temperature for 18 h. The solution was concentrated. The residue was dissolved in H_2O and made basic (pH 11) with 10% NaOH; the solution was filtered through a fiberglass pad and neutralized to pH 7.5. The precipitate which formed was filtered, washed with H_2O, and dried to give 0.61 g (73%) of the title compound: mp 148–150 °C; IR 1726, 1632 cm^{-1}; NMR (DMSO-d_6) δ 1.04 (m, 10 H, 4 cyclopropyl plus $NHCH_2CH_3$ plus CH_3 on pyrrolidine), 1.58 (m, 1 H, pyrrolidine), 2.00 (m, 2 H, pyrrolidine), 2.48 (m, 2 H, $CHNHCH_2CH_3$), 2.67 (m, 1 H, CHNHEt), 3.33 (m, 1 H, pyrrolidine), 3.66 (m, 3 H, pyrrolidine), 3.95 (m, 1 H, cyclopropyl), 7.10 (bs, 2 H, NH_2 at C-5), 8.41 (s, 1 H, C_2H).

The three remaining stereoisomers—**32H**-*R,R*, **32H**-*S,R*, and **32H**-*S,S*—were prepared in an identical fashion, giving rise to the following physical data.

32H-*R,R*: NMR (DMSO-d_6) δ 1.03 (m, 10 H), 1.60 (m, 1 H), 2.11 (m, 2 H), 2.65 (m, 2 H), 3.48 (m, 4 H), 3.80 (m, 1 H), 4.05 (m, 1 H), 7.13 (bs, 2 H), 8.44 (s, 1 H).

32H-*S,R*: NMR (DMSO-d_6) δ 1.05 (m, 10 H), 1.59 (m, 1 H), 1.97 (m, 1 H), 2.07 (m, 1 H), 2.51 (m, 2 H), 2.69 (m, 1 H), 3.61 (m, 4 H), 3.96 (m, 1 H), 7.10 (bs, 2 H), 8.41 (s, 1 H).

32H-*S,S*: NMR (DMSO-d_6) δ 1.03 (m, 10 H), 1.63 (m, 1 H), 2.10 (m, 2 H), 2.51–2.67 (m, 3 H), 3.55 (m, 3 H), 3.77 (m, 1 H), 3.97 (m, 1 H), 7.12 (bs, 2 H), 8.43 (s, 1 H).

Compounds **30H**-*R,R*, **30H**-*R,S*, **30H**-*S,R*, **30H**-*S,S*, **34H**-*R,R*, and **34H**-*R,S* were synthesized in identical fashion to **32H**-*R,S*. For compound **32H**-*R,S*, the product obtained from the isoelectric precipitation was dissolved in concentrated HCl; the solution was concentrated and the residue triturated with 2-PrOH to give the desired product as the HCl salt. In many cases, the target compounds precipitated from the cooled CH_3CN solution in excellent purity, and for these compounds (**31H**-*R,R*, **31H**-*R,S*, **31H**-*S,R*, **31H**-*S,S*, **34D**, and **36H**-*R,R*) no further purification was necessary. For the quinolone **36H**-*R,S* the reaction mixture was concentrated to a paste which was triturated with H_2O to give the desired product.

General Method C. Preparation of (3*R*,1*S*)-7-[3-(1-Aminoethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic Acid (35F-*R,S*). A solution of 0.53 g (1.6 mmol) of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid–boron difluoride complex (**28**, R_1 = c-C_3H_5), 0.40 g (1.9 mmol) of pyrrolidine **14**, 0.60 g (4.7 mmol) of diisopropylethylamine, and 25 mL of CH_3CN was stirred at room temperature for 18 h. The solution was concentrated to a paste which was dissolved in 20 mL of EtOH and 5 mL of Et_3N. The solution was refluxed for 5 h and stirred at room temperature for 18 h. The solvent was evaporated, and the residue was chromatographed on silica gel, eluting with 90:10 CH_2Cl_2/MeOH, to give a yellow foam, mp 161–163 °C.

