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Synthesis and Antibacterial Activities of Optically Active Ofloxacin and Its Fluoromethyl Derivative

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Two optically active (100% enantiomeric excess) isomers (**13a** and **13b**) of ofloxacin (**1**) [(±)-ofloxacin; DL-8280; (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid] and their fluoromethyl derivatives (**14a** and **14b**) were prepared *via* their optically active intermediates resolved by use of high-performance liquid chromatography (HPLC). The isomers (**13a** and **13b**) were also obtained efficiently by an alternative route *via* separation of the diastereoisomers (**18**, **19**) prepared in the reaction of benzoxazine (**17**) with L-prolinyl chloride.

The (−)-isomers of **1** and its fluoromethyl derivative (**2**) were approximately twice as active as the corresponding racemates, while the (+)-isomers were considerably less active than the racemates.

The absolute configuration at the C_3 position in the oxazine ring in a series of (−)-compounds (**b**) was confirmed by X-ray analysis of the hydrochloride of the (−)-benzoxazine derivative (**15b**) to be *S*.

Keywords——ofloxacin; DL-8280; optically active ofloxacin; 7,8-difluoro-2,3-dihydro-3-methyl-4*H*-[1,4]benzoxazine; *N*-*p*-toluenesulfonyl-L-proline; X-ray analysis; absolute configuration; antibacterial activity; DNA gyrase

Considerable progress in the activity of quinolone carboxylic acid antibacterial agents (quinolones) has recently been achieved by the development of new quinolones having both a fluorine atom and a piperazinyl group in their parent compounds. The active center of this class of compounds is considered to be the N-1 substituted 1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety, and a variety of N-1 substituents having no asymmetric center have been reported. The roles of these substituents have also been discussed.[1] However, the new quinolone ofloxacin (**1**),[2] which is characterized by a tricyclic structure, possesses a methyl group at the C_3 position in the oxazine ring, and so has an asymmetric center at this position.

In this paper, we describe the synthesis of optically active (+)- and (−)-ofloxacin (**13a** and **13b**)[3] and their 3-fluoromethyl derivatives (**14a** and **14b**) through optical resolution of racemic intermediates of **1** both by high-performance liquid chromatography (HPLC) and by an alternative route consisting of derivatization from the diastereomers using an optically active proline analogue.

The Riker Laboratories group[4] has recently disclosed that the *S*-(−)-isomer of 6,7-dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic acid (S-25930) had potent antibacterial activity *in vitro* while the *R*-(+)-isomer showed much weaker activity.

Chemistry

The (±)-3,5-dinitrobenzoyl derivative (**4**) of the racemic alcohol (**3**)[5] was resolved

completely by HPLC to yield optically pure benzoyl esters (**5a** and **5b**). The benzoyl ester (**5b**) was hydrolyzed with an ethanolic aqueous $NaHCO_3$ solution to yield an alcohol (**6b**), which was converted to an iodide (**7b**) with triphenylphosphite methiodide[6] in *N*,*N*-dimethylformamide (DMF). Optically active carboxylic acid (**9b**) was obtained by reduction of **7b** with tri-*n*-butyltin hydride in EtOH, followed by hydrolysis of the resulting ester (**8b**) in concentrated HCl–AcOH. Reaction of **9b** with boron trifluoride etherate in Et_2O[7] gave a chelate compound (**12b**), which was reacted with 1-methylpiperazine in dimethyl sulfoxide (DMSO), and then with triethylamine in 95% MeOH to give (−)-ofloxacin (**13b**) in good yield. Similarly, the benzoyl ester (**5a**)was converted through **6a**, **7a**, **8a**, **9a** and **12a** to (+)-ofloxacin (**13a**).

Optically active fluoromethyl derivatives (**14a** and **14b**) were synthesized as follows: fluorination of **6a** and **6b** with diethylaminosulfur trifluoride (DAST)[8] in dry CH_2Cl_2 afforded **10a** and **10b**, which were hydrolyzed in concentrated HCl–AcOH to give the carboxylic acids (**11a** and **11b**). The acids (**11a** and **11b**) were reacted with 1-methylpiperazine by heating in DMSO to afford the desired compounds (**14a** and **14b**) in good yield.

