Efficient Synthesis of a Key Intermediate of DV-7751 *via* Optical Resolution or Microbial Reduction

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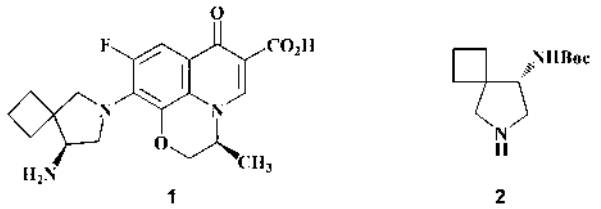
Two efficient and practical methods of synthesis of the C-10 substituent of DV-7751 (1), a novel quinolone carboxylic acid, were established. The first method utilizes an optical resolution of racemic 8-amino-6-benzyl-6-azaspiro[3.4]octane (13), while the second employs an enantioselective microbial reduction of 6-benzyl-5,8-dioxo-6-azaspiro[3.4]octane (8b). The enantiomeric excess of (*S*)-8-amino-6-benzyl-6-azaspiro[3.4]octane (11) with each method of synthesis is greater than 96%.

Key words DV-7751; antibacterial quinolone carboxylic acid; optical resolution; enantioselective microbial reduction

A large number of 4-piridone-3-carboxylic acid derivatives, so-called 4-quinolones, have been synthesized since the discovery of nalidixic acid.[1] Of them, DV-7751 (**1**), 10-[8(*S*)-amino-6-azaspiro[3.4]octane-6-yl]-9-fluoro-2,3-dihydro-3(*S*)-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1.4]benzoxazine-6-carboxylic acid, exhibits marked antibacterial activity against both Gram-negative and Gram-positive bacteria.[2] These characteristics of **1** correlate well with its (*S*)-amino-6-azaspiro[3.4]octane moiety.

To perform a clinical trial of **1**, we needed to prepare large quantities of (*S*)-(*tert*-butoxycarbonylamino)-6-azaspiro[3.4]-octane (**2**), which is easily introduced as the C-10 substituent of **1**. The reported method[2] utilizing the separation of a 1 : 1 diastereomeric mixture of (*R*)- and (*S*)-8-amino-6-[(*R*)-1-phenylethyl]-5-oxo-5-azaspiro[3.4]octanes with silica gel column chromatography seemed unsuitable for large-scale manufacturing.



1 2

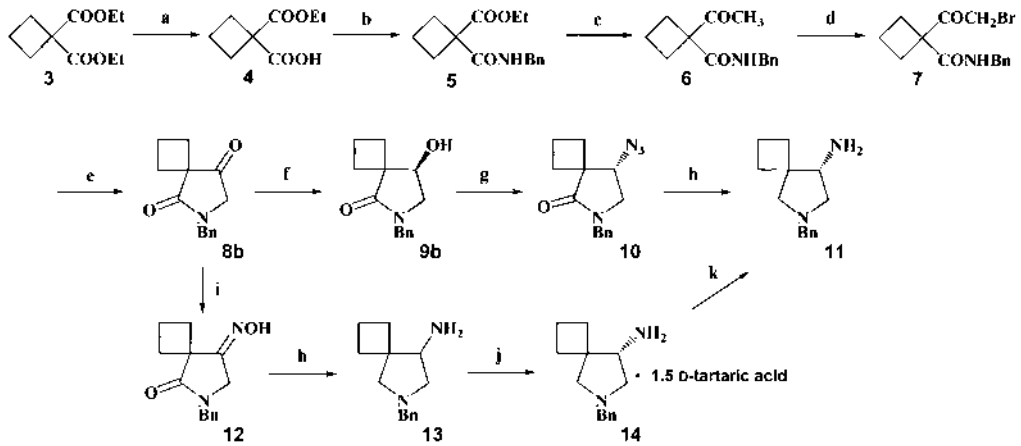
Chart 1

In this paper, we describe, as shown in Chart 2, the practical synthesis of the key chiral compound **2** using optical resolution or microbial reduction.