The penultimate intermediate was dissolved in 25 mL of $CHCl_3$, cooled to 5 °C, and treated with gaseous HCl for 10 min. The mixture was allowed to warm to room temperature and was concentrated to a paste. The residue was triturated with EtOAc, and the solids were filtered, washed with ether, and dried to give 0.57 g (89% overall) of the title compound as the hydrochloride salt: mp 236–238 °C; IR 1718, 1621 cm^{-1}; NMR (TFA) δ 1.21 (m, 1 H, cyclopropyl), 1.33 (m, 1 H, cyclopropyl), 1.50 (m, 1 H, cyclopropyl), 1.61 (m, 1 H, cyclopropyl), 1.69 (d, J = 5.5 Hz, 3 H, CH_3 on pyrrolidine), 2.21 (m, 1 H, pyrrolidine), 2.57 (m, 1 H, pyrrolidine), 3.09 (m, 1 H, pyrrolidine), 3.93 (s, 4 H, OCH_3 plus $CHNH_2$), 4.14 (m, 2 H, pyrrolidine), 4.32 (m, 2 H, pyrrolidine), 4.55 (m, 1 H, cyclopropyl), 8.18 (d, 1 H, C_5H), 9.41 (s, 1 H, C_2H).

The remaining stereoisomers **35F**-*R,R* and **35F**-*S,R* were prepared in an identical fashion, giving rise to the following physical data.

35F-*R,R*: NMR (DMSO-d_6) δ 0.98–1.17 (m, 4 H), 1.26 (d, J = 6.0 Hz, 3 H), 1.78 (m, 1 H), 2.21 (m, 1 H), 2.45 (m, 1 H), 3.26 (m, 1 H), 3.56 (bs, 6 H), 3.77 (m, 1 H), 4.14 (m, 1 H), 7.66 (d, J = 14 Hz, 1 H), 8.66 (s, 1 H); IR 1728, 1622 cm^{-1}.

35F-*S,R*: NMR (DMSO-d_6) δ 0.93 (m, 1 H), 1.05–1.25 (m, 3 H), 1.31 (d, J = 6.7 Hz, 3 H), 1.74 (m, 1 H), 2.10 (m, 1 H), 2.50 (m, 1 H), 3.30 (m, 1 H), 3.56 (bs, 5 H), 3.70 (m, 2 H), 4.14 (m, 1 H), 7.67 (d, J = 14 Hz, 1 H), 8.66 (s, 1 H).

Compounds **35G**-*R,S*, **37F**-*R,S*, **37G**-*R,S*, **35B**, and **37A** were prepared in the same manner as was **35F**-*R,S*. For compounds **35G**-*R,R* and **35A**, the crude HCl salt was dissolved in water, filtered, and neutralized to pH 7.2. The solids that formed were filtered, redissolved in 6 N HCl, and concentrated. Trituration of this residue with 2-PrOH gave the desired products in pure form.

Pyrrolidine **16** does not contain a *tert*-butoxycarbonyl protecting group and therefore did not undergo acid hydrolysis. Rather, the crude product obtained in the initial coupling was simply refluxed in 20 mL of EtOH and 5 mL of Et_3N and then cooled to room temperature. The reaction mixtures containing **35H**-*R,R* and **35H**-*R,S* were concentrated, and the desired products were chromatographed on silica gel, eluting with a solution of CH_2Cl_2/2.6% NH_3 in CH_3OH/H_2O (1900 mL:95 mL:5 mL).

Preparation of 7-Chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acid[27] (17). A solution of 9.8 g (35 mmol) of ethyl (2,6-dichloro-5-fluoronicotinyl)acetate,[29] triethyl orthoformate (7.8 g, 53 mmol), and Ac_2O (50 mL) was refluxed for 3 h. The solution was cooled to room temperature and concentrated. The residue was dissolved in 150 mL of Et_2O, cooled to 5 °C, and treated dropwise with 2.2 g (38 mmol) of cyclopropylamine. The suspension was stirred at 5–10 °C for 1 h and then at room temperature for 2 h. Concentration gave a tan solid which was dissolved in 30 mL of dry DMSO. This solution was treated with 14 g (135 mmol) of Et_3N, stirred at room temperature overnight, and poured into H_2O (200 mL). The suspension was extracted with CH_2Cl_2, and the extract was washed (H_2O), dried, and concentrated. This residue was suspended in 100 mL of 6 N HCl, refluxed for 5 h, cooled to room temperature, and filtered. The solids were washed with H_2O and dried to give 5.1 g of the title compound: mp 210–212 °C; NMR (DMSO-d_6) δ 1.18–1.26 (m, 4 H, cyclopropyl), 3.83 (m, 1 H, cyclopropyl), 8.70 (d, 1 H, C_5H), 8.84 (s, 1 H, C_2H); IR (KBr) 1735, 1613 cm^{-1}.

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