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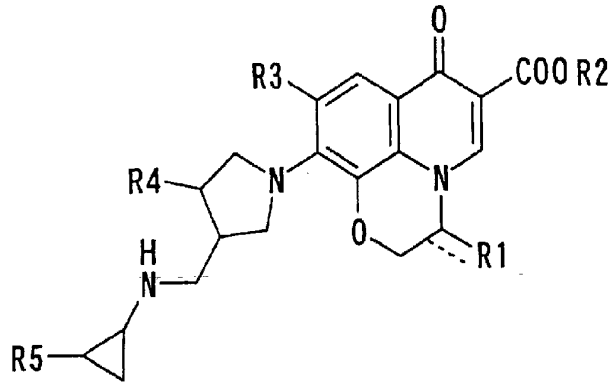
(54) Title: 10-(3-CYCLOPROPYLAMINOMETHYL-1-PYRROLIDINYL)PYRIDOBENZOXAZINECARBOXYLIC ACID DERIVATIVE EFFECTIVE AGAINST RESISTANT BACTERIUM

(54) 発明の名称: 耐性菌に有効な１０−（３−シクロプロピルアミノメチル−１−ピロリジニル）ピリドベンズオキサジンカルボン酸誘導体



(I)

(57) Abstract: A compound represented by the general formula (I): (I) wherein R1 represents methyl, fluoromethyl, methoxymethyl, acetoxymethyl, hydroxymethyl, or methylene; R2 represents hydrogen, C_{1-3} alkyl, or a pharmaceutically acceptable ester group of a cation and a prodrug; R3 represents hydrogen or halogeno; R4 represents hydrogen, C_{1-3} alkyl, fluoromethyl, trifluoromethyl, or fluorine; and R5 represents hydrogen or fluorine. It has excellent antibacterial activity against gram-positive bacteria, in particular, resistant bacteria such as MRSA, PRSP, and VRE.

[続葉有]

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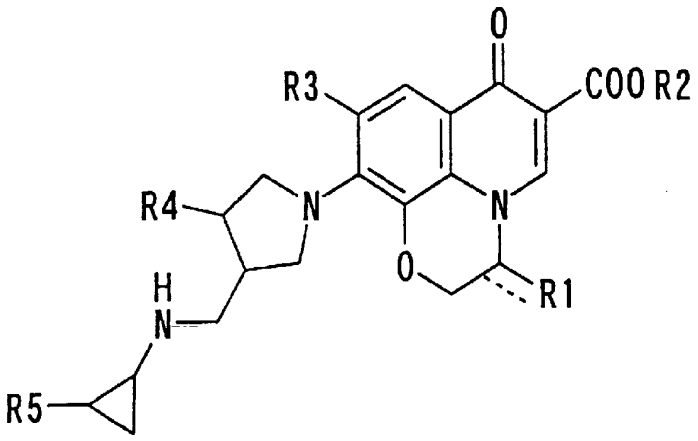
(57) 要約:

一般式（Ｉ）



(I)

(式中、Ｒ１はメチル基、フルオロメチル基、メトキシメチル基、アセトキシメチル基、ヒドロキシメチル基またはメチレン基を、Ｒ２は水素原子、炭素数１から３の低級アルキルまたは医薬的に許容される陽イオンおよびプロドラッグのエステル基を、Ｒ３は水素原子またはハロゲン原子を、Ｒ４は水素原子、炭素数１から３の低級アルキル基、フルオロメチル基、トリフルオロメチル基またはフッ素原子をおよびＲ５は水素原子またはフッ素原子を示す。)で表される化合物がグラム陽性菌、特にＭＲＳＡ、ＰＲＳＰ、ＶＲＥ等の耐性菌に対し優れた抗菌力を示す。

DESCRIPTION

10-(3-Cyclopropylaminomethyl-1-pyrrolidinyl)pyridobenzoxazine carboxylic acid derivatives effective against drug-resistant bacteria

TECHNICAL FIELD

The present invention relates to novel 10-(3-cyclopropylaminomethyl-1-pyrrolidinyl)pyridobenzoxazine carboxylic acid derivatives, salts and hydrates thereof that, in addition to being safe and exhibiting strong antibacterial activities, are effective against drug-resistant bacteria that are less susceptible to conventional antibacterial agents.

TECHNICAL BACKGROUND

Reference should be made to the following articles: Japanese Patent Laid-Open Publication No. Sho 57-46986 (Patent Article 1); Japanese Patent Laid-Open Publication No. Sho 61-204188 (Patent Article 2); Japanese Patent Laid-Open Publication No. Sho 62-155282 (Patent Article 3).

Since the development of norfloxacin, considerable effort has been made worldwide to develop quinolone carboxylic acid-based antibacterial agents, which are also known as new quinolones and have now become important cures for infectious diseases.

The recent emergence of drug-resistant bacteria, such as

Methicillin-Resistant Staphylococcus aureus (MRSA), Penicillin-Resistant Streptococcus pneumoniae (PRSP), and Vancomycin-Resistant Enterococcus (VRE), most of which are gram-positive bacteria, has posed a serious threat to the treatment of patients. Traditional quinolone carboxylic acid-based antibacterial agents have relatively weak antibacterial activities against gram-positive bacteria and thus are not considered as effective cures for the drug-resistant bacteria. Furthermore, the increasing incidence of Quinolone-Resistant Staphylococcus aureus (QRSA) makes the use of these drugs even more difficult.

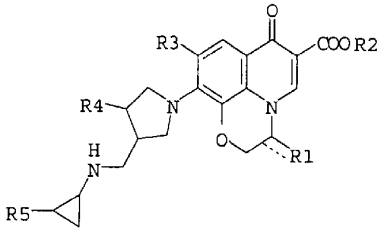
While pyridobenzoxazine carboxylic acid-based antibacterial agents similar to the ones claimed in the present invention are described in, for example, Patent Articles 1, 2, and 3, none of these agents offer sufficient antibacterial activity against gram-positive bacteria, nor are they described to have antibacterial activity against drug-resistant bacteria such as those described above.

DISCLOSURE OF THE INVENTION

The present invention relates to novel pyridobenzoxazine carboxylic acid-based compounds that, in addition to being safe and exhibiting strong antibacterial activities, are effective against drug-resistant bacteria that are less susceptible to conventional antibacterial agents.

In view of the above-described problems, the present inventors have devoted a significant amount of effort to seeking quinolone carboxylic acid derivatives that are effective against gram-positive bacteria, in particular, such drug-resistant bacteria as MRSA, PRSP, and VRE, which are less susceptible to traditional quinolone carboxylic acid-based antibacterial agents. The effort was rewarded by the discovery of the compounds of the present invention, which proved to be effective against gram-positive bacteria, in particular, such drug-resistant bacteria as MRSA, PRSP, and VRE, and exhibit higher antibacterial activity as compared not only with traditional quinolone carboxylic acid-based antibacterial agents, but also with various other antibacterial agents. The discovery ultimately led the present inventors to complete the present invention.

According to the present invention, there is provided a compound as represented by the following general formula (I), or a salt or a hydrate thereof:



I

wherein R1 is a fluoromethyl group; R2 is a hydrogen atom, a lower alkyl group having 1 to 3 carbon atoms, or a pharmaceutically acceptable cation and an ester of a prodrug; R3 is a hydrogen atom or a halogen atom; R4 is a hydrogen atom, a lower alkyl group having 1 to 3 carbon atoms, a fluoromethyl group, a trifluoromethyl group or a fluorine atom; and R5 is a hydrogen atom or a fluorine atom.

Examples of the lower alkyl group in the general formula (I) include a methyl group, an ethyl group, a propyl group, an isopropyl group, and a cyclopropyl group. Examples of the pharmaceutically acceptable cation include sodium ion, potassium ion, magnesium ion, calcium ion, and ammonium ion. Examples of the ester of a prodrug include a pivaloyloxymethyl group, an acetoxymethyl group, a phthalidinyl group, an indanyl group, a methoxymethyl group, and a 5-methyl-2-oxo-1,3-dioxolene-4-yl group. Examples of the halogen atom include fluorine, chlorine, bromine, and iodine.

Further according to the present invention there is provided an antibacterial agent containing as an active ingredient the compound as described above, a salt or a hydrate thereof.

The present invention also provides a pharmaceutical composition comprising the compound as described above, a salt or a hydrate thereof and a pharmaceutically acceptable carrier.

The present invention further provides a method of treating a bacterial infection comprising administering an effective amount of the compound as described above, a salt or a hydrate thereof to a subject in need thereof.

The present invention still further provides a use of the compound as described above, a salt or a hydrate thereof in the manufacture of a medicament for treating a bacterial infection.

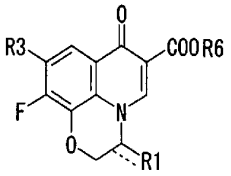
The present invention even further provides use of the compound as described above, a salt or a hydrate thereof for treating a bacterial infection.

BEST MODE FOR CARRYING OUT THE INVENTION

An exemplary production process of the compound of the present invention will now be described.

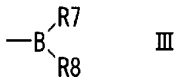
The compound of the present invention may be produced by reacting a compound represented by the following general

formula (II):



II

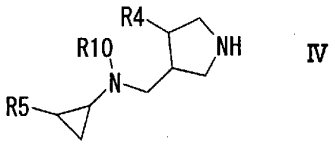
[wherein R1 and R3 are the same as in the general formula (I); and R6 is represented by the following general formula (III):



III

[wherein R6 and R7 are each independently a fluorine atom, or a lower alkylcarbonyloxy group]]

with a compound represented by the following general formula (IV), or an acid addition salt thereof:



IV

[wherein R4 and R5 are the same as in the general formula (I); and R10 is a hydrogen atom or a protective group of nitrogen atom such as t-butoxycarbonyl]

and then removing the boron chelate and, if necessary, the protective group of nitrogen atom.

The reaction of the compound of the general formula (II) with the compound of the general formula (IV) may be carried out in the absence or presence of a solvent, such as an

alcohol, acetonitrile, dimethylsulfoxide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, tetrahydrofuran, dioxane, benzene, or toluene, and in the presence of an acid receptor. The acid receptor may be a carbonate or a hydrogen carbonate of an alkali metal or an alkaline earth metal, or a basic organic compound, such as triethylamine, diazabicyclo-7-undecene, or pyridine. The reaction is typically carried out at a temperature in the range of room temperature to 200°C and preferably in the range of 25°C to 150°C. The reaction takes from 30min to 48 hours and is typically complete within 30min to 15 hours.

If desired, the compound of the general formula (I) may be converted to its salt using an ordinary technique. Examples of such salts include salts formed with an inorganic acid, such as hydrochloric acid, sulfuric acid, and phosphoric acid, salts formed with an organic acid, such as methanesulfonic acid, lactic acid, oxalic acid, and acetic acid, and salts formed with sodium, potassium, magnesium, calcium, aluminum, cerium, chromium, cobalt, copper, iron, zinc, platinum, silver, or the like.

The compound of the present invention may be administered to humans or animals in a pharmaceutically known form through a pharmaceutically known route. For example, the compound may be prepared in the form of powders, tablets, capsules, ointments, injections, syrups, solutions, eye drops, and

suppositories for oral or parenteral administration.

EXAMPLES

Exemplary tests as well as production processes for the compound of the present invention will now be described in detail with reference to examples.

Reference Example 1

Bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron

To a mixture of boric acid (12.8g) and acetic anhydride (63.4g), zinc chloride (236mg) was added and the resulting mixture was stirred at room temperature for 0.5 hours. To this mixture, (3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester (22.6g) was added and the mixture was stirred at 60°C for 2.5 hours. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was dissolved in ethyl acetate (300mL). The solution was sequentially washed with a saturated aqueous solution of sodium hydrogen carbonate (2 x 200mL) and then with water (100mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (dichloromethane: acetone

= 7:1), and the eluted yellow amorphous product was crystallized in an acetone/diethyl ether mixture to give 24.5g of bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron as a white powder.

^{1}H NMR($CDCl_3$): δ 1.85 (s, 3H), 2.05 (s, 3H), 4.62 (ddd, J = 2.9 Hz, 3.9 Hz, 12.2 Hz, 1H), 4.74 (ddd, J = 7.8 Hz, 10.3 Hz, 46.4 Hz, 1H), 4.90 (ddd, J = 4.9 Hz, 10.3 Hz, 45.4 Hz, 1H), 4.92 (dd, J = 1.0 Hz, 12.7 Hz, 1H), 5.35-5.38 (m, 1H), 7.92 (dd, J = 7.3 Hz, 9.3 Hz, 1H), 9.22 (s, 1H).

Elementary analysis (%): Calcd for $C_{17}H_{13}BF_3NO_8 \cdot 0.75H_2O$: C 46.34, H 3.32, N 3.18; found: C 46.30, H 3.34, N 3.30.

Reference Example 2

Synthesis of bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron

Step 1:

(3S)-9,10-Difluoro-2,3-dihydro-3-hydroxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester (1.30g) was suspended in anhydrous dimethylformamide (40mL). Silver oxide (I) (4.63g) and methyl iodide (1.25mL) were then added to the suspension. The resulting mixture was stirred at room temperature for 21 hours. Subsequently, insoluble materials were removed from the reaction mixture by

filtration and the filtrate was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (dichloromethane: acetone= 5: 1) to give 740mg of (3S)-9,10-difluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester as a white powder.

MS(EI)m/z: 339(M^+).

Elementary analysis (%): Calcd for $C_{16}H_{15}F_2NO_5$: C 56.64, H 4.46, N 4.13; found: C 56.56, H 4.71, N 4.26.

Step 2:

In a similar manner to Reference Example 1, (3S)-9,10-difluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester (679mg) was reacted to give 830mg of bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron as a colorless amorphous product.

