Synthesis and Antibacterial Activity of Novel Pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid Derivatives Carrying the 3-Cyclopropylaminomethyl-4-substituted-1-pyrrolidinyl Group as a C-10 Substituent

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Novel pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid derivatives **5**−**9** carrying a 3-cyclopropylaminomethyl-4-substituted-1-pyrrolidinyl moiety at the C-10 position were synthesized and their in vitro antibacterial activity, intravenous single-dose toxicity, convulsion inductive ability, and phototoxicity were evaluated. It appeared evident that compounds **5a**, **6a**, **8a**, and **9a**, which have a cis-oriented 4-methyl or 4-fluoro-3-cyclopropylaminomethyl-1-pyrrolidinyl moiety at the C-10 position, exhibited 2- to 16-fold more potent in vitro antibacterial activity than clinafloxacin against quinolone-resistant Gram-positive clinical isolates. Furthermore, it was obvious that introduction of a fluorine atom to the C-4 position of the 3-cyclopropylaminomethyl-1-pyrrolidinyl moiety reduced intraveneous single-dose acute toxicity and the convulsion inductive ability, and introduction of a fluorine atom to the C-3 methyl group of the pyridobenzoxazine nucleus eliminated the phototoxicity.

Introduction

Multidrug-resistant Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA)[a], vancomycin-resistant *Enterococcus* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP), which are spread worldwide, have become a serious problem in the treatment of infectious diseases. In the field of fluoroquinolone antibacterial agents, since the development of the first new quinolone, norfloxacin,[1] a great deal of effort has been put into developing more potent quinolones for multidrug-resistant Gram-positive bacteria for more than 10 years. As a result of these efforts, gatifloxacin,[2] moxifloxacin,[3] trovafloxacin,[4] gemifloxacin,[5] etc. have been developed, and gatifloxacin and moxifloxacin in particular are being used for the treatment of community-acquired pneumonia caused by *Streptococcus pneumoniae*, including PRSP (Figure 1). However, the frequency of quinolone-resistant Gram-positive bacteria, particularly, quinolone-resistant methicillin-resistant *Staphylococcus aureus* (QMRSA), has been increasing.[6] Therefore, urgent development of more effective fluoroquinolones against multidrug-resistant Gram-positive bacteria is still required.

A number of syntheses of fluoroquinolone derivatives have been reported, together with the corresponding structure−activity relationship (SAR) studies.[7] As a result of these SAR studies, it appears evident that the N-1, C-7, and C-8 substituents of the fluoroquinolones play an important role in the antibacterial potency, antibacterial spectrum, and toxicity of fluoroquinolones. For example, a 3-ethylaminomethylpyrrolidinyl group, found in the C-7 substituent of **1** (CI-934),[8] showed enhancement of antibacterial potency against Gram-positive bacteria. However, it has also been reported that the 1-cyclopropylquinolone derivatives such as **2** (PD117558),[9] bearing the 3-ethylaminomethylpyrrolidinyl group as the C-7 substituent show strong cytotoxicity against mammalian cells.[10] To find the more effective fluoroquinolones against multidrug-resistant Gram-positive bacteria, we focused on the fluoroquinolone derivatives bearing the 3-alkylaminomethylpyrrolidinyl group as the C-7 (C-10 in the case of pyrido[1,2,3-*de*][1,4]benzoxazine derivatives) substituent, especially 10-(3-cyclopropylamionomethyl-1-pyrrolidinyl)pyrido[1,2,3-*de*][1,4]benzoxazine derivatives **4**. We found that compound **4**[11] exhibited 32- to 64-fold more potent in vitro antibacterial activity against QMRSA and VRE than 10-(3-ethylamionomethyl-1-pyrrolidinyl)pyrido[1,2,3-*de*][1,4]benzoxazine derivatives **3**.[9a] Despite the pyrido[1,2,3-*de*][1,4]benzoxazinecarboxylic acid derivative, in vitro antibacterial activity of **4** against QMRSA and VRE was superior to that of clinafloxacin, which has been reported as one of the most active fluoroquinolones against Gram-positive bacteria.[9a,12] Thus, we designed and synthesized the compounds **5**−**9**, into which were introduced a methyl group or a fluorine atom to the C-4 position of the 3-cyclopropylaminomethyl-1-pyrrolidinyl moiety and a fluorine atom to a C-3 methyl group of the pyrido[1,2,3-*de*][1,4]benzoxazine nucleus, with the intent of increasing the in vitro antibacterial activity[13] and reducing the toxicity[14] of **4** (Figure 2).

The results of the present study indicate that the introduction of a fluorine atom to the C-4 position of a 3-cyclopropylaminomethylpyrrolidinyl moiety and a C-3 methyl group served to reduce the phototoxicity of **4** without loss of highly potent antibacterial activity. In this paper, we present the synthesis, in vitro antibacterial activity, and toxicological properties of a series of the pyrido[1,2,3-*de*][1,4]benzoxazinecarboxylic acid derivatives **5**−**9** carrying a (3*S*)-3-cyclopropylaminomethyl-1-pyrrolidinyl, (3*S*)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl, or (3*S*)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl group at the C-10 position.

Chemistry

We planned to synthesize the compounds **5**−**9** using a nucleophilic substitution reaction from diacetoxyboron chelate[15]

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[a] Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; QMRSA, quinolone-resistant methicillin-resistant *Staphylococcus aureus*; DAST, diethylaminosulfur trifluoride; MICs, minimum inhibitory concentrations.

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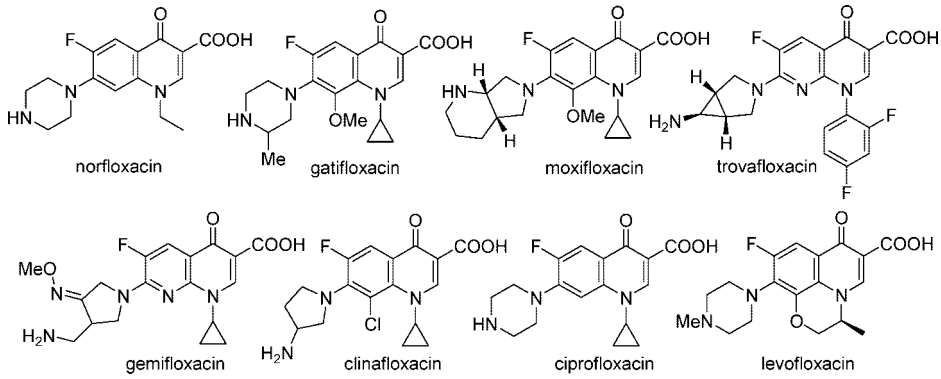


Figure 1. Structure of reference quinolones.

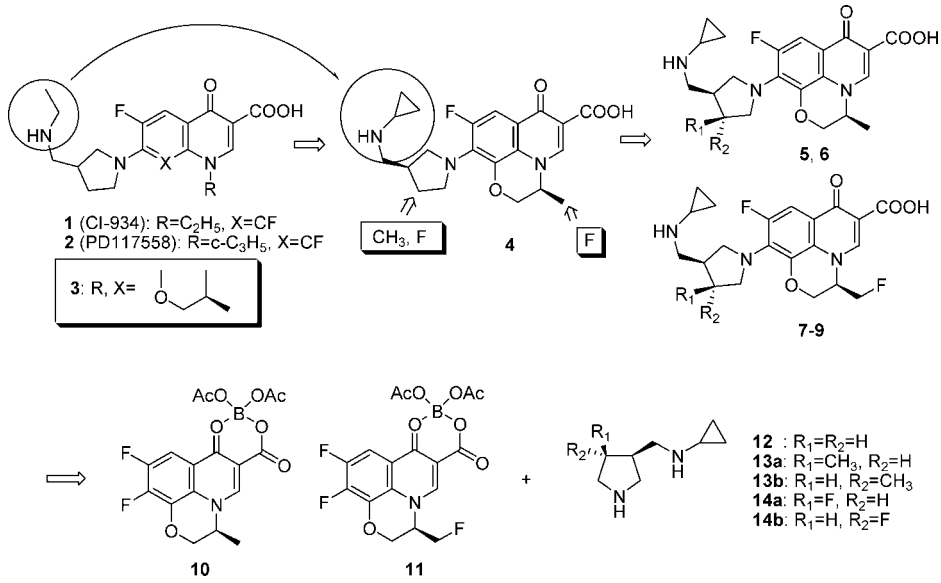
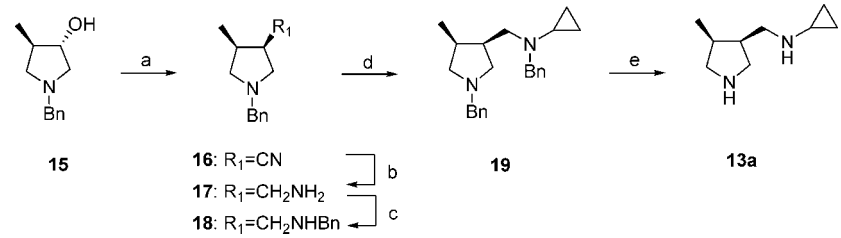


Figure 2

Scheme 1[a]



[a] Reagents and conditions: (a) (1) MsCl, Et_3N, CH_2Cl_2; (2) NaCN, Bu_4NCN, DMF, 69%; (b) $LiAlH_4$, Et_2O, 83%; (c) PhCHO, molecular sieves 4A, MeOH, then BH_3-pyridine, 78%; (d) [1-(ethoxycyclopropyl)oxy]trimethylsilane, $NaBH_3CN$, AcOH, molecular sieves 3A, MeOH, 98%; (e) H_2, Pd−C, EtOH, 67%.

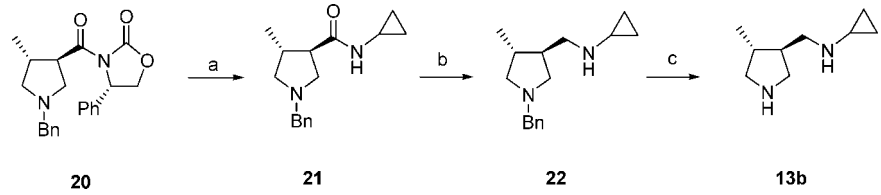
of the (3*S*)-methyl- and (3*R*)-fluoromethyl-pyrido[1,2,3-*de*][1,4]benzoxazinecarboxylic acid derivatives **10** and **11**, and appropriate (3*S*)-cyclopropylaminomethylpyrrolidine derivatives **12**,[16] **13**, and **14**, followed by removal of the chelate (Figure 1).

The synthesis of cis-oriented (3*R*,4*S*)-3-cyclopropylaminomethyl-4-methylpyrrolidine (**13a**) is shown in Scheme 1. Treatment of (3*S*,4*R*)-1-benzyl-4-methyl-3-pyrrolidinol (**15**)[17] with methanesulfonyl chloride in the presence of triethylamine, followed by sodium cyanide and tetrabutylammonium cyanide, gave cyanopyrrolidine **16**.[18] Reduction of a cyano group of **16** with lithium aluminum hydride, followed by reductive benzylation of the resulting amino group, provided the benzylaminomethyl derivative **18**. Cyclopropanation of a benzylamino group of **18** with [(1-ethoxycyclopropyl)oxy]trimethylsilane in the presence of sodium cyanoborohydride,[19] followed by removal of two benzyl groups of **19** using catalytic hydrogenation, gave the desired cis-oriented **13a**.

The synthesis of trans-oriented (3*R*,4*R*)-3-cyclopropylaminomethyl-4-methylpyrrolidine (**13b**) is shown in Scheme 2. Treatment of **20**[20] with excess cyclopropylamine gave *N*-cyclopropylamide **21** directly. Reduction of a carbamoyl group of **21** with a borane−dimethyl sulfide complex, followed by removal of a benzyl group of **22** using catalytic hydrogenation in the presence of trifluoroacetic acid, provided the desired trans-oriented **13b**.