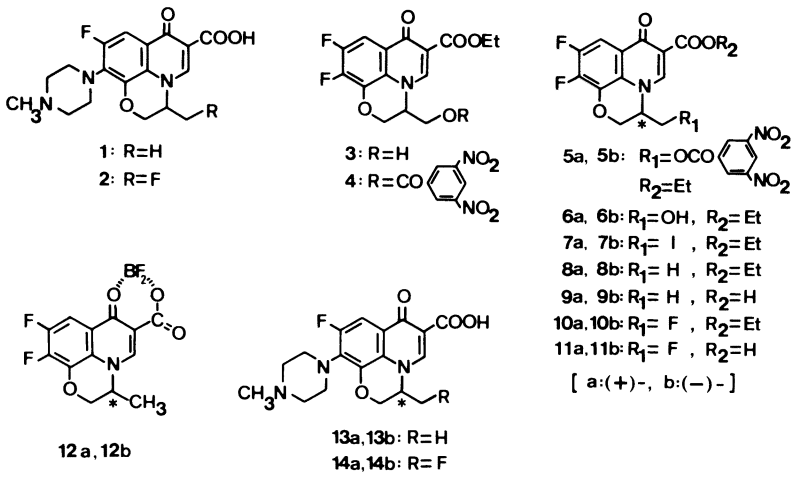

Chart 1

In order to obtain a large quantity of optically active ofloxacin, we investigated effective methods of preparing optical isomers of (±)-7,8-difluoro-2,3-dihydro-3-methyl-4*H*-[1,4]benzoxazine (**17**),[2] a key intermediate of **1**. We have already reported[9] an asymmetric hydrolysis of (±)-7,8-difluoro-2,3-dihydro-3-acetoxymethyl-4*H*-[1,4]benzoxazine with enzymes to give (+)- and (−)-7,8-difluoro-2,3-dihydro-3-methyl-4*H*-[1,4]benzoxazine, (**15a** and **15b**), from which optically active esters (**8a** and **8b**) were prepared in a manner similar to that reported in the previous paper.[2]

An alternative method of obtaining **15a** and **15b** that was similar to the technique reported by Gerster and his co-workers[4] was employed. Reaction of **17** with *N*-*p*-toluenesulfonyl-L-prolinyl chloride[10] in the presence of pyridine in dry CH_2Cl_2 afforded amides (**18** and **19**). Most of **19** was removed by fractional crystallization of the mixture of **18** and **19** from AcOEt–hexane. Then, the desired pure **18** was easily obtained from the filtrate by silica gel column chromatography. Hydrolysis of the first eluate (**18**) was carried out by heating in 1 N NaOH–EtOH solution to give **15b** in excellent yield. The physical data of the optically active compounds are shown in Table I.

The hydrochloride of **15b** was crystallized from EtOH–AcOEt to give colorless prisms

