An Efficient Synthesis of a Key Intermediate of DU-6859a *via* Asymmetric Microbial Reduction

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An efficient synthetic method for the C-7 substituent of DU-6859a (1), which is a new-generation antibacterial quinolone carboxylic acid, was established by utilizing an enantioselective microbial reduction of 5-benzyl-4,7-dioxo-5-azaspiro[2.4]heptane (7) to the corresponding chiral alcohol (8) as the key reaction. This synthetic method was based on use of AIPHOS (Artificial Intelligence for Planning and Handling Organic Synthesis), which is a synthesis design system that generates suitable retrosynthetic routes from the standpoints of both novelty and practicality.

Key words DU-6859a; enantioselective microbial reduction; AIPHOS; antibacterial quinolone carboxylic acid; synthesis design system

DU-6859a (**1**) is a new-generation antibacterial quinolone carboxylic acid with broad-spectrum antibacterial activity.[1] The characteristics of **1** are closely correlated with its (*S*)-7-amino-5-azaspiro[2.4]heptane moiety.[2] (*S*)-7-[(*tert*-Butoxycarbonyl)amino]-5-azaspiro[2.4]heptane (**2**), which is easily introduced as the C-7 substituent of **1**, has been prepared by means of the separation of a 1 : 1 diastereomeric mixture of (*R*)- and (*S*)-7-amino-5-[(*R*)-1-phenylethyl]-4-oxo-5-azaspiro[2.4]-heptanes with silica gel column chromatography (Fig. 1).[2] However, this synthetic method is inefficient, because one of the two diastereomers of **2** is discarded.

AIPHOS (Artificial Intelligence for Planning and Handling Organic Synthesis),[3] a synthesis design system, was employed as a tool to explore effective synthetic routes to **2**. AIPHOS proposes suitable retrosynthetic routes from standpoints of both novelty and practicality. Retrosynthetic paths generated by AIPHOS are rational and prospective ones because each of them is evaluated as to whether it can proceed or not with an unique reaction knowledge base[4] of AIPHOS, constructed by the SYNLIB (SYNthesis LIBrary)[5] reaction database, and is proposed to users only when AIPHOS guarantees that it can proceed.

In the course of evaluation of the proposed routes, a novel synthetic method of **2** including an asymmetric microbial reduction as the key reaction was found. We herein report the asymmetric synthesis of **2** using stereoselective microbial reduction as the key reaction.

Results and Discussion

Retrosynthetic Analysis of (*S*)-7-[(*tert*-Butoxycarbonyl)amino]-5-azaspiro[2.4]heptane (2) with AIPHOS (*S*)-7-Amino-5-azaspiro[2.4]heptane (I) was employed as the input target molecule, since AIPHOS, which is continuously under development, has no function to consider protecting groups. In evaluating the retrosynthetic paths proposed by AIPHOS, one was attractive because it involved the synthesis of a single enantiomer by means of asymmetric microbial reduction (Chart 1). Further, this retrosynthetic analysis generated by AIPHOS was a rational and prospective one because it was assured its