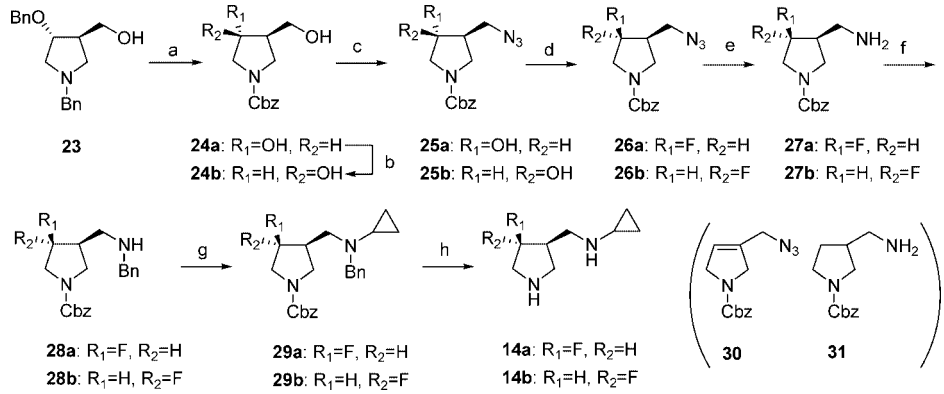
The synthesis of cis-oriented (3*R*,4*S*)-3-cyclopropylaminomethyl-4-fluoropyrrolidine (**14a**) and trans-oriented (3*R*,4*R*)-3-

Scheme 2[a]



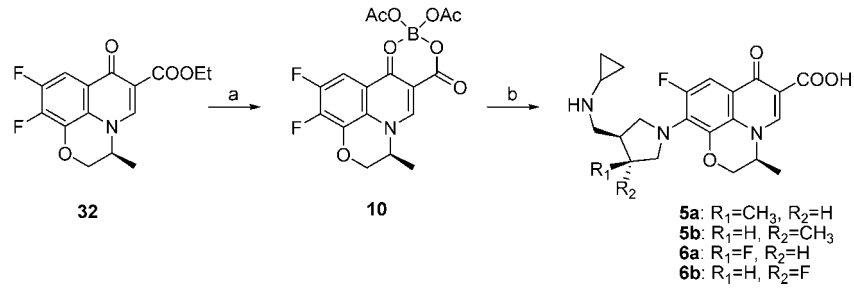
[a] Reagents and conditions: (a) cyclopropylamine, 49%; (b) BH_3-dimethyl sulfide, toluene, 94%; (c) H_2, 10% Pd−C, CF_3COOH, EtOH, then aqueous NaOH, 76%.

Scheme 3[a]



[a] Reagents and conditions: (a) (1) $HCOONH_4$, 10% Pd−C, aqueous MeOH; (2) ClCOOBn, Et_3N, DMF, 91%; (b) (1) PhCOOH, DEAD, PPh_3, THF, 99%; (2) K_2CO_3, aqueous EtOH, 83%; (c) NaN_3, CBr_4, PPh_3, DMF, 89% (for **25a**), 85% (for **25b**); (d) DAST, CH_2Cl_2, 60% (for **26a**); (e) H_2,PtO_2, EtOH, 88% (for **27a**), 26% (for **27b** from **25a**), and 15% (for **31** from **25a**); (f) PhCHO, molecular sieves 4A, MeOH, then BH_3-pyridine, 82% (for **28a**), 77% (for **28b**); (g) [1-(ethoxycyclopropyl)oxy]trimethylsilane, $NaBH_3CN$, AcOH, molecular sieves 3A, MeOH, 99% (for **29a**), 100% (for **29b**); (h) H_2, 10% Pd−C, EtOH, 82% (for **14a**), 77% (for **14b**).

Scheme 4[a]



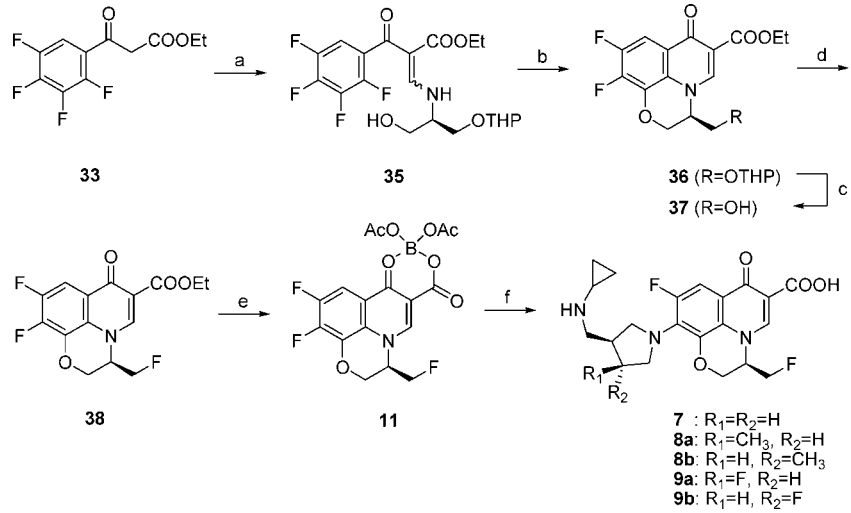
[a] Reagents and conditions: (a) H_3BO_3, Ac_2O, cat. $ZnCl_2$, 88%; (b) **13a**,**b** or **14a**,**b**, Et_3N, MeCN, then 5% aqueous AcOH, 47% (for **5a**), 71% (for **5b**), 71% (for **6a**), 70% (for **6b**).

cyclopropylaminomethyl-4-fluoropyrrolidine (**14b**) is illustrated in Scheme 3. Removal of both of the two benzyl groups of [(3*R*,4*R*)-1-benzyl-4-benzyloxypyrrolidin-3-yl]methanol (**23**)[21] using the catalytic hydrogen transfer reaction, followed by treatment with benzyl chloroformate in the presence of triethylamine, afforded **24a**. The selective azidation of the primary alcohol group of **24a** was achieved by treatment with sodium azide, carbon tetrabromide, and triphenylphosphine to give **25a**. Fluorination of the secondary alcohol group of **25a** using diethylaminosulfur trifluoride (DAST) and catalytic hydrogenation of an azide group of **26a** in the presence of platinum dioxide provided **27a**. Reductive benzylation of an amino group of **27a**, cyclopropanation of the resulting benzylamino group of **28a** with [(1-ethoxycyclopropyl)oxy]trimethylsilane, and removal of both of the benzyl group and benzyloxycarbonyl group of **29a** by catalytic hydrogenation afforded cis-oriented **14a**. In contrast, the synthesis of trans-oriented **14b** was achieved by way of inversion of the secondary alcohol of **24a**. The Mitsunobu reaction[22] of **24a**, followed by basic hydrolysis of the two resulting benzoyl ester groups, gave cis-oriented **24b**. After conversion of **24b** to azidomethyl derivative **25b**, fluorination of the secondary alcohol was performed by using DAST to give **26b**. Unfortunately, opposite to fluorination of **25a**, the fluorinated product **26b** was obtained along with eliminated product **30** as an inseparable mixture. ^{1}H NMR spectra of the mixture indicated that the ratio of **26b** and **30** was approximately 3:2. After reduction of the azido group of the mixture by catalytic hydrogenation, separation of **31** by silica gel column chromatography provided the desired **27b**. According to the method for the synthesis of **14a**, **27b** was converted to trans-oriented **14b**.

The reaction of the pyrrolidines **13** and **14** with diacetoxyboron chelate **10**, which was prepared from the ester **32**[23,24] by treatment with boric acid and acetic anhydride, followed by removal of the chelate under acidic conditions, gave the 3-methyl derivatives **5** and **6** (Scheme 4).

Synthesis of the 3-fluoromethyl derivatives **7**−**9** is shown in Scheme 5. Treatment of benzoylacetate **33** with triethyl ortho-

Scheme 5[a]



[a] Reagents and conditions: (a) (1) $HC(OEt)_3$, Ac_2O; (2) (2*R*)-2-amino-3-(2-tetrahydropyranyl)oxypropanol (**34**), 83%; (b) KF, DMSO, 62%; (c) p-TsOH.H_2O, EtOH, 92%; (d) DAST, CH_2Cl_2, 56%; (e) H_3BO_3, Ac_2O, cat. $ZnCl_2$, 83%; (f) **12**, **13a**,**b**, or **14a**,**b**, Et_3N, MeCN, then 5% aqueous AcOH, 50% (for **7**), 83% (for **8a**), 61% (for **8b**), 72% (for **9a**), 69% (for **9b**).

formate and acetic anhydride, followed by the reaction with optically active aminopropanol **34**, gave enaminoester **35**. Cyclization of **35** using potassium fluoride gave the optically active pyrido[1,2,3-*de*][1,4]benzoxazine derivative **36**. Removal of a tetrahydropyranyl group under acidic conditions followed by treatment with DAST gave the optically active 3-fluoromethyl derivative **38**.[23] Compound **38** was converted to the diacetoxyboron chelate **11**, which reacted with the pyrrolidines **12**−**14**, and removal of the chelate occurred in the same manner described for the synthesis of the 3-methyl derivatives **5** and **6** to give the 3-fluoromethyl derivatives **7**−**9**.

Results and Discussion

The minimum inhibitory concentrations (MICs) of the compounds **3**−**9** against four Gram-positive standard strains (*Staphylococcus aureus* Smith, *Streptococcus pneumoniae* Type III, *Streptococcus pyogenes* IID692, and *Enterococcus faecalis* IID682) and four Gram-negative standard strains *(Escherichia coli* NIHJ JC-2, *Klebsiella pneumoniae* IID5209, *Haemophilus influenzae* IID983, and *Pseudomonas aeruginosa* IID1210) are shown in Table 1, along with those of ciprofloxacin, levofloxacin, and clinafloxacin. The synthesized compounds **4**−**9**, except for **6b** and **9b**, exhibited 2- to 128-fold more potent in vitro antibacterial activity against Gram-positive standard strains than ciprofloxacin, levofloxacin, and the racemic 10-(3-ethylaminomethypyrrolidinyl) derivative **3**. In contrast, against Gram-negative strains such as *E. coli* and *K. pneumoniae*, the in vitro antibacterial activity of **4**−**9** was 2- to 16-fold less potent than those of ciprofloxacin, levofloxacin, and clinafloxacin. However, **4**−**9** exhibited comparable in vitro antibacterial activity to ciprofloxacin and levofloxacin against *H. influenzae*, which is an important pathogen of respiratory tract infection.

The MICs of **3**−**9** against three quinolone-resistant clinical isolates (*S. aureus* OITI MR1-1002, *S. pneumoniae* No. 55, and *E. faecalis* No. 15) and two VREs [*E. faecalis* KU1856 (*vanA*) and *E. faecalis* KU1866 (*vanB*)], along with those for ciprofloxacin, levofloxacin, and clinafloxacin, are summarized in Table 2. These results indicate that the cis-oriented derivatives **5a**, **6a**, **8a**, and **9a** exhibited in vitro antibacterial activity against quinolone-resistant clinical isolates and VREs, which was superior to that of **4** and **7**, as well as the trans-oriented derivatives **5b**, **6b**, **8b**, and **9b**. In addition, these cis-oriented derivatives, **5a**, **6a**, **8a**, and **9a**, were found to show 2- to 16-fold more potent activity than clinafloxacin.