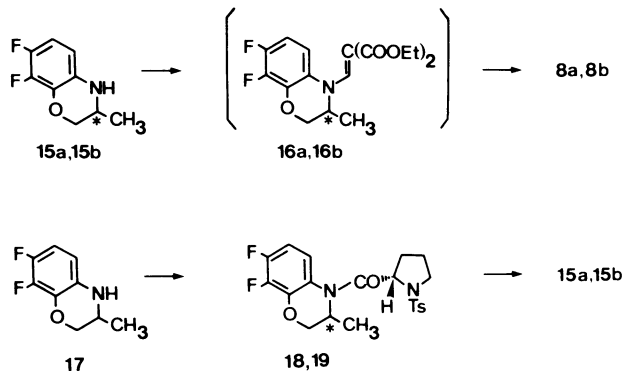

Chart 2

TABLE I. Physical Data for Optically Active Compounds

Compds. No.	mp (°C)	$[\alpha]_D^{23}$ (c, solvent)	Compds. No.	mp (°C)	$[\alpha]_D^{23}$ (c, solvent)
5a	235—240	+90.8° (0.852, DMF)	**11a**	>300	+92.4° (0.675, DMSO)
5b	244—249	−92.5° (0.889, DMF)	**11b**	>300	−92.7° (0.550, DMSO)
6a	231—234	+125.9° (0.715, DMF)	**12a**	>300	+9.6° (0.540, DMSO)
6b	235—240	−125.9° (0.918, DMF)	**12b**	>300	−9.4° (0.490, DMSO)
8a	259—260	+68.9° (0.700, AcOH)	**13a**	222—225 (dec.)	+76.7° (0.350, 0.05 N NaOH)
8b	258—259	−68.1° (0.520, AcOH)	**13b**	225—227 (dec.)	−76.9° (0.385, 0.05 N NaOH)
9a	>300	+65.6° (0.903, DMSO)	**14a**	225—235 (dec.)	+109.4° (0.435, DMSO)
9b	>300	−65.6° (0.950, DMSO)	**14b**	228—239 (dec.)	−109.6° (0.500, DMSO)
10a	275—278	+102.5° (0.640, AcOH)	**18**	107—108	+70.7° (0.953, $CHCl_3$)
10b	277—279	−102.1° (0.425, AcOH)	**19**	194—196	−277.2° (2.04, $CHCl_3$)

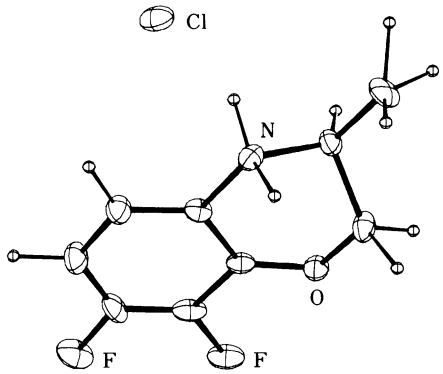

Fig. 1. The Molecular Structure of the Hydrochloride of **15b**

[mp 90—105 °C; $[\alpha]_D^{23}$ −33.2° (c=0.68, EtOH)], which belong to the orthorhombic system and have unit cell dimensions of a=10.8282(3), b=14.2060(6) and c=6.6090(4) Å. The space group was $P2_12_12_1$ and Z=4. The lattice parameters and intensities were measured on a Philips four-circle diffractometer with monochromated CuK_α radiation. A total of 764 independent reflections were obtained by the θ–2θ scan method. The crystal was sealed in a glass capillary, since it easily decomposed. The size of the crystal was approximately 0.3×0.2×0.2 mm.

The structure was solved by the direct method with the program MULTAN. Refinement

TABLE II. *In Vitro* Antibacterial Activities

Organism	MIC (μg/ml)					
	13b	**13a**	**1**	**14b**	**14a**	**2**
Staphylococcus aureus 209P	0.20	25.0	0.39	0.10	12.5	0.20
S. aureus SMITH	0.10	12.5	0.20	0.10	6.25	0.20
S. epidermidis 56500	0.78	>100	1.56	0.39	50.0	0.78
S. epidermidis 56556	0.39	25.0	0.39	0.39	50.0	0.39
Streptococcus pyogenes G-36	1.56	>100	3.13	1.56	100	1.56
S. faecalis ATCC 19433	1.56	>100	3.13	1.56	>100	1.56
Escherichia coli NIHJ	≦0.05	0.39	≦0.05	≦0.05	1.56	≦0.05
E. coli 05136	≦0.05	0.78	≦0.05	≦0.05	3.13	≦0.05
Salmonella enteritidis IID 604	≦0.05	1.56	0.10	≦0.05	3.13	≦0.05
Proteus vulgaris 08601	≦0.05	0.39	≦0.05	≦0.05	3.13	≦0.05
P. morganii IID 602	≦0.05	1.56	0.10	≦0.05	6.25	0.10
Klebsiella pneumoniae type 2	≦0.05	1.56	0.10	≦0.05	6.25	0.10
Enterobacter cloacae 03400	≦0.05	0.78	≦0.05	≦0.05	3.13	≦0.05
Serratia marcescens 10100	≦0.05	1.56	0.10	≦0.05	6.25	0.10
Pseudomonas aeruginosa 32104	0.39	12.5	0.78	0.78	100	0.78
P. aeruginosa 32121	0.10	6.25	0.20	0.10	12.5	0.10
P. maltophilia IID 1275	0.39	12.5	0.78	0.39	50.0	0.78

was carried out by the block-diagonal least-squares method using anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms. The final R value was 0.096. The absolute configuration was determined by the use of the anomalous scattering effect of the chlorine atom for CuK_α radiation.