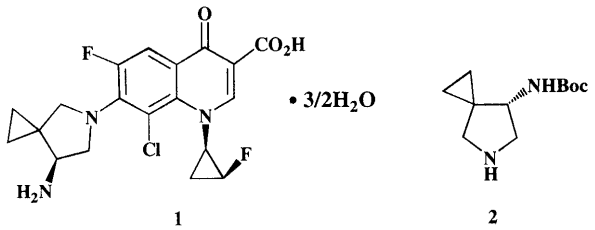

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Fig. 1

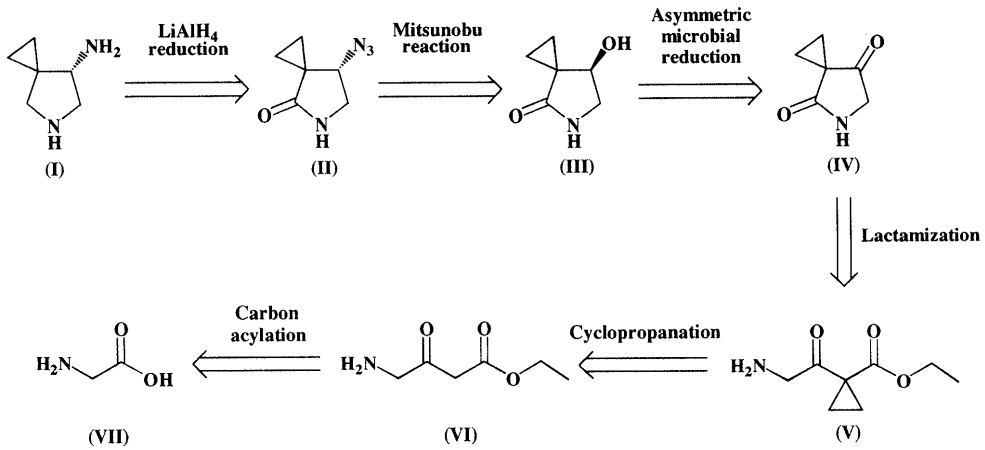

Chart 1

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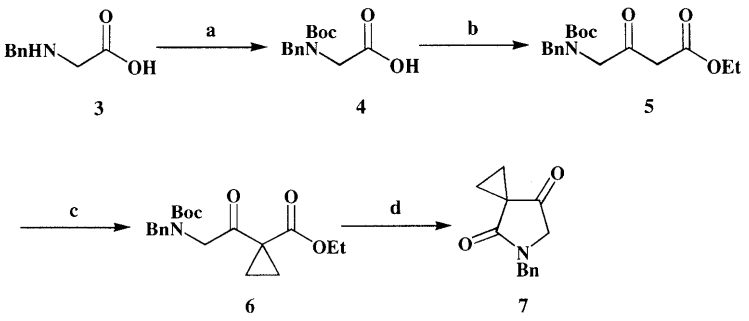


(a) Boc_2O; (b) $CO(Im)_2$; $KO_2CCH_2CO_2Et$, $MgCl_2$; (c) $(CH_2Br)_2$, K_2CO_3; (d) CF_3CO_2H; toluene, reflux

Chart 2

Table 1. Results of Transformation of **7** to **8** by Microorganisms

Microorganism	Yield (%)[c]	e.e.(%)[d]
Phaeocreopsis sp. JSM 1880[a]	8.0	>98 (*R*)
Absidia sp. DSC 185[a]	1.9	—
Lactobacillus sp. JSM 1667[b]	7.4	22 (*R*)
Bacillus sp. JSM 2509[b]	2.7	—

a) 5 ml of 2% glucose and 1% polypeptone solution (pH 7.0) for 30 h. *b*) 5 ml of 1% nutrient broth solution (pH 7.0) for 30 h. *c*) Determined by HPLC analysis with a column of Inertsil ODS-2 (GL Science) employing 0.05 M phosphate buffer (pH 2.4): CH_3CN (4:1) as the solvent system. *d*) The conditions are described in the experimental section.

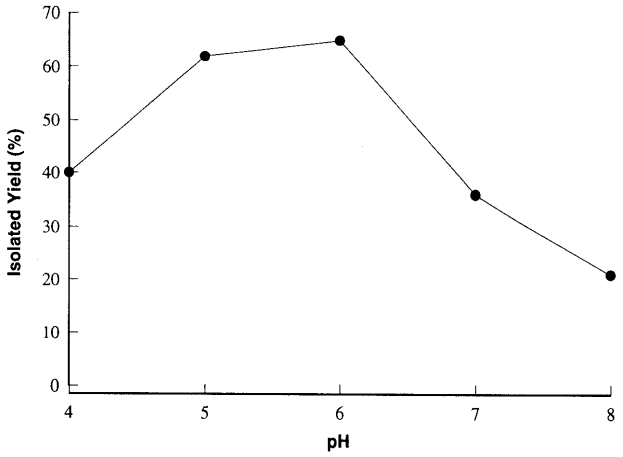


Fig. 2. Relationship between Yield of **8** and pH
The reaction conditions in the experimental section.

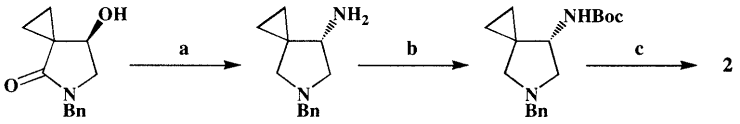
implementation in laboratories of known reactions.