Table 1. In Vitro Antibacterial Activity of Compounds **3**−**9** against Standard Strains

	MIC (μg/mL)[a]							
	Gram-positive organisms				Gram-negative organisms			
compd	Sa	Spn	Spy	Ef	Ec	Kpn	Hi	Pa
4	0.008	0.016	0.016	0.063	0.008	0.063	0.016	2
5a	0.008	0.016	0.016	0.063	0.016	0.063	0.004	2
5b	0.002	0.016	0.016	0.031	0.016	0.125	0.008	4
6a	0.008	0.016	0.031	0.063	0.008	0.031	0.004	2
6b	0.016	0.063	0.125	0.125	0.016	0.125	0.008	4
7	0.008	0.016	0.031	0.063	0.004	0.031	0.004	2
8a	0.008	0.016	0.031	0.063	0.016	0.063	0.004	2
8b	0.008	0.031	0.031	0.063	0.016	0.125	0.008	4
9a	0.008	0.016	0.031	0.063	0.008	0.031	0.004	2
9b	0.016	0.063	0.125	0.125	0.031	0.125	0.008	4
3	0.125	0.063	0.063	0.25	0.031	NT[c]	NT[c]	4
CPFX[b]	0.25	0.5	0.5	0.5	≤0.001	0.004	0.008	0.125
LVFX[b]	0.125	0.5	0.5	1	≤0.001	0.016	0.008	0.5
CLFX[b]	0.016	0.031	0.063	0.063	0.002	0.004	≤0.001	0.125

[a] Organisms selected for inclusion in the table: Sa, *S. aureus* Smith; Spn, *S. pneumoniae* Type III; Spy, *S. pyogenes* IID692; Ef, *E. faecalis* IID682; Ec, *E. coli* NIHJJC-2; Kpn, *K. pneumoniae* IID5209; Hi, *H. influenzae* IID983; Pa, *P. aeruginosa* IID1210. [b] Abbreviations are as follows: CPFX = ciprofloxacin; LVFX = levofloxacin; CLFX = clinafloxacin. [c] Not tested.

We next evaluated intravenous single-dose acute toxicity, convulsion inductive ability, and phototoxicity of **4**, **5a**,**b**, **6a**, **7**, **8a**,**b**, and **9a**. The results along with those of clinafloxacin are summarized in Table 3. Concerning the single-dose acute toxicity, **5a** and **8a** possessing the (3*S*,4*S*)-4-methyl-3-cyclopropylaminomethyl-1-pyrrolidinyl moiety were slightly more toxic than **4**. In contrast, **6a** and **9a** bearing the (3*S*,4*S*)-4-fluoro-3-cyclopropylaminomethyl-1-pyrrolidinyl moiety were less toxic than **4**. In contrast, less convulsion inductive ability was observed in compounds having a 3-methyl group rather than a 3-fluoromethyl group (**4** vs **7** and **5** vs **8**). However, **9a**, which has the (3*S*,4*S*)-4-fluoro-3-cyclopropylaminomethylpyrrolidinyl moiety, showed less convulsion inductive ability, similar to the 3-methyl derivative **6a**. Furthermore, the phototoxicity was completely eliminated by the introduction of a fluorine atom to a C-3 methyl group (**4**−**6** vs **7**−**9**). Introduction of a fluorine atom to the C-4 position of a pyrrolidine ring was effective in reducing the single-dose acute toxicity and convulsion inductive

Table 2. In Vitro Antibacterial Activity of Compounds **3**−**9** against Quinolone-Resistant Clinical Isolates and VRE

	MIC (μg/mL)				
	quinolone-resistant clinical isolates			VRE	
compd	*S. aureus* OITI MR1−1002	*S. pneumoniae* no. 55	*E. faecalis* no. 15	*E. faecalis* KU1856	*E. faecalis* KU1866
4	2	0.25	1	0.5	0.5
5a	0.5	0.125	1	1	1
5b	2	0.5	1	1	1
6a	1	0.125	0.5	0.5	0.5
6b	16	2	4	2	4
7	2	0.25	1	0.5	0.5
8a	0.5	0.125	1	1	1
8b	2	0.5	1	1	1
9a	1	0.25	1	0.5	0.5
9b	16	2	4	2	4
3	32	2	32	16	16
CPFX[a]	64	64	64	64	64
LVFX[a]	>128	32	64	32	32
CLFX[a]	8	0.5	8	2	4

[a] Abbreviations are as follows: CPFX = ciprofloxacin; LVFX = levofloxacin; CLFX = clinafloxacin.

Table 3. Intravenous Single-Dose Acute Toxicity, Convulsive Ability, and Phototoxicity of Compounds **4**, **5a**,**b**, **6a**, **7**, **8a**,**b**, and **9a**

	intravenous single-dose acute toxicity[a](mortality, dead/tested)		convulsive activity[b] (μg/mouse)		phototoxicity (100 mg/kg, po)
compd	100 mg/kg	200 mg/kg	-FB	+FB	score[c]
4	0/5	3/3	20	20	2.0
5a	3/3	NT[d]	>40	>40	2.0
5b	0/5	3/3	>40	>40	1.3
6a	0/5	0/5	>40	>40	1.7
7	0/5	4/5	10	10	0
8a	1/5	3/3	20	10	0
8b	0/5	4/5	40	20	0
9a	0/5	0/5	>40	>40	0
CLFX[d]	0/5	0/5	10	5	3.0

[a] Intravenous single-dose acute toxicity was characterized by a single-dose toxicity after intravenous administration. [b] Convulsive activity was characterized by a minimum inducible dose of behavioral convulsion after intraventricular administration without Fenbufen (-FB) and with Fenbufen (+FB). [c] Score is the mean value for three animals. Score is as follows: 0 = nil; 1 = slight; 2 = moderate; 3 = marked change. [d] Abbreviations are as follows: CLFX = clinafloxacin.

Table 4. In Vitro Inhibition Potency of **9a** against Topoisomerase IV and DNA Gyrase Isolated from Wild and Resistant *S. aureus* MS5935

	IC_{50} (μg/mL)			
	DNA gyrase		topoisomerase IV	
compd	wild	resistant	wild	resistant
9a	1.06	13.6	1.27	7.00
CLFX[a]	1.15	16.2	1.89	13.9
LVFX[a]	9.31	772	5.44	319

[a] Abbreviations are as follows: LVFX = levofloxacin; CLFX = clinafloxacin.

ability, and introduction of a fluorine atom to a C-3 methyl group of a pyridobenzoxazine ring contributed to complete elimination of the phototoxicity.

On the basis of the results regarding in vitro antibacterial activity, intravenous single-dose acute toxicity, convulsion inductive ability, and phototoxicity in this study, we selected **9a** as the candidate compound and carried out further evaluation of the in vitro and in vivo antibacterial activity of **9a**. The results regarding the inhibition potency of **9a** against bacterial DNA gyrase and bacterial topoisomerase IV isolated from a wild and resistant strain of *S. aureus* MS5935[25] are shown in Table 4, along with those of clinafloxacin and levofloxacin. The compound **9a** showed 4- to 9-fold smaller IC_{50} values against both the wild-type DNA gyrase and topoisomerase IV, and over 40-fold smaller IC_{50} values against both the resistant type DNA gyrase and topoisomerase IV compared with levofloxacin, which has the same pyridobenzoxazine nucleus as **9a**. Furthermore, because the IC_{50} values of **9a** against both the resistant type DNA gyrase and topoisomerase IV were slightly smaller than those of clinafloxacin, we can conclude that **9a** more strongly inhibited both of the target enzymes.

Table 5. In Vitro Antibacterial Activity of **9a** against Sequentially Acquired Quinolone-Resistant Mutants of *S. aureus* MS5935

	MIC (μg/mL)		
strain	**9a**	CLFX[e]	LVFX[e]
wild	0.016	0.016	0.125
first-step mutant[a]	0.031	0.063	0.5
second-step mutant[b]	0.25	0.5	8
third-step mutant[c]	0.5	2	32
fourth-step mutant[d]	4	8	>128

[a] The first-step mutant possessed a single *grlA* mutation. [b] The second-step mutant possessed single *grlA* and single *gyrA* mutations. [c] The third-step mutant possessed double *grlA* and single *gyrA* mutations. [d] The fourth-step mutant possessed double *grlA* and double *gyrA* mutations. [e] Abbreviations are as follows: LVFX = levofloxacin; CLFX = clinafloxacin.

Table 6. In Vivo Antibacterial Activity of Compound **9a** against Systemic Infection in Mice

		ED_{50} (mg/kg)	
infected bacteria	compd	sc	po
S. aureus Smith	**9a**	0.12	0.50
	CLFX[a]	0.35	2.1
S. pneumoniae Type III	**9a**	2.9	4.0
	CLFX[a]	45	15

[a] Abbreviation is as follows: CLFX = clinafloxacin.

The in vitro antibacterial activity of **9a** against sequentially acquired quinolone-resistant mutants of *S. aureus* MS5935[26] along with those of clinafloxacin and levofloxacin is shown in Table 5. Indicative of the strong in vitro inhibition potency against both the target enzymes, **9a** exhibited 2- to 4-fold more potent in vitro antibacterial activity than clinafloxacin, with particular potency being seen against third- and fourth-step mutants.[26]

Finally, we evaluated the in vivo efficacy of **9a** by oral and subcutaneous administration against experimental systemic infections in mice caused by *S. aureus* Smith and *S. pneumonia* Type III. The ED_{50} values of **9a** along with those of clinafloxacin are shown in Table 6. The in vivo efficacy of **9a** against both experimental systemic infections in mice caused by *S. aureus*

Smith and *S. pneumoniae* Type III was superior to that of clinafloxacin, especially by oral administration.

Conclusions

As described above, we synthesized a series of the novel pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid derivatives **5**−**9** carrying a 3-cyclopropylaminomethyl-4-substituted-1-pyrrolidinyl moiety at the C-10 position and evaluated the in vitro antibacterial activity, the intravenous single-dose acute toxicity, the convulsion inductive ability, and the phototoxicity of the compounds **5**−**9**. Despite the pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid derivatives, the in vitro antibacterial activity of compounds **5**−**9** was superior to that of clinafloxacin due to the replacement of the ethyl group of a 3-ethylaminomethylpyrrolidinyl moiety with a cyclopropyl group. In particular, the cis-oriented derivatives **5a**, **6a**, **8a**, and **9a** exhibited 2- to 16-fold more potent in vitro antibacterial activity than clinafloxacin against quinolone-resistant Gram-positive clinical isolates. In contrast, introduction of the cis-oriented fluorine atom to the C-4 position of the 3-cyclopropylaminomethylpyrrolidinyl moiety brought about a reduction in the intravenous single-dose acute toxicity, similar to Inagaki's study[14]. Furthermore, our results revealed that the cis-oriented fluorine atom at the C-4 position of the 3-cyclopropylaminomethylpyrrolidinyl moiety contributes to reduced convulsion inductive ability, and the introduction of a fluorine atom to the C-3 methyl group of the pyridobenzoxazine nucleus contributes to a complete elimination of phototoxicity. As a result of these antibacterial and toxicological evaluations, we selected the compound **9a**, which has cis-oriented (3*S*,4*S*)-3-cyclopropylaminomethyl-4-fluoropyrrolidinyl moiety at the C-10 position and a fluoromethyl group at the C-3 position, as a candidate. Further investigations of **9a** for clinical trials are in progress.

Experimental Section

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Elemental analyses are within ±0.4% of the theoretical values and were determined by a Yanaco CHN MT-5 instrument. Infrared spectra (IR) were recorded with a JASCO FT/IR-5300 spectrometer. Measurements of mass spectra (MS) and high resolution MS (HRMS) were performed with a JEOL JMS SX-102A or a JEOL JMS-T100LP mass spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured with a JEOL EX-90 (90 MHz), a JEOL JMN-EX400 (400 MHz), or a JEOL JMN-ECA-400 (400 MHz) spectrometer. The chemical shifts are expressed in parts per million (δ value) downfield from tetramethylsilane, using tetramethylsilane ($\delta = 0$) and/or residual solvents such as chloroform ($\delta = 7.26$) as an internal standard. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br; broad peak. Column chromatography was carried out with silica gel [silica gel 60 (Kanto)] as an absorbent. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for the TLC analysis. Solutions were dried over sodium sulfate and the solvent was removed by rotary evaporation under reduced pressure.