Results and Discussion

Preparation of 6-Benzyl-5,8-dioxo-6-azaspiro[3.4]octane (8b) We selected a commercially available diethyl 1,1-cyclobutane dicarboxylate (**3**) as a starting material. After treatment of compound **3** with an equimolar amount of 10% aqueous KOH, the resulting half-ester **4** was condensed with benzylamine using ethylchloroformate to afford cyclobutyl carboxamide **5**. Compound **5** was treated with trimethylsilyl methyllithium to obtain the β-keto carboxamide derivative **6**. After bromination of **6**, the resulting bromide **7** was cyclized with sodium hydride to afford **8b**.

Optical Resolution of 8-Amino-6-benzyl-6-azaspiro-[3.4]octane (13) Compound **8b** was derived to oxime **12** by treatment with hydroxylamine, followed by hydrogenation, which yielded amine **13** as a racemate. The optical resolution of **13** was easily performed with D-tartaric acid in ethanol,



(a) 10% aq.KOH, MeOH; (b) $ClCO_2Et$, Et_3N, $CHCl_3$ then benzylamine; (c) TMS-CH_2Li; (d) Br_2, 1,4-dioxane;
(e) NaH, DMF; (f) fungi (JCM 1880), phosphate buffer(pH 6.0); (g) Ph_3P, $EtO_2CN_2CO_2Et$, DPPA, THF;
(h) $LiAlH_4$, THF; (i) NH_2OH-HCl, Et_3N, EtOH; (j) D-tartaric acid; (k) 10% aq. NaOH

Chart 2

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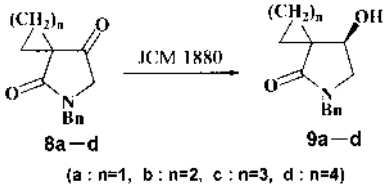

Chart 3

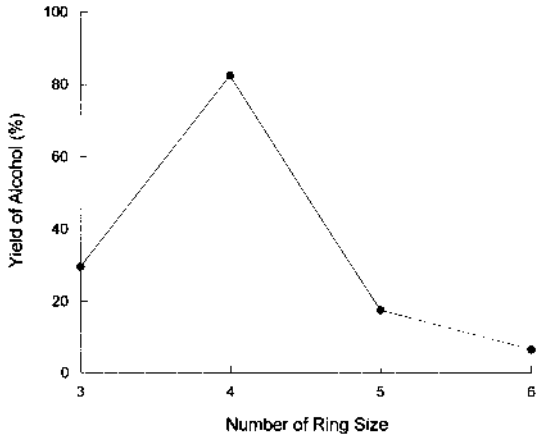

Fig. 1. Relationship between Yield and Ring Size of Substrate
The reaction conditions are described in the Experimental section.

yielding the desired diastereomeric salt, (*S*)-**13**·1.5 D-tartaric acid was precipitated as a less soluble salt in 34% yield (>98% de). The resulting salt, **14**, was treated with 10% aqueous NaOH to obtain the requisite (*S*)-amine **11** (>98% ee). The optical purity of **11** was determined by HPLC analysis using Sumichiral OA-4400 after derivation to the 3,5-dinitrobenzamide. Although we found an alternative approach to **11** *via* the optical resolution of **13**, we turned our attention to employing the microbial reduction of **8b** to establish a more efficient process.

Synthesis of (*S*)-8-Amino-6-benzyl-6-azaspiro[3.4]octane (11) *via* Stereoselective Microbial Reduction In our previous paper, we reported that *Phaeocrepsis* sp. could perform the stereoselective transformation of 5-benzyl-4,7-dioxo-5-azaspiro[2.4]heptane (**8a**).[3] Four substrates with a spiro ring in their structure were examined for the transformation of **8a**—**d** to **9a**—**d** by *Phaeocrepsis* sp. As shown in Fig. 1, compound **8b** was the most reactive among the four substrates examined. The amine **11** was obtained from **9b** in 63% yield and 96% enantiomeric excess *via* a Mitsunobu reaction[4] using diphenylphosphoryl azide (DPPA), followed by reduction with lithium aluminum hydride. *Phaeocreopsis* sp. JCM 1880 was found to significantly induce stereoselective transformation.