^{1}H NMR($CDCl_3$):δ 1.86 (s, 3H), 2.06 (s, 3H), 3.39 (s, 3H), 3.70 (dd, J= 8.3 Hz, 10.3 Hz, 1H), 3.82 (dd, J = 5.4 Hz, 10.3 Hz, 1H), 4.56 (dd, J= 2.9 Hz, 12.2 Hz, 1H), 4.86 (dd, J = 1.0 Hz, 12.2 Hz, 1H), 5.10-5.13 (m, 1H), 7.89 (dd, J = 7.3 Hz, 9.3 Hz, 1H), 9.13 (s, 1H).

Elementary analysis (%): Calcd for $C_{18}H_{16}BF_2NO_9 \cdot 1.5H_2O$: C 46.38, H 4.11, N 3.00; found: C 46.18, H 3.74, N 3.15.

Reference Example 3

Synthesis of bis(acetato-O)[(3S)-3-acetoxymethyl-9,10-difluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron

Step 1:

(3S)-9,10-Difluoro-2,3-dihydro-3-hydroxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester (976mg) was suspended in anhydrous dichloromethane (30mL). To the suspension, acetic anhydride (368mg) and 4-dimethylaminopyridine (5.0mg) were added and the resulting mixture was refluxed for 1.5 hours while heated. Subsequently, the mixture was allowed to cool and was washed with water. This was followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was suspended in ethanol and the suspension was filtrated to give 1.04g of (3R)-3-acetoxymethyl-9,10-difluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester as a white powder.

MS(EI)m/z: 367(M^+).

Elementary analysis (%): Calcd for $C_{17}H_{15}F_2NO_6$: C 55.59, H 4.12, N 3.81; found: C 56.25, H 4.15, N 3.93.

Step 2:

In a similar manner to Reference Example 1, (3S)-3-

acetoxymethyl-9,10-difluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid ethyl ester (918mg) was reacted to give 1.00g of bis(acetato-O)[(3S)-3-acetoxymethyl-9,10-difluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron as a colorless amorphous product.

^{1}H NMR($CDCl_3$): δ 1.83 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 4.44-4.54 (m, 2H), 4.63 (dd, J = 2.9 Hz, 12.2 Hz, 1H), 4.89 (dd, J = 1.0 Hz, 12.7 Hz, 1H), 5.27-5.30 (m, 1H), 7.88 (dd, J = 7.3 Hz, 9.3 Hz, 1H), 9.17 (s, 1H).

Elementary analysis (%): Calcd for $C_{19}H_{16}BF_2NO_{10}\cdot 1.75H_2O$: C 45.76, H 3.94, N 2.81; found: C 45.94, H 3.82, N 2.95.

Reference Example 4

Synthesis of trans-3-cyclopropylaminomethyl-4-methylpyrrolidine

Step 1:

trans-1-Benzyl-4-methyl-3-pyrrolidinecarboxylic acid (4.04g) was dissolved in dichloromethane (50mL). To this solution, 1,1'-carbonylbis-1H-imidazole (3.58g) was added and the mixture was stirred at room temperature for 1 hour. While the reaction mixture was cooled on an ice bath, a dichloromethane solution (15mL) of cyclopropylamine (1.53mL) was added dropwise and the mixture was stirred at room temperature for 3 hours. Subsequently, the reaction mixture

was washed with water, was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was crystallized in a hexane/diisopropyl ether mixture and the formed crystal was filtrated. The collected crystal was then washed with a hexane/diisopropyl ether mixture and was dried under reduced pressure to obtain 4.07g of trans-1-benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide as a white crystal.

Melting point: 81-83°C.

MS (EI) m/z: 258(M^+).

Step 2:

trans-1-Benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide (3.80g) was suspended in anhydrous tetrahydrofuran (85mL). To this suspension, a 1mol/L tetrahydrofuran solution of borane-tetrahydrofuran complex (58.8mL) was added and the mixture was refluxed for 8 hours while heated. Subsequently, a 2mol/L aqueous solution of sodium hydroxide (35mL) was added to the reaction mixture and the mixture was refluxed for 4 hours while heated. After concentration under reduced pressure, the resultant residue was extracted with toluene (2 x 100mL) and the toluene extracts were combined. The combined extract was washed with water, was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was

dissolved in dichloromethane (50mL). To this solution, di-*tert*-butyl dicarbonate (3.53g) was added and the mixture was stirred at room temperature for 4 hours. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was purified on a silica gel column (hexane: ethyl acetate = 4:1 shifted to 1:1) to obtain 3.07g of trans-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine as a colorless oil.

MS (FAB^+)m/z: 345 (MH^+).

HRMS (FAB^+): Calcd for $C_{21}H_{33}N_2O_2$ (MH^+): 345.2542; found: 345.2505.

Step 3:

trans-1-Benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine (3.00g) was dissolved in ethanol (50mL). To this solution, 7.5% palladium carbon (300mg) was added and the mixture was stirred at room temperature for 6 hours under a hydrogen pressure of 3.9 x 10^5Pa. Subsequently, the catalyst was removed from the reaction mixture by filtration and the collected catalyst was washed with ethanol. The filtrate and the washing solution were combined and the resulting residue was dried under reduced pressure to obtain 2.12g of trans-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine

as a pale brown oil.

MS (FAB^+) m/z: 255 (MH^+).

HRMS (FAB^+): Calcd for $C_{14}H_{27}N_2O_2$ (MH^+): 255.2073; found: 255.2079.

Step 4:

trans-3-[[(N-tert-Butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine (2.07g) was dissolved in dichloromethane (10mL). While this solution was cooled on an ice bath, trifluoroacetic acid (5mL) was added and the mixture was stirred at room temperature for 2 hours. After concentration under reduced pressure, the resulting residue was dissolved in tetrahydrofuran (6mL) and the solution was allowed to stand for 13 hours at room temperature. The separated crystal was collected by filtration, followed by washing with tetrahydrofuran and drying under reduced pressure, to give 2.47g of trans-3-cyclopropylaminomethyl-4-methylpyrrolidine trifluoroacetic acid salt. The salt product (2.37g) was dissolved in water (5mL), followed by addition of a 20% aqueous solution of sodium hydroxide to adjust the pH to 14. The solution was then extracted with diethyl ether (2 x 50mL) and the extracts were combined. The combined extract was then dried over anhydrous sodium sulfate and was concentrated under reduced pressure. The resulting residue was purified by distillation under reduced pressure to obtain 660mg of trans-

3-cyclopropylaminomethyl-4-methylpyrrolidine.

^{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), 1.66-1.76(m, 4H), 2.08-2.13(m, 1H), 2.46(dd, J =7.3 Hz, 10.7 Hz, 1H), 2.57(dd, J = 8.3 Hz, 11.7 Hz, 1H), 2.63(dd, J =6.3 Hz, 10.7 Hz, 1H), 2.80 (dd, J = 5.4 Hz, 11.7 Hz, 1H), 3.10 (dd, J =6.8 Hz, 10.7Hz, 1H), 3.14 (dd, J = 7.3 Hz, 10.7 Hz, 1H).

Elementary analysis (%): Calcd for $C_9H_{18}N_2 \cdot 2CF_3COOH$: C 40.84, H 5.27, N 7.33; found: C 40.90, H 5.47, N 7.37.

Reference Example 5

Synthesis of (3R,4R)-3-cyclopropylaminomethyl-4-methylpyrrolidine

Step 1:

(3R,4R)-1-Benzyl-4-methyl-3-pyrrolidinecarboxylic acid (6.27g) was suspended in dichloromethane (250mL). To this suspension, cyclopropylamine (1.76mL) and hydrochloric acid 1-ethyl-(3-dimethylaminopropyl)carbodiimide (12.2g) were sequentially added and the mixture was stirred at room temperature for 4 hours. Subsequently, the reaction mixture was washed with water, was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (ethyl acetate: methanol = 10:1) to give 3.32g of (3R,4R)-1-benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide as a white

crystal.

MS (EI) m/z: 258 (M^+).

Elementary analysis (%): Calcd for $C_{16}H_{22}N_2O$: C 74.38, H 8.58, N 10.84; found: C 74.46, H 8.67, N 10.72.

Step 2:

In a similar manner to Step 2 in Reference Example 4, (3R,4R)-1-benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide (5.52g) was reacted to give 4.16g of (3R,4R)-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine as a pale brown oil.

MS (FAB^+) m/z: 345 (MH^+).

HRMS (FAB^+): Calcd for $C_{21}H_{33}N_2O_2$ (MH^+): 345.2542; found 345.2585.

Step 3:

In a similar manner to Step 3 in Reference Example 4, (3R,4R)-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine (4.00g) was reacted to give 2.88g of (3R,4R)-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine.

MS (FAB^+) m/z: 255 (MH^+).

HRMS (FAB^+): Calcd for $C_{14}H_{27}N_2O_2$(MH^+): 255.2073; found: 255.2070.

Step 4:

In a similar manner to Step 4 in Reference Example 4, (3R,4R)-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine (2.78g) was reacted to give 730mg of (3R,4R)-3-cyclopropylaminomethyl-4-methylpyrrolidine.

Specific rotation: +74.6°(c=0.648, methanol).

Elementary analysis (%): Calcd for $C_9H_{18}N_2 \cdot 2CF_3COOH$: C 40.84, H 5.27, N 7.33; found: C 40.73, H 5.26, N 7.36.

Reference Example 6

Synthesis of (3S,4S)-3-cyclopropylaminomethyl-4-methylpyrrolidine

Step 1:

In a manner similar to Step 1 in Reference Example 5, (3S,4S)-1-benzyl-4-methyl-3-pyrrolidinecarboxylic acid (14.5g) was reacted to give 6.33g of (3S,4S)-1-benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide as a pale brown crystal.

MS (EI) m/z: 258 (M^+).

Elementary analysis (%): Calcd for $C_{16}H_{22}N_2O$: C 74.38, H 8.58, N 10.84; found: C 74.64, H 8.66, N 10.71.

Step 2:

In a manner similar to Step 2 in Reference Example 4, (3S,4S)-1-benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide (6.13g) was reacted to give 4.67g of

(3S,4S)-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine as a pale brown oil.

MS (FAB^+) m/z: 345 (MH^+).

HRMS (FAB^+): Calcd for $C_{21}H_{33}N_2O_2$ (MH^+): 345.2542; found: 345.2547.

Step 3:

In a similar manner to Step 3 in Reference Example 4, (3S,4S)-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine (4.47g) was reacted to give 3.05g of (3S,4S)-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine.

MS (FAB^+) m/z: 255 (MH^+).

HRMS (FAB^+): Calcd for $C_{14}H_{27}N_2O_2$ (MH^+): 255.2073; found 255.2075.

Step 4:

In a similar manner to Step 4 in Reference Example 4, (3S,4S)-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-methylpyrrolidine (2.85g) was reacted to give 1.21g of (3S,4S)-3-cyclopropylaminomethyl-4-methylpyrrolidine.

Specific rotation: -74.5°(c=0.62, methanol).

Elementary analysis (%): Calcd for $C_9H_{18}N_2 \cdot 2CF_3COOH$: C 40.84, H 5.27, N 7.33; found: C 40.80, H 5.18, N 7.39.

Reference Example 7

Synthesis of cis-3-cyclopropylaminomethyl-4-methylpyrrolidine

Step 1:

cis-1-Benzyl-3-hydroxy-4-methylpyrrolidine (6.81g) was dissolved in dichloromethane (70mL). While this solution was cooled on a dry ice/acetone bath, triethylamine (5.21mL) was added. Methanesulfonyl chloride (2.89mL) was then added dropwise and the mixture was further stirred for 1 hour. Following addition of water (50mL), the temperature of the mixture was allowed to rise to room temperature and the dichloromethane layer was separated. The aqueous layer was extracted with dichloromethane (50mL) and the extract was combined with the dichloromethane layer. The combined dichloromethane layer was then washed with water, followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was dissolved in acetonitrile (180mL). To this solution, tetrabutylammonium cyanide (23.9g) was added and the mixture was refluxed for 7 hours while heated. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was dissolved in ethyl acetate (300mL). The solution was washed with water, was dried over anhydrous sodium sulfate, and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (hexane: ethyl

acetate = 1:1) to give 4.61g of cis-1-benzyl-4-methyl-3-pyrrolidinecarbonitrile as a brown oil.

IR (neat): 2240, 1496, 1454 cm^{-1}.

MS (EI) m/z: 200 (M^+).

Step 2:

Lithium aluminum hydride (80%, 3.89g) was suspended in diethyl ether (90mL). While the suspension was cooled on an ice bath, a diethyl ether solution (25mL) of cis-1-benzyl-4-methyl-3-pyrrolidinecarbonitrile (4.11g) was added dropwise and the mixture was stirred at room temperature for 1 hour. While the reaction mixture was cooled on an ice bath, a saturated aqueous solution of sodium hydrogen carbonate (8mL) was carefully added dropwise. Following dilution with diethyl ether (100mL), insoluble materials were collected by filtration and were washed with diethyl ether. The filtrate and the washing solution were combined and the combined solution was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (hexane: ethyl acetate = 1:1 shifted to ethyl acetate: methanol = 10:1) to give 2.35g of cis-1-benzyl-4-methyl-3-aminomethylpyrrolidine as a pale yellow oil.

^1H NMR($CDCl_3$): δ 0.94 (d, J = 7.3 Hz, 3H), 1.09-1.66 (br, 2H), 2.03 (dd, J = 7.3 Hz, 9.3 Hz, 1H), 2.11-2.26 (m, 2H), 2.31-2.42 (m, 1H), 2.58 (dd, J = 8.3 Hz, 12.2 Hz, 1H), 2.82 (dd, J

= 5.9 Hz, 12.2 Hz, 1H), 2.96-3.02 (m, 2H), 3.60 (s, 2H), 7.21-7.35 (m, 5H).

