dimethylacetamide; nitriles such as acetonitrile and propionitrile; halogenated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, and 1,2-dichloroethane; and dimethylsulfoxide. One of these solvents may be used, or two or more of the solvents may be used as a mixture.

In particular, preferred examples of the crystallization solvent include polar solvents, for example, alcohols such as methanol, ethanol, *n*-propyl alcohol, isopropyl alcohol, *n*-butanol, and *tert*-butanol; ketones such as acetone, 2-butanone, and methylisobutyl ketone; esters such as methyl acetate, ethyl acetate, and isopropyl acetate; amides such as *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetamide; nitriles such as acetonitrile and propionitrile; ~~amides such as *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetamide; nitriles such as acetonitrile and propionitrile;~~ and dimethylsulfoxide; since these show high solubility and high purification efficiency. Among these, alcohols such as methanol, ethanol, *n*-propyl alcohol, isopropyl alcohol, *n*-butanol, and *tert*-butanol; ketones such as acetone, 2-butanone, and methylisobutyl ketone; esters such as methyl acetate, ethyl acetate, and isopropyl acetate; and nitriles such as acetonitrile and propionitrile; are more preferred since these have low boiling points, and can be easily removed by vacuum drying.

For improvement of the recovery, it is preferred to add, as a poor solvent(s), one or more of low-polarity solvents to these polar solvents. Examples of the low-polarity solvents include aromatic hydrocarbons such as benzene, toluene, xylene, chlorobenzene, and trifluoromethylbenzene; and aliphatic hydrocarbons such as *n*-pentane, *n*-hexane, *n*-heptane, and cyclohexane.

[0167]

<Step (vii)>

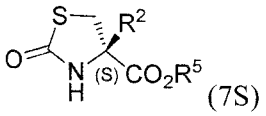
The step (vii) is described below.

The step (vii) is a step of allowing an acid or a base to act on a compound represented by General Formula (7-2):

D-cysteine represented by the General Formula (1S) or the salt thereof.

[0188]

In other words, by allowing the reaction to proceed through the step (vi-1), elimination of the *tert*-butyl group, which generally requires a long time, can be allowed to proceed quickly, and the compound represented by General Formula (7S-1) can be obtained in a short time. Since the compound represented by General Formula (7S-2) obtained in the subsequent step (vi-2) has high crystallinity, and low solubility in water at a pH of not more than the point of neutralization and in nonpolar solvents, both water-soluble impurities including inorganic salts and water-insoluble impurities including reaction intermediates can be easily removed by crystallization. Therefore, after the subsequent step (vii), the α-substituted-D-cysteine represented by General Formula (1S) or the salt thereof can be obtained with high purity. Moreover, since the compound represented by General Formula (7S-2) has higher crystallinity than the racemic body of the compound, its optical purity can be easily increased by crystallization. Therefore, the compound represented by the General Formula (7S) below:



(7S)

(wherein $\underline{R^2}$~~R2~~ has the same meaning as in General Formula (6); and $\underline{R^5}$~~R5~~ represents a hydrogen atom, or has the same meaning as $\underline{R^1}$~~R1~~ in the General Formula (6S)) can be said to be an important intermediate for producing a high-quality α-substituted cysteine represented by General Formula (1S) or a salt thereof in a short time.

Here, General Formula (7S) includes General Formula (7S-1) and General Formula (7S-2).

[0189]

Specific examples of the compound represented by the General Formula (7S) include the following compounds.

is contained in alkali metal hydrides, is not required. Potassium *tert*-butoxide is more preferred since it is highly soluble in organic solvents and has low nucleophilicity.

[0217]

The amount of the base may be appropriately set within the range of, for example, 0.7 molar equivalent to 10 molar equivalents with respect to the amount of the compound represented by General Formula (8). In order to quantitatively obtain the compound represented by General Formula (2), the amount is preferably 0.9 molar equivalent to 5 molar equivalents. From the viewpoint of the cost and reduction of side reactions, the amount is more preferably 0.9 molar equivalent to 1.5 molar equivalents.

[0218]

In this step, a reaction solvent may be used. Examples of the reaction solvent include aromatic hydrocarbons such as benzene, toluene, xylene, chlorobenzene, and trifluoromethylbenzene; aliphatic hydrocarbons such as *n*-pentane, *n*-hexane, *n*-heptane, and cyclohexane; ethers such as diethyl ether, di-*n*-propyl ether, di-*n*-butyl ether, methyl isopropyl ether, methyl-*tert*-butyl ether, ethyl-*tert*-butyl ether, cyclopentyl methyl ether, tetrahydrofuran, dioxane, and 1,2-dimethoxyethane; amides such as *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetamide; halogenated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, and 1,2-dichloroethane; and dimethylsulfoxide.