Conclusion

We have demonstrated the efficient syntheses of compound **11**, which can easily be converted to compound **2** in two steps (debenzylation followed by *tert*-butoxycarbonylation), employing optical resolution or asymmetric microbial reduction. We established practical methods of synthesis of the key intermediate for an important quinolone antibacterial agent. The present methods are more suitable than the previously reported method[1] for large-scale production of DV-7751 (**1**).

Experimental

All melting points were measured using a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a JEOL JNM-EX 270 spectrometer. All signals are expressed in ppm (δ) with tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with JEOL HX110 and JEOL AX505W mass spectrometers. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Column chromatography was performed on silica gel (Kiesel gel 60, 70—230 mesh, Merck). Unless otherwise noted, all reactions were carried out in anhydrous solvents. The starting material, 1,1-cyclobutane dicarboxylic acid diethy ester, was purchased from Tokyo Kasei Co., Ltd. (Japan).

1,1-Cyclobutanedicarboxylic Acid Monoethyl Ester (4) To a solution of 1,1-cyclobutane dicarboxylic acid diethy ester (**3**, 85 ml, 0.45 mol) in methanol (110 ml) was added 10% aqueous potassium hydroxide (290 ml) at 0 °C over a period of 1 h. The mixture was stirred at room temperature for 14 h. After evaporation of methanol, the residue was washed with CH_2Cl_2 and the aqueous layer was acidified (pH 2) with 10% hydrochloric acid, then extracted with ethyl acetate. The extract was washed with brine, dried over sodium sulfate and concentrated to obtain **4** (77.1 g, 99.5%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.2 Hz, $C\underline{H}_3CH_2O$), 1.9—2.2 (2H, m, cyclobutane), 2.5—2.8 (4H, m, cyclobutane), 4.25 (2H, q, J=7.2 Hz, $CH_3C\underline{H}_2O$). MS *m/z*: 172 (M^+).

1-Benzylaminocarbonyl-1-ethoxycarbonyl Cyclobutane (5) Ethyl chloroformate (50 ml, 0.50 mol) was added dropwise at 0 °C to a mixture of **4** (77.1 g, 0.45 mol) and triethylamine (78.1 ml, 0.56 mol) in chloroform (420 ml). The mixture was stirred at room temperature for 1 h. After the addition of a solution of benzylamine (49.2 ml, 0.45 mol) in chloroform (140 ml) at 0 °C, the reaction mixture was stirred for 35 min at room temperature. The mixture was washed with 10% aqueous citric acid and brine. The organic layer was dried over sodium sulfate and concentrated to give **5** (113.3 g, 96.3%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7.2 Hz, $C\underline{H}_3CH_2O$), 1.7—2.2 (2H, m, cyclobutane), 2.4—2.7 (4H, m, cyclobutane), 4.20 (2H, q, J=7.2 Hz, $CH_3C\underline{H}_2O$), 4.44, 4.50 (2H, each s, ArH_2CN), 7.30 (5H, br s, aromatic H). MS *m/z*: 261 (M^+).

1-Acetyl-1-benzylaminocarbonyl Cyclobutane (6) A one molar solution of trimethylsilyl methyl lithium in pentane (76.5 ml, 76.5 mmol) was added dropwise to a solution of **5** (5.01 g, 19.2 mmol) in pentane–tetrahydrofuran (THF) (17 : 3, 100 ml) at 0 °C. The reaction mixture was stirred for an additional 45 min at 0 °C. Methanol was added to the solution, then the mixture was stirred at room temperature for 1 h. After water was poured into the mixture, it was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated to yield a white solid. The solid was washed with pentane to obtain **6** (3.50 g, 78.9%). mp 74—77 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.7—2.2 (2H, m, cyclobutane), 2.1 (3H, s, CH_3CO), 2.4—2.7 (4H, m, cyclobutane), 4.44, 4.50 (2H, each s, ArH_2CN), 7.30 (5H, br s, aromatic H). MS *m/z*: 231 (M^+). *Anal*. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.54; H, 7.51; N, 6.34.