The structure of the resulting hydrochloride of **15b** is shown in Fig. 1, and confirms that the absolute configuration of the chiral center is *S*. Consequently, the C_3 position in the oxazine ring in a series of the (−)-compounds (**b**) derived from **15b** was of the *S* configuration.

Biological Activities

Antibacterial activities of the optically active ofloxacins (**13a** and **13b**) and the corresponding fluoromethyl derivatives (**14a** and **14b**), together with their racemates (**1** and **2**),[11] were tested *in vitro*. The results are summarized in Table II in terms of the minimum inhibitory concentration (MIC, μg/ml) of the compounds, as determined by the serial dilution method.[12] *S*-(−)-Ofloxacin (**13b**) was 8 to 128 times more active than *R*-(+)-ofloxacin (**13a**), and about twice as active as the racemate (**1**) against both the gram-positive and the gram-negative bacteria tested. In the case of fluoromethyl derivatives, the *S*-(−)-isomer, **14b**, also showed an activity 32 to 128 times higher than the *R*-(+)-isomer, **14a**. These results indicate that the configuration of the methyl or fluoromethyl group at the C_3 position of **1** or **2** has a significant effect on the antibacterial activity.

Quinolone antibacterial agents are generally called desoxyribonucleic acid (DNA) gyrase inhibitors because of their mode of action.[13] In relation to this, a study on the activities of **13a** and **13b** toward bacterial DNA gyrase has recently been reported.[14]

Experimental

Melting points were taken in a Yanagimoto micromelting point apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a JEOL FX-90Q (90 MHz) or a Varian XL-200 (200 MHz) spectrometer. The optical rotations were recorded with a Horiba SEPA-200 polarimeter. The description in this experimental section refers to both of the optically active compounds.

(±)-Ethyl 9,10-Difluoro-2,3-dihydro-3-(3,5-dinitrobenzoyloxy)methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benz-

oxazine-6-carboxylate (4)——A mixture of **3** (1.0 g), 3,5-dinitrobenzoyl chloride (1.6 g) and pyridine (0.5 g) in dry tetrahydrofuran (THF) (100 ml) was heated under reflux for 1.5 h. Precipitate was formed around the end of the reaction. Removal of the solvent gave a solid, which was washed with MeOH and Et_2O to give **4** (1.2 g, 75%) as a colorless solid, mp 240—242 °C. ^{1}H-NMR ($CDCl_3$–5%DMSO-d_6) δ: 1.30 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 4.26 (2H, q, J=7 Hz, $\underline{CH_2}CH_3$), 4.4—5.4 (5H, m, 2×CH_2 and CH), 7.76 (1H, dd, J=11, 7 Hz, C_8-H), 8.8 (1H, s, C_5-H), 9.0 (2H, d, J=3 Hz, 2×ArH), 9.2 (1H, t, J=3 Hz, ArH). *Anal.* Calcd for $C_{22}H_{15}F_2N_3O_{10}$: C, 50.88; H, 2.91; N, 8.09. Found: C, 50.75; H, 2.95; N, 8.30.

Optical Resolution of the (±)-Benzoate (4)——The (±)-benzoate (**4**) (6 mg) was dissolved in DMF (0.6 ml), and the solution was subjected to HPLC to give the optically pure isomers (**5a**) (2.5 mg), and (**5b**) (2.5 mg). Each isomer (250 mg) was obtained by repeating this operation. HPLC: SUMIPAX OA-4200 column (20×250 mm) (Sumitomo Chemical Co., Ltd.). Solvent: hexane–1,2-dichloroethane–EtOH=6:3:1. Flow rate: 8 ml/min. Retention times: **5a**, 56—76 min; **5b**, 78—98 min.