Among these, aprotic polar solvents such as tetrahydrofuran, dioxane, 1,2-dimethoxyethane, *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide~~-dimethylsulfoxide~~, and dimethylsulfoxide are preferred since the alkaline metal salt produced by the reaction between the compound represented by General Formula (8) and the base is highly soluble in these solvents, and the solvents therefore have an effect to promote the reaction. Tetrahydrofuran is more preferred since it is

diethyl ether, di-*n*-propyl ether, diisopropyl ether, di-*n*-butyl ether, methyl isopropyl ether, methyl-*tert*-butyl ether, ethyl-*tert*-butyl ether, and cyclopentyl methyl ether; and halogenated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, and 1,2-dichloroethane.

Among these, *n*-pentane, *n*-hexane, *n*-heptane, cyclohexane, diethyl ether, di-*n*-propyl ether, diisopropyl ether, di-*n*-butyl ether, methyl isopropyl ether, methyl-*tert*-butyl ether, ethyl-*tert*-butyl ether, and dichloromethane are preferred since these have low boiling points, and show high removal efficiencies. Diethyl ether, di-*n*-propyl ether, diisopropyl ether, di-*n*-butyl ether, methyl isopropyl ether, methyl-*tert*-butyl ether, and ethyl-*tert*-butyl ether are more preferred since they show high extraction efficiencies.

[0240]

[Novel Compounds]

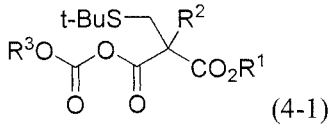
By the present invention, the following novel compounds can be provided.

The novel compounds of the present invention can be produced by the production methods of the present invention, and can also be synthesized using ordinary techniques of organic chemistry.

Specific examples of $\underline{R^1}$~~R1~~, $\underline{R^2}$~~R2~~, and $\underline{R^3}$~~R3~~ in the following General Formulae (4-1), (5), (6), and (7S), and specific examples of the compounds represented by General Formulae (4-1), (5), (6), and (7S) are the same as those described in the [Method for Producing α-Substituted Cysteine Represented by General Formula (1) or Salt Thereof] and the [Method for Producing α-Substituted Cysteine Represented by General Formula (1S) or Salt Thereof] mentioned above.

<Compound Represented by General Formula (4-1)>

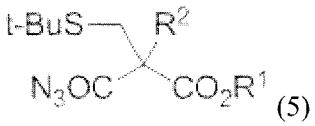
A compound represented by General Formula (4-1):


(4-1)

(wherein R¹R1 and R³R3 each independently represent a C_1-C_{10} alkyl group which is optionally substituted, a C_7-C_{20} aralkyl group which is optionally substituted, or a C_6-C_{12} aryl group which is optionally substituted, and R²R2 represents a C_1-C_4 alkyl group).

<Compound Represented by General Formula (5)>

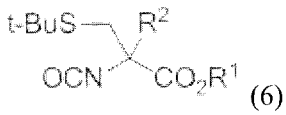
A compound represented by General Formula (5):


(5)

(wherein R¹R1 represents a C_1-C_{10} alkyl group which is optionally substituted, a C_7-C_{20} aralkyl group which is optionally substituted, or a C_6-C_{12} aryl group which is optionally substituted, and R²R2 represents a C_1-C_4 alkyl group).

<Compound Represented by General Formula (6)>

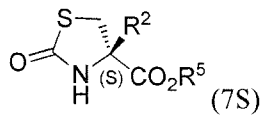
A compound represented by General Formula (6):


(6)

(wherein R¹R1 represents a C_1-C_{10} alkyl group which is optionally substituted, a C_7-C_{20} aralkyl group which is optionally substituted, or a C_6-C_{12} aryl group which is optionally substituted, and R²R2 represents a C_1-C_4 alkyl group).

<Compound Represented by General Formula (7S)>

A compound represented by General Formula (7S):


(7S)

(wherein R²R2 represents a C_1-C_4 alkyl group, and R⁵R5 represents a hydrogen atom, or a C_1-C_{10} alkyl group which is optionally substituted, a C_7-C_{20} aralkyl group which is

by BLAST. As a result, no homology was found.

[0254]

[Table 1]

Amino acid sequence of hydrolase	SEQ ID NO	Homology to *Bacillus subtilis* IFO3108-derived carboxyesterase NP (SEQ ID NO:4)
CENP-1	4	100%
CENP-2	6	99%
CENP-3	8	99%
CENP-4	10	99%
CENP-5	12	99%
CENP-6	14	99%
CENP-7	16	99%
CENP-8	18	99%

[0255]

[Example 12] Screening for Hydrolase for Step (i)

In an aqueous solution containing 100 mmol/L potassium phosphate, cells of each of the eight kinds of bacteria obtained in Example 11 and 30 g/L diethyl 2-[(*tert*-butylthio)methyl]-2-methylmalonate were mixed, and the reaction was allowed to proceed at 30°C at pH 7 overnight with shaking. *Bacillus licheniformis*-derived protease (manufactured by Sigma-Aldrich) was also allowed to react under the same conditions overnight with shaking.

To 200 μL of each solution after the reaction, 100 μL of 1 mol/L hydrochloric acid was added to stop the reaction, and the reaction solution was then centrifuged at 10,000 rpm. After mixing 50 μL of the supernatant obtained after the centrifugation with 50 vol% acetonitrile and 950 μL of 50 mmol/L trifluoroacetic acid solution, the optical purity was analyzed under the conditions of <HPLC-2>.