(3*R*,4*S*)-1-Benzyl-4-methyl-3-pyrrolidinecarbonitrile (16). Methanesulfonyl chloride (1.70 mL, 22.0 mmol) was added dropwise to a solution of **15** (4.00 g, 20.9 mmol) and triethylamine (3.06 mL, 22.0 mmol) in anhydrous CH_2Cl_2 (40 mL) at −78 °C, and the whole mixture was stirred at −78 °C for 1 h. After quenching the reaction by adding water (40 mL), the CH_2Cl_2 layer was separated and the aqueous layer was extracted with CH_2Cl_2 (40 mL). The combined CH_2Cl_2 extracts were washed with water (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. To a solution of the residue in anhydrous DMF (120 mL), sodium cyanide (2.05 g, 41.8 mmol) and tetrabutylammonium cyanide (5.53 g, 20.9 mmol) were added; the whole mixture was stirred at 80 °C for 13 h and then concentrated in vacuo. After addition of water (50 mL) to the residue, the resulting mixture was extracted with diethyl ether (2 × 100 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 4:1) of the residue gave **16** (3.32 g, 79%). ^{1}H NMR ($CDCl_3$) δ: 1.22 (d, J = 7.3 Hz, 3H), 2.12 (dd, J = 9.3, 8.3 Hz, 1H), 2.46−2.57 (m, 1H), 2.60−2.67 (m, 1H), 2.99 (dd, J = 9.3, 7.3 Hz, 1H), 3.09−3.19 (m, 2H), 3.62 (s, 2H), 7.25−7.35 (m, 5H). MS (CI^+) *m*/*z*: 201 ($M^+ + H$). HRMS (CI^+) for $C_{13}H_{17}N_2$ ($M^+ + H$): calcd, 201.13918; found, 201.13734. $[\alpha]_D^{23}$ −16.8° (*c* 0.742, $CHCl_3$).

(3*S*,4*S*)-3-Aminomethyl-1-benzyl-4-methylpyrrolidine (17). A solution of **16** (3.20 g, 16.0 mmol) in anhydrous diethyl ether (20 mL) was added dropwise to a suspension of $LiAlH_4$ (2.43 g, 64.0 mmol) in anhydrous diethyl ether (66 mL) under cooling with ice, and the whole mixture was stirred at room temperature for 1 h. After quenching the reaction by adding saturated aqueous $NaHCO_3$ solution (6.6 mL) under cooling with ice, the mixture was diluted with diethyl ether (75 mL). The insoluble materials were filtered off and washed with diethyl ether (75 mL). The combined filtrate and the washing were dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 1:1 → AcOEt/MeOH = 10:1) of the residue gave **17** (2.98 g, 91%). ^{1}H NMR ($CDCl_3$) δ: 0.94 (d, J = 7.3 Hz, 3H), 2.03 (dd, J = 9.3, 1.5 Hz, 1H), 2.11−2.26 (m, 2H), 2.31−2.43 (m, 1H), 2.58 (dd, J = 12.2, 8.8 Hz, 1H), 2.82 (dd, J = 12.2, 5.9 Hz, 1H), 2.97−3.02 (m, 2H), 3.60 (s, 2H), 7.22−7.33 (m, 5H). MS (CI^+) *m*/*z*: 205 ($M^+ + H$). HRMS (CI^+) for $C_{13}H_{21}N_2$ ($M^+ + H$): calcd, 205.17048; found, 205.16855. $[\alpha]_D^{28}$ +10.2° (*c* 0.703, $CHCl_3$).

(3*R*,4*S*)-1-Benzyl-3-benzylaminomethyl-4-methylpyrrolidine (18). A mixture of **17** (2.80 g, 13.7 mmol), molecular sieves 4A (1.23 g), and benzaldehyde (1.39 mL, 13.7 mmol) in anhydrous MeOH (60 mL) was stirred at room temperature for 1 h. Borane−pyridine complex (1.39 mL, 13.8 mmol) was added to the above mixture, and the whole mixture was stirred at room temperature for 3 h. After addition of 6 mol/L HCl (23.2 mL), the whole mixture was stirred at room temperature for 1 h. After the reaction mixture was adjusted to pH 14 by addition of 6 mol/L aqueous NaOH solution, the resulting mixture was extracted with diethyl ether (2 × 100 mL). The combined organic extracts were washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 4:1) of the residue gave **18** (3.49 g, 87%). ^{1}H NMR ($CDCl_3$) δ: 0.92 (d, J = 6.8 Hz, 3H), 2.00 (dd, J = 8.8, 6.8 Hz, 1H), 2.13 (dd, J = 9.3, 7.8 Hz, 1H), 2.29−2.40 (m, 2H), 2.51 (dd, J = 11.2, 8.8 Hz, 1H) 2.71 (dd, J = 11.2, 5.4 Hz, 1H), 2.96−3.02 (m, 2H), 3.59 (s, 2H), 3.78 (s, 2H), 7.21−7.34 (m, 10H). MS (EI) *m*/*z*: 294 (M^+). HRMS (EI) for $C_{20}H_{26}N_2$ (M^+): calcd, 294.2096; found, 294.2072. $[\alpha]_D^{24}$ +6.1° (*c* 1.03, MeOH).

(3*R*,4*S*)-1-Benzyl-3-(*N*-benzyl-*N*-cyclopropyl)aminomethyl-4-methylpyrrolidine (19). A mixture of **18** (3.40 g, 11.5 mmol), molecular sieves 3A (3.53 g), AcOH (6.58 mL, 115 mmol), [(1-ethoxycyclopropyl)oxy]trimethylsilane (9.25 mL, 46.0 mmol), and $NaBH_3CN$ (2.17 g, 34.5 mmol) in anhydrous MeOH (40 mL) was heated under reflux for 3 h. After the insoluble materials were filtered off, the filtrate was adjusted to pH 14 by addition of 6 mol/L aqueous NaOH solution, and the mixture was extracted by diethyl ether (2 × 100 mL). The combined extracts were washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 4:1) of the residue gave **19** (3.72 g, 96%). ^{1}H NMR ($CDCl_3$) δ: 0.31−0.47 (m, 4H), 0.85 (d, J = 7.3 Hz, 3H), 1.64−1.69 (m, 1H), 1.91 (dd, J = 9.3, 7.3 Hz, 1H), 2.06 (t, J = 9.3 Hz, 1H), 2.23−2.34 (m, 1H), 2.39 (dd, J = 11.7, 9.3 Hz, 1H), 2.55 (dd, J = 11.7, 5.9 Hz, 1H), 2.53−2.66 (m, 1H), 2.90−2.96 (m, 2H), 3.49 (d, J = 13.2 Hz, 1H), 3.59 (d, J = 12.7 Hz, 1H), 3.63 (d, J = 13.6 Hz, 1H), 3.74 (d, J = 13.2 Hz, 1H), 7.21−7.32 (m, 10H). MS (FAB^+) *m*/*z*: 335 ($M^+ + H$). HRMS (FAB^+) for $C_{23}H_{31}N_2$ ($M^+ + H$): calcd, 335.2487; found, 335.2503. $[\alpha]_D^{25}$ +11.2° (*c* 1.03, MeOH).

(3*R*,4*S*)-3-Cyclopropylaminomethyl-4-methylpyrrolidine (13a). A suspension of **19** (3.60 g, 10.8 mmol), 10% Pd−C (3.60 g), and $CHCl_3$ (3.44 mL, 43.0 mmol) in EtOH (40 mL) was stirred at 50 °C for 10 h under H_2 atmosphere (4 kg/cm^2). After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. The suspension of the residue and 10% Pd−C (3.60 g) in EtOH (40 mL) was stirred at 50 °C for 6 h under H_2 atmosphere (4 kg/cm^2). After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. The suspension of the residue and 10% Pd−C (2.00 g) in EtOH (100 mL) was stirred at 50 °C for 10 h under H_2 atmosphere (4 kg/cm^2). After the insoluble materials were filtered off, the filtrate was concentrated in vacuo and then the residue was dissolved in water (30 mL). After the mixture was adjusted to pH 14 by addition of 6 mol/L aqueous NaOH solution, the mixture was extracted with diethyl ether (4 × 50 mL). The combined organic extracts were concentrated in vacuo. Flash chromatography (CH_2Cl_2/MeOH = 25:1) of the residue gave **13a** (1.29 g, 79%). ^{1}H NMR ($CDCl_3$) δ: 0.29−0.36 (m, 2H), 0.41−0.46 (m, 2H), 0.93 (d, J = 7.3 Hz, 3H), 2.10−2.24 (m, 3H), 2.53−2.65 (m, 3H), 2.77 (dd, J = 11.7, 6.3 Hz, 1H), 3.09 (dd, J = 6.3, 2.9 Hz, 1H), 3.12 (dd, J = 6.3, 2.9 Hz, 1H). MS (CI$^+$) *m*/*z*: 155 (M$^+$ + H). HRMS (CI$^+$) for $C_9H_{19}N_2$ (M$^+$ + H): calcd, 155.1548; found, 155.1539. $[\alpha]_D^{25}$ −3.4° (*c* 1.14, MeOH).

(3*R*,4*R*)-1-Benzyl-*N*-cyclopropyl-4-methylpyrrolidine-3-carboxamide (21). A mixture of **20** (150 g, 0.412 mol) and cyclopropylamine (650 mL, 9.39 mol) was stirred at room temperature for 23 h and concentrated in vacuo. After the addition of diisopropyl ether (800 mL) to the residue, the resulting precipitates were collected by filtration. The obtained crystals were dissolved in CH_2Cl_2 (800 mL), and the CH_2Cl_2 solution was extracted with 1 mol/L HCl (2 × 400 mL). The combined aqueous extracts were adjusted to pH 13 by addition of 30% aqueous NaOH solution under cooling with ice. The resulting precipitates were collected by filtration, washed with water and diisopropyl ether, and then dried in vacuo to give **21** (52.2 g, 49%); mp: 99−100 °C (hexane/AcOEt). ^{1}H NMR ($CDCl_3$) δ: 0.36−0.44 (m, 2H), 0.71−0.77 (m, 2H), 1.11 (d, J = 6.8 Hz, 3H), 1.89 (dd, J = 8.8, 7.3 Hz, 1H), 2.28−2.41 (m, 3H), 2.65−2.71 (m, 1H), 2.84 (dd, J = 9.8, 2.0 Hz, 1H), 3.13 (t, J = 8.8 Hz, 1H), 3.53 (d, J = 12.7 Hz, 1H), 3.65 (d, J = 12.7 Hz, 1H), 7.00 (br s, 1H), 7.25−7.35 (m, 5H). MS (EI) *m*/*z*: 258 (M$^+$). $[\alpha]_D^{26}$ +26.8° (*c* 0.552, MeOH). Anal. ($C_{16}H_{22}N_2O$) C, H, N.

(3*S*,4*R*)-1-Benzyl-3-cyclopropylaminomethyl-4-methylpyrrolidine (22). Boran−dimethylsulfide complex (90%, 34.3 mL, 0.326 mol) was added dropwise to a suspension of **21** (70.0 g, 0.271 mol) in anhydrous toluene (700 mL) under cooling with ice. The reaction mixture was stirred for 15 min under cooling with ice and heated under reflux for 5 h. After cooling to room temperature, 10% aqueous Na_2CO_3 solution (400 mL) was added to the reaction mixture and the whole mixture was stirred at 100 °C for 2 h. After cooling to room temperature, the toluene layer was separated, washed with water (2 × 250 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Vacuum distillation (140−150 °C, 0.4 mmHg) of the residue gave **22** (62.1 g, 94%). ^{1}H NMR ($CDCl_3$) δ: 0.26−0.33 (m, 2H), 0.37−0.43 (m, 2H), 1.05 (d, J = 6.3 Hz, 3H), 1.74−1.87 (m, 2H), 2.06−2.14 (m, 2H), 2.40 (dd, J = 9.3, 5.4 Hz, 1H), 2.60−2.68 (m, 2H), 2.74−2.80 (m, 2H), 3.52 (d, J = 13.2 Hz, 1H), 3.63 (d, J = 13.0 Hz, 1H), 7.21−7.35 (m, 5H). MS (CI$^+$) *m*/*z*: 245 (M$^+$ + H). HRMS (CI$^+$) for $C_{16}H_{25}N_2$ (M$^+$ + H): calcd, 245.20178; found, 245.20057. $[\alpha]_D^{25}$ +41.2° (*c* 1.075, MeOH).