(−)-Ethyl 9,10-Difluoro-2,3-dihydro-3-hydroxymethyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (6b)——A suspension of **5b** (120 mg), EtOH (10 ml) and satd. $NaHCO_3$ solution (4 ml) was stirred with heating at 50—60 °C for 2 h. The mixture was concentrated to give the residue, which was washed with water, 95% EtOH and Et_2O to afford **6b** (68 mg, 91%) as colorless crystals. *Anal.* Calcd for $C_{15}H_{13}F_2NO_5$: C, 55.39; H, 4.03; N, 4.31. Found: C, 55.44; H, 4.01; N, 4.49. The NMR spectrum and thin-layer chromatography (TLC) behavior were identical with those of the racemate (**3**).[5] In the same manner as described above, the (+)-compound (**6a**) was synthesized from **5a**. *Anal.* Calcd for $C_{15}H_{13}F_2NO_5$: C, 55.39; H, 4.03; N, 4.31. Found: C, 55.48; H, 4.11; N, 4.20.

(−)-Ethyl 9,10-Difluoro-2,3-dihydro-3-iodomethyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (7b)——A suspension of **6b** (63 mg) and triphenylphosphite methiodide (341 mg) in DMF (12 ml) was stirred at room temperature for 1.5 h. After evaporation of the solvent, the residue was dissolved in $CHCl_3$, washed with aqueous $Na_2S_2O_3$ solution and brine, then dried over $MgSO_4$. Evaporation of the solvent gave a semi-solid, which was triturated with Et_2O to afford **7b** (78 mg, 93%) as a colorless powder, mp 214—217 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 3.38—3.70 (2H, m, CH_2), 4.36—4.58 (2H, m, 2×CH), 4.42 (2H, q, J=7 Hz, $\underline{CH_2}CH_3$), 5.04 (1H, dd, J=13, 2 Hz, CH), 7.86 (1H, dd, J=11, 7 Hz, C_8-H), 8.44 (1H, s, C_5-H). *Anal.* Calcd for $C_{15}H_{12}F_2INO_4$: C, 41.40; H, 2.78; N, 3.22. Found: C, 41.16; H, 2.58; N, 2.99. The (+)-compound (**7a**) was obtained in the same manner as described above.

(−)-Ethyl 9,10-Difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (8b)——A mixture of **7b** (78 mg) and tri-*n*-butyltin hydride (0.2 ml) in EtOH (30 ml) was stirred at 50—60 °C for 1 h. Evaporation of the solvent gave a pale yellow powder, which was purified by preparative TLC on silica gel (developing solvent: $CHCl_3$–MeOH=20:1) and recrystallized from EtOH to afford **8b** (40 mg, 73%) as colorless needles. The NMR spectrum and TLC behavior were identical with those of the racemate.[2] *Anal.* Calcd for $C_{15}H_{13}F_2NO_4$: C, 58.26; H, 4.24; N, 4.53. Found: C, 58.49; H, 4.40; N, 4.49. The (+)-compound (**8a**) was obtained in the same manner as described above. *Anal.* Calcd for $C_{15}H_{13}F_2NO_4$: C, 58.26; H, 4.24; N, 4.53. Found: C, 58.25; H, 4.23; N, 4.58.

(−)-9,10-Difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (9b)——A solution of **8b** (40 mg) in concd. HCl–AcOH (2:1) (6 ml) was refluxed for 40 min. Concentration of the solution to 2 ml gave crystals, which were filtered, washed with water, EtOH and Et_2O, then dried *in vacuo* to afford **9b** (22 mg, 61%) as colorless needles. *Anal.* Calcd for $C_{13}H_9F_2NO_4$: C, 55.52; H, 3.23; N, 4.98. Found: C, 55.79; H, 3.20; N, 4.91. The NMR spectrum and TLC behavior were identical with those of the racemate.[2] The (+)-compound (**9a**) was obtained in the same manner as described above. *Anal.* Calcd for $C_{13}H_9F_2NO_4$: C, 55.52; H, 3.23; N, 4.98. Found: C; 55.32; H, 3.25; N, 4.94.