Step 3:

cis-1-Benzyl-4-methyl-3-aminomethylpyrrolidine (1000mg) was dissolved in methanol (10mL). While this solution was cooled on an ice bath, benzaldehyde (0.50mL) was added dropwise and the mixture was stirred at room temperature for 1 hour. Subsequently, sodium cyanoborohydride (184mg) was added and the mixture was stirred at room temperature for 1.5 hours. This was followed by a second addition of sodium cyanoborohydride (123mg) and stirring for additional 5.5 hours. Subsequently, a 2mol/L aqueous solution of sodium hydroxide (5mL) was added to the reaction mixture and the mixture was refluxed for 2 hours while heated. Following concentration under reduced pressure, the resulting residue was extracted with toluene (2 x 30mL) and the toluene extracts were combined. The combined toluene layer was then washed with water, followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (hexane: ethyl acetate = 4:1) to give 690mg of cis-1-benzyl-3-benzylaminomethyl-4-methylpyrrolidine as a pale yellow oil.

MS (EI) m/z: 294 (M^+).

HRMS (EI): Calcd for $C_{20}H_{26}N_2(M^+)$: 294.2096; found: 294.2110.

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Step 4:

cis-1-Benzyl -3-benzylaminomethyl-4-methylpyrrolidine (680mg) was dissolved in methanol (7mL). To this solution, molecular sieves 3A (700mg), acetic acid (1.32mL), [1-(ethoxycyclopropyl)oxy]trimethylsilane (1.85mL), and sodium cyanoborohydride (435mg) were added and the mixture was refluxed for 4 hours while heated. Insoluble materials were collected by filtration and were washed with methanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. To the resulting residue, water was added (5mL), followed by addition of a 2mol/L aqueous solution of sodium hydroxide to make the mixture basic. The mixture was then extracted with toluene (2 x 50mL) and the extracts were combined. The combined toluene layer was then washed with water, was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (hexane: ethyl acetate = 4:1) to give 648mg of cis-1-benzyl-3-(N-benzyl-N-cyclopropyl)aminomethyl-4-methylpyrrolidine as a colorless oil.

MS (EI) m/z: 334 (M^+).

HRMS (EI): Calcd for $C_{23}H_{30}N_2$(M^+): 334.2409; found: 334.2403.

Step 5:

cis-1-Benzyl-3-(N-benzyl-N-cyclopropyl)aminomethyl-4-methylpyrrolidine (640mg) was dissolved in ethanol (10mL). To this solution, 10% palladium carbon (500mg) and chloroform (0.77mL) were added and the mixture was stirred at 50°C for 7 hours under a hydrogen pressure of 3.9×10^5Pa. From the reaction mixture, the catalyst was collected by filtration and was washed with ethanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. To the resulting residue, water (2mL) was added, followed by addition of a 2mol/L aqueous solution of sodium hydroxide to make the mixture basic. Sodium chloride was then added to the mixture for salting out and the mixture was extracted with diethyl ether (2 x 25mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (hexane: ethyl acetate = 4:1 shifted to dichloromethane: methanol = 10:1) to give 124mg of cis-3-cyclopropylaminomethyl-4-methylpyrrolidine as a pale brown oil.

MS (CI^+) m/z: 155 (MH^+).

HRMS (CI^+): Calcd for $C_9H_{19}N_2$(MH^+): 155.1548; found: 155.1553.

Reference Example 8

Synthesis of (3R,4S)-3-cyclopropylaminomethyl-4-methylpyrrolidine

Step 1:

(3R,4S)-1-Benzyl-3-hydroxy-4-methylpyrrolidine (4.00g) was dissolved in dichloromethane (40mL). While this solution was cooled on a dry ice/acetone bath, triethylamine (3.06mL) was added. Methanesulfonyl chloride (1.70mL) was then added dropwise and the mixture was further stirred for 1 hour. Following addition of water (40mL), the temperature of the mixture was allowed to rise to room temperature and the dichloromethane layer was separated. The aqueous layer was extracted with dichloromethane (40mL) and the extract was combined with the dichloromethane layer. The combined dichloromethane layer was then washed with water, followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was dissolved in N,N-dimethylformamide (120mL). To this solution, tetrabutylammonium cyanide (5.53g) and sodium cyanide (2.05g) were added and the mixture was stirred at 80°C for 13 hours. Subsequently, the reaction mixture was concentrated under reduced pressure and water (50mL) was added to the resulting residue. The mixture was extracted with diethyl ether (2 x 200mL). The diethyl ether extracts were combined and the combined extract was washed with a saturated aqueous solution of sodium chloride, followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (hexane:

ethyl acetate = 4:1) to give 3.32g of (3R,4S)-1-benzyl-4-methyl-3-pyrrolidinecarbonitrile as a brown oil.

^{1}H NMR($CDCl_3$):δ 1.22 (d, J = 7.3 Hz, 3H), 2.12 (dd, J = 8.3 Hz, 9.3 Hz, 1H), 2.45-2.57 (m, 1H), 2.60-2.67 (m, 1H), 2.99 (dd, J = 7.3 Hz, 9.3 Hz, 1H), 3.09-3.19 (m, 2H), 3.62 (s, 2H), 7.25-7.35 (m, 5H).

MS(EI)m/z: 200 (M^+).

Step 2:

In a similar manner to Step 2 in Reference Example 7, (3R,4S)-1-benzyl-4-methyl-3-pyrrolidinecarbonitrile (3.20g) was reacted to obtain 2.98g of (3S,4S)-1-benzyl-4-methyl-3-aminomethylpyrrolidine.

^{1}H NMR($CDCl_3$):δ 0.94 (d, J = 7.3 Hz, 3H), 2.03 (dd, J = 7.3 Hz, 9.3 Hz, 1H), 2.11-2.26 (m, 2H), 2.31-2.43 (m, 1H), 2.58 (dd, J = 8.3 Hz, 12.2 Hz, 1H), 2.82 (dd, J = 5.9 Hz, 12.2 Hz, 1H), 2.97-3.02 (m, 2H), 3.60 (s,2H), 7.22-7.33 (m, 5H).

Step 3:

In a similar manner to Step 3 in Reference Example 7, (3S,4S)-1-benzyl-4-methyl-3-aminomethylpyrrolidine (2.80g) was reacted to give 3.49g of (3R,4S)-1-benzyl-3-benzylaminomethyl-4-methyl-pyrrolidine.

MS (EI) m/z: 294 (M^+).

HRMS (EI): Calcd for $C_{20}H_{26}N_2$(M^+): 294.2096; found: 294.2072.

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Step 4:

In a similar manner to Step 4 in Reference Example 7, (3R,4S)-1-benzyl-3-benzylaminomethyl-4-methylpyrrolidine (3.40g) was reacted to give 3.72g of (3R,4S)-1-benzyl-3-(N-benzyl-N-cyclopropyl)aminomethyl-4-methylpyrrolidine.

MS (FAB^+) m/z: 335 (MH^+).

HRMS (EI): Calcd for $C_{23}H_{31}N_2(MH^+)$: 335.2487; found: 335.2503.

Step 5:

In a similar manner to Step 5 in Reference Example 7, (3R,4S)-1-benzyl-3-(N-benzyl-N-cyclopropyl)aminomethyl-4-methylpyrrolidine (3.60g) was reacted to give 1.29g of (3R,4S)-3-cyclopropylaminomethyl-4-methylpyrrolidine.

MS (CI^+)m/z: 155 (MH^+).

HRMS (CI^+): Calcd for $C_9H_{19}N_2(MH^+)$: 155.1548; found: 155.1539.

Reference Example 9

Synthesis of (3S,4R)-3-cyclopropylaminomethyl-4-methylpyrrolidine

Step 1:

In a similar manner to Step 1 in Example 8, (3S,4R)-1-benzyl-3-hydroxy-4-methylpyrrolidine (4.62g) was reacted to give 3.07g of (3S,4R)-1-benzyl-4-methyl-3-pyrrolidinecarbonitrile.

^{1}H NMR($CDCl_3$):δ 1.22 (d, J = 6.8 Hz, 3H), 2.13 (t,J = 9.3 Hz, 1H), 2.45-2.55 (m, 1H), 2.61-2.65 (m, 1H), 2.99 (dd, J = 6.8 Hz, 9.3 Hz, 1H), 3.09-3.19 (m, 2H), 3.62 (s, 2H), 7.27-7.34 (m, 5H).

Step 2:

In a similar manner to Step 2 in Reference Example 7, (3S,4R)-1-benzyl-4-methyl-3-pyrrolidinecarbonitrile (3.00g) was reacted to give 1.44g of (3R,4R)-1-benzyl-4-methyl-3-aminomethylpyrrolidine.

MS (EI)m/z: 204 (M^+).

HRMS (EI): Calcd for $C_{13}H_{20}N_2$(M^+): 204.1626; found: 204.1614.

Step 3:

In a similar manner to Step 3 in Reference Example 7, (3R,4R)-1-benzyl-4-methyl-3-aminomethylpyrrolidine (1.06g) was reacted to give 1.20g of (3S,4R)-1-benzyl-3-benzylaminomethyl-4-methylpyrrolidine.

MS (EI) m/z: 294 (M^+).

HRMS (EI): Calcd for $C_{20}H_{26}N_2$(M^+): 294.2096; found: 294.2106.

Step 4:

In a similar manner to Step 4 in Reference Example 7, (3S,4R)-1-benzyl-3-benzylaminomethyl-4-methylpyrrolidine (1.40g) was reacted to give 1.55g of (3S,4R)-1-benzyl-3-(N-

benzyl-N-cyclopropyl)aminomethyl-4-methylpyrrolidine.

MS (FAB^+) m/z: 335 (MH^+).

HRMS (EI): Calcd for $C_{23}H_{31}N_2(MH^+)$: 335.2487; found: 335.2498.

Step 5:

In a similar manner to Step 5 in Reference Example 7, (3S,4R)-1-benzyl-3-(N-benzyl-N-cyclopropyl)aminomethyl-4-methylpyrrolidine (700mg) was reacted to give 215mg of (3S,4R)-3-cyclopropylaminomethyl-4-methylpyrrolidine.

MS (CI^+)m/z: 155 (MH^+).

HRMS (CI^+): Calcd for $C_9H_{19}N_2(MH^+)$: 155.1548; found: 155.1510.

Reference Example 10

Synthesis of trans-3-cyclopropylaminomethyl-4-trifluoromethylpyrrolidine

Step 1:

In a similar manner to Step 1 in Example 4, trans-1-benzyl-4-trifluoromethyl-3-pyrrolidinecarboxylic acid (3.00g) was reacted to give 3.32g of trans-1-benzyl-4-trifluoromethyl-3-pyrrolidinecarboxamide.

^1H NMR($CDCl_3$):δ 0.42-0.46 (m, 2H), 0.75-0.79 (m, 2H), 2.64-2.78 (m, 4H), 2.82-2.86 (m, 1H), 2.95 (t, J = 9.3 Hz, 1H), 3.10-3.22 (m,1H), 3.59 (d, J = 13.2 Hz, 1H), 3.68 (d, J = 12.7 Hz, 1H), 6.34-6.53 (br, 1H), 7.26-7.36 (m, 5H).

Step 2:

In a similar manner Step 2 in Example 4, trans-1-benzyl-4-trifluoromethyl-3-pyrrolidinecarboxamide (3.21g) was reacted to give 3.37g of trans-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-trifluoromethylpyrrolidine.

MS (FAB^+) m/z: 399 (MH^+).

HRMS (FAB^+): Calcd for $C_{21}H_{30}F_3N_2O_2$ (MH^+): 399.2259; found: 399.2254.

Step 3:

In a similar manner to Step 3 in Example 4, trans-1-benzyl-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-trifluoromethylpyrrolidine (3.27g) was reacted to give 2.38g of trans-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-trifluoromethylpyrrolidine.

MS (FAB^+) m/z: 309 (MH^+).

HRMS (FAB^+): Calcd for $C_{14}H_{24}F_3N_2O_2$(MH^+): 309.1790; found: 309.1783.

Step 4:

In a similar manner to Step 4 in Example 4, trans-3-[[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl]-4-trifluoromethylpyrrolidine (2.30g) was reacted to give 992mg of trans-3-cyclopropylaminomethyl-4-trifluoromethylpyrrolidine.

^1H NMR($CDCl_3$): δ 0.29-0.33 (m, 2H), 0.42-0.46 (m, 2H), 2.10-

2.15 (m, 1H), 2.30-2.39 (m, 1H), 2.41-2.53 (m, 1H), 2.62-2.71 (m, 2H), 2.83 (dd, J= 6.3 Hz, 11.7 Hz, 1H), 3.10 (d, J = 6.8 Hz, 2H), 3.18 (dd, J= 7.8 Hz, 11.7 Hz, 1H).

Elementary analysis (%): Calcd for $C_9H_{15}F_3N_2 \cdot 2CF_3COOH$: C 35.79, H 3.93, N 6.42; found: C 35.82, H 3.90, N 6.59.

Reference Example 11

Synthesis of (3R,4S)-3-cyclopropylaminomethyl-4-fluoropyrrolidine (Process (I))

Step 1:

(E)-3-Benzyloxypropenyl-(1R)-camphorsultam (21.6g) was dissolved in dichloromethane (300mL) containing trifluoroacetic acid (0.116mL). To this solution, N-methoxymethyl-N-(trimethylsilyl)benzylamine (15.0g) was added dropwise and the mixture was further stirred for 2 hours. The mixture was sequentially washed with a saturated aqueous solution of sodium hydrogen carbonate (2 x 200mL) and then with water (200mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting pale yellow oil was dissolved in diethyl ether (150mL) and the solution was allowed to stand for 18 hours at room temperature. The crystal formed was collected by filtration, was washed with diethyl ether, and was then dried under reduced pressure to give 11.5g of N-[[(3S,4R)-benzyl-4-benzyloxypyrrolidin-3-yl]carbonyl]-(2'S)-bornane-10,2-sultam

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as a white crystal. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = cyclohexane: ethyl acetate = 4:1) to obtain additional 8.48g of N-[[(3S,4R)-benzyl-4-benzyloxypyrrolidin-3-yl]carbonyl]-(2'S)-bornane-10,2-sultam. ^{1}H NMR($CDCl_3$): δ 0.95 (s, 3H), 1.02 (s, 3H), 1.32-1.45 (m, 2H), 1.86-1.96 (m, 3H), 2.00-2.10 (m, 2H), 2.57 (dd, J=9.3 Hz, 5.3 Hz), 2.69 (dd, J= 9.8 Hz, 3.9 Hz, 1H), 2.93 (dd, J= 10.3 Hz, 6.3 Hz, 1H), 3.20 (t, J=9.3Hz), 3.42-3.51 (m, 3H), 3.69-3.74 (m, 2H), 3.90 (d, J=11.7 Hz), 4.54 (d, J= 11.7 Hz), 4.63-4.66 (m, 1H), 7.22-7.31 (m, 10H).