Bacterial cells of each of commercially available enzymes, *Bacillus*

3.02 (1H, d, J = 11.9 Hz), 3.08 (1H, d, J = 11.9 Hz), 4.24 (2H, q, J = 7.1 Hz)

[0260]

[Example 16] Step (iv): Production of Ethyl (*S*)-2-[(*tert*-Butylthio)methyl]-2-isocyanatopropanoate

Under nitrogen gas flow, 45 mL of toluene was placed in a 500-mL four-necked flask, and heated to 85°C. To the flask, the solution containing 23.3 g (85.3 mmol) of ethyl (*S*)-2(azidocarbonyl)~~3-(azidocarboxy)~~-2-[(*tert*-butylthio)methyl]propanoate in toluene produced in Example 15 was added dropwise at an inner temperature within the range of 84°C to 91°C for 4 hours. Thereafter, the resulting mixture was stirred at 85°C for 1 hour, to obtain a solution of ethyl (*S*)-2-[(*tert*-butylthio)methyl]-2-isocyanatopropanoate in toluene. As a result of ^{1}H-NMR analysis, the amount of ethyl (*S*)-2-[(*tert*-butylthio)methyl]-2-isocyanatopropanoate contained in this solution in toluene was found to be 21.5 g (87.6 mmol; yield from ethyl (*S*)-2-[(*tert*-butylthio)methyl]-2-methylmalonate, 97.2%). The measurement results obtained were as follows.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, t, J = 7.1 Hz), 1.30 (9H, s), 1.52 (3H, s), 2.78 (1H, d, J = 12.1 Hz), 2.98 (1H, d, J = 12.1 Hz), 4.23 (2H, q, J = 7.1 Hz)

[0261]

[Example 17] Step (vi-1) and Step (vi-2): Production of Ethyl (*S*)-4-Methyl-2-oxo-1,3-thiazolidine-4-carboxylate and (*S*)-4-Methyl-2-oxo-1,3-thiazolidine-4-carboxylic Acid

Under nitrogen gas flow, 39.2 g of a solution containing 11.2 g (45.6 mmol) of ethyl (*S*)-2-[(*tert*-butylthio)methyl]-2-isocyanatopropanoate in toluene was placed in a 200-mL four-necked flask. To the flask, 9.19 g (88.4 mmol) of 35 wt% hydrochloric acid was added, and the resulting mixture was stirred at 25°C for 45 minutes. As a result of ^{1}H-NMR analysis of the toluene layer of this reaction solution, the layer was found to contain ethyl (*S*)-4-methyl-2-oxo-1,3-thiazolidine-4-

carboxylate.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, J = 7.1 Hz), 1.60 (3H, s), 3.28 (1H, d, J = 11.4 Hz), 3.80 (1H, d, J = 11.4 Hz), 4.26 (2H, q, J = 7.1 Hz)

Subsequently, the inner temperature of this reaction solution was increased to 60°C to 70°C, and the reaction solution was then stirred for additional 1 hour. After removing toluene by distillation, 22.6 g of water and 2.40 g (23.0 mmol) of 35 wt% hydrochloric acid were added to the solution, and the resulting mixture was stirred at an inner temperature within the range of 70°C to 90°C for 4 hours. After allowing the reaction solution to cool to room temperature, precipitated crystals were collected by filtration. The obtained crystals were washed with 23 mL of toluene and 23 mL of water, to obtain 5.97 g of white crystals. As a result of HPLC analysis under the conditions of <HPLC-3>, the white crystals were found to contain 5.56 g (34.5 mol; yield, 75.7%) of (*S*)-4-methyl-2-oxo-1,3-thiazolidine-4-carboxylic acid. The measurement results on the compound obtained were as follows.

^{1}H-NMR (400 MHz, D_2O) δ 1.52 (3H, s), 3.40 (1H, d, J = 11.6 Hz), 3.69 (1H, d, J = 11.6 Hz)

[0262]

[Example 18] Step (vi-1) and Step (vi-2): Production of Ethyl (*S*)-4-Methyl-2-oxo-1,3-thiazolidine-4-carboxylate and (*S*)-4-Methyl-2-oxo-1,3-thiazolidine-4-carboxylic Acid~~: Production of (*S*)-4-Methyl-2-oxo-1,3-thiazolidine-4-carboxylic Acid~~

Under nitrogen gas flow, 4.62 g of a solution containing 1.33 g (5.4 mmol) of ethyl (*S*)-2-[(*tert*-butylthio)methyl]-2-isocyanatopropanoate in toluene was placed in a 50-mL test tube. To the test tube, 1.52 g (5.4 mmol) of *p*-toluenesulfonic acid monohydrate was added, and the resulting mixture was stirred at room temperature for 60 minutes, followed by adding 8 ml (16.0 mmol) of 2 mol/L aqueous sodium hydroxide solution thereto at the same temperature. After stirring the resulting mixture for 1.5 hours, the toluene layer was removed by separation. To the obtained