(−)-9,10-Difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid BF_2-chelate (12b)——Boron trifluoride etherate (0.3 ml) was added to a suspension of **9b** (21 mg) in Et_2O (5 ml) and the resulting mixture was stirred at room temperature for 1 h. The precipitate was filtered, washed with Et_2O and dried *in vacuo* to afford **12b** (24 mg, 98%) as pale yellow prisms. ^{1}H-NMR (DMSO-d_6) δ: 1.57 (3H, d, J=7 Hz, CH_3), 4.61 (1H, dd, J=12, 2 Hz, 1H of OCH_2), 4.87 (1H, d, J=12 Hz, 1H of OCH_2), 5.37 (1H, m, C_3-H), 8.21 (1H, dd, J=12, 8 Hz, C_8-H), 9.72 (1H, s, C_5-H). *Anal.* Calcd for $C_{13}H_8BF_4NO_4$: C, 47.45; H, 2.45; N, 4.26. Found: C, 47.43; H, 2.48; N, 4.33. The (+)-compound (**12a**) was obtained in the same manner as described above. *Anal.* Calcd for $C_{13}H_8BF_4NO_4$: C, 47.45; H, 2.45; N, 4.26. Found: C, 47.11; H, 2.55; N, 4.25.

(−)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (13b)——A solution of **12b** (24 mg) and 1-methylpiperazine (50 mg) in DMSO (3 ml) was stirred at room temperature for 17 h. After evaporation of the solvent, the residual oil was triturated with Et_2O to give a solid, which was dissolved in 95% MeOH (10 ml) and triethylamine (0.2 ml), and the solution was refluxed for 6 h. Evaporation of the solvent gave a residue, which was purified by preparative TLC on silica gel (developing solvent: lower layer solution of $CHCl_3$–MeOH–H_2O=15:3:1) to afford **13b** (14 mg, 52%) as a pale yellow powder. Crystallization from EtOH–Et_2O gave needles. ^{1}H-NMR ($CDCl_3$) δ: 1.63 (3H, d, J=7 Hz, C_3-CH_3), 2.38 (3H, s, N-CH_3), 2.54—2.60 (4H, m, 2×CH_2N), 3.40—3.44 (4H, m, 2×CH_2N), 4.35—4.52 (3H, m, CH and CH_2), 7.76 (1H, d, aromatic ring C_8-H), 8.64 (1H, s, C_5-H). *Anal.* Calcd for $C_{18}H_{20}FN_3O_4 \cdot 1/2H_2O$: C, 58.37 H, 5.72; N, 11.35. Found:

C, 58.17; H, 5.58; N, 11.27. (+)-Ofloxacin (**13a**) was obtained in the same manner as described above. *Anal.* Calcd for $C_{18}H_{20}FN_3O_4 \cdot 1/2H_2O$: C, 58.37; H, 5.72; N, 11.35. Found: C, 58.50; H, 5.64; N, 11.44.

(−)-Ethyl 9,10-Difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (10b)——An excess of diethylaminosulfur trifluoride was added to a suspension of **6b** (70 mg) in dry CH_2Cl_2 (60 ml) and the solution was stirred at room temperature for 2 h. After addition of EtOH (2 ml), the reaction mixture was stirred at room temperature for 30 min, and washed with brine, then dried over Na_2SO_4. Evaporation of the solvent gave a pale yellow oil, which was chromatographed on silica gel using $CHCl_3$–MeOH (50 : 1 v/v) to yield **10b** (59 mg) as a powder. The (+)-compound (**10a**) was obtained in the same manner as described above.

(−)-9,10-Difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (11b)——A solution of **10b** (57 mg) in concd. HCl–AcOH (2 : 1) (9 ml) was refluxed for 1 h. Concentration of the solvent to 1 ml gave a residue, which was filtered, washed with water, EtOH and Et_2O, and dried *in vacuo* to afford **11b** (43 mg, 83%) as a colorless powder. The (+)-compound (**11a**) was obtained in the same manner as described above.