Step 2:

Lithium aluminum hydride (80%, 5.56g) was suspended in tetrahydrofuran (170mL). While the suspension was cooled on a sodium chloride/ice bath, a tetrahydrofuran solution (300mL) of N-[[(3S,4R)-benzyl-4-benzyloxypyrrolidin-3-yl]carbonyl]-(2'S)-bornane-10,2-sultam (19.9g) was added dropwise and the mixture was stirred at -5°C or below for 1 hour. Subsequently, water (34mL) was carefully added dropwise to the mixture. Insoluble materials were collected by filtration and were washed with ethyl acetate (2 x 400mL). The filtrate and the washing solutions were combined and the combined organic layer was extracted with 1mol/L hydrochloric acid (2 x 500mL). The

hydrochloric acid extracts were combined and a 30% aqueous solution of sodium hydroxide was added to make the combined solution basic (pH 14). The mixture was then extracted with diethyl ether (2 x 500mL) and the diethyl ether extracts were combined. The combined diethyl ether layer was concentrated under reduced pressure and the resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 1:1) to give 9.91g of (3R,4R)-(1-benzyl-4-benzyloxypyrrolidin-3-yl)methanol as a pale yellow oil.

^{1}H NMR($CDCl_3$): δ 2.29-2.34(m, 1H), 2.40 (dd, J=10.3 Hz, 4.4 Hz, 1H), 2.68 (dd, J=9.3 Hz, 2.4 Hz, 1H), 2.75 (dd, J= 9.8 Hz, 6.3 Hz, 1H), 3.18 (dd, J= 9.8 Hz, 6.8 Hz, 1H), 3.61 (s, 2H), 3.65 (dd, J=10.3 Hz, 4.4 Hz, 1H), 3.73 (dd, J=10.3 Hz, 4.4 Hz, 1H), 4.07 (ddd, J= 6.3 Hz, 4.4 Hz, 2.0 Hz, 1H), 4.48 (s, 2H), 7.25-7.35 (m, 10H).

Step 3:

Process (A): (3R,4R)-(1-benzyl-4-benzyloxypyrrolidin-3-yl)methanol (9.80g) was dissolved in ethanol (100mL). To this solution, 10% palladium carbon (2.00g) was added and the mixture was stirred at 50°C for 21 hours under a hydrogen pressure of 3.9 x 10^5Pa. Subsequently, the catalyst was collected from the reaction mixture by filtration through a Celite pad. The collected catalyst and the Celite pad were washed with ethanol. The filtrate and the washing solution

were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was dissolved in ethanol (100mL), followed by addition of 10% palladium carbon (2.00g). The mixture was then stirred at 50°C for 20 hours under a hydrogen pressure of 3.9 x 10^5Pa. Subsequently, the catalyst was collected from the reaction mixture by filtration through a Celite pad. The collected catalyst and the Celite pad were washed with ethanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was dried under reduced pressure to give 3.77g of (3R,4R)-(4-hydroxypyrrolidin-3-yl)methanol.

^{1}H NMR(DMSO-d_6): δ 1.96-2.03 (m, 1H), 2.61 (dd, J=11.6 Hz, 5.5 Hz, 1H), 2.68 (dd, J=11.6 Hz, 3.1 Hz, 1H), 2.91 (dd, J= 11.1 Hz, 5.5 Hz, 1H), 3.06 (dd, J= 11.0 Hz, 7.3 Hz, 1H), 3.26 (dd, J=10.4 Hz, 7.3 Hz, 1H), 3.37 (dd, J=10.4 Hz, 6.1 Hz), 3.90-3.93 (m, 1H).

Sodium hydroxide (2.70g) was dissolved in water (25mL) and dioxane (15mL) was added. To this solution, (3R,4R)-(4-hydroxypyrrolidin-3-yl)methanol (1.00g) was dissolved. While the solution was cooled on an ice bath, carbobenzoxy chloride (0.97mL) was added dropwise. The mixture was stirred at 5°C or below for 1 hour, followed by dropwise addition of carbobenzoxy chloride (0.97mL). The mixture was further stirred at 5°C or below for additional 1 hour and carbobenzoxy

chloride (0.97mL) was subsequently added dropwise. This was followed by stirring for 1 hour at 5°C or below and another 1 hour at room temperature. Subsequently, the reaction mixture was extracted with dichloromethane (2 x 100mL). The dichloromethane extracts were combined, and the combined dichloromethane layer was dried over anhydrous sodium sulfate and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 1:1 shifted to ethyl acetate: methanol = 20:1) to give 1.18g of (3R,4R)-[1-benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol as a milky white tar-like product.

MS (EI) m/z: 251 (M^+).

^{1}H NMR($CDCl_3$): δ 2.08-2.40 (br +m, 2H), 2.58-2.79 (br, 1H), 3.20 (dd, J=11.0 Hz, 7.3 Hz, 1H), 3.32 (dt, J=11.1Hz, 5.5 Hz, 1H), 3.59-3.76 (m, 4H), 4.23-4.33 (br, 1H), 5.12 (s, 2H), 7.28-7.36 (m, 5H).

Process (B): (3R,4R)-[1-benzyl-4-benzyloxypyrrolidin-3-yl]methanol (10.0g) was dissolved in methanol (200mL). To this solution, 10% palladium carbon (3.00g) suspended in water (60mL) and ammonium formate (21.2g) were sequentially added, and the mixture was heat-refluxed for 4 hours while being stirred. Subsequently, the catalyst was collected from the reaction mixture by filtration through a Celite pad. The collected catalyst and the Celite pad were washed with a

methanol/water mixture (80:20). The filtrate and the washing solution were combined and the combined solution was concentrated under reduced pressure. The resulting pale brown, tar-like material was dissolved in N,N-dimethylformamide (100mL). While this solution was cooled on an ice bath, triethylamine (9.40mL) was added, followed by dropwise addition of carbobenzoxy chloride (6.00mL). While being cooled on an ice bath, the resulting mixture was stirred for 1.5 hours and was subsequently concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (400mL) and the solution was washed with a saturated aqueous solution of sodium chloride (2 x 100mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate, shifted to ethyl acetate: methanol = 20:1) to give 7.66g of (3R,4R)-[1-benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol as a milky white tar-like product.

This compound was identical to the compound obtained by Process (A).

Step 4:

Process (A): (3R,4R)-(1-benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl)methanol (3.19g) was dissolved in N,N-dimethylformamide (91mL). While this solution was cooled on an

ice bath, imidazole (6.05g) and tert-butylchlorodimethylsilane (5.74g) were sequentially added and the mixture was stirred at room temperature for 3 hours. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was dissolved in diethyl ether (400mL). The diethyl ether layer was washed with a saturated aqueous solution of sodium chloride (2 x 100mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1) to give 5.46g of (3R,4R)-1-benzyloxycarbonyl-3-(tert-butyldimethylsilyl)oxymethyl-4-(tert-butyldimethylsilyl)oxypyrrolidine as a colorless oil.

MS (CI^+): m/z=480(MH^+).

^1H NMR($CDCl_3$): δ 0.03 (s, 3H), 0.05 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.87 (s, 9H), 0.88 (s, 9H), 2.17-2.27 (m, 1H), 3.21-3.28 (m, 2H), 3.48-3.67 (m, 4H), 4.21-4.28 (m, 1H), 5.13 (s, 2H), 7.31-7.37 (m, 5H).

(3R,4R)-1-Benzyloxycarbonyl-3-(tert-butyldimethylsilyl)oxymethyl-4-(tert-butyldimethylsilyl)oxypyrrolidine (5.46g) was dissolved in tetrahydrofuran (23mL). While this solution was cooled on an ice bath, water (23mL) and acetic acid (68mL) were sequentially added and the mixture was stirred at room temperature for 8 hours. Subsequently, the reaction mixture

was concentrated under reduced pressure and the resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1 shifted to 1:1) to give 2.74g of (3R,4R)-1-benzyloxycarbonyl-3-hydroxymethyl-4-(tert-butyldimethylsilyloxy)pyrrolidine as a colorless oil.

MS (CI^+): m/z=366(MH^+).

^1H NMR($CDCl_3$): δ 0.07-0.08 (m, 6H), 0.88 (s, 9H), 2.23-2.35 (m, 1H), 3.21-3.30 (m, 2H), 3.58-3.72 (m, 4H), 4.17-4.25 (m, 1H), 5.128 (s, 1H), 5.135 (s, 1H), 7.31-7.37 (m, 5H).

(3R,4R)-1-Benzyloxycarbonyl-3-hydroxymethyl-4-(tert-butyldimethylsilyloxy)pyrrolidine (2.73g) was dissolved in dichloromethane (60mL). While this solution was cooled on a sodium chloride/ice bath, triethylamine (1.21mL) was added, which was followed by dropwise addition of methanesulfonyl chloride (0.71mL) at -5°C or below. The reaction mixture was then stirred at -5°C or below for 1 hour, was washed with water (2 x 25mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was dissolved in N,N-dimethylformamide (60mL), followed by addition of sodium azide (1.14g) and stirring at 100°C for 2 hours. The reaction mixture was then concentrated under reduced pressure and water (30mL) was added to the resulting residue. The mixture was then extracted with diethyl ether (2 x 100mL) and the diethyl ether extracts were combined. The combined diethyl ether layer was dried over anhydrous

sodium sulfate and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1) to give 3.06g of (3R,4R)-3-azidomethyl-1-benzyloxycarbonyl-4-(tert-butyldimethylsilyl)oxypyrrolidine as a colorless oil.

MS (CI^+):m/z=391 (MH^+).

^1H NMR($CDCl_3$): δ 0.07-0.09 (m, 3H), 2.23-2.34 (m, 1H), 3.19-3.25 (m, 2H), 3.27-3.40 (m, 2H), 3.60-3.71 (m, 2H), 4.11-4.17 (m, 1H), 5.13 (s, 2H), 7.31-7.37 (m, 5H).

(3R,4R)-3-Azidomethyl-1-benzyloxycarbonyl-4-(tert-butyldimethylsilyl)oxypyrrolidine (3.05g) was dissolved in tetrahydrofuran (50mL). While this solution was cooled on an ice bath, tetrabutylammonium fluoride (1mol/L tetrahydrofuran solution, 13.3mL) was added dropwise and the mixture was stirred for additional 1 hour. Subsequently, a saturated aqueous solution of sodium chloride 70mL) was added and the mixture was extracted with ethyl acetate (150mL, 100mL). The ethyl acetate extracts were combined and the combined solvent was dried over anhydrous sodium sulfate and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate) to give 2.01g of (3R,4R)-3-azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine as a milky white syrup-like product.

MS (CI^+):m/z=277 (MH^+).

^1H NMR ($CDCl_3$): δ 2.18-2.30 (br, 1H), 2.32-2.40 (m, 1H), 3.24

(dd, J=11.6 Hz, 6.1 Hz, 1H), 3.30-3.47 (m, 3H), 3.68-3.75 (m, 2H), 4.18-4.24 (m, 1H), 5.13 (s, 2H), 7.31-7.37 (m, 5H).

Process (B): (3R,4R)-[1-Benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol (3.00g), sodium azide (2.32g), triphenylphosphine (3.43g) and N,N-dimethylformamide (60mL) were mixed with each other. While the mixture was cooled on an ice bath, a dichloromethane solution (14mL) of carbon tetrabromide (4.34g) was added dropwise. The reaction mixture was stirred for 25 hours at room temperature and additional 2 hours at 60°C, followed by addition of methanol (5mL) and concentration under reduced pressure. The resulting residue was dissolved in ethyl acetate (200mL) and was washed with a saturated aqueous solution of sodium chloride (2 x 50mL), followed by drying over anhydrous sodium sulfate and concentration under reduce pressure. The resulting residue was purified on silica gel column (eluant = ethyl acetate: hexane = 2:1) to give 2.94g of (3R,4R)-3-azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine as a pale brown syrup-like product. This compound was identical to the compound obtained by Process (A).

Process (C): (3R,4R)-[1-Benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol (150mg) was dissolved in dichloromethane (12mL) and 2,4,6-collidine (0.79mL) was added. While this solution was cooled on an ice bath, methanesulfonyl chloride (46.2μL) was added dropwise. The mixture was then

stirred for 2 hours on the ice bath and was allowed to stand for 15 hours in a refrigerator (3°C). Subsequently, the reaction mixture was sequentially washed with water (2mL), 1mol/L hydrochloric acid (2 x 2mL), and a saturated aqueous solution of sodium chloride (2 x 2mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 1:2 shifted to ethyl acetate) to give 38.7mg of (3R,4R)-1-benzyloxycarbonyl-3-methanesulfonyloxy-4-methanesulfonyloxymethylpyrrolidine as a pale yellow syrup-like product and 133mg of (3R,4R)-1-benzyloxycarbonyl-3-hydroxy-4-methanesulfonyloxymethylpyrrolidine as a white syrup-like product.

(3R,4R)-1-Benzyloxycarbonyl-3-hydroxy-4-methanesulfonyloxymethylpyrrolidine (125mg) was dissolved in N,N-dimethylformamide (3mL) and sodium azide (50.0mg) was added. The mixture was stirred at 100°C for 1 hour and was then concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (5mL) and the solution was washed with water (2 x 1mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate) to give 91.0mg of (3R,4R)-3-azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine as a

milky white syrup-like product. The compound was identical to the compound obtained by Process (A).