1-Benzylaminocarbonyl-1-bromomethylcarbonyl Cyclobutane (7) Bromine (9.1 g, 57.1 mmol) was added to 1,4-dioxane (24 ml) and the mixture was stirred for 20 min at room temperature. A solution of **6** (12.0 g, 51.9 mmol) in CH_2Cl_2 (120 ml) was added to the mixture, which was stirred for 4 h at room temperature. The reaction mixture was then diluted with CH_2Cl_2, and washed with 5% aqueous sodium thiosulfate, brine and water. The organic layer was dried over sodium sulfate and concentrated to afford **7** (13.2 g, 82.0%) as a yellow oil which was subjected to the next batch without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.7—2.1 (2H, m, cyclobutane), 2.4—2.8 (4H, m, cyclobutane), 4.12 (2H, s, $BrCH_2CO$), 4.41, 4.50 (2H, each s, ArH_2CN), 7.32 (5H, br s, aromatic H). MS *m/z*: 310 (M^+).

6-Benzyl-5,8-dioxo-6-azaspiro[3.4]octane (8b) Sodium hydride in mineral oil (60%, 2.3 g, 57.5 mmol) was added portionwise to a solution of **7** (12.2 g, 39.3 mmol) in dry *N*,*N*-dimethylformamide (DMF) (500 ml) at 0 °C. After the mixture was stirred for 75 min at the same temperature, the reaction mixture was poured into ice-water, and extracted with ether. The ether extract was washed with 10% aqueous citric acid and water, then dried over sodium sulfate. After the organic layer was evaporated *in vacuo*, the residue was chromatographed on silica gel using toluene : AcOEt (4 : 1), affording **8b** (6.53 g, 72.4%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 2.0—2.7 (6H, m, cyclobutane), 3.64 (2H, s, NCH_2CO), 4.63 (2H, s, ArH_2CN), 7.15—7.49 (5H, m, aromatic H). MS *m/z*: 229 (M^+).

7-Benzyl-6,9-dioxo-7-azaspiro[4.4]nonane (8c) The synthesis of **8c** was performed under the same conditions as for **8b**. Compound **8c** (66% from the corresponding cyclopentyl β-keto carboxamide derivative) was obtained as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.6—2.2 (8H, m, cyclopen-

tane), 3.69 (2H, s, NCH_2CO), 4.87 (2H, s, ArH_2CN), 7.16—7.52 (5H, m, aromatic H). MS *m/z*: 244 ($M^+ + 1$).

8-Benzyl-7,10-dioxo-8-azaspiro[5.4]decane (8d) The synthesis of **8d** was performed under the same conditions as for **8b**. Compound **8d** (62% from the corresponding cyclohexyl β-keto carboxamide derivative) was obtained as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.5—2.0 (10H, m, cyclohexane), 3.63 (2H, s, NCH_2CO), 4.85 (2H, s, ArH_2CN), 7.19—7.46 (5H, m, aromatic H). MS *m/z*: 258 ($M^+ + 1$).