(3*R*,4*R*)-3-Cyclopropylaminomethy-4-methylpyrrolidine (13b). A suspension of **22** (25.0 g, 0.102 mol), 10% Pd−C (12.5 g), and trifluoroacetic acid (15.7 mL, 0.204 mol) in EtOH (200 mL) was stirred at room temperature for 20 h under H_2 atmosphere (4 kg/cm^2). After the insoluble materials ware filtered off, the filtrate was concentrated in vacuo. After the addition of THF (100 mL) to the residue, the resulting precipitates were collected by filtration, washed with THF, and then dried in vacuo to give ditrifluoroacetic acid salt of **13b** (34.1 g, 87%). A solution of the above salt (116 g, 0.303 mol) in water (350 mL) was made strongly basic (pH >12) with solid NaOH, and the mixture was extracted with diethyl ether (3 × 300 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and then concentrated in vacuo. Vacuum distillation of the residue gave **13b** (40.7 g, 87%); bp: 60−68 °C (0.7 mmHg). ^{1}H NMR ($CDCl_3$) δ: 0.30−0.37 (m, 2H), 0.41−0.45 (m, 2H), 1.04 (d, J = 6.3 Hz, 3H), 1.68−1.75 (m, 2H), 2.08−2.13 (m, 1H), 2.46 (dd, J = 10.7, 7.3 Hz, 1H), 2.57 (dd, J = 11.7, 8.3 Hz, 1H), 2.64 (dd, J = 10.7, 6.3 Hz, 1H), 2.81 (dd, J = 11.7, 4.9 Hz, 1H), 3.11 (dd, J = 10.7, 6.8 Hz, 1H), 3.15 (dd, J = 10.7, 7.3 Hz, 1H). MS (CI$^+$) *m*/*z*: 155 (M$^+$ + H). HRMS (CI$^+$) for $C_9H_{19}N_2$ (M$^+$ + H): calcd, 155.15483; found, 155.15414. $[\alpha]_D^{25}$ −74.6° (*c* 0.648, MeOH). Anal. ($C_9H_{18}N_2 \cdot 2CF_3COOH$) C, H, N.

(3*R*,4*R*)-1-Benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol (24a). To a solution of **23** (10.0 g, 33.6 mmol) in MeOH (200 mL), a suspension of 10% Pd−C (3.00 g) in water (60 mL) and ammonium formate (21.2 g, 0.336 mol) was added; the whole mixture was heated under reflux for 4 h. After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. Benzyl chloroformate (6.00 mL, 40.6 mmol) was added to a solution of the residue and triethylamine (9.40 mL, 63.4 mmol) in anhydrous DMF (100 mL) under cooling with ice, the whole mixture was stirred under the same condition for 1.5 h, and then concentrated in vacuo. After dilution of the residue with AcOEt (400 mL), the mixture was washed with brine (2 × 100 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (AcOEt/MeOH = 20:1) of the residue gave **24a** (7.66 g, 91%). ^{1}H NMR ($CDCl_3$) δ: 1.91−2.16 (br, 1H), 2.30−2.39 (m, 1H), 2.44−2.60 (br, 1H), 3.20 (dd, J = 11.0, 6.7 Hz, 1H), 3.32 (dt, J = 11.0, 5.5 Hz, 1H), 3.59−3.77 (m, 4H), 4.23−4.35 (br, 1H), 5.12 (s, 2H), 7.29−7.36 (m, 5H). MS (CI$^+$) *m*/*z*: 252 (M$^+$ + H). HRMS (CI$^+$) for $C_{13}H_{18}NO_4$ (M$^+$ + H): calcd, 252.12358; found, 252.12256. $[\alpha]_D^{25}$ +11.6° (*c* 1.14, MeOH).

[(3*R*,4*S*)-4-Benzoyloxy-1-benzyloxycarbonylpyrrolidin-3-yl]methanol (24b). Diethyl azodicarboxylate (40% toluene solution, 9.53 mL, 21.9 mmol) was added dropwise to a mixture of **24a** (2.50 g, 9.95 mmol), triphenylphosphine (5.74 g, 21.9 mmol), and benzoic acid (2.55 g, 20.9 mmol) in anhydrous THF (60 mL) under cooling with NaCl−ice. The whole mixture was stirred at 0 °C for 1 h, further stirred at room temperature for 2 h, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 2:1) of the residue gave pale-brown oil (4.52 g, 99%). To a solution of the above oil (4.51 g, 9.82 mmol) in EtOH (60 mL) was added a solution of K_2CO_3 (4.07 g, 29.4 mmol) in water (30 mL), the whole mixture was heated under reflux for 3 h, and concentrated in vacuo. After dilution of the residue with CH_2Cl_2 (200 mL), the mixture was washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (AcOEt/MeOH = 10:1) of the residue gave **24b** (2.04 g, 83%). ^{1}H NMR ($CDCl_3$) δ: 2.29−2.40 (m, 1H), 2.52 (t, J = 4.9 Hz, 0.5H), 2.58 (t, J = 4.9 Hz, 0.5H), 2.95 (d, J = 3.7 Hz, 0.5H), 3.00 (d, J = 3.1 Hz, 0.5H), 3.41−3.47 (m, 1H), 3.53−3.61 (m, 3H), 3.82−3.96 (m, 2H), 4.43−4.56 (br, 1H), 5.09−5.17 (m, 2H), 7.29−7.36 (m, 5H). MS (CI$^+$) *m*/*z*: 252 (M$^+$ + H). HRMS (CI$^+$) for $C_{13}H_{18}NO_4$ (M$^+$ + H): calcd, 252.12358; found, 252.12085. $[\alpha]_D^{25}$ +2.6° (*c* 0.835, MeOH).

(3*R*,4*R*)-3-Azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine (25a). A solution of CBr_4 (4.34 g, 13.1 mmol) in anhydrous CH_2Cl_2 (14 mL) was added dropwise to a mixture of **24a** (3.00 g, 11.9 mmol), NaN_3 (2.32 g, 35.7 mmol), and triphenylphosphine (3.43 g, 13.1 mmol) in anhydrous DMF (60 mL) under cooling with ice. The whole mixture was stirred at room temperature for 25 h and further stirred at 60 °C for 2 h. After quenching the reaction by adding MeOH (5 mL), the mixture was concentrated in vacuo. After dilution of the residue with AcOEt (200 mL), the mixture was washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 1:2) of the residue gave **25a** (2.94 g, 89%). ^{1}H NMR ($CDCl_3$) δ: 2.07 (d, J = 4.3 Hz, 1H), 2.29−2.40 (m, 1H), 3.24 (dd, J = 11.0, 6.1 Hz, 1H), 3.30−3.48 (m, 3H), 3.68−3.77 (m, 2H), 4.09−4.26 (m, 1H), 5.13 (s, 2H), 7.31−7.37 (m, 5H). MS (CI$^+$) *m*/*z*: 277 (M$^+$ + H). HRMS (CI$^+$) for

$C_{13}H_{17}N_4O_3$ ($M^+ + H$): calcd, 277.13007; found, 277.13275. $[\alpha]_D^{24}$ +13.3° (*c* 0.506, MeOH).

(3*R*,4*S*)-3-Azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine (25b). The compound **25b** (2.18 g, 85%) was prepared from **24b** (2.33 g, 9.27 mmol) by the same method as that used for **25a**. ^{1}H NMR ($CDCl_3$): δ 1.94−2.07 (br, 1H), 2.33−2.44 (m, 1H), 3.24 (t, J = 11.0 Hz, 1H), 3.37−3.48 (m, 1H), 3.55−3.69 (m, 4H), 4.33−4.49 (br, 1H), 5.09−5.17 (m, 2H), 7.31−7.36 (m, 5H). MS (CI^+) *m/z*: 277 ($M^+ + H$). HRMS (CI^+) for $C_{13}H_{17}N_4O_3$ ($M^+ + H$): calcd, 277.13007; found, 277.12616. $[\alpha]_D^{25}$ +3.6° (*c* 0.613, MeOH).

(3*S*,4*S*)-3-Aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (27a). Diethylaminosulfur trifluoride (DAST, 1.20 mL, 9.08 mmol) was added dropwise to a solution of **25a** (1.20 g, 4.34 mmol) in anhydrous CH_2Cl_2 (40 mL) under cooling with NaCl−ice, and the whole mixture was stirred at room temperature for 3 h. Diethylaminosulfur trifluoride (0.57 mL, 4.31 mmol) was added dropwise to the reaction mixture and then stirred at room temperature additional 2 h. The reaction mixture was quenched by adding saturated aqueous $NaHCO_3$ solution (40 mL) under cooling with ice. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution (2 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 2:1) of the residue gave **26a** (726 mg, 60%). A suspension of **26a** (4.99 g, 17.9 mmol) and PtO_2 (406 mg, 1.80 mmol) in EtOH (100 mL) was stirred at room temperature for 7 h under H_2 atmosphere (1 atm). After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. Flash chromatography (AcOEt/MeOH = 10:1) of the residue gave **27a** (3.99 g, 88%). ^{1}H NMR ($CDCl_3$) δ: 2.21−2.36 (m, 1H), 2.83−2.92 (m, 1H), 2.98−3.03 (m, 1H), 3.22 (t, J = 11.0 Hz, 1H), 3.52−3.67 (m, 1H), 3.70−3.90 (m, 2H), 5.10−5.26 (m, 3H), 7.31−7.37 (m, 5H). MS (CI^+) *m/z*: 253 ($M^+ + H$). HRMS (CI^+) for $C_{13}H_{18}FN_2O_2$ ($M^+ + H$): calcd, 253.13523; found, 253.13479. $[\alpha]_D^{24}$ −0.8° (*c* 0.487, MeOH).

(3*S*,4*R*)-3-Aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (27b). Diethylaminosulfur trifluoride (DAST, 2.10 mL, 15.9 mmol) was added dropwise to a solution of **25b** (1.49 g, 5.39 mmol) in anhydrous CH_2Cl_2 (30 mL) under cooling with ice, the whole mixture was stirred at room temperature for 9 h, and then allowed to stand at room temperature for overnight. The reaction mixture was quenched by adding saturated aqueous $NaHCO_3$ solution (30 mL) under cooling with ice. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 2:1) of the residue gave a mixture of **26a** and **30** (929 mg). A suspension of the above mixture (925 mg) and PtO_2 (185 mg, 0.815 mmol) in EtOH (20 mL) was stirred at room temperature for 4.5 h under H_2 atmosphere (1 atm). After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. Flash chromatography (AcOEt/MeOH = 20:1) of the residue gave **27b** (359 mg, 26%) and **31** (196 mg, 15%).

27b. ^{1}H NMR ($CDCl_3$) δ: 2.39−2.49 (m, 1H), 2.61−2.72 (m, 2H), 3.37−3.43 (m, 1H), 3.57−3.79 (m, 3H), 5.01−5.18 (m, 3H), 7.29−7.37 (m, 5H). MS (CI^+) *m/z*: 253 ($M^+ + H$). HRMS (CI^+) for $C_{13}H_{18}FN_2O_2$ ($M^+ + H$): calcd, 253.1352; found, 253.1330. $[\alpha]_D^{25}$ −3.0° (*c* 0.717, MeOH).

