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Enantioselective synthesis of D- and L-α-methylcysteine with hydantoinase

Takahiro Ohishi,[a,*] Hirokazu Nanba,[b] Masanobu Sugawara,[b] Masashi Izumida,[b] Tatsuya Honda,[b] Kohei Mori,[b] Satohiro Yanagisawa,[b] Makoto Ueda,[b] Nobuo Nagashima[b] and Kenji Inoue[b]

[a]*Frontier Materials Development Laboratories, Frontier Materials R&D Center, Corporate Research and Development Division, KANEKA Corporation, 5-1-1, Torikai-Nishi, Settsu, Osaka 566-0072, Japan*
[b]*Fine Chemicals Research Laboratories, Fine Chemicals Division, KANEKA Corporation, 1-8, Miyamae-machi, Takasago-cho, Takasago, Hyogo 676-8688, Japan*

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Abstract—A scalable and cost-effective synthesis of D- and L-α-methylcysteine is described. A key step is D-selective cyclization of *N*-carbamoyl *S*-*tert*-butyl-D,L-α-methylcysteine catalyzed by hydantoinase. D-5-*tert*-Butylthiomethyl-5-methylhydantoin and *N*-carbamoyl *S*-*tert*-butyl-L-α-methylcysteine were obtained with excellent yield and optical purity, and these compounds were easily separated by filtration. After hydrolysis and cleavage of the *tert*-butyl group, D- and L-α-methylcysteine hydrochloride were obtained.

Optically pure α,α-disubstituted amino acids have played a special role in the design of peptides. They severely restrict rotation around the N–C^{α}(φ) and C^{α}–C(O)(ψ) bonds of the amino acid and stabilize preferred conformations of the peptide backbone. Among them, α-methylcysteine is an attractive target molecule because it can form a constrained cyclic peptide via disulfide bridge formation. In addition, α-methylcysteine is a valuable building block for the synthesis of a number of natural products such as mirabazoles A–C,[1a–e] tantazoles A–F,[1f,g] and thiangazole.[1h,i] Several members of these unique thiazoline/thiazole-containing alkaloids have been shown to exhibit unusual high inhibitory against HIV-1 protease in vitro.[2]

Because of the difficulty of constructing tetrasubstituted α-carbon in optically pure form, asymmetric synthesis of α-methylcysteine has been rather limited. The first reported synthesis of an optically pure α-methylcysteine derivative used L-valine as a chiral auxiliary for asymmetric alkylation of chiral bis-lactim ether.[3] More recently, Singh et al. reported a method of asymmetric methylation to 2-phenylthiazoline using camphorsultam as a chiral auxiliary.[4] Two other methods utilize Seebach's 'self-reproduction of chirality' approach to stereoselectively thiomethylate oxazolidinone derived from alanine[1c] or to methylate a thiazoline derivative of cysteine.[5,6] Since these strategies based on asymmetric alkylation require expensive bases such as butyl lithium and must be carried out under strict conditions at −78 °C, their application to industrial processes for the mass production of optically pure α-methylcysteine might not be straightforward.

Some completely different approaches have been reported. One is a method using the regioselective ring opening of chiral aziridine[7] or β-lactone[8,9] with a thiolate nucleophile. Kedrowski synthesized α-methylcysteine from dimethyl 2-methylmalonate via enzymatic resolution followed by Curtius rearrangement.[10] These methods involve some difficult-to-secure and/or hazardous reagents for industrial scale production, such as azide compounds, Sharpless catalyst, and PLE. Asano and co-workers reported L-stereoselective hydrolysis of racemic α-methylcysteinamide by amidase from *Xanthobacter flavus* NR303 strain.[11] However, no other practical and efficient process for α-methylcysteine production using enzymatic resolution is known.

Keywords: Enantioselective synthesis; α-Methylcysteine; Hydantoinase; *tert*-Butyl group; Scalable process.
* Corresponding author. Tel.: +81 72 653 8227; fax: +81 72 653 8291; e-mail: takahiro_ohishi@kn.kaneka.co.jp