Step 5:

Process (A): (3R,4R)-3-Azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine (1.20g) was dissolved in dichloromethane (40mL). While this solution was cooled on a sodium chloride/ice bath, diethylaminosulfur trifluoride (1.20mL) was added dropwise and the mixture was stirred at room temperature for 3 hours. The reaction vessel was again cooled on a sodium chloride/ice bath and diethylaminosulfur trifluoride (0.57mL) was again added dropwise. The mixture was then stirred at room temperature for 2 hours. While the reaction mixture was cooled on an ice bath, a saturated aqueous solution of sodium hydrogen carbonate (40mL) was added dropwise and the dichloromethane layer was separated. The dichloromethane layer was sequentially washed with a saturated aqueous solution of sodium hydrogen carbonate (2 x 20mL) and water (20mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 2:1) to give 726mg of (3R,4S)-3-azidomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a pale brown oil.
MS (CI^+):m/z=279 (MH^+).
^1H NMR($CDCl_3$): δ 2.34-2.54 (m, 1H), 3.22 (dt, J=11.0 Hz, 2.4 Hz,

1H), 3.39-3.49 (m, 1H), 3.54-3.69 (m, 2H), 3.73-3.91 (m, 2H), 5.14 (s, 2H), 5.16 (dt, J=53.2 Hz, 3.7 Hz, 1H), 7.32-7.37 (m, 5H).

Process (B): (3R,4R)-3-Azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine (1.79g) was dissolved in toluene (56mL). While this solution was cooled on an ice bath, 1,8-diazabicyclo[5.4.0]undec-7-ene (2.03mL) was added. This was followed by dropwise addition of perfluoro-1-octanesulfonyl fluoride (2.80mL) and stirring for another 1 hour. Insoluble materials were removed from the reaction mixture by filtration and were washed with toluene. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was then purified on a silica gel column (eluant = hexane: ethyl acetate = 2:1) to give 1.58g of (3R,4S)-3-azidomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a pale brown syrup-like product. The compound was identical to the compound obtained by Process (A).

Step 6:

(3R,4S)-3-Azidomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (1.35g) was dissolved in ethanol (30mL). To this solution, platinum oxide (IV) (190mg) was added and the mixture was stirred at room temperature for 2 hours in a stream of hydrogen (provided from a balloon). Subsequently,

the catalyst was collected from the reaction mixture by filtration through a Celite pad. The collected catalyst and the Celite pad were washed with ethanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate: methanol = 10:1) to give 1.13g of (3S,4S)-3-aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a pale brown oil. MS (CI^+):m/z=253(MH^+).

Step 7:

(3S,4S)-3-Aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (1.10g) was dissolved in methanol (13mL). To this solution, molecular sieves 4A (440mg) and benzaldehyde (0.44mL) were sequentially added and the mixture was stirred at room temperature for 1 hour. Subsequently, a borane-pyridine complex (0.44mL) was added and the mixture was further stirred at room temperature for 3.5 hours. This was followed by addition of 6mol/L hydrochloric acid (7.3mL) and stirring at room temperature for 1 hour. Subsequently, a 30% aqueous solution of sodium hydroxide was added to make the mixture basic and the mixture was extracted with diethyl ether (2 x 100mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure. The

resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1 shifted to 1:1) to give 1.18g of (3S,4S)-3-benzylaminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a colorless tar-like product.

MS (CI^+):m/z=343 (MH^+).

Step 8:

(3S,4S)-3-Benzylaminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (1.15g) was dissolved in methanol (21mL). To this solution, molecular sieves 3A (1.05g), acetic acid (1.92mL), [(1-ethoxycyclopropyl)oxy]trimethylsilane (2.70mL), and sodium cyanoborohydride (633mg) were added and the mixture was heat-refluxed for 2 hours while being stirred. Subsequently, insoluble materials were removed from the reaction mixture by filtration through a Celite pad. The insoluble materials and the Celite pad were washed with methanol. The filtrate and the washing solution were combined and a 2mol/L aqueous solution of sodium hydroxide was added to make the combined organic layer basic (pH14). Methanol was then removed under reduced pressure and the residue was extracted with diethyl ether (2 x 100mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl

acetate = 4:1) to give 1.26g of (3S,4S)-3-(N-benzyl-N-cyclopropyl)aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a colorless tar-like product.

MS (EI) m/z:=382 (M^+).

Step 9:

(3S,4S)-3-(N-Benzyl-N-cyclopropyl)aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (1.22g) was dissolved in ethanol (14mL). To this solution, 10% palladium carbon (150mg) was added and the mixture was stirred at room temperature for 4 hours in a stream of hydrogen (provided from a balloon). Subsequently, the catalyst was collected from the reaction mixture by filtration through a Celite pad. The collected catalyst and the Celite pad were washed with ethanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate: methanol = 20:1). The eluate was distilled under reduced pressure to give 414mg of (3R,4S)-3-cyclopropylaminomethyl-4-fluoropyrrolidine as a colorless oil.

MS (CI^+): m/z=159 (MH^+).

HRMS (CI^+): Calcd for $C_8H_{16}FN_2$: 159.1298; found: 159.1316.

Reference Example 12

Synthesis of (3R,4S)-3-cyclopropylaminomethyl-4-

fluoropyrrolidine (Process (II))

Step 1:

(3R,4R)-(4-Hydroxypyrrolidin-3-yl)methanol (1.18g) was dissolved in ethanol (25mL) and triethylamine (1.40mL) was added to the solution. While this mixture was cooled on a sodium chloride/ice bath, benzyl bromide (1.10mL) was added dropwise. The mixture was then stirred at room temperature for 1 hour and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate: methanol = 20:1) to give 1.02g of (3R,4R)-(1-benzyl-4-hydroxypyrrolidin-3-yl)methanol as a milky white syrup-like product.

MS (EI^+): m/z=207 (M^+).

HRMS (EI^+): Calcd for $C_{12}H_{17}NO_2$: 207.1259; found: 207.1237.

Step 2:

(3R,4R)-(1-Benzyl-4-hydroxypyrrolidin-3-yl)methanol (1.36g) was dissolved in dichloromethane (14mL). While this solution was cooled on an dry ice/acetone bath, triethylamine (0.83mL) was added, followed by dropwise addition of methanesulfonyl chloride (0.46mL) and stirring for 30min. Water (10mL) was then added to the reaction mixture and the temperature of the mixture was allowed to rise to room temperature. The mixture was then diluted with dichloromethane (20mL) and the dichloromethane layer was collected. The

collected dichloromethane layer was washed with water (2 x 10mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (hexane: ethyl acetate = 1:1 shifted to ethyl acetate: methanol = 20:1). From a fraction eluted at hexane: ethyl acetate = 1:1, 585mg of (3R,4R)-1-benzyl-3-methanesulfonyloxy-4-methanesulfonyloxymethylpyrrolidine was obtained as a milky white syrup-like product.

MS (EI^+): m/z=363 (M^+).

HRMS (EI^+): Calcd for $C_{14}H_{21}NO_6S_2$: 363.0810; found: 363.0804.

Also, 840mg of (3R,4R)-1-benzyl-3-hydroxy-4-methanesulfonyloxymethylpyrrolidine was obtained as a white crystal from a fraction eluted at ethyl acetate: methanol = 20:1.

MS (EI^+): m/z=285 (M^+).

HRMS (EI^+): Calcd for $C_{13}H_{19}NO_4S$: 285.1035; found: 285.1045.

Step 3:

(3R,4R)-1-Benzyl-3-hydroxy-4-methanesulfonyloxymethylpyrrolidine (835mg), sodium azide (381mg), and N,N-dimethylformamide (12mL) were mixed with one another and the mixture was stirred at 120°C for 1 hour. Subsequently, the reaction mixture was concentrated under reduced pressure. To the resulting residue, water (10mL) was

added and the mixture was extracted with diethyl ether (2 x 30mL). The diethyl ether extracts were combined and the combined extract was dried over anhydrous sodium sulfate and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate: methanol = 20:1) to give 576mg of (3R,4R)-3-azidomethyl-1-benzyl-4-hydroxypyrrolidine as a pale brown oil.

MS (EI^+): m/z=232 (M^+).

HRMS (EI^+): Calcd for $C_{12}H_{16}N_4O$: 232.1324; found: 232.1309.

Step 4:

(3R,4R)-3-Azidomethyl-1-benzyl-4-hydroxypyrrolidine (566mg) was dissolved in dichloromethane (9mL). While this solution was cooled on an ice bath, diethylaminosulfur trifluoride (0.39mL) was added dropwise and the mixture was stirred at room temperature for 2 hours. While the reaction vessel was cooled on an ice bath, a saturated aqueous solution of sodium hydrogen carbonate (9mL) was added, and the mixture was diluted with dichloromethane (15mL). The dichloromethane layer was collected and the collected dichloromethane layer was washed with a saturated aqueous solution of sodium hydrogen carbonate (10mL) and then water (10mL), was dried over anhydrous sodium sulfate, and was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1). From

the first half fraction, 76.7mg of (3R,4R)-3-azidomethyl-1-benzyl-4-fluoropyrrolidine was obtained as a pale brown oil.

MS (EI^+):m/z=234 (M^+).

HRMS (EI^+): Calcd for $C_{12}H_{15}FN_4$: 234.1281; found: 234.1263.

From the second half fraction, 220mg of (3R,4S)-3-azidomethyl-1-benzyl-4-fluoropyrrolidine was obtained as a pale brown oil.

MS (EI^+): m/z=234 (M^+).

HRMS (EI^+): Calcd for $C_{12}H_{15}FN_4$: 234.1281; found: 234.1269.

Step 5:

(3R,4S)-3-Azidomethyl-1-benzyl-4-fluoropyrrolidine (215mg) was dissolved in ethanol (3mL). To this solution, platinum oxide (IV) (30.0mg) was added and the mixture was stirred at room temperature for 5 hours in a stream of hydrogen (provided from a balloon). Subsequently, the catalyst was removed from the reaction mixture by filtration through a Celite pad. The removed catalyst and the Celite pad were washed with ethanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure to obtain 191mg of (3S,4S)-3-aminomethyl-1-benzyl-4-fluoropyrrolidine as a brown oil.

MS (CI^+): m/z=209 (MH^+).

HRMS (CI^+): Calcd for $C_{12}H_{18}FN_2$: 209.1454; found: 209.1465.

Step 6:

(3S,4S)-3-Aminomethyl-1-benzyl-4-fluoropyrrolidine (186mg) was dissolved in methanol (4mL). To this solution, molecular sieves 4A (80.0mg) and benzaldehyde (90.8μL) were sequentially added and the mixture was stirred at room temperature for 1 hour. Subsequently, a borane-pyridine complex (90.2μL) was added and the mixture was further stirred at room temperature for 3 hours. This was followed by addition of 6mol/L hydrochloric acid (1.5mL) and stirring for 1 hour. Subsequently, a 6mol/L aqueous solution of sodium hydroxide was added to make the mixture basic and the mixture was extracted with diethyl ether (3 x 10mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1) to give 179mg of (3S,4S)-1-benzyl-3-benzylaminomethyl-4-fluoropyrrolidine as a pale brown oil.
MS (CI^+): m/z = 299 (MH^+).
HRMS (CI^+): Calcd for $C_{19}H_{24}FN_2$: 299.1924; found: 299.1960.

Step 7:

(3S,4S)-1-Benzyl-3-benzylaminomethyl-4-fluoropyrrolidine (175mg) was dissolved in methanol (2mL). To this solution, molecular sieves 3A (180mg), acetic acid (0.36mL), [(1-

ethoxycyclopropyl)oxy]trimethylsilane (0.47mL) and sodium cyanoborohydride (110mg) were added and the mixture was heat-refluxed for 3 hours while being stirred. Subsequently, insoluble materials were removed from the reaction mixture by filtration through a Celite pad. The insoluble materials and the Celite pad were washed with methanol. The filtrate and the washing solution were combined and a 2mol/L aqueous solution of sodium hydroxide was added to make the combined organic layer basic (pH14). Methanol was then removed under reduced pressure and the residue was extracted with diethyl ether (3 x 100mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1) to give 172mg of (3R,4S)-3-(N-benzyl-N-cyclopropyl)aminomethyl-1-benzyl-4-fluoropyrrolidine as a colorless tar-like product.

MS (CI^+):m/z=339 (MH^+).

HRMS (CI^+): Calcd for $C_{22}H_{28}FN_2$: 339.2237; found: 339.2285.

Step 8:

(3R,4S)-3-(N-Benzyl-N-cyclopropyl)aminomethyl-1-benzyl-4-fluoropyrrolidine (170mg) was dissolved in ethanol (10mL). To this solution, 10% palladium carbon (200mg) and chloroform (0.17mL) were added and the mixture was stirred at 50°C for 23

hours under a hydrogen pressure of 3.9×10^5Pa. Subsequently, palladium carbon was removed from the reaction mixture by filtration through a Celite pad. The removed palladium carbon and the Celite pad were washed with ethanol. The filtrate and the washing solution were then combined and the combined organic layer was concentrated under reduced pressure. To the resulting residue, a 30% aqueous solution of sodium hydroxide (approximately 1mL) was added. Subsequently, sodium chloride was added to saturation and the mixture was extracted with diethyl ether (3 x 10mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure to give 65.4mg of (3R,4S)-3-cyclopropylaminomethyl-4-fluoropyrrolidine as a pale brown oil. This compound was identical to the compound obtained in Reference Example 11 (Process (I)).