(*R*)-8-Hydroxy-6-benzyl-5-oxo-6-azaspiro[3.4]octane (9b) *Phaeocreopsis* sp. JCM 1880 was grown in a complex medium consisting of 2% (w/v) glucose and 1% (w/v) polypeptone. The medium was adjusted to pH 6.0 with 0.1% K_2HPO_4 buffer and 0.1% KH_2PO_4 buffer, placed in a Sakaguchi flask, sterilized (121 °C, 15 min), and inoculated with the preincubated culture. The cultivation was performed for 48 h at 30 °C with shaking. Then, **8b** (800 mg, 3.49 mmol) was added to eight flasks (100 mg×8) and the reaction mixtures were shaken for 14 h at 30 °C. After filtration through Celite, the filtrate was extracted with AcOEt. The organic layer was dried over sodium sulfate and evaporated *in vacuo*. The residue was purified by silica gel column chromatography with toluene : AcOEt = 2 : 1 to afford **9b** (658 mg, 82.3%) as a pale yellow solid, which was 96% ee by HPLC analysis using chiralcel OJ; mobile phase, hexane : isopropanol = 10 : 1; flow rate, 1.0 ml/min; detector, UV (230 nm). Retention time for racemate: 15.1 min [50%, (*R*)-form], 17.0 min [50%, (*S*)-form]. Retention time for **8b**: 15.0 min (98%), 16.9 min (2%), 96% ee. mp 107—108 °C. $[\alpha]_D^{25}$ +65.3° (c = 0.540, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.90—2.41 (6H, m, cyclobutane), 3.02—3.43 (2H, m, NCH_2CO), 4.22—4.27 (1H, m, 8-H), 4.46 (2H, s, ArH_2CN), 7.18—7.38 (5H, m, aromatic H). MS *m/z*: 231 ($M^+ + 1$); *Anal*. Calcd for $C_{14}H_{17}NO_2$: C, 72.73; H, 7.36; N, 6.06. Found: C, 72.87; H, 7.42; N, 6.02.

Comparison of the Rate of Microbial Reduction Compounds **8a**—**d** (5 mg) were added to the culture (5 ml) of JCM 1880. The resulting suspension was stirred for 8 h at 30 °C. Conversion was observed by HPLC analysis [Inertsil ODS-2 column (GL Science), 4.6×150 mm; eluent, 35% acetonitrile containing 50 mM phosphate buffer (pH 6.0); flow rate, 1.0 ml/min; UV detection, 230 nm].

6-Benzyl-8-hydroxyimino-5-oxo-6-azaspiro[3.4]octane (12) A mixture of **8b** (11.1 g, 48.4 mmol), hydroxylamine hydrochloride (10.1 g, 145.2 mmol) and triethylamine (20.2 ml, 145.2 mmol) in ethanol (440 ml) was stirred for 2 h at room temperature. After the mixture was evaporated *in vacuo*, the residue was dissolved in AcOEt. The organic layer was washed with 10% aqueous citric acid and brine and dried over sodium sulfate. The solvent was removed *in vacuo*, and the residue was chromatographed on silica. Elution with toluene: AcOEt (1 : 1) yielded **12** (10.3 g, 87.3%) as a white solid, mp 173—178 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.7—2.8 (6H, m, cyclobutane), 3.94 (2H, s, NCH_2CN), 4.53 (2H, s, ArH_2CN), 7.32 (5H, br s, aromatic H). MS *m/z*: 244 (M^+); *Anal*. Calcd for $C_{14}H_{16}N_2O_2 \cdot 1/4H_2O$: C, 67.58; H, 6.68; N, 11.25. Found: C, 67.38; H, 6.77; N, 11.54.

8-Amino-6-benzyl-6-azaspiro[3.4]octane (13) A one molar solution of lithium aluminum hydride in THF (2 ml, 2 mmol) was added to the solution of **12** (3.0 g, 12.3 mmol) in THF (30 ml) with ice-water cooling. The whole mixture was refluxed for 1 h, then water and 10% aqueous NaOH were carefully added under ice-water cooling. The precipitate was filtered off and the filtrate was concentrated *in vacuo* to afford **13** (2.6 g, 98.2%) as a pale yellow oil.