Preparation of 5-Benzyl-4,7-dioxo-5-azaspiro[2.4]heptane (7) Our initial efforts were focused on synthesis of the 5-azaspiro[2.4]heptane skeleton from *N*-benzylglycine **3**. However, an attempt at direct introduction of a C_2 unit into **3**, by treatment with *N*,*N*′-carbonyldiimidazole and magnesium enolate failed. Then, we turned our attention to using *N*-benzyl-*N*-(*tert*-butoxycarbonyl)glycine **4**[6] as the starting material. Compound **4** obtained by a reported method was treated with *N*,*N*′-carbonyldiimidazole followed by carbon acylation *in situ* using the magnesium enolate of hydrogen ethyl malonate to afford the corresponding β-keto-γ-amino ester **5** in 84% yield. Cyclopropanation of **5** with 1,2-dibromoethane and potassium carbonate in acetone gave **6** in 72% yield. Treatment of **6** with trifluoroacetic acid in methylene chloride followed by heating under reflux in toluene afforded the desired compound **7** in 72% yield (Chart 2).

Synthesis of (*R*)-5-Benzyl-7-hydroxy-4-oxo-5-azaspiro-[2.4]heptane (8) *via* Stereoselective Microbial Reduction At this retrosynthetic step, AIPHOS proposed utilizing baker's yeast, which is widely used for efficient and stereoselective reduction of β-ketoesters. Therefore, the reduction of **7** to **8** by baker's yeast was examined, but the reduction did not proceed. In order to obtain **8** in high enantiomeric excess and high yield, about four hundred strains of microorganisms (molds, bacteria and yeasts) were screened. The reduction of the ketone moiety of **7** was proceeded with four strains of microorganisms. Investigation of their enantioselectivity showed that two of them could transform **7** to **8**. It turned out that *Phaeocreopsis* sp. JCM 1880 had a high ability of stereoselective transformation (>98% e.e.) (Table 1). The enantiomeric excess of the resulting **8** was determined by HPLC analysis utilizing a chiralcel OJ column. Evaluation of the optimal pH conditions established that the alcohol **8** was obtained in 65% yield at pH 6.0 (Fig. 2).

Derivation of 8 to (*S*)-7-[(*tert*-Butoxycarbonyl)amino]-5-azaspiro[2.4]heptane (2) The amine **9** was obtained from **8** in 48% yield and 98% enantiomeric excess *via* Mitsunobu reaction[7] using diphenylphosphoryl azide (DPPA), followed by reduction with lithium aluminum hydride. The optical purity of **9** was determined by HPLC analysis using Sumichiral OA-4600 after derivation to the 3,5-dinitrobenzamide with 3,5-dinitrobenzoyl chloride. The obtained **9** was treated with di-*tert*-butyl dicarbonate (Boc_2O) to provide **10** in 92% yield. Hydrogenolysis of **10** in the presence of a catalytic amount of palladium on carbon (Pd–C) afforded the desired compound **2** in 90% yield, mp 56—58 °C and −68.7° (c=1.700, $CHCl_3$) [lit.[2a] mp 56—59 °C and −68.5° (c=1.742, $CHCl_3$)] (Chart 3). The optical purity of **2** was determined as 98% enantiomeric excess by chiral HPLC analysis using Sumichiral OA-4400 in a similar manner to that mentioned for **9**.

In conclusion, we have developed an efficient and stereoselective route for the synthesis of (*S*)-7-[(*tert*-butoxycarbonyl)amino]-5-azaspiro[2.4]heptane **2**, a key intermediate for DU-6859a **1**, employing the asymmetric microbial reduction of 5-benzyl-4,7-dioxo-5-azaspiro-[2.4]heptane **7**. This is the first synthetic example based on a proposal of the synthesis design system AIPHOS and



(a) Ph_3P, $EtO_2CN_2CO_2Et$, DPPA; then $LiAlH_4$ (b) Boc_2O; (c) 5%Pd-C, H_2

Chart 3

confirms that AIPHOS is an useful tool for designing syntheses of organic compounds.

Further applications of AIPHOS to various medicinal intermediates to develop efficient synthetic methods is in progress.

Experimental

All melting points were measured with 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. Infrared (IR) spectra were recorded on Horiba FT-720 and Perkin-Elmer 1600 FT-IR spectrometers. 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, *N*-benzylglycine, was purchased from Senn Chemicals AG (Switzerland).