Reference Example 13

Synthesis of (3R,4R)-3-cyclopropylaminomethyl-4-fluoropyrrolidine

Step 1:

(3R,4R)-[1-Benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol (2.50g), triphenylphosphine (5.74g), and benzoic acid (2.55g) were dissolved in tetrahydrofuran (60mL). While this solution was cooled on a sodium chloride/ice bath,

azodicarboxylic acid diethyl ester (40% toluene solution, 9.53mL) was added dropwise. The mixture was stirred for 1 hour at 0°C or below and then for additional 2 hours at room temperature and was subsequently concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 2:1). The eluted pale brown tar-like material was dissolved in ethanol (60mL). To this solution, potassium carbonate (4.07g) dissolved in water (30mL) was added and the mixture was heat-refluxed for 3 hours while being stirred. Subsequently, the reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved in dichloromethane (200mL). The dichloromethane solution was washed with a saturated aqueous solution of sodium chloride (2 x 50mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate: methanol = 10:1) to give 2.04g of (3R,4S)-[1-benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol as a milky white syrup-like product.

MS (EI)m/z=251 (M^+).

Step 2:

(3R,4S)-[1-Benzyloxycarbonyl-4-hydroxypyrrolidin-3-yl]methanol (2.33g), sodium azide (1.81g), triphenylphosphine (2.67g), and N,N-dimethylformamide (46mL) were mixed with one

another. While this mixture was cooled on an ice bath, a dichloromethane solution (10mL) of carbon tetrabromide (3.38g) was added dropwise. The reaction mixture was stirred for 13 hours at room temperature and additional 3 hours at 60°C, followed by addition of methanol (3mL) and concentration under reduced pressure. The resulting residue was dissolved in ethyl acetate (200mL) and was washed with a saturated aqueous solution of sodium chloride (2 x 50mL), followed by drying over anhydrous sodium sulfate and concentration under reduce pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate: hexane = 2:1) to give 2.18g of (3R,4S)-3-azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine as a milky white syrup-like product.

MS (FAB^+): m/z=277 (MH^+).

Step 3:

(3R,4S)-3-Azidomethyl-1-benzyloxycarbonyl-4-hydroxypyrrolidine (300mg) was dissolved in dichloromethane (6mL). While this solution was cooled on an ice bath, diethylaminosulfur trifluoride (0.43mL) was added dropwise. The mixture was stirred at room temperature for 4 hours. While the reaction vessel was cooled on an ice bath, a saturated aqueous solution of sodium hydrogen carbonate (6mL) was added and the dichloromethane layer was collected. The collected dichloromethane layer was washed with a saturated aqueous

solution of sodium chloride (2 x 2mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 2:1) to give 211mg of a mixture of (3R,4R)-3-azidomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine and 3-azidomethyl-1-benzyloxycarbonyl-3-pyrroline.

Step 4:

Platinum oxide (IV) (50.0mg) was suspended in ethanol (7mL) and the suspension was stirred at room temperature for 30min in a stream of hydrogen (provided from a balloon). To this suspension, an ethanol solution (3mL) of a mixture (551mg) of (3R,4R)-3-azidomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine and 3-azidomethyl-1-benzyloxycarbonyl-3-pyrroline was added, and the mixture was stirred at room temperature for 5 hours in a stream of hydrogen (provided from a balloon). Subsequently, the catalyst was removed from the reaction mixture by filtration and was washed with ethanol. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was then purified on a silica gel column (eluant = ethyl acetate shifted to ethyl acetate: methanol = 10:1) to give 313mg of a mixture of (3S,4R)-3-aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine and 3-aminomethyl-1-

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benzyloxycarbonyl-3-pyrroline.

Step 5:

A mixture (310mg) of (3S,4R)-3-aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine and 3-aminomethyl-1-benzyloxycarbonyl-3-pyrroline was dissolved in methanol (4mL). To this solution, molecular sieves 4A (130mg) and benzaldehyde (0.13mL) were sequentially added and the mixture was stirred at room temperature for 1 hour. Subsequently, a borane-pyridine complex (0.19mL) was added and the mixture was further stirred at room temperature for 4 hours. This was followed by addition of 6mol/L hydrochloric acid (2mL) and stirring at room temperature for 1 hour. Subsequently, a 30% aqueous solution of sodium hydroxide was added to make the mixture basic and the mixture was extracted with diethyl ether (3 x 10mL). The diethyl ether extracts were combined and the combined diethyl ether layer was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = dichloromethane: methanol = 10:1) to give 177mg of (3S,4R)-3-benzylaminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a pale yellow oil.

MS (FAB^+): m/z = 343 (MH^+).

HRMS (FAB^+): Calcd for $C_{20}H_{24}FN_2O_2$: 343.1822; found: 343.1815.

Step 6:

(3S,4R)-3-Benzylaminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (170mg) was dissolved in methanol (5mL). To this solution, molecular sieves 3A (160mg), acetic acid (0.29mL), [(1-ethoxycyclopropyl)oxy]trimethylsilane (0.40mL), and sodium cyanoborohydride (93.5mg) were added and the mixture was heat-refluxed for 3 hours while being stirred. Subsequently, insoluble materials were removed from the reaction mixture by filtration through a Celite pad. The insoluble materials and the Celite pad were washed with methanol. The filtrate and the washing solution were combined and a 2mol/L aqueous solution of sodium hydroxide was added to make the combined organic layer basic (pH>12). Methanol was then removed under reduced pressure and the residue was extracted with diethyl ether (3 x 10mL). The diethyl ether extracts were combined and the combined diethyl ether layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 2:1) to give 166mg of (3S,4R)-3-(N-benzyl-N-cyclopropyl)aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine as a colorless tar-like product.

MS (FAB^+): m/z = 383 (MH^+).

HRMS (FAB^+): Calcd for $C_{23}H_{28}FN_2O_2$:383.2135; found:383.2119.

Step 7:

(3S,4R)-3-(N-Benzyl-N-cyclopropyl)aminomethyl-1-benzyloxycarbonyl-4-fluoropyrrolidine (160mg) was dissolved in ethanol (3mL). To this solution, 10% palladium carbon (20.0mg) was added and the mixture was stirred at room temperature for 5 hours in a stream of hydrogen (provided from a balloon). Subsequently, the catalyst was collected from the reaction mixture by filtration through a Celite pad. The collected catalyst and the Celite pad were washed with ethanol. The filtrate and the washing solution were combined and the resulting residue was purified on a silica gel column (eluant = ethyl acetate : methanol = 20:1, shifted to dichloromethane: methanol = 10:1) to give 50.7mg of (3R,4R)-3-cyclopropylaminomethyl-4-fluoropyrrolidine as a colorless oil.

MS (FAB^+): m/z = 159 (MH^+).

HRMS (FAB^+): Calcd for $C_8H_{16}FN_2$: 159.1298; found: 159.1286.

Reference Example 14

Synthesis of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethylpyrrolidine

Step 1:

(1S,5R)-7-[(1R)-1-Phenylethyl]-3-oxa-7-azabicyclo[3.3.0]octane-2-one (7.73g, 33.4mmol) was dissolved in ethanol (92mL). To this solution, cyclopropylamine (46.3ml) was added, and the mixture was stirred at 80°C for 44 hours and was subsequently concentrated under reduced pressure. The

resulting residue was dissolved in ethyl acetate (300mL) and the solution was washed with water (2 x 50mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. To the resulting residue, diisopropyl ether (300mL) was added and the mixture was heated to form crystal and was then concentrated to approximately 1/2. The formed crystal was collected by filtration and the collected crystal was washed with diisopropyl ether and was dried under reduced pressure to give 4.41g of (3R,4S)-N-cyclopropyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine-3-carboxamide as a white crystal. The filtrate and the washing solution were combined and the combined solvent was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 1:1, shifted to ethyl acetate) to obtain additional 1.50g of (3R,4S)-N-cyclopropyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine-3-carboxamide. The total amount of the compound was 5.91g.

MS (EI)m/z = 288 (M^+).

Elementary analysis (%): Calcd for $C_{17}H_{24}N_2O_2 \cdot 0.2H_2O$: C 69.93, H 8.42, N 9.59; found: C 70.16, H 8.32, N 9.60.

Step 2:

(3R,4S)-N-Cyclopropyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine-3-carboxamide (7.54g) was dissolved in

N,N-dimethylformamide (180mL). While this solution was cooled on an ice bath, imidazole (2.67g) and tert-butylchlorodimethylsilane (4.72g) were sequentially added. The mixture was stirred at room temperature for 90min and was subsequently concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (300mL) and the solution was washed with water (2 x 100mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was purified on a silica gel column (eluant = ethyl acetate) to give 7.05g of (3R,4S)-N-cyclopropyl-4-(tert-butyldimethylsilyl)oxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine-3-carboxamide as a pale yellow tar-like product.

MS (EI) m/z: = 402 (M^+).

Step 3:

(3R,4S)-N-Cyclopropyl-4-(tert-butyldimethylsilyl)oxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine-3-carboxamide (7.00g) was dissolved in toluene (70mL). To this solution, borane-dimethyl sulfide complex (2.20mL) was added and the mixture was heat-refluxed for 5 hours while being stirred. Subsequently, the reaction mixture was allowed to cool to room temperature. Following addition of a 10% aqueous solution of sodium carbonate (42mL), the mixture was stirred at 100°C for 1 hour and the toluene

layer was separated. The toluene layer was washed with water (2 x 30mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1) to give 4.78g of (3S,4S)-4-(tert-butyldimethylsilyl)oxymethyl-3-cyclopropylaminomethyl-1-[(1S)-1-phenylethyl]pyrrolidine as a colorless oil.

Step 4:

(3S,4S)-4-(tert-Butyldimethylsilyl)oxymethyl-3-cyclopropylaminomethyl-1-[(1S)-1-phenylethyl]pyrrolidine (4.70g) was dissolved in dichloromethane (70mL). To this solution, di-tert-butyldicarbonate (2.77g) was added and the mixture was stirred at room temperature for 2 hours. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1, shifted to 1:1) to give 5.28g of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-(tert-butyldimethylsilyl)oxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine as a colorless oil.

Step 5:

Process (A): (3R,4S)-N-Cyclopropyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine-3-carboxamide (1.49g) was

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dissolved in toluene (15mL). To this solution, a borane-dimethyl sulfide complex (0.65mL) was added and the mixture was heat-refluxed for 6 hours while being stirred. Subsequently, the reaction mixture was allowed to cool to room temperature. Following addition of a 10% aqueous solution of sodium carbonate (12.4mL), the mixture was stirred at 100°C for 1 hour and the toluene layer was separated. The toluene layer was then washed with water (10mL) and was dried over anhydrous sodium sulfate. Following addition of di-tert-butyldicarbonate (1.13g), the mixture was stirred at room temperature for 30min and was subsequently allowed to stand overnight. The reaction mixture was then concentrated under reduced pressure and the resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 1:1) to give 1.50g of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine as pale brown crystal.

Process (B): (3R,4S)-3-[(N-tert-Butoxycarbonyl-N-cyclopropyl)amino]methyl-4-(tert-butyldimethylsilyl)oxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine (3.02g) was dissolved in tetrahydrofuran (45mL). While this solution was cooled on an ice bath, tetrabutylammonium fluoride (1mol/L tetrahydrofuran solution, 7.42ml) was added dropwise and the mixture was stirred at room temperature for 2 hours. Subsequently, a saturated aqueous solution of sodium chloride (60mL) was added

and the mixture was extracted with ethyl acetate (2 x 150mL). The ethyl acetate extracts were combined and the combined ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (2 x 100mL), followed by drying over anhydrous sodium sulfate and concentration under reduced pressure. The resulting residue was dissolved in ethyl acetate (10mL) and the formed crystal was collected by filtration, was washed with a small amount of ethyl acetate, and was then dried under reduced pressure to give 781mg of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine as a white crystal. The filtrate and the washing solution were combined and the combined organic layer was concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 1:1) to give additional 1.43g of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine. The total amount of the compound was 2.21g.

MS (EI) m/z: = 374 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{34}N_2O_3$: C 70.55, H 9.15, N 7.48; found: C 70.56, H 9.29, N 7.52.

Step 6:

(3R,4S)-3-[(N-tert-Butoxycarbonyl-N-

cyclopropyl)amino]methyl-4-hydroxymethyl-1-[(1S)-1-phenylethyl]pyrrolidine (2.66g) was dissolved in dichloromethane (40mL). While this solution was cooled on a sodium chloride/ice bath, triethylamine (1.05mL) was added. This was followed by dropwise addition of methanesulfonyl chloride (0.58mL). After being stirred at -5°C for 30min, the reaction mixture was washed with water, was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was dissolved in tetrahydrofuran (21mL). To this solution, tetrabutylammonium fluoride (1mol/L tetrahydrofuran solution, 21.3mL) was added and the mixture was heat-refluxed for 2 hours while being stirred. The reaction mixture was concentrated under reduced pressure and the resulting residue was dissolved in ethyl acetate (200mL). The ethyl acetate solution was washed with water (2 x 50mL), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = hexane: ethyl acetate = 4:1, shifted to 1:1) to give 1.13g of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethyl-1-[(1S)-1-phenylethyl]pyrrolidine as a pale brown tar-like product.

MS (EI) m/z = 376 (M^+).

Step 7:

(3R,4S)-3-[(N-tert-Butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethyl-1-[(1S)-1-phenylethyl]pyrrolidine (1.10g) was dissolved in methanol (20mL). To this solution, a suspension of 10% palladium carbon (230mg) in water (4mL) and ammonium formate (921mg) were sequentially added and the mixture was heat-refluxed for 90min while being stirred. Subsequently, the catalyst was removed from the reaction mixture by filtration through a Celite pad. The removed catalyst and the Celite pad were washed with ethanol containing 20% water. The filtrate and the washing solution were combined and the combined solution was concentrated under reduced pressure. Water (20mL) was then added to the resulting residue. While this mixture was cooled on an ice bath, a 30% aqueous solution of sodium hydroxide was added to make the mixture basic (pH14). The mixture was subsequently extracted with dichloromethane (50mL x 2). The dichloromethane extracts were combined, washed with water (2 x 20mL), and the combined dichloromethane layer was dried over anhydrous sodium sulfate and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (eluant = dichloromethane: methanol = 20:1) to give 684mg of (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethylpyrrolidine as a pale brown tar-like product.

MS (EI) m/z = 272 (M^+).