(−)-9-Fluoro-3-fluoromethyl-2,3-dihydro-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (14b)——A solution of **11b** (41 mg) and 1-methylpiperazine (50 mg) in DMSO (5 ml) was stirred at 110—120°C for 40 min. The solvent was evaporated *in vacuo*, and the residue was triturated with Et_2O to give a powder, which was purified by preparative TLC on silica gel (developing solvent: lower layer solution of $CHCl_3$–MeOH–H_2O = 7 : 3 : 1) to yield a yellow oil. The oil was dissolved in water, and the solution was lyophilized to yield **14b** (40 mg, 77%) as an amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (3H, s, N-CH_3), 2.45—2.7 (4H, m, 2×CH_2N), 3.3—3.5 (4H, m, 2×CH_2N), 4.40, 4.75 (each 1H, br d, J=12 Hz, 2×C_2-H), 4.46, 4.85 (each 1H, dm, J=36 Hz, CH_2F), 4.9—5.1 (1H, m, C_3-H), 7.70 (1H, d, J=12 Hz, C_8-H), 8.63 (1H, s, C_5-H). *Anal.* Calcd for $C_{18}H_{19}F_2N_3O_4$: C, 56.99; H, 5.05; N, 11.08. Found: C, 56.67; H, 5.19; N, 10.82. The (+)-compound (**14a**) was obtained in the same manner as described above. *Anal.* Calcd for $C_{18}H_{19}F_2N_3O_4$: C, 56.99; H, 5.05; N, 11.08. Found: C, 56.76; H, 5.23; N, 10.80.

(+)- and (−)-7,8-Difluoro-2,3-dihydro-3-methyl-4-[(*S*)-*N*-*p*-toluenesulfonyl]prolinyl-4*H*-[1,4]benzoxazine (18) and (19)——(*S*)-*N*-*p*-Toluenesulfonylprolinyl chloride, prepared from (*S*)-*N*-*p*-toluenesulfonylproline[10] (61.9 g) and thionyl chloride (50 ml) in benzene (350 ml), was added to a solution of **17** (32.8 g) and pyridine (28 ml) in CH_2Cl_2 (300 ml) at room temperature, and the solution was stirred for an additional 2 h. The reaction mixture was washed with 10% HCl, satd. $NaHCO_3$ and brine, then dried over $MgSO_4$. Evaporation of the solvent gave an oil, which was crystallized from AcOEt–hexane to afford the (−)-amide (**19**). Concentration of the mother liquor gave an oil, which was chromatographed on silica gel using benzene–AcOEt (25 : 1, v/v) as an eluent to afford an oil. Crystallization from EtOH–Et_2O yielded the (+)-amide (**18**) (33.4 g, 86%) as colorless crystals.

18: ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, d, J=7.2 Hz, CH_3), 2.42 (3H, s, ArMe), 6.78 (1H, dt, J=7.2, 11 Hz, C_5-H), 7.32 (2H, d, J=9 Hz, 2×ArH), 7.42—7.66 (1H, m, C_6-H), 7.78 (2H, d, J=9 Hz, 2×ArH). *Anal.* Calcd for $C_{21}H_{22}F_2N_2O_4S$: C, 57.79; H, 5.08; N, 6.42. Found: C, 58.05; H, 5.14; N, 6.47.

19: ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, d, J=7 Hz, CH_3), 2.40 (3H, s, ArMe), 6.5—6.9 (2H, m, C_5-H, C_6-H), 7.0—7.4 (4H, m, 4×ArH). *Anal.* Calcd for $C_{21}H_{22}F_2N_2O_4S$: C, 57.79; H, 5.08; N, 6.42. Found: C, 57.90; H, 5.03; N, 6.40.

(−)-7,8-Difluoro-2,3-dihydro-3-methyl-4*H*-[1,4]benzoxazine (15b)[9]——A solution of **18** (32.8 g) in EtOH (1000 ml) and 1 N NaOH (500 ml) was refluxed for 2 h. The solvent was evaporated and the residue was dissolved in benzene; this solution was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave an oil, which was chromatographed on silica gel using benzene as an eluent to afford the (−)-benzoxazine (**15b**) (12.2 g, 88%) as a colorless oil. $[\alpha]_D^{23}$ −7.8° (c=6.80, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, d, J=6 Hz, CH_3), 3.53 (1H, m, C_3-H), 3.81 (1H, dd, J=8, 10 Hz, C_2-H), 4.31 (1H, dd, J=3, 10 Hz, C_2-H), 6.30 (1H, m, ArH), 6.60 (1H, m, ArH).

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