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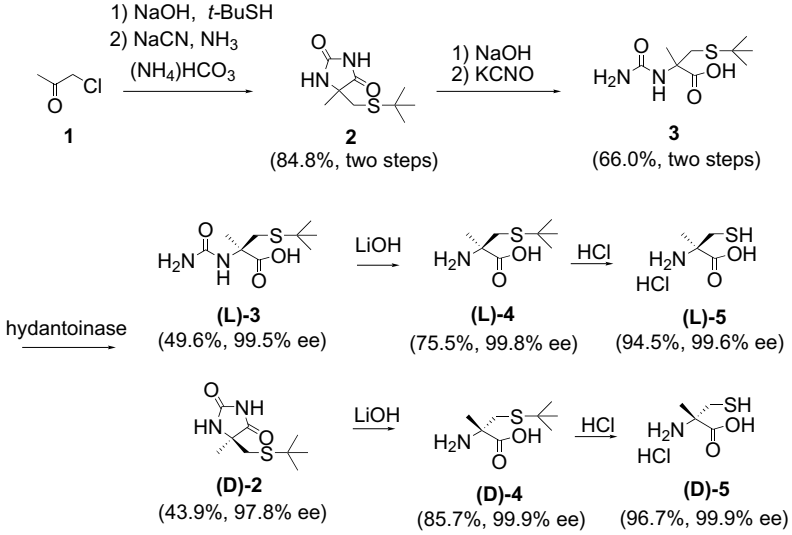
Enzymatic production of optically pure amino acid from 5-substituted hydantoin, called the hydantoinase process, is one of the most practical, scalable and environmentally benign methods that are applicable for this purpose. The process was first introduced in the 1970s for the production of D-amino acids such as D-phenylglycine and D-*p*-OH-phenylglycine.[12a–c] We screened several routes using the hydantoinase process to synthesize optically pure α-methylcysteine, and found that hydantoinase catalyzed D-stereoselective cyclization of *N*-carbamoyl *S*-*tert*-butyl-D,L-α-methylcysteine derivative **3** (Scheme 1). Fortunately, the reaction gave (D)-**2** and (L)-**3** with high optical purity and excellent yields. Herein we wish to report a short, cost-effective and scalable method of synthesizing optically pure α-methylcysteine using the hydantoinase process.

Hydantoin (**2**) was easily prepared from chloroacetone (**1**) by a previously described procedure.[13] This compound was then hydrolyzed by 10 wt % aqueous NaOH solution and *N*-carbamoylated with KCNO to give *N*-carbamoyl D,L-α-methylcysteine derivative **3**. The hydrolysis and N-carbamoylation were carried out in one pot.

The next step is enzymatic resolution of **3** with hydantoinase. It is worth noting that our process uses the enantioselective cyclization of *N*-carbamoyl amino acid (**3**) (the reverse reaction). The hydantoinase process generally uses enantioselective hydrolysis of hydantoin (the normal reaction).[12a–c] We first investigated the hydrolysis of **2**, but the reaction gave (D)-**3** in very low yield; otherwise no desired product was detected because of the low solubility of **2** in an aqueous medium. On the other hand, **3** has good solubility in aqueous NaOH (pH of the resulting solution >6), a fact which encouraged us to examine the reverse reaction. It is known that the hydantoinase reaction (hydrolysis of hydantoin) is reversible, but only a few examples of an enantioselective reverse reaction have been reported.[14] To our surprise, the reverse reaction of **3** catalyzed by hydantoinase gave (L)-**3** and (D)-**2** with excellent selectivity ((D)-**2**: 97.8% ee, (L)-**3**: 99.5% ee) and yield ((D)-**2**: 43.9%, (L)-**3**: 49.6%).

Compound **3** was treated with an immobilized hydantoinase in an aqueous medium, stirred at 40 °C, and kept a pH at 6.5. Immobilized hydantoinase was prepared from *Bacillus* sp. KNK245 strain according to the culture method and the method for enzyme immobilization in a patent application.[15] The difference of solubility in the reaction solution between **3** and **2** gave our process an advantage for scale-up. During the reaction, (D)-**2** appeared as a white precipitate and was easily separated from (L)-**3** by filtration. The filtrate was acidified to give (L)-**3** as white crystals. We prepared multigram quantities of (L)-**3** and (D)-**2** to demonstrate the possibility of our method for large scale production.[16] (L)-**3** was dissolved in aqueous LiOH solution and refluxed for 41 h. After cooling to room temperature, the insoluble substance was filtered off and the filtrate was neutralized to give (L)-**4** as white crystals (75.5%, 99.8% ee). The mixture of (D)-**2** and the enzyme was dissolved in aqueous LiOH solution. The immobilized hydantoinase was removed by filtration, and the resultant mixture was refluxed for hydrolysis of (D)-**2**. After the mixture was cooled to room temperature, it was neutralized and cooled to give (D)-**4** as white crystals (85.7%, 99.9% ee). The optical purity of (D)-**2** and (L)-**3** were increased by crystallization of (D)-**4** and (L)-**4**.

Finally, the *tert*-butyl group was cleaved from **4** to give **5**. Generally, alkyl thioethers are difficult to cleave and only a few methods for the deprotection of *S*-*tert*-butyl-cysteine derivatives are known. Yajima et al. reported that the combination of a hard acid (TMSOTf[17,18] or TMSBr[19]) and a soft nucleophile such as thioanisole has the ability to cleave the *tert*-butyl group of cysteine derivatives. They also reported that $Tl(OTf)_3$ in TFA can cleave various S-protecting groups



Scheme 1.

of cysteine.[17,20] Kiso and co-workers used HBF_4 as a useful deprotecting reagent for *S*-*tert*-butyl-cysteine derivatives.[21] None of the methods described above are practical for production on an industrial scale, because they use expensive and/or hazardous reagents. So, we investigated many reagents and conditions for the deprotection of the *tert*-butyl group of **4**. As a result, fortunately, we found that *tert*-butyl group was cleaved efficiently simply by heating with concd HCl.[22]

Thus, **4** was dissolved in concd HCl, and the resulting solution was refluxed for 45 h. After the reaction mixture was cooled to room temperature, it was concentrated and the remaining water was removed by azeotropic distillation with toluene to give **5** as white crystals.