31. ^{1}H NMR ($CDCl_3$) δ: 1.56−1.65 (m, 1H), 1.95−2.07 (m, 1H), 2.18−2.31 (m, 1H), 2.67−2.78 (m, 2H), 3.05−3.13 (m, 1H), 3.34−3.43 (m, 1H), 3.49−3.65 (m, 2H), 5.13 (s, 2H), 7.28−7.38 (m, 5H). MS (CI^+) *m/z*: 235 ($M^+ + H$).

(3*S*,4*S*)-3-Benzylaminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (28a). The compound **28a** (3.64 g, 82%) was prepared from **27a** (3.26 g, 12.9 mmol) by the same method as that used for **18**. ^{1}H NMR ($CDCl_3$) δ: 2.31−2.42 (m, 1H), 2.71−2.80 (m, 1H), 2.93 (ddd, J = 11.6, 8.6, 3.7 Hz, 1H), 3.23 (dt, J = 11.0, 4.3 Hz, 1H), 3.49−3.65 (m, 1H), 3.70−3.88 (m, 4H), 5.13 (d, J = 2.4 Hz, 1H), 5.14 (d, J = 2.4 Hz, 1H), 5.14 (ddd, J = 53.2, 6.1, 3.1 Hz), 7.22−7.37 (m, 10H). MS (CI^+) *m/z:* 343 ($M^+ + H$). HRMS (CI^+) for $C_{20}H_{24}FN_2O_2$ ($M^+ + H$): calcd, 343.18218; found, 343.18449. $[\alpha]_D^{24}$ −3.3° (*c* 0.516, MeOH).

(3*S*,4*R*)-3-Benzylaminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (28b). The compound **28b** (329 mg, 77%) was prepared from **27b** (313 mg, 1.24 mmol) by the same method as that used for **18**. ^{1}H NMR ($CDCl_3$) δ: 2.26−2.47 (m, 3H), 3.40 (t, J = 11.0 Hz, 1H), 3.52−3.81 (m, 5H), 5.02−5.20 (br, 1H), 5.13 (s, 2H), 7.24−7.37 (m, 5H). MS (FAB^+) *m/z*: 343 ($M^+ + H$). HRMS (FAB^+) for $C_{20}H_{24}FN_2O_2$ ($M^+ + H$): calcd, 343.1822; found, 343.1815. $[\alpha]_D^{25}$ −9.0° (*c* 0.519, MeOH).

(3*S*,4*S*)-3-(*N*-Benzyl-*N*-cyclopropyl)aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (29a). The compound **29a** (3.97 g, 99%) was prepared from **28a** (3.59 g, 10.5 mmol) by the same method as that used for **19**. ^{1}H NMR ($CDCl_3$) δ: 0.36−0.50 (m, 4H), 1.78−1.82 (m, 1H), 2.37−2.56 (m, 1H), 2.61−2.70 (m, 1H), 2.85 (dd, J = 12.8, 7.3 Hz, 1H), 3.06 (dt, J = 17.1, 11.0 Hz, 1H), 3.50 (ddt, J = 40.0, 12.8, 3.7 Hz, 1H), 3.59−3.82 (m, 4H), 5.00 (ddt, J = 53.2, 4.9, 3.1 Hz, 1H), 5.09−5.16 (m, 2H), 7.21−7.38 (m, 10H). MS (CI^+) *m/z*: 383 ($M^+ + H$). HRMS (CI^+) for $C_{23}H_{28}FN_2O_2$ ($M^+ + H$): calcd, 383.21348; found, 383.21378. $[\alpha]_D^{24}$ −4.4° (*c* 0.462, MeOH).

(3*S*,4*R*)-3-(*N*-Benzyl-*N*-cyclopropyl)aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (29b). The compound **29b** (336 mg, 100%) was prepared from **28b** (302 mg, 0.811 mmol) by the same method as that used for **19**. ^{1}H NMR ($CDCl_3$) δ: 0.34−0.56 (m, 4H), 1.76−1.80 (m, 1H), 2.04−2.37 (m, 2H), 2.74−2.85 (m, 1H), 3.06−3.32 (m, 2H), 3.47−3.68 (m, 3H), 3.79 (dd, J = 13.4, 7.3 Hz, 1H), 4.94 (dd, J = 52.6, 4.3 Hz, 1H), 5.09−5.16 (m, 2H), 7.19−7.36 (m, 10H). MS (FAB^+) *m/z*: 383 ($M^+ + H$). HRMS (FAB^+) for $C_{23}H_{28}FN_2O_2$ ($M^+ + H$): calcd, 383.2135; found, 383.2119. $[\alpha]_D^{27}$ −21.7° (*c* 0.409, MeOH).

(3*S*,4*S*)-3-Cyclopropylaminomethyl-4-fluoropyrrolidine (14a). A suspension of **29a** (1.22 g, 3.19 mmol) and 10% Pd−C (150 mg) in EtOH (14 mL) was stirred at room temperature for 4 h under H_2 atmosphere (1 atm). After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. Flash chromatography (CH_2Cl_2/MeOH = 10:1) of the residue gave **14a** (414 mg, 82%). ^{1}H NMR ($CDCl_3$) δ: 0.30−0.46 (m, 4H), 2.12−2.18 (m, 1H), 2.19−2.35 (m, 1H), 2.76−2.82 (m, 2H), 2.96−3.03 (m, 2H), 3.07−3.12 (m, 2H), 3.23 (dd, J = 25.1, 13.5 Hz, 1H), 5.10 (dt, J = 55.0, 3.7 Hz, 1H). MS (CI^+) *m/z:* 159 ($M^+ + H$). HRMS (CI^+) for $C_8H_{16}N_2$ ($M^+ + H$): calcd, 159.1298; found, 159.1316. $[\alpha]_D^{25}$ −26.6° (*c* 0.662, MeOH).

(3*S*,4*R*)-3-Cyclopropylaminomethyl-4-fluoropyrrolidine (14b). The compound **14b** (50.7 mg, 77%) was prepared from **29b** (160 mg, 0.418 mmol) by the same method as that used for **14a**. ^{1}H NMR ($CDCl_3$) δ: 0.28−0.45 (m, 4H), 2.09−2.13 (m, 1H), 2.33−2.52 (m, 2H), 2.63 (ddd, J = 12.2, 7.3, 1.8 Hz, 1H), 2.69 (dd, J = 11.6, 7.9 Hz, 1H), 2.86 (ddd, J = 35.5, 13.4, 3.7 Hz, 1H), 3.21 (ddd, J = 22.0, 13.4, 1.2 Hz, 1H), 3.34 (dd, J = 11.6, 7.9 Hz, 1H), 4.93 (dd, J = 54.4, 3.7 Hz, 1H). MS (FAB^+) *m/z*: 159 ($M^+ + H$). HRMS (FAB^+) for $C_8H_{16}FN_2$ ($M^+ + H$): calcd, 159.1298; found, 159.1286. $[\alpha]_D^{25}$ +22.0° (*c* 0.239, MeOH).

(2*R*)-2-Amino-3-(tetrahydropyran-2-yl)oxypropanol (34). To a suspension of L-serine methyl ester hydrochloride (195 g, 1.25 mol) in anhydrous CH_2Cl_2 (973 mL) was added 3,4-dihydro-1*H*-pyran (158 g, 1.88 mol) and *p*-toluenesulfonic acid monohydrate (4.76 g, 25.0 mmol), the whole mixture was stirred at room temperature for 20 h. The resulting precipitates were collected by filtration, washed with CH_2Cl_2, and dried in vacuo to give O^3-(tetrahydropyran-2-yl)-L-serine methyl ester hydrochloride (201 g, 67%).

To a suspension of $LiAlH_4$ (91.1 g, 2.40 mol) in anhydrous THF (2400 mL) was added O^3-(tetrahydropyran-2-yl)-L-serine methyl ester hydrochloride (288 g, 1.20 mol) portionwise under cooling with ice, the whole mixture was stirred at room temperature for 0.5 h, and then heated under reflux for additional 2 h. After quenching the reaction by adding aqueous KOH solution [KOH (34.6 g) in water (173 mL)] under cooling with ice, the resulting insoluble materials were filtered off and washed with THF (1000 mL). The combined filtrate and washing were concentrated in vacuo. After dilution of the residue with CH_2Cl_2 (1000 mL), the mixture was washed with aqueous 5 mol/L KOH solution (300 mL) and brine (300 mL), dried over anhydrous Na_2SO_4, and then concen-

trated in vacuo to give **34** (149 g, 71%). ^{1}H NMR ($CDCl_3$) δ: 1.47−1.87 (m, 6H), 3.05−3.11 (m, 1H), 3.46 (dd, J = 9.8, 4.4 Hz, 1H), 3.50−3.56 (m, 1H), 3.53 (dd, J = 10.8, 5.9 Hz, 1H), 3.67 (dd, J = 10.8, 4.9 Hz, 1H), 3.74 (dd, J = 9.8, 5.9 Hz, 1H), 3.83−3.89 (m, 1H), 4.58 (dd, J = 4.9, 4.4 Hz, 1H). MS (CI^+) m/z: 176 ($M^+ + H$). $[\alpha]_D^{23}$ −70.6° (*c* 1.09, MeOH).

Ethyl 3-[(2*R*)-1-Hydroxy-3-(tetrahydropyran-2-yloxy)prop-2-ylamino]-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (35). A mixture of **33** (220 g, 0.833 mol), triethyl orthoformate (208 mL, 1.25 mol), and acetic anhydride (197 mL, 2.08 mol) was stirred at 115−120 °C for 3 h and concentrated in vacuo. To a solution of the residue in EtOH (1000 mL) was added a solution of **34** (161 g, 0.916 mol) in EtOH (300 mL) dropwise under cooling with ice, the whole mixture was stirred at room temperature for 1 h, and then concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2 → CH_2Cl_2/MeOH = 10:1) gave **35** (312 g, 83%). ^{1}H NMR ($CDCl_3$) δ: 0.84−1.26 (m, 3H), 1.33−2.12 (m, 6H), 2.40−2.79 (m, 1H), 3.19−4.31 (m, 9H), 4.46−4.78 (m, 1H), 6.84−7.15 (m, 1H), 8.12−8.32 (m, 1H), 9.36−9.93 (m, 0.2H), 10.5−11.4 (m, 0.8H). MS (EI) m/z 449 (M^+). $[\alpha]_D^{24}$ −67.7° (*c* 0.514, MeOH).

Ethyl (3*R*)-9,10-Difluoro-2,3-dihydro-3-[(tetrahydropyran-2-yloxy)methyl]-7-oxo-7*H*-pyrido[1,2,3-*de*]benzoxazine-6-carboxylate (36). A mixture of **35** (310 g, 0.690 mol), KF (spray dried, 140 g, 2.41 mol), and anhydrous DMSO (1200 mL) was stirred at 115−120 °C for 5 h. After cooling, MeOH (600 mL) was added to the reaction mixture; the whole mixture was allowed to stand at room temperature for 2 h. The resulting precipitates were collected by filtration. The filtered precipitates were suspended in MeOH (1000 mL) and then filtered. The filtered precipitates were suspended in water (1000 mL), filtered, washed with MeOH (500 mL), and then dried in vacuo to give **36** (176 g, 62%); mp: 228−231 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25−1.29 (m, 1H), 1.27 (t, J = 6.8 Hz, 3H), 1.37−1.60 (m, 5H), 3.10−3.16 (m, 1H), 3.19−3.23 (m,1H), 3.73 (dd, J = 10.3, 9.8 Hz, 1H), 3.82 (dd, J = 10.3, 4.4 Hz, 1H), 4.20−4.27 (m, 2H), 4.52 (dd, J = 11.7, 2.4 Hz, 1H), 4.71 (br, 1H), 4.83 (d, J = 11.2 Hz, 1H), 4.94−4.96 (m, 1H), 7.65 (dd, J = 10.8, 7.8 Hz, 1H), 8.61 (s, 1H). MS (EI) m/z 409 (M^+). $[\alpha]_D^{22}$ −103° (*c* 0.516, $CHCl_3$). Anal. ($C_{20}H_{21}F_2NO_6$) C, H, N.

