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

Akihiko Miyadera,* Koji Satoh, and Akihiro Imura

Chemical Technology Research Laboratories, Daiichi Pharmaceutical Co., Ltd., Edogawa-ku, Tokyo 134-8630, Japan.

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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(5)-amino-6-azaspiro[3.4]octane-6-yl]-9-fluoro-2,3-dihydro-3(5)-methyl-7-oxo-7H-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 (5)-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 method2 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.

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 b-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,
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 Phaeocresis 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 Phaeocresis 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 using diphenylphosphoryl azide (DPPA), followed by reduction with lithium aluminum hydride. Phaeocresis 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-butoxyxycarbonylation), 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 11) for large-scale production of DV-7751 (1).
CO2H), 4.46, 4.55 (each 1H, d, J = 1.5 Hz, aromatic H), 3.63 (2H, s, NCH2CO), 4.85 (2H, s, ArH 2CN), 7.19—7.46 (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 carbamido derivative) was obtained as a pale yellow oil. 1H-NMR (CDCl3) δ: 1.5—2.0 (10H, m, cyclohexane), 3.63 (2H, s, NCH2CO), 4.79—4.86 (2H, s, aromatic H), 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) Phaenecopsis 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% KH2PO4 buffer and 0.1% KH2PO4 buffer, placed in a Sankaku 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 mg) 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 OD; mobile phase, hexane:iso-propanol=10:1; flow rate, 1.0 ml/min; detector, UV (230 nm). Retention time for racemate: 15.1 min [50%, (S)-form, 17.0 min [50%, (R)-form]. Retention time for 9b: 15.0 min (98%), 16.9 min (2%), 96% ee. mp 107—108 °C. [C14H17NO2: 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 ms 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—175 °C. 1H-NMR (CDCl3) δ: 1.7—2.8 (6H, m, cyclobutane), 3.94 (2H, s, NCH3CN), 4.53 (2H, s, ArH2CN), 7.32 (5H, br, aromatic H). MS m/z = 244 (M+); Anal. Calcd for C14H17N2O: C, 72.73; H, 7.36; N, 11.25. Found: C, 67.38; H, 6.77; N, 11.54.

Amino-6-benzyl-6-azaspiro[3.4]octane (13a) A one molar solution of lithium aluminium 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 AcOEt. The precipitate was washed with brine, dried and evaporated in vacuo to obtain 13 (491 mg, 95.9%) as a pale yellow oil. In order to determine the optical purity of 11, the 3,5-dinitrobenzamide was prepared as follows: Triethylamine (9 ml) was added to a solution of 12 (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 NaHCO3 was added, and the resulting mixture was stirred vigorously for 30 min. The mixture was diluted with CH2Cl2 (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, Shim-pack 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. [C14H17N2O: C, 67.38; H, 6.77; N, 11.54. The organic layer was washed with brine, dried and evaporated in vacuo to obtain 11 (941 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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References