In order to determine the optical purity, **5** was converted to Cbz-α-MeCys(Cbz)–OH and analyzed by chiral HPLC.[23] The optical purity of (L)-**5** was 99.6% ee, and that of (D)-**5** was 99.9% ee. These results proved that (D)-**5** and (L)-**5** were converted from (D)-**4** and (L)-**4** without any decrease of optical purity.

Optical rotation of (L)-**5** was measured to confirm absolute stereochemistry. Since the sign of the optical rotation coincided with the value in the lit.,[5] it was confirmed that the obtained (L)-**5** was the L-stereoisomer. The optical rotation of (D)-**5** was also measured and confirmed to be opposite in sign.

In summary, an efficient asymmetric synthesis of α-methylcysteine from chloroacetone, a commercially available and cheap compound, was successfully achieved with excellent optical purity and in overall good yield. In this new method, no expensive and/or difficult-to-secure reagents are used, and all steps proceed under mild conditions. In addition, no chromatographic purification was required. Thus, we believe that our method is suitable for large-scale production.

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16. *Multigram syntheses of* (L)-**3** *and* (D)-**2**. Compound **3** (267 g, 1.14 mol) was added to a 3.2% aq NaCl solution (1405 g), and the resultant mixture was adjusted to pH 6.5 with aq NaOH solution. Then, immobilized hydantoinase (total 87 g, wet weight) and manganese sulfate pentahydrate (0.44 g) were added to the mixture, and the reaction was performed by stirring at 40 °C for 41.5 h. During the reaction, the pH was kept at 6.5 using 10% aqueous sulfuric acid solution. After the reaction, a mixture of the precipitate and the immobilized hydantoinase was filtered off and washed with water (300 g). (D)-**2** was obtained in the mixture (132.6 g, yield 43.9%, optical purity 97.8% ee). The filtrate was washed with ethyl acetate (300 g) twice, and cooled to 20 °C. Then the filtrate was adjusted to pH 3 with concd HCl (59 g) and stirred for 30 min. The precipitated solid was filtered off, washed with water, and (L)-**3** was obtained as white crystals. (132.6 g, yield 49.6%) The chemical purity was 97.3 area% by HPLC (column, COSMOSIL 5C8-MS (produced by Nacalai Tesque Inc), mobile phase, potassium dihydrogen phosphate—phosphoric acid solution (pH 2.0)/acetonitrile = 80/20, flow rate, 1.0 ml/min, detection wavelength, 210 nm, column temperature, 40 °C). The optical purity was 99.5% ee by HPLC (column, CHIRALPAK AS (produced by Daicel Chemical Industries, Ltd), mobile phase, hexane/2-propanol/trifluoroacetic acid = 80/20/0.1, flow rate, 0.5 ml/min, detection wavelength, 210 nm, column temperature, 30 °C). The yield and optical purity of (D)-**2** were determined by HPLC (For yield: column, COSMOSIL 5C18-AR (produced by Nacalai Tesque Inc), mobile phase, potassium dihydrogen phosphate–phosphoric acid solution (pH 2.0)/acetonitrile = 70/30, flow rate, 1.0 ml/min, detection wavelength, 210 nm, column temperature, 40 °C), For optical purity: column, CHIRALPAK AS (produced by Daicel Chemical Industries, Ltd), mobile phase, hexane/2-propanol/trifluoroacetic acid = 85/15/0.1, flow rate, 1.0 ml/min, detection wavelength, 210 nm, column temperature, 30 °C.
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23. *Method for determining optical purity of* L-α-*methycysteine hydrochloride* (L)-**5** *(preparation of Cbz*-L-α-*MeCys-(Cbz)–OH)*. Compound (L)-**5** (74.9 mg, 0.44 mmol) obtained by the above method was dissolved in water (3 mL), and sodium hydrogen carbonate (197.7 mg) and ethanol (3 mL) were added to the resultant solution. After a nitrogen purge, benzyl chlorocarbonate (0.17 mL, 1.10 mmol) was added to the resultant mixture, followed by stirring at room temperature for 1 h. Then, concd HCl was added to the solution to adjust the pH to 1.9, and extraction was performed with ethyl acetate. Then, the organic phase was dried over anhydrous sodium sulfate, and the solvent was distilled off under reduced pressure. As a result of the PTLC (hexane/ethyl acetate = 1/1 with a small amount of acetic acid) purification and ^{1}H NMR analysis of the residue, it was confirmed that the desired compound (106 mg, yield 60%) had been produced. The HPLC analysis (column, CHIRALCEL OD–RH (produced by Daicel Chemical Industries, Ltd), mobile phase, potassium dihydrogen phosphate–phosphoric acid solution (pH 2.0)/acetonitrile = 6/4, flow rate, 1.0 ml/min, detection wavelength, 210 nm, column temperature, 30 °C, retention time, 19.15 min (D), 22.92 min (L)) of the compound showed an optical purity of 99.6% ee.