(*S*)-8-Amino-6-benzyl-6-azaspiro[3.4]octane·1.5 D-Tartaric Acid (14) The solution of D-tartaric acid (108 mg, 0.72 mmol) in ethanol (1 ml) was added dropwise to a solution of **13** (280 mg, 1.30 mmol) in ethanol (4 ml) at 0 °C. After stirring of the mixture at the same temperature for 30 min, the mixture was refluxed for 30 min and stirred for 30 min at room temperature. The precipitate was collected and washed with ethanol. The resulting white crystals were filtered to obtain **14** (196 mg, 34.0%), mp 215—221 °C (dec.). $[\alpha]_D^{25}$ −56.3° (c = 1.00, water). ^{1}H-NMR (D_2O) δ: 1.87—2.32 (6H, m, cyclobutane), 3.56 (1H, dd, J = 4.8, 13 Hz, 8-H), 3.69—3.78 (2H, m, CCH_2N), 4.0—4.11 (2H, m, NCH_2CN), 4.51 (3H, s, $CHCO_2H$), 4.46, 4.55 (each 1H, d, J = 13 Hz, ArH_2CN), 7.56 (5H, br s, aromatic H). *Anal.* Calcd for $C_{20}H_{29}N_2O_9$: C, 54.41; H, 6.62; N, 6.34. Found: C, 54.23; H, 6.59; N, 6.27.

(*S*)-8-Amino-6-benzyl-6-azaspiro[3.4]octane (11) i) An ice-cooled solution of **9b** (230 mg, 1.0 mmol), triphenylphosphine (341 mg, 1.3 mmol) and diethylazodicarboxylate (226 mg, 1.3 mmol) in THF (5 ml) was stirred for 30 min, then a solution of DPPA (358 mg, 1.3 mmol) was added over a period of 15 min. Stirring was then continued at room temperature for 24 h. After evaporation of the solvent, Et_2O was added to the residue. After removing the precipitate by filtration, the filtrate was concentrated *in vacuo* to obtain crude **10**. The residue was dissolved in THF (5 ml). The mixture was added to an ice-cooled solution of 1 M lithium aluminum hydride in THF (2 ml, 2 mmol), and the entire mixture was refluxed for 1 h. Water and 10% aqueous NaOH were then carefully added under ice cooling. The grainy precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography with $CHCl_3$: MeOH = 10 : 1 to afford **11** (135 mg, 62.5%) as a pale yellow oil. In order to determine the optical purity of **11**, 3,5-dinitrobenzamide was prepared as follows: Triethylamine (9 μl) was added to a solution of **11** (2.0 mg) and 3,5-dinitrobenzoyl chloride (9.2 mg) in THF (3 ml) at room temperature. The reaction mixture was stirred for 1 h, then saturated aqueous $NaHCO_3$ was added, and the resulting mixture was stirred vigorously for 30 min. The mixture was diluted with $CHCl_3$ (3 ml), then dried over sodium sulfate, and filtered through a pad of silica gel to obtain a chloroform solution of the 3,5-dinitrobenzamide usable for chiral HPLC analysis. The conditions for HPLC analysis were as follows : column, Sumichiral OA-4400; mobile phase, hexane : 1,2-dichloroethane : ethanol : trifluoroacetic acid = 80 : 20 : 5 : 0.2; flow rate, 1.0 ml/min; detection, UV (254 nm). Retention time for racemate: 19.3 min [50%, (*S*)-form], 22.1 min [50%, (*R*)-form]. Retention time for **11**: 19.1 min (98%), 21.8 min (2%), 96% ee. $[\alpha]_D^{25}$ −56.8° (c = 1.00, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.70—2.40 (9H, m, cyclobutane, $-NH_2$, 8-H), 2.70 (2H, s, CCH_2N), 2.84—3.20 (2H, m, NCH_2CN), 3.62 (2H, s, ArH_2CN), 7.33 (5H, br s, aromatic H). MS *m/z*: 214 ($M^+ + 1$).

ii) 10% aqueous sodium hydroxide (20 ml) was added to **14** (1.05 g, 2.40 mmol). The solution was extracted with $CHCl_3$: MeOH (9 : 1). The organic layer was washed with brine, dried and evaporated *in vacuo* to obtain **11** (491 mg, 95.9%) as a pale yellow oil. In order to determine the optical purity of **11**, the 3,5-dinitrobenzamide was prepared in the same fashion. Retention time for racemate: 19.3 min [(*S*)-form], 22.1 min [(*R*)-form]. Retention time for 11: 19.1 min (99%), 21.8 min (1%), 98% ee.

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