Ethyl 4-[Benzyl(*tert*-butoxycarbonyl)amino]-3-oxobutyrate (5) *N*,*N*′-carbonyldiimidazole (458.9 mg, 2.83 mmol) was added portionwise to a solution of compound **4** (500 mg, 1.88 mmol) in tetrahydrofuran (THF) (5 ml) at 0 °C under an argon atmosphere and the reaction mixture was stirred at room temperature for 2 h. In another flask, a suspension of $MgCl_2$ (173.3 mg, 1.82 mmol) and the potassium salt of hydrogen ethyl malonate (481.1 mg, 2.83 mmol) in THF (7 ml) was stirred at 50 °C for 6 h under an argon atmosphere. To the suspension, the above-mentioned imidazolide solution was added *via* a cannula at room temperature. After the addition, stirring was continued for 16 h. Saturated aqueous $KHSO_4$ was added to the reaction mixture and the whole was extracted with AcOEt. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography with hexane/AcOEt = 3/1 to afford **5** (530.8 mg, 84%) as a colorless oil. IR (neat): 2933, 1750, 1729, 1699 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J = 7.2 Hz, $C\underline{H}_3CH_2O$), 1.46, 1.47 (9H, each s, $C(CH_3)_3$), 3.34, 3.41 (2H, each s, $COCH_2CO$), 3.93, 4.07 (2H, each s, NCH_2CO), 4.16 (2H, q, J = 7.2 Hz, $CH_3C\underline{H}_2O$), 4.46, 4.51 (2H, each s, ArH_2CN), 7.26—7.38 (5H, m, aromatic H). MS *m*/*z*: 336 ($M^+ + 1$), 280, 236, 234, 91.

Ethyl 1-[*N*-Benzyl-*N*-(*tert*-butoxycarbonyl)glycyl]-1-cyclopropanecarboxylate (6) Solid K_2CO_3 (329.7 mg, 2.39 mmol) was added portionwise to a solution of **5** (100 mg, 0.30 mmol) and 1,2-dibromoethane (224.0 mg, 1.19 mmol) in acetone (6 ml) at room temperature. The reaction mixture was refluxed for 6 h. After evaporation of the solvent, water was added to the residue and the resulting mixture was extracted with AcOEt. The organic layer was washed with saturated aqueous NH_4Cl and brine, then dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by column chromatography with toluene/AcOEt = 3/1 to afford the crude **6**. Further purification by column chromatography with hexane/AcOEt = 3/1 gave **6** (77.9 mg, 72%) as a colorless oil. IR (neat): 2933, 1717, 1709, 1699 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J = 7.2 Hz, $C\underline{H}_3CH_2O$), 1.38—1.60 (4H, m, cyclopropane), 1.46 (9H, s, $C(CH_3)_3$), 4.13 (2H, q, J = 7.2 Hz, $CH_3C\underline{H}_2O$), 4.40, 4.45 (2H, each s, NCH_2CO), 4.48 (2H, s, ArH_2CN), 7.20—7.38 (5H, m, aromatic H). MS *m*/*z*: 362 ($M^+ + 1$), 306, 262, 260, 91.

5-Benzyl-4,7-dioxo-5-azaspiro[2.4]heptane (7) Trifluoroacetic acid (1.82 ml, 23.6 mmol) was added dropwise to an ice-cooled solution of **6** (131.5 mg, 0.36 mmol) in CH_2Cl_2 (1 ml) and the reaction mixture was stirred at room temperature for 1.5 h. The mixture was concentrated *in vacuo*. The residue was partitioned between 1 N aqueous NaOH and AcOEt, and the organic layer was washed with brine, then dried over $MgSO_4$, and evaporated *in vacuo*. The residue was dissolved in toluene (5 ml) and the mixture was refluxed for 30 min. After removal of the solvent, the residue was purified by column chromatography with hexane/AcOEt = 3/1 to afford **7** (56.3 mg, 72%) as colorless needles. mp 94—96 °C. IR (KBr): 2923, 1751, 1677 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.78 (4H, m, cyclopropane), 3.79 (2H, s, NCH_2CO), 4.68 (2H, s, ArH_2CN), 7.26—7.42 (5H, m, aromatic H). *Anal.* Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.41; H, 6.03; N, 6.46.