Ethyl (3*S*)-9,10-Difluoro-2,3-dihydro-3-hydroxymethyl-7-oxo-7*H*-pyrido[1,2,3-*de*]benzoxazine-6-carboxylate (37). A mixture of **36** (174 g, 0.424 mol), *p*-toluenesulfonic acid monohydrate (4.03 g, 21.2 mmol), and EtOH (3000 mL) was heated under reflux for 3 h and then allowed to stand at room temperature for 16 h. The resulting precipitates were collected by filtration, washed with EtOH (300 mL), and then dried in vacuo to give **37** (127 g, 92%); mp: 235−237 °C. ^{1}H NMR (DMSO-d_6) δ: 1.27 (t, J = 6.8 Hz, 3H), 3.53−3.59 (m, 1H), 3.73−3.78 (m, 1H), 4.16−4.28 (m, 2H), 4.44 (dd, J = 11.7, 2.9 Hz, 1H), 4.62−4.66 (m, 1H), 4.77 (d, J = 11.7 Hz, 1H), 5.32 (t, J = 5.4 Hz, 1H), 7.62 (dd, J = 11.2 Hz, 8.3 Hz, 1H), 8.54 (s, 1H). MS (EI) m/z 325 (M^+). $[\alpha]_D^{25}$ −124° (*c* 1.03, DMF). Anal. ($C_{15}H_{13}F_2NO_5 \cdot H_2O$) C, H, N.

Ethyl (3*R*)-9,10-Difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*]benzoxazine-6-carboxylate (38). To a suspension of **37** (40.7 g, 125 mmol) in anhydrous THF (1667 mL) was added diethylaminosulfur trifluoride (19.8 mL, 150 mmol), and the whole mixture was heated under reflux for 0.5 h. After cooling, the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution (1000 mL). The resulting mixture was extracted with CH_2Cl_2 (700 mL). The organic extracts were washed with water, dried over anhydrous Na_2SO_4, and then concentrated in vacuo. A mixture of the residue and THF (1500 mL) was heated under reflux for 1 h. The resulting precipitates were collected by filtration to give crude **38** (17.6 g). The filtrate was concentrated in vacuo. Flash chromatography (CH_2Cl_2/acetone = 10:1) of the residue gave additional crude **38** (12.5 g). A mixture of the combined crude **38** (30.1 g) and EtOH (500 mL) was heated under reflux for 0.5 h, and the resulting precipitates were collected by filtration. The filtered precipitates were washed with EtOH and dried in vacuo to give **38** (26.5 g, 65%); mp: 254−257 °C. ^{1}H NMR (DMSO-d_6) δ: 1.27 (t, J = 7.3 Hz, 3H), 4.17−4.28 (m, 2H), 4.51 (ddd, J = 12.2, 4.9, 2.9 Hz, 1H), 4.70 (ddd, J = 46.5, 10.3, 7.8 Hz, 1H), 4.86 (ddd, J = 46.0, 10.3, 4.9 Hz, 1H), 4.86 (d, J = 12.3 Hz, 1H), 5.06−5.13 (m, 1H), 7.64 (dd, J = 10.6 Hz, 7.8 Hz, 1H), 8.64 (s, 1H). MS (EI) m/z 327 (M^+). $[\alpha]_D^{25}$ −101° (*c* 1.03, AcOH). Anal. ($C_{15}H_{12}F_3NO_4$) C, H, N.

bis(Acetato-*O*)[(3*S*)-9,10-difluoro-2,3-dihydro-3-methyl-7-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylato-*O*^{6},*O*^{7}]boron (10). A mixture of boric acid (23.9 g, 0.387 mol), Ac_2O (110 mL, 1.17 mol), and $ZnCl_2$ (440 mg, 3.23 mmol) was stirred at room temperature for 30 min. To the reaction mixture was added **32** (40.0 g, 0.129 mol), the whole mixture was stirred at 60 °C for 2 h, and then concentrated in vacuo. A solution of the residue in AcOEt (1000 mL) was washed with saturated aqueous $NaHCO_3$ solution (2 × 500 mL) and water (500 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. After treatment, the residue was treated with diisopropyl ether (500 mL) and the resulting precipitates were collected by filtration, washed with diisopropyl ether, and then dried in vacuo to give **10** (46.8 g, 88%); mp: >300 °C. ^{1}H NMR ($CDCl_3$) δ: 1.74 (d, J = 6.8 Hz, 3H), 1.90 (s, 3H), 2.05 (s, 3H), 4.55 (dd, J = 12.2, 2.4 Hz, 1H), 4.62 (dd, J = 12.2, 2.4 Hz, 1H), 5.11−5.19 (m, 1H), 7.90 (dd, J = 9.8, 7.3 Hz, 1H), 9.25 (s, 1H). Anal. ($C_{17}H_{14}BF_2NO_8$) C, H, N.

bis(Acetato-*O*)[(3*R*)-9,10-difluoro-3-(2-fluoromethyl)-2,3-dihydro-7-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylato-*O*^{6},*O*^{7}]boron (11). The compound **11** (24.5 g, 83%) was prepared from **38** (22.6 g, 69.0 mmol) by the same method as that used for **10**. ^{1}H NMR ($CDCl_3$) δ: 1.85 (s, 3H), 2.05 (s, 3H), 4.62 (ddd, J = 12.2, 3.9, 2.9 Hz, 1H), 4.74 (ddd, J = 46.4, 10.3, 7.8 Hz, 1H), 4.90 (ddd, J = 45.4, 10.3, 4.9 Hz, 1H), 4.92 (dd, J = 12.7, 1.0 Hz 1H), 5.35−5.38 (m, 1H), 7.92 (dd, J = 9.3, 7.3 Hz, 1H), 9.22 (s, 1H). Anal. ($C_{17}H_{13}BF_3NO_8 \cdot 0.75H_2O$) C, H, N.

(3*S*)-10-[(3*S*)-3-Cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (4). A mixture of **10** (1.20 g, 2.93 mmol), **12** (493 mg, 3.51 mmol), and triethylamine (0.49 mL, 3.52 mmol) in anhydrous CH_3CN (20 mL) was stirred at 60 °C for 3 h and then concentrated in vacuo. Flash chromatography (AcOEt/MeOH = 5:1) of the residue gave the product as a yellow foam. A mixture of the above product in 5% aqueous AcOH solution (20 mL) was stirred at 80 °C for 2 h. The reaction mixture was washed with AcOEt (2 × 5 mL) and adjusted to pH 7 by addition of 2 mol/L aqueous NaOH solution. The resulting precipitates were collected by filtration and washed with water. Recrystallization of the precipitates from EtOH gave **4** (719 mg, 61%); mp: 178−179 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.33−0.36 (m, 2H), 0.44−0.49 (m, 2H), 1.60 (d, J = 6.7 Hz, 3H), 1.63−1.65 (m, 1H), 2.06−2.12 (m, 1H), 2.13−2.18 (m, 1H), 2.36−2.47 (m, 1H), 2.81 (d, J = 6.7 Hz, 2H), 3.57 (ddd, J = 10.4 Hz, 7.9, 3.1 Hz, 1H), 3.67−3.73 (m, 1H), 3.77 (ddd, J = 9.8, 7.9, 1.8 Hz, 1H), 3.84−3.91 (m, 1H), 4.27 (dd, J = 11.6 Hz, 2.4 Hz, 1H), 4.38−4.46 (m, 2H), 7.67 (d, J = 14.1 Hz, 1H), 8.54 (s, 1H). MS (EI) m/z: 401 (M^+). IR (KBr) cm^{-1}: 1709, 1622. $[\alpha]_D^{25}$ −107° (*c* 0.512, 0.05 mol/L aqueous NaOH). Anal. ($C_{21}H_{24}FN_3O_4$) C, H, N.

(3*S*)-10-[(3*S*,4*S*)-3-Cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (5a). The compound **5a** (474 mg, 47%) was prepared from **10** (1.00 g, 2.44 mmol) and **13a** (452 mg, 2.93 mmol) by the same method as that used for **4**; mp: 186−188 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.30−0.38 (m, 2H), 0.42−0.49 (m, 2H), 1.03 (d, J = 6.8 Hz, 3H), 1.60 (d, J = 6.8 Hz, 3H), 2.12−2.17 (m, 1H), 2.32−2.44 (m, 2H), 2.73 (dd, J = 11.7, 7.8 Hz, 1H), 2.84 (dd, J = 11.7, 6.4 Hz, 1H), 3.41 (dt, J = 10.7, 3.4 Hz, 1H), 3.72 (dd, J = 7.3, 2.4 Hz, 1H), 3.98−4.03 (m, 1H), 4.26 (dd, J = 11.2, 2.4 Hz, 1H), 4.34 (dd, J = 11.2, 2.4 Hz, 1H), 4.40−4.45 (m, 1H), 7.66 (d, J = 14.2 Hz, 1H), 8.53 (s, 1H). MS (EI) m/z: 415 (M^+). HRMS (EI) for $C_{22}H_{26}FN_3O_4$ (M^+): calcd, 415.1907; found, 415.1892. IR (KBr) cm^{-1}: 1719, 1621. $[\alpha]_D^{25}$ −115° (*c* 0.526, 0.05 mol/L aqueous NaOH). Anal. ($C_{22}H_{26}FN_3O_4 \cdot 0.25H_2O$) C, H, N.

(3*S*)-10-[(3*S*,4*R*)-3-Cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (5b). The compound **5b** (362 mg, 71%) was prepared from **10** (500 mg, 1.22 mmol) and **13b**

(226 mg, 1.47 mmol) by the same method as that used for **4**; mp: 163−165 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.32−0.39 (m, 2H), 0.45−0.51 (m, 2H), 1.13 (d, J = 5.9 Hz, 3H), 1.61 (d, J = 6.4 Hz, 3H), 1.89−1.99 (m, 2H), 2.13−2.18 (m, 1H), 2.67 (dd, J = 11.7, 8.3 Hz, 1H), 2.98 (dd, J = 11.7, 3.9 Hz, 1H), 3.53−3.59 (m, 1H), 3.67−3.84 (m, 3H), 4.26 (dd, J = 11.2, 2.0 Hz, 1H), 4.40 (dd, J = 11.7, 2.0 Hz, 1H), 4.42−4.47 (m, 1H), 7.66 (d, J = 14.2 Hz, 1H), 8.54 (s, 1H). MS (EI) *m/z*: 415 (M^+). IR (KBr) cm^{-1}: 1730, 1625. $[\alpha]_D^{25}$ −123° (*c* 0.507, 0.05 mol/L aqueous NaOH). Anal. ($C_{22}H_{26}FN_3O_4$) C, H, N.

(3*S*)-10-[(3*S*,4*S*)-3-Cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (6a). The compound **6a** (217 mg, 71%) was prepared from **10** (300 mg, 0.733 mmol) and **14a** (128 mg, 0.806 mmol) by the same method as that used for **4**; mp: 202−204 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.33−0.51 (m, 4H), 1.61 (d, J = 6.7 Hz, 3H), 2.15−2.20 (m, 1H), 2.37−2.55 (m, 1H), 2.93 (dd, J = 12.2, 6.7 Hz, 1H), 3.09 (dd, J = 12.2, 7.9 Hz, 1H), 3.73−3.86 (m, 2H), 3.93 (dt, J = 10.5, 3.3 Hz, 1H), 4.22−4.48 (m, 4H), 5.21 (dt, J = 54.0, 3.2 Hz, 1H), 7.70 (d, J = 14.1 Hz, 1H), 8.55 (s, 1H). MS (FAB$^+$) *m/z*: 420 (M^+ + H). HRMS (FAB$^+$) for $C_{21}H_{24}F_2N_3O_4$ (M^+ + H): calcd, 420.1735; found, 420.1778. IR (KBr) cm^{-1}: 1728, 1625. $[\alpha]_D^{24}$ −196° (*c* 0.257, 0.05 mol/L aqueous NaOH). Anal. ($C_{21}H_{23}F_2N_3O_4$) C, H, N.