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Reference Example 15

Synthesis of (3R,4R)-3-cyclopropylaminomethyl-4-methylpyrrolidine·trifluoroacetate

Step 1:

1-Benzyl-4-(R)-methyl-3-(R)-[(4-(S)-phenyl-2-oxazolidinone-3-yl)carbonyl]pyrrolidine (150g) was dissolved in cyclopropylamine (650mL). The mixture was stirred at room temperature for 23 hours and was subsequently concentrated under reduced pressure. To the resulting residue, diisopropyl ether (800mL) was added and the mixture was stirred at room temperature for 70min. The resulting crystal was then collected by filtration. The collected crystal was then dissolved in dichloromethane (800mL) and the solution was extracted with 1mol/L hydrochloric acid (2 x 400mL). The 1mol/L hydrochloric acid extracts were combined. While the combined solution was cooled on an ice bath, a 30% aqueous solution of sodium hydroxide was added to make the solution basic (pH13). The resulting crystal was collected by filtration, was sequentially washed with water and diisopropyl ether, and was then dried under reduced pressure to give 52.2g of (3R,4R)-1-1-benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide as a white crystal.

Step 2:

(3R,4R)-1-Benzyl-N-cyclopropyl-4-methyl-3-pyrrolidinecarboxamide (70.0g) was dissolved in toluene (700mL). While this solution was cooled on an ice bath, a borane-dimethyl sulfide complex (90%, 34.3mL) was added dropwise. The mixture was then stirred for 15min and was heat-refluxed. After the reaction mixture was cooled to room temperature, a 10% aqueous solution of Na_2CO_3 (400mL) was added and the mixture was stirred at 100°C for 2 hours. The mixture was cooled to room temperature and the toluene layer was separated. The toluene layer was then washed with water (2 x 250mL), was dried over anhydrous sodium sulfate, and was concentrated under reduced pressure. The resulting residue was purified by distillation under reduced pressure to obtain (3S,4R)-1-benzyl-3-cyclopropylaminomethyl-4-methylpyrrolidine (62.1g) as a colorless oil.

Step 3:

(3S,4R)-1-Benzyl-3-cyclopropylaminomethyl-4-methylpyrrolidine (25.0g) was dissolved in ethanol (200mL). To this solution, trifluoroacetic acid (15.7mL) and 10% palladium carbon (12.5g) were added and the mixture was stirred at room temperature for 9 hours under a hydrogen pressure of 3.9×10^5Pa. The catalyst was collected from the reaction mixture by filtration and was washed with ethanol containing 25% water (300mL). The filtrate and the washing solution were combined

and the combined solution was concentrated under reduced pressure. The resultant pale brown crystal was suspended in tetrahydrofuran (100mL) and was collected by filtration. The collected crystal was washed with tetrahydrofuran and was dried under reduced pressure to give 34.1g of (3R,4R)-3-cyclopropylaminomethyl-4-methylpyrrolidine·trifluoroacetate as a white crystal.

Example 1

Synthesis of (3R)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

Process (A): [(3R)-9,10-Difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]difluoroboron (51.0g), 3(R)-cyclopropylaminomethylpyrrolidine (24.7g), triethylamine (24.6mL) and dimethylsulfoxide (500mL) were mixed with one another and the mixture was stirred at 70°C for 1 hour. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was purified on a silica gel column (eluant = dichloromethane: methanol = 10:1). The eluates were combined and the combined solution was concentrated under reduced pressure. To the resulting residue, 80% ethanol (2500mL) and triethylamine (25.0mL) were added and the mixture was heat-refluxed for 2 hours while being stirred.

Subsequently, the reaction mixture was left on an ice bath for 2 hours and the resulting crystal was collected by filtration. The collected crystal was washed with ethanol, was suspended in purified water (300ml), and was then collected by filtration. The collected crystal was dried under reduced pressure and was purified on a silica gel column (eluant = dichloromethane: methanol = 10:1). The eluates were combined and the combined solution was concentrated under reduced pressure. The resulting residue was dissolved in ethanol (2000mL) by heating and the solution was allowed to stand for 14 hours at room temperature. The resultant crystal was collected by filtration and the collected crystal was washed with ethanol and was dried under reduced pressure to give 27.7g of (3R)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow powder.

Process B: To a dichloromethane solution (273mL) of bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (22.6g), (3R)-3-cyclopropylaminomethylpyrrolidine (8.41g) and triethylamine (7.59g) were added and the mixture was allowed to stand at room temperature for 13 hours. Subsequently, the reaction mixture was sequentially washed with water (200mL) and a saturated aqueous solution of sodium

chloride (50ml), was dried over anhydrous sodium sulfate, and was then concentrated under reduced pressure. The resulting residue was purified on a silica gel column (dichloromethane: methanol = 15:1) to obtain a yellow amorphous product. To this product, a 5% aqueous solution of acetic acid (100mL) was added and the mixture was stirred at 80°C for 3 hours. Subsequently, the reaction mixture was washed with ethyl acetate (100mL). While the mixture was cooled on an ice bath, a 1mol/L aqueous solution of sodium hydroxide was added to adjust the pH to 7.01 and the mixture was further stirred for 0.5 hours. The resultant crystal was collected by filtration, was washed with purified water (2 x 50mL), and was then dissolved in ethanol (1200mL) by heating. The solution was allowed to stand at room temperature for 12 hours. Subsequently, the resulting crystal was collected by filtration, followed by washing with ethanol and drying under reduced pressure, to give 11.2g of (3R)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (EI)m/z: 419 (M^+).

Elementary analysis (%): Calcd for $C_{21}H_{23}F_2N_3O_4$: C 60.14, H 5.53, N 10.02; found: C 60.01, H 5.47, N 9.94.

Example 2

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Synthesis of (3R)-10-[(3R)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (982mg) was reacted with (3S)-3-cyclopropylaminomethylpyrrolidine (335mg) to give 587mg of (3R)-10-[(3R)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (FAB^+)m/z: 420 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{23}F_2N_3O_4 \cdot 0.25H_2O$: C 59.50, H 5.59, N 9.91; found: C 59.68, H 5.47, N 9.97.

Example 3

Synthesis of (3R)-10-[3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (513mg) was reacted with 3-cyclopropylaminomethylpyrrolidine (185mg)to give 231mg of (3R)-10-(3-cyclopropylaminomethyl-1-pyrrolidinyl)-9-fluoro-3-

fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (FAB^+)m/z: 420 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{23}F_2N_3O_4 \cdot 0.25H_2O$: C 59.50, H 5.59, N 9.91: found: C 59.41, H 5.41, N 9.89.

Example 4

Synthesis of (3S)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (790mg) was reacted with (3R)-3-cyclopropylaminomethylpyrrolidine (303mg) to give 602mg of (3S)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methoxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (FAB^+) m/z: 432 (MH^+).

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_5$: C 61.24, H 6.07, N 9.74; found: C 61.01, H 6.04, N 9.73.

Example 5

Synthesis of (3S)-3-acetoxymethyl-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-7-

oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-3-acetoxymethyl-9,10-difluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron(934mg) was reacted with (3R)-3-cyclopropylaminomethylpyrrolidine(337mg)to give 612mg of (3S)-3-acetoxymethyl-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (FAB^+) m/z: 460 (MH^+).

Elementary analysis (%): Calcd for $C_{23}H_{26}FN_3O_6 \cdot H_2O$: C 57.85, H 5.91, N 8.80; found: C 57.94, H 5.83, N 8.89.

Example 6

Synthesis of (3S)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-hydroxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

A 1mol/L aqueous solution of sodium hydroxide (8.0mL) containing (3S)-3-acetoxymethyl10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid (368mg) was stirred at 50°C for 2 hours. While this mixture was cooled on an ice bath, 1mol/L hydrochloric acid was added to adjust the pH to 7.05 and the mixture was further stirred for 0.5 hours. The resultant crystal was collected by

filtration, was washed with purified water, and was then dissolved in ethanol (50mL) by heating. The solution was allowed to stand at room temperature for 2 hours. Subsequently, the resulting crystal was collected by filtration, was washed ethanol, and was then dried under reduced pressure to give 251mg of (3S)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-hydroxymethyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (FAB^+) m/z: 418 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{24}FN_3O_5 \cdot 0.5H_2O$: C 59.15, H 5.91, N 9.85; found: C 59.16, H 5.92, N 9.88.

Example 7

Synthesis of 10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methylene-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

(3R)-10-[(3S)-3-Cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid (252mg) was suspended in ethanol (1mL). To this suspension, a 1mol/L aqueous solution of sodium hydroxide (6mL) was added and the mixture was stirred at room temperature for 2 hours. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was dissolved in purified water (10ml).

While this solution was cooled on an ice bath, 1mol/L hydrochloric acid was added to adjust the pH to 7.03 and the mixture was further stirred for 0.5 hours. The resultant crystal was collected by filtration to obtain 214mg of 10-[(3S)-cyclopropylmethylamino-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methylene-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow powder.

MS (FAB^+)m/z: 400 (MH^+).

Elementary analysis (%): Calcd for $C_{22}H_{23}FN_3O_4 \cdot 1.75H_2O$: C 58.53, H 5.96, N 9.75; found: C 58.62, H 5.79, N 9.76.

Example 8

Synthesis of (3R)-10-[(3S)-cyclopropylaminomethyl-1-pyrrolidinyl]-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-10-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (500mg) was reacted with (3R)-3-cyclopropylaminomethylpyrrolidine (240mg) to give 335mg of (3R)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow needle-shaped product.

MS (FAB^+) m/z: 402 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{24}FN_3O_4$: C 62.83, H 6.03, N 10.47; found: C 62.56, H 5.94, N 10.40.

Example 9

Synthesis of (3S)-10-[(3S)-cyclopropylaminomethyl-1-pyrrolidinyl]-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-10-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (1000mg) was reacted with (3R)-3-cyclopropylaminomethylpyrrolidine (431mg) to give 335mg of (3S)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow needle-shaped product.

MS (EI^+) m/z: 383 (M^+).

Elementary analysis (%): Calcd for $C_{21}H_{25}N_3O_4$: C 65.78, H 6.57, N 10.96; found: C; 65.58, H 6.61, N 10.91.

Example 10

Synthesis of (3S)-10-(trans-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-

7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (300mg) was reacted with trans-3-cyclopropylaminomethyl-4-methylpyrrolidine (136mg) to give 166mg of (3S)-10-(trans-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as yellow crystal.

MS (EI^+) m/z: 415 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_4 \cdot 0.5H_2O$: C 62.25, H 6.41, N 9.90; found: C 62.30, H 6.17, N 10.06.

Example 11

Synthesis of (3S)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (500mg) was reacted with (3R,4R)-3-cyclopropylamino-4-methylpyrrolidine (226mg) to give 362mg of (3S)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (EI^+) m/z:415 (M^+)

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_4$: C 63.60, H 6.31, N 10.11; found: C 63.41, H 6.30, N 10.17.

Example 12

Synthesis of (3S)-10-[(3R,4S)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (500mg) was reacted with (3S,4S)-3-cyclopropylamino-4-methylpyrrolidine (226mg) to give 276mg of (3S)-10-[(3R,4S)-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a pale yellow crystal.

MS (EI^+) m/z: 415 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_4 \cdot 0.5\ H_2O$: C 62.25, H 6.41, N 9.90; found: C 62.23, H 6.06, N 9.92.

Example 13

Synthesis of (3S)-10-(cis-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (220mg) was reacted with cis-3-cyclopropylaminomethyl-4-

methylpyrrolidine (100mg) to give 109mg of (3S)-10-(cis-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (EI^+) m/z: 415 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_4 \cdot H_2O$: C 60.96, H 6.51, N 9.69; found: C 61.27, H 6.69, N 9.52.

Example 14

Synthesis of (3S)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (1000mg) was reacted with (3R,4S)-cyclopropylamino-4-methylpyrrolidine (452mg) to give 474mg of (3S)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (EI^+) m/z: 415 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_4 \cdot 0.25H_2O$: C 62.92, H 6.36, N 10.01; found: C 62.69, H 6.52, N 9.98.

Example 15

Synthesis of (3S)-10-[(3R,4R)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (250mg) was reacted with (3S,4R)-3-cyclopropylamino-4-methylpyrrolidine (113mg) to give 33mg of (3S)-10-[(3R,4R)-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a pale yellow crystal.

MS (EI^+) m/z: 415 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{26}FN_3O_4 \cdot 0.5H_2O$: C 62.25, H 6.41, N 9.90; found: C 61.98, H 6.57, N 9.91.

Example 16

Synthesis of (3R)-10-(trans-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl)-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (300mg) was reacted with trans-3-cyclopropylaminomethyl-4-methylpyrrolidine (130mg) to give 110mg of (3R)-10-(trans-3-cyclopropylaminomethyl-4-methyl-1-

pyrrolidinyl)-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid.

MS (EI^+) m/z: 433 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{25}F_2N_3O_4$: C 60.96, H 5.81, N 9.69; found: C 60.81, H 5.85, N 9.66.

Example 17

Synthesis of (3R)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (1000mg) was reacted with (3R,4R)-cyclopropylamino-4-methylpyrrolidine (397mg) to give 620mg of (3R)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (EI^+) m/z: 433 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{25}F_2N_3O_4$: C 60.96, H 5.81, N 9.69; found: C 60.81, H 5.86, N 9.63.

Example 18

Synthesis of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-

oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (500mg) was reacted with (3R,4R)-cyclopropylamino-4-methylpyrrolidine (199mg) to give 422mg of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid as a yellow crystal.

MS (EI^+) m/z: 433 (M^+).

Elementary analysis (%): Calcd for $C_{22}H_{25}F_2N_3O_4$: C 60.96, H 5.81, N 9.69; found: C 60.79, H 5.91, N 9.77.