(*R*)-7-Hydroxy-4-oxo-5-azaspiro[2.4]heptane (8) *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, 30 min) and inoculated with the preincubated culture. The cultivation was carried out for 48 h at 30 °C with shaking. Then **7** (100 mg, 0.47 mmol) was added and the reaction mixture was shaken for 10 d at 30 °C. After filtration, the filtrate was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography with hexane/AcOEt = 2/1 to afford **8** (65.8 mg, 65%) as a white solid, which was >98% e.e. by HPLC analysis using chiralcel OJ; mobile phase, hexane : isopropanol = 10 : 1; flow rate, 0.8 ml/min; detector, UV (230 nm). Retention time for racemate: 17.3 min [50%, (*R*)-form], 18.9 min [50%, (*S*)-form]. Retention time for **8**: 17.1 min (>99%), 18.9 min (<1%), >98% e.e. mp 94—96 °C. +75.7° (c = 1.000, MeOH). IR (KBr): 3301, 2921, 1668 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88—1.18 (4H, m, cyclopropane), 3.12—3.63 (3H, m, 7-H, NCH_2CO), 4.46 (2H, s, ArH_2CN), 7.19—7.35 (5H, m, aromatic H). *Anal.* Calcd for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.87; H, 6.75; N, 6.62.

(*S*)-7-Amino-5-benzyl-5-azaspiro[2.4]heptane (9) An ice-cooled solution of **8** (217.3 mg, 1 mmol), triphenylphosphine (341 mg, 1.3 mmol) and diethylazodicarboxylate (226.4 mg, 1.3 mmol) in THF (5 ml) was stirred for 30 min, then a solution of DPPA (357.8 mg, 1.3 mmol) was added over a period of 15 min. After the addition, stirring was continued at room temperature for 24 h. After evaporation of the solvent, Et_2O was added to the residue. After filtration of the precipitate, the filtrate was concentrated *in vacuo* and the residue was dissolved in THF (5 ml). The resulting mixture was added to an ice-cooled solution of 1 M lithium aluminum hydride in THF (2 ml, 2 mmol), and the whole was refluxed for 1 h. Water and 10% aqueous NaOH were 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 **9** (97.3 mg, 48%) as a pale yellow oil. In order to determine the optical purity of **9**, the 3,5-dinitrobenzamide was prepared as follows: Triethylamine (9 μl) was added to a solution of **9** (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. It was diluted with $CHCl_3$ (3 ml), then dried over $MgSO_4$, and filtered through a pad of silica gel to give a chloroform solution of the 3,5-dinitrobenzamide usable for chiral HPLC analysis. The analysis conditions for HPLC were as follows: column, Sumichiral OA-4600; mobile phase, hexane : 1,2-dichloroethane : ethanol : trifluoroacetic acid = 80 : 20 : 5 : 0.2; flow rate, 1.2 ml/min; detecter, UV (254 nm). Retention time for racemate: 15.3 min [50%, (*S*)-form], 17.8 min [50%, (*R*)-form]. Retention time for **9**: 15.1 min (99%), 17.8 min (1%), 98% e.e. −60.0° (c = 1.076, MeOH). IR (neat): 3374, 3290, 2948, 1563 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.06—1.82 (4H, m, cyclopropane), 2.36 (2H, br s, NH_2), 2.20—3.16 (5H, m, CH_2NCH_2, 7-H), 3.61 (2H, s, ArH_2CN), 7.21—7.40 (5H, m, aromatic H). MS *m*/*z*: 203 ($M^+ + 1$), 186, 111, 91.

(*S*)-5-Benzyl-7-[(*tert*-butoxycarbonyl)amino]-5-azaspiro[2.4]heptane (10) A solution of di-*tert*-butyl dicarboxylate (Boc_2O) (785.7 mg,

3.6 mmol) in toluene (5 ml) was added to a solution of **9** (606.9 mg, 3 mmol) in toluene (10 ml) at room temperature, and the mixture was stirred for 18 h. After evaporation of the solvent, the resultant residue was partitioned between saturated aqueous NH_4Cl and AcOEt. The organic layer was washed with water and brine, then dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography with hexane/AcOEt = 1/1 to afford **10** (835.1 mg, 92%) as colorless needles. mp 73—75 °C. −33.2° (c = 1.208, MeOH). IR (KBr): 3374, 2933, 1685, 1529 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.40—0.88 (4H, m, cyclopropane), 1.42 (9H, s, $C(CH_3)_3$), 2.32 (1H, d, J = 8.9 Hz, 4-H), 2.58—2.75 (1H, m, 6-H), 2.66 (1H, d, J = 8.9 Hz, 4-H), 2.91 (1H, dd, J = 9.6, 5.9 Hz, 6-H), 3.56, 3.64 (2H, each d, J = 12.9 Hz, ArH_2CN), 3.70—3.91 (1H, m, 7-H), 4.95 (1H, br d, $CNHCO_2$), 7.21—7.40 (5H, m, aromatic H). *Anal.* Calcd for $C_{18}H_{26}N_2O_4$: C, 71.49; H, 8.67; N, 9.26. Found: C, 71.29; H, 8.74; N, 9.44.