(3*S*)-10-[(3*S*,4*R*)-3-Cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (6b). The compound **6b** (50.8 mg, 70%) was prepared from **10** (71.0 mg, 0.174 mmol) and **14b** (30.0 mg, 0.190 mmol) by the same method as that used for **4**; mp: 201−203 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.30−0.35 (m, 2H), 0.45−0.48 (m, 2H), 1.62 (d, J = 6.7 Hz, 3H), 2.11−2.16 (m, 1H), 2.58−2.80 (m, 3H), 3.48 (dt, J = 10.4, 2.4 Hz, 1H), 3.83 (dd, J = 25.1, 12.2 Hz, 1H), 4.04−4.19 (m, 2H), 4.31 (dd, J = 11.6, 2.4 Hz, 1H), 4.41 (dd, J = 11.6, 2.4 Hz, 1H), 4.44−4.47 (m, 1H), 5.10 (dt, J = 53.2, 2.4 Hz, 1H), 7.70 (d, J = 13.4 Hz, 1H), 8.56 (s, 1H). MS (FAB$^+$) *m/z*: 420 (M^+ + H). HRMS (FAB$^+$) for $C_{21}H_{24}F_2N_3O_4$ (M^+ + H): calcd, 420.1735; found, 420.1719. IR (KBr) cm^{-1}: 1707, 1625. $[\alpha]_D^{26}$ −7.9° (*c* 0.242, 0.05 mol/L aqueous NaOH). Anal. ($C_{21}H_{23}F_2N_3O_4$) C, H, N.

(3*R*)-10-[(3*S*)-3-Cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (7). A mixture of **11** (22.6 g, 53.0 mmol), **12** (8.41 g, 60.0 mmol), and triethylamine (7.59 g, 75.0 mmol) in anhydrous CH_2Cl_2 (273 mL) was allowed to stand at room temperature for 13 h. The reaction mixture was washed with water (200 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (CH_2Cl_2/MeOH = 15:1) of the residue gave the product as a yellow foam. A mixture of the above product in 5% aqueous AcOH solution (100 mL) was stirred at 80 °C for 3 h. After washing of the reaction mixture with AcOEt (100 mL), the aqueous solution was adjusted to pH 7 by addition of 1 mol/L aqueous NaOH solution. The resulting precipitates were collected by filtration and washed with water. Recrystallization of the precipitates from EtOH gave **7** (11.2 g, 50%); mp: 198−199 °C (EtOH). ^{1}H NMR (DMSO−d_6) δ: 0.15−0.26 (m, 2H), 0.28−0.40 (m, 2H), 1.50−1.59 (m, 1H), 1.95−2.00 (m, 1H), 2.02−2.07 (m, 1H), 2.25−2.35 (m, 1H), 2.58−2.67 (m, 2H), 3.31 (br, 1H), 3.44−3.49 (m, 1H), 3.60−3.80 (m, 3H), 4.34 (ddd, J = 12.2, 4.9, 2.9 Hz, 1H), 4.73 (ddd, J = 47.0, 9.8, 8.3 Hz, 1H), 4.77 (d, J = 12.2 Hz, 1H), 4.89 (ddd, J = 45.5, 9.8, 4.9 Hz, 1H), 5.12−5.19 (m, 1H), 7.53 (d, J = 12.2 Hz, 1H), 8.80 (s, 1H). MS (EI) *m/z*: 419 (M^+). IR (KBr) cm^{-1}: 1717, 1623. $[\alpha]_D^{25}$ −139° (*c* 0.518, 0.05 mol/L aqueous NaOH). Anal. ($C_{21}H_{23}F_2N_3O_4$) C, H, N.

(3*R*)-10-[(3*S*,4*S*)-3-Cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (8a). The compound **8a** (422 mg, 83%) was prepared from **11** (500 mg, 1.17 mmol) and **13a** (199 mg, 1.29 mmol) by the same method as that used for **4**; mp: 200−201 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.30−0.37 (m, 2H), 0.42−0.50 (m, 2H), 1.02 (d, J = 6.4 Hz, 3H), 2.12−2.18 (m, 1H), 2.31−2.42 (m, 2H), 2.72 (dd, J = 11.7, 7.8 Hz, 1H), 2.84 (dd, J = 11.7, 6.4 Hz, 1H), 3.41 (dt, J = 10.3, 3.4 Hz, 1H), 3.67−3.76 (m, 2H), 3.98 (ddd, J = 10.3, 6.4 Hz, 3.4 Hz, 1H), 4.31 (ddd, J = 11.2, 4.9, 2.9 Hz, 1H), 4.59−4.86 (m, 4H), 7.66 (d, J = 14.2 Hz, 1H), 8.54 (s, 1H). MS (EI) *m/z*: 433 (M^+). HRMS (EI) for $C_{22}H_{25}F_2N_3O_4$ (M^+): calcd, 433.1813; found, 433.1788. IR (KBr) cm^{-1}: 1723, 1626. $[\alpha]_D^{24}$ −162° (*c* 0.243, 0.05 mol/L aqueous NaOH). Anal. ($C_{22}H_{25}F_2N_3O_4$) C, H, N.

(3*R*)-10-[(3*S*,4*R*)-3-Cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (8b). The compound **8b** (620 mg, 61%) was prepared from **11** (1000 mg, 2.34 mmol) and **13b** (397 mg, 2.57 mmol) by the same method as that used for **4**; mp: 183−184 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.32−0.38 (m, 2H), 0.44−0.49 (m, 2H), 1.12 (d, J = 6.4 Hz, 3H), 1.90−1.98 (m, 2H), 2.12−2.17 (m, 1H), 2.65 (dd, J = 11.7, 8.3 Hz, 1H), 2.98 (dd, J = 11.7, 3.9 Hz, 1H), 3.52−3.58 (m, 1H), 3.66−3.72 (m, 1H), 3.73−3.84 (m, 2H), 4.30 (ddd, J = 11.7, 4.4, 2.4 Hz, 1H), 4.59−4.87 (m, 4H), 7.66 (d, J = 14.2 Hz, 1H), 8.55 (s, 1H). MS (EI) *m/z*: 433 (M^+). HRMS (EI) for $C_{22}H_{25}F_2N_3O_4$ (M^+): calcd, 433.1813; found, 433.1824. IR (KBr) cm^{-1}: 1721, 1625. $[\alpha]_D^{24}$ −143° (*c* 0.449, 0.05 mol/L aqueous NaOH). Anal. ($C_{22}H_{25}F_2N_3O_4$) C, H, N.

(3*R*)-10-[(3*S*,4*S*)-3-Cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (9a). The compound **9a** (1399 mg, 72%) was prepared from **11** (1900 mg, 4.45 mmol) and **14a** (774 mg, 4.89 mmol) by the same method as that used for **4**; mp: 197−198 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.33−0.39 (m, 2H), 0.46−0.50 (m, 2H), 2.15−2.20 (m, 1H), 2.37−2.54 (m, 1H), 2.92 (dd, J = 12.2, 6.7 Hz, 1H), 3.08 (dd, J = 12.2, 7.9 Hz, 1H), 3.71−3.85 (m, 2H), 3.91 (dt, J = 10.4, 3.1 Hz, 1H), 4.20−4.36 (m, 2H), 4.61−4.87 (m, 2H), 5.20 (dt, J = 53.8, 3.1 Hz, 1H), 7.69 (d, J = 14.1 Hz, 1H), 8.57 (s, 1H). MS (FAB$^+$) *m/z*: 438 (M^+ + H). HRMS (FAB$^+$) for $C_{21}H_{23}F_3N_3O_4$ (M^+ + H): calcd, 438.1641; found, 438.1656. IR (KBr) cm^{-1}: 1733, 1625. $[\alpha]_D^{28}$ −207° (*c* 0.260, 0.05 mol/L aqueous NaOH). Anal. ($C_{21}H_{22}F_3N_3O_4$) C, H, N.

(3*R*)-10-[(3*S*,4*R*)-3-Cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (9b). The compound **9b** (71.0 mg, 69%) was prepared from **11** (100 mg, 0.234 mmol) and **14b** (40.6 mg, 0.257 mmol) by the same method as that used for **4**; mp: 198−200 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.31−0.34 (m, 2H), 0.45−0.48 (m, 2H), 2.10−2.15 (m, 1H), 2.58−2.78 (m, 3H), 3.48 (dt, J = 10.4, 2.4 Hz, 1H), 3.81 (dd, J = 24.4, 12.2 Hz, 1H), 4.04−4.19 (m, 2H), 4.35 (ddd, J = 12,2, 4.3, 2.4 Hz, 1H), 4.58−4.86 (m, 4H), 5.10 (dt, J = 53.8, 2.4 Hz, 1H), 7.72 (d, J = 13.4 Hz, 1H), 8.56 (s, 1H). MS (FAB$^+$) *m/z*: 438 (M^+ + H). HRMS (FAB$^+$) for $C_{21}H_{23}F_3N_3O_4$ (M^+ + H): calcd, 438.1641; found, 438.1664. IR (KBr) cm^{-1}: 1709, 1624. $[\alpha]_D^{25}$ −38.9° (*c* 0.118, 0.05 mol/L aqueous NaOH). Anal. ($C_{21}H_{22}F_3N_3O_4$) C, H, N.

In Vitro Antibacterial Activity. The MIC (μg/mL) was determined by the agar dilution method[27] with Muller−Hinton agar (Difco Laboratories, Detroit, MI). The MIC was defined as the lowest concentration of an antibacterial agent that inhibited visible growth after incubation at 35 °C for 18 h.

In Vivo Antibacterial Activity. Male ICR mice (4 weeks) weighting about 20 g were infected intraperitoneally with bacterial suspensions. The bacteria used for infection were *S. aureus* Smith (2.7×10^6 CFU per mouse) and *S. pneumoniae* Type III (660 CFU per mouse). The test compounds were administered orally or subcutaneously 1 h after bacterial challenge. Untreated and treated groups at each dose were composed of five mice each. The 50% effective dose (ED50s) were calculated by the least-squares method or the probit method on the basis of the number of survivals at 7 days after infection.

Inhibitory Activity against DNA Gyrase and Topoisomerase IV of *S. aureus*. The supercoiling activity of DNA gyrase and the decatenation activity of topoisomerase IV were determined by a previous reported procedure.[25] The inhibitory activity was assayed

by determining the concentration required to inhibit 50% of the enzyme reaction.

Intravenous Single-Dose Toxicity. The test compounds were dissolved in 0.1 mol/L NaOH in saline at different concentrations. The solution was administered intravenously to three or five four-week-old male ICR mice at the speed of 0.1 mL/1 min. The total volume of administration was adjusted 10 mL/kg of body weight. The number of dead mice was counted 7 days after administration.

Convulsive Activity Test. Ten μL of the test compounds solution was intracerebroventricularly injected into four-week-old male ICR mice. In experiments with concurrent NSAID, 20 min before intracerebroventricular injection of the test article solution, γ-oxo-(1,1′-bipenyl)-4-butanoic acid (fenbufen) was orally administered at a dose of 200 mg/kg. Development of clonic convulsion was examined until 2 h after injection.

Phototoxicity Test. The back of mice was shaved and then ultraviolet A was irradiated for 3 h (4.59 J/cm^2) 60 min after oral administration of the test compounds. The erythema of skin was evaluated 72 h after completion of the irradiation by scoring (0, nil; 1, slight; 2, moderate; 3, marked change).

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Supporting Information Available: Purity data for compounds **4**−**11**, **13b**, **21**, **36**−**38**. This material is available free of charge via the Internet at http://pubs.acs.org.

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