Example 19

Synthesis of (3S)-10-(trans-3-cyclopropylaminomethyl-4-trifluoromethyl-1-pyrrolidinyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (300mg) was reacted with trans-3-cyclopropylaminomethyl-4-trifluoromethylpyrrolidine (198mg) to give 87mg of (3S)-10-(trans-3-cyclopropylaminomethyl-4-trifluoromethyl-1-pyrrolidinyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid.

MS (FAB^+) m/z: 470 (MH^+).

Elementary analysis (%): Calcd for $C_{22}H_{23}F_4N_3O_4$: C 56.29, H 4.94, N 8.95; found: C 55.97, H 4.84, N 9.00.

Example 20

Synthesis of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (500mg) was reacted with (3R,4S)-3-cyclopropylaminomethyl-4-fluoropyrrolidine (204mg) to give 387mg of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid as a pale yellow crystal.

MS (FAB^+):m/z=438 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{22}F_3N_3O_4$: C 57.66, H 5.07, N 9.61; found: C 57.47, H 5.07, N 9.57.

Example 21

Synthesis of (3S)-10-[(3S,4S)-3-cyclopropylamino-4-fluoro-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3S)-9,10-difluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (64.6mg) was reacted with (3R,4S)-3-cyclopropylaminomethyl-4-fluoropyrrolidine (25.0mg) to give 18.5mg of (3R)-10-[(3S,4S)-3-cyclopropylamino-4-fluoro-1-pyrrolidinyl]-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid as a yellow powder.

MS (FAB^+) m/z=420 (MH^+).

HRMS (FAB^+): Calcd for $C_{21}H_{24}F_2N_3O_4$: 420.1735; found: 420.1747.

Example 22

Synthesis of (3R)-10-[(3S,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethyl-1-pyrrolidine]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid

Bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (912mg), (3R,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethylpyrrolidine (640mg), triethylamine (0.33mL) and acetonitrile (17mL) were mixed with one another and the mixture was stirred at 60°C for 90min. Subsequently, the reaction mixture was concentrated under reduced pressure and the resulting residue was purified on a silica gel column

(eluant = ethyl acetate: methanol = 20:1). To the eluate, a 5% aqueous solution of acetic acid (17mL) and ethanol (10mL) were added and the mixture was stirred at 80°C for 2 hours. The mixture was allowed to cool and the resulting crystal was collected by filtration, was washed with a mixture of water and ethanol, and was then dried under reduced pressure to give 915mg of (3R)-10-[(3S,4S)-3-[(N-tert-butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethyl-1-pyrrolidine]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido [1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid as a yellow powder.

MS (EI) m/z = 551 (M^+).

Elementary analysis (%): Calcd for $C_{27}H_{32}F_3N_3O_6 \cdot 0.5H_2O$: C 57.85, H 5.93, N 7.50; found: C 57.90, H 5.80, N 7.49.

Example 23

Synthesis of (3R)-10-[(3S,4S)-3-cyclopropyl]amino]methyl-4-fluoromethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid hydrochloride

(3R)-10-[(3S,4S)-3-[(N-tert-Butoxycarbonyl-N-cyclopropyl)amino]methyl-4-fluoromethyl-1-pyrrolidine]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid (860mg) was dissolved in ethanol (9mL) saturated with hydrogen chloride. The mixture was stirred at room temperature for 1 hour and was

subsequently concentrated under reduced pressure. To the resulting residue, ethanol (50mL) was added and the mixture was concentrated under reduced pressure. After repeating the ethanol addition and concentration once, ethanol (50mL) was added to the resultant residue, and the mixture was heated to 70°C and was then allowed to stand at room temperature for 1 hour. The resulting crystal was collected by filtration, followed by washing with ethanol and drying under reduced pressure, to give 762mg of (3R)-10-[(3S,4S)-3-cyclopropyl]amino]methyl-4-fluoromethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid hydrochloride as a yellow crystal.

MS (FAB^+): m/z=452 (MH^+).

Elementary analysis (%): Calcd for $C_{22}H_{24}F_3N_3O_4 \cdot HCl \cdot H_2O \cdot 0.5C_2H_5OH$: C 52.23, H 5.72, N 7.94; found: C 52.17, H 5.38, N 8.20.

Example 24

Synthesis of (3R)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid

In a similar manner to Process (B) in Example 1, bis(acetato-O)[(3R)-9,10-difluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylato-O^6,O^7]boron (100mg) was reacted with (3R,4R)-3-

cyclopropylaminomethyl-4-fluoropyrrolidine (40.6mg) to give 71.0mg of (3R)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid as a pale yellow crystal.

MS (FAB^+): m/z=438 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{22}F_3N_3O_4$: C 57.66, H 5.07, N 9.61; found: C 57.50, H 5.18, N 9.22.

Example 25

Synthesis of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid methanesulfonate

(3R)-10-[(3S,4S)-3-Cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid (50.0mg) was suspended in ethanol (2mL). To this suspension, methanesulfonic acid (15.0μL) was added and the mixture was stirred at room temperature for 1 hour. The resulting crystal was collected by filtration, was washed with ethanol, and was then dried under reduced pressure to give 50.4mg of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid methanesulfonate as a

pale yellow crystal.

MS (FAB^+): m/z = 438 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{22}F_3N_3O_4 \cdot CH_3SO_3H \cdot 0.25H_2O$: C 49.11, H 4.96, N 7.81; found: C 49.18, H 4.86, N 7.42.

Example 26

Synthesis of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid hydrochloride

(3R)-10-[(3S,4S)-3-Cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid (50.0mg) was suspended in ethanol (2mL). To this suspension, ethanol (60.0μL) saturated with hydrogen chloride was added and the mixture was stirred at room temperature for 1 hour. The resulting crystal was collected by filtration, was washed with ethanol, and was then dried under reduced pressure to give 52.9mg of (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d.e][1,4]benzoxazine-6-carboxylic acid hydrochloride as a pale yellow crystal.

MS (FAB^+): m/z=438 (MH^+).

Elementary analysis (%): Calcd for $C_{21}H_{22}F_3N_3O_4 \cdot HCl \cdot 0.25H_2O$: C 52.73, H 4.95, N 8.78; found: C 52.68, H 5.04, N 8.28.

(Antibacterial activity)

Test Example 1: *in vitro* antibacterial activity

The *in vitro* antibacterial activity (as measured by the minimum inhibitory concentration (MIC)) was determined for each of the compounds of the present invention by the agar dilution method according to NCCLS (National Committee for Clinical Laboratory Standard (1997), Methods for Dilution Antibacterial Susceptibility Tests for Bacteria that grow Aerobically-Forth Edition: Approved Standard m7-A4. NCCLS, Villanova, Pa.), which involved the use of Muller-Hinton agar medium. For pneumococci and enterococci, MIC was determined by using Muller-Hinton agar medium containing 5% defibrinated equine blood. The results are shown in Table 1 below.

Table 1: *in vitro* antibacterial activity

Strain	MIC (mg/mL)				
	Example 1	Example 2	Example 4	Example 5	Example 6
S. aureus Smith	0.016	0.031	0.031	0.25	0.25
S. aureus MR5867	0.016	0.016	0.031	0.25	0.25
S. aureus MS16401	0.125	0.25	0.5	4	4
S. pneumoniae Type III	0.032	0.125	0.125	0.125	0.125
E. faecalis IID682	0.063	0.25	0.25	0.25	0.25

Strain	MIC (mg/mL)				
	Example 7	Example 10	Example 11	Example 12	Example 13
S. aureus Smith	≤0.008	0.016	0.008	0.016	0.008
S. aureus MR5867	≤0.008	0.016	0.008	0.016	0.008
S. aureus MS16401	0.063	0.063	0.031	0.063	0.031
S. pneumoniae Type III	0.016	0.031	0.016	0.063	0.031
E. faecalis IID682	0.125	0.125	0.063	0.25	0.125

Strain	MIC (mg/mL)				
	Example 14	Example 15	Example 16	Example 17	Example 18
S. aureus Smith	0.008	0.031	0.016	0.008	0.008
S. aureus MR5867	0.008	0.031	0.016	0.008	0.016
S. aureus MS16401	0.031	0.063	0.063	0.063	0.063
S. pneumoniae Type III	≤0.008	0.063	0.032	0.016	0.016
E. faecalis IID682	0.063	0.125	0.063	0.063	0.063

Strain	MIC (mg/mL)			
	Example 19	Example 20	Example 21	Ciprofloxacin
S. aureus Smith	0.016	0.008	0.016	0.25
S. aureus MR5867	0.016	0.016	0.016	0.25
S. aureus MS16401	0.125	0.063	0.031	8
S. pneumoniae Type III	0.125	0.016	0.016	0.5
E. faecalis IID682	0.25	0.063	0.063	0.5

S. aureus MR5867: methicillin-resistant *S. aureus*
S. aureus MS16401: quinolone-resistant *S. aureus*

INDUSTRIAL APPLICABILITY

The novel 10-(3-cyclopropylaminomethyl-1-pyrrolidinyl)pyridobenzoxazine carboxylic acid derivatives, salts and hydrates thereof, which are compounds of the present invention, are not only safe and exhibit strong antibacterial activities, but they are also effective against drug-resistant bacteria that are less susceptible to conventional antibacterial agents.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

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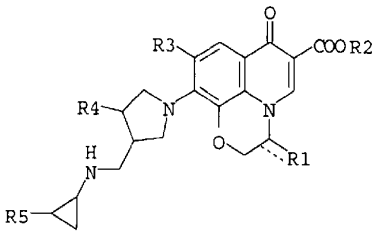
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pyridobenzoxazine carboxylic acid derivative as represented by the following general formula (I), or a salt or a hydrate thereof:



I

wherein R1 is a fluoromethyl group; R2 is a hydrogen atom, a lower alkyl group having 1 to 3 carbon atoms, or a pharmaceutically acceptable cation and an ester of a prodrug; R3 is a hydrogen atom or a halogen atom; R4 is a hydrogen atom, a lower alkyl group having 1 to 3 carbon atoms, a fluoromethyl group, a trifluoromethyl group or a fluorine atom; and R5 is a hydrogen atom or a fluorine atom.

2. The compound according to claim 1, a salt or a hydrate thereof, wherein in the general formula (I), R3 is a fluorine atom.

3. The compound according to claim 1 or 2, a salt or a hydrate thereof, wherein in the general formula (I), R3 is a fluorine atom, and R4 is a hydrogen atom, a methyl group, a fluoromethyl group or a fluorine atom.

4. The compound according to any one of claims 1 to 3, a salt or a hydrate thereof, wherein the compound of the general formula (I) has a single stereochemistry.

5. The compound according to claim 1, a salt or a hydrate thereof, wherein the compound of the general formula (I) is (3R)-10-[(3S,4R)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid, a salt or a hydrate thereof.

6. The compound according to claim 1, a salt or a hydrate thereof, wherein the compound of the general formula (I) is (3R)-10-[(3S,4S)-3-cyclopropylaminomethyl-4-methyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid, a salt or a hydrate thereof.

7. The compound according to claim 1, a salt or a hydrate thereof, wherein the compound of the general formula (I) is (3R)-10-[(3S)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid, a salt or a hydrate thereof.

8. The compound according to claim 1, a salt or a hydrate thereof, wherein the compound of the general formula (I) is (3R)-10-[(3R)-3-cyclopropylaminomethyl-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid, a salt or a hydrate thereof.

9. The compound according to claim 1, a salt or a hydrate thereof, wherein the compound of the general formula (I) is (3R)-10-[(3S,4R)-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid, a salt or a hydrate thereof.

10. The compound according to claim 1, a salt or a hydrate thereof, wherein the compound of the general formula (I) is (3R)-10-[(3S,4S)-cyclopropylaminomethyl-4-fluoro-1-pyrrolidinyl]-9-fluoro-3-fluoromethyl-2,3-dihydro-7-oxo-7H-pyrido[1,2,3-d,e][1,4]benzoxazine-6-carboxylic acid, a salt or a hydrate thereof.

11. An antibacterial agent containing as an active ingredient the compound according to any one of claims 1 to 11, a salt or a hydrate thereof.

12. A pharmaceutical composition comprising the compound according to any one of claims 1 to 11, a salt or a hydrate thereof and a pharmaceutically acceptable carrier.

13. A method of treating a bacterial infection comprising administering an effective amount of the compound according to any one of claims 1 to 11, a salt or a hydrate thereof to a subject in need thereof.

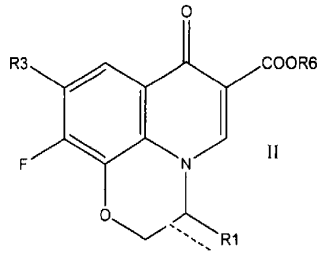
14. Use of the compound according to any one of claims 1 to 11, a salt or a hydrate thereof in the manufacture of a medicament for treating a bacterial infection.

15. Use of the compound according to any one of claims 1 to 11, a salt or a hydrate thereof for treating a bacterial infection.

16. The method according to claim 14 or the use according to claim 15 or 16, wherein the bacterial infection is a gram-positive bacterial infection.

17. The method according to claim 14 or the use according to claim 15 or 16, wherein the bacterial infection is drug-resistant bacterial infection.

18. A process for the production of the compound according to any one of claims 1 to 11, a salt or hydrate thereof comprising reacting a compound represented by the following general formula (II):



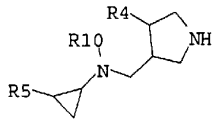
II

[wherein R1 and R3 are the same as in the claim 1; and R6 is represented by the following general formula (III):

III

[wherein R7 and R8 are each independently a fluorine atom, or a lower alkylcarbonyloxy group]

with a compound represented by the following general formula (IV), or an acid addition salt thereof:



IV

[wherein R4 and R5 are the same as in claim 1; and R10 is a hydrogen atom or a protective group of nitrogen atom such as t-butoxycarbonyl]

and then removing the boron chelate and, if necessary, the

protective group from the nitrogen atom.

19. Pyridobenzoxazine carboxylic acid derivatives as represented by the general formula (I), processes for their production, antibacterial agents or pharmaceutical compositions containing them or methods or uses involving them, substantially as herein described with reference to the accompanying examples.