(*S*)-7-[(*tert*-Butoxycarbonyl)amino]-5-azaspiro[2.4]heptane (2) A mixture of **10** (604.8 mg, 2 mmol) and 5% palladium on carbon (50% wet) (480 mg) in EtOH (30 ml) was hydrogenated at 60 °C under a hydrogen pressure of 20—25 atm for 5 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography with $CHCl_3$/MeOH = 20/1 to afford **2** (383.3 mg, 90%) as a hygroscopic white powder. In order to determine the optical purity of **2**, it was acylated with 3,5-dinitrobenzoyl chloride in a similar manner to that described for the preparation of the 3,5-dinitrobenzamide of **9**. The analysis conditions for HPLC were as follows: column, Sumichiral OA-4400; mobile phase, hexane : 1,2-dichloroethane : ethanol = 60 : 40 : 5; flow rate, 1.0 ml/min; detector, UV (254 nm). Retention time for racemate: 16.2 min [50%, (*S*)-form], 18.3 min [50%, (*R*)-form]. Retention time for **2**: 16.0 min (99%), 18.3 min (1%), 98% e.e. mp 56—58 °C. −68.7° (c = 1.700, $CHCl_3$). IR (KBr): 3367, 3208, 2979, 1685 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.40—0.88 (4H, m, cyclopropane), 1.43 (9H, s, $C(CH_3)_3$), 2.09 (1H, br s, 5-H), 2.73 (1H, d, J = 10.8 Hz, 4-H), 2.94 (1H, dd, J = 11.5, 5.9 Hz, 6-H), 3.03 (1H, d, J = 10.8 Hz, 4-H), 3.35 (1H, dd, J = 11.5, 5.9 Hz, 6-H), 3.56—3.76 (1H, m, 7-H), 4.75 (1H, br d, $CNHCO_2$). MS m/z: 213 ($M^+ + 1$), 157, 155, 57. *Anal.* Calcd for $C_{11}H_{20}N_2O_2 \cdot 1/10H_2O$: C, 61.71; H, 9.51; N, 13.08. Found: C, 61.86; H, 9.60; N, 12.86.

Acknowledgments The authors are grateful to Associate Prof. Kimito Funatsu (Toyohashi University of Technology) for his allowance to use AIPHOS.

References and Notes

1) *a*) Hayakawa I., Atarashi S., Kimura Y., Kawakami K., Saito T., Yafune T., Sato K., Une K.,Sato M., The 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 1991, Abstract No. 1504; *b*) Hayakawa I., Kimura Y., European Patent 0341493A2 (1989) [*Chem. Abstr.*, **113**, 40473*q* (1990)].
2) *a*) Hayakawa I., Atarashi S., Imamura M., Kimura Y., European Patent 0357047A1 (1990) [*Chem. Abstr.*, **113**, 132157*t* (1990)]; *b*) Kimura Y., Atarashi S., Kawakami K., Sato K., Hayakawa I., *J. Med. Chem.*, **37**, 3344—3352 (1994).
3) Funatsu K., Sasaki S., *Tetrahedron Comput. Method.*, **1**, 27—38 (1988).
4) Satoh H., Funatsu K., *J. Chem. Inf. Comput. Sci.*, **35**, 34—44 (1995).
5) Distributed Chemical Graphics, Inc. permitted us to use SYNLIB for research work on AIPHOS.
6) Mouna A. M., Nguyen C., Rage I., Xie J., Née G., Mazaleyrat J. P., Wakselman M., *Syn. Commun.*, **24**, 2429—2435 (1994).
7) Lal B., Pramanik B. N., Manhas M. S., Bose A. K., *Tetrahedron Lett.*, **1977**, 1977—1980.