Synthesis of *threo*-3-Methylcysteine from Threonine[1)]

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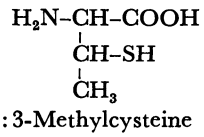
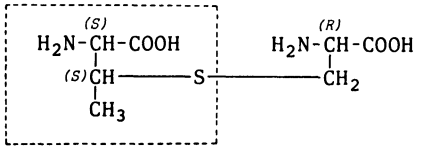
threo-3-Methyl-D-cysteine as a moiety of β-methyllanthionine in the peptide antibiotic nisin was synthesized stereospecifically. The reaction of (2*R*,3*R*)-3-methyl-2-aziridinecarboxylic acid derivative prepared from D-threonine with thiobenzoic acid gave the β-mercapto α-amino acid derivative of the same configuration as the starting material. A stereochemistry of the product was retained as a result of double inversion mechanism through the reactions. Thus, we could prepare *threo*-3-methyl-D-cysteine, *i.e.*, (2*S*,3*S*)-2-amino-3-mercaptobutanoic acid which is required for the synthetic study of nisin.

3-Methylcysteine (**1**)[2)] is a moiety of β-methyllanthionine (**2**) which is a constituent amino acid of the peptide antibiotic nisin.[3)] The absolute structure of 3-methylcysteine in natural β-methyllanthionine in nisin can be represented as (2*S*,3*S*)-2-amino-3-mercaptobutanoic acid[4,6)] whose stereochemistry corresponds to D-threonine. A convenient preparative method of this



1: 3-Methylcysteine



2: β-Methyllanthionine

amino acid is required for the purpose of the synthesis of nisin which contains four cyclic peptide parts involving sulfide bridge of β-methyllanthionine. One reasonable method for the synthesis of this amino acid could be a conversion of β-hydroxy α-amino acid into β-mercapto α-amino acid by use of substitution reaction of β-tosylate with thioacetic acid.[5,6)] However, a configuration of the carbon atom carrying hydroxyl group in β-hydroxy α-amino acid must be inverted through the reaction.[6)] Therefore, by application of this reaction, the required *threo* form of β-mercapto α-amino acid can not be obtained from threonine which is mostly available as starting material.

On the other hand, when 2-aziridinecarboxylic acid derivative prepared from *threo*-β-hydroxy α-amino acid was treated with ammonia or carboxylic acid, β-amino or β-*O*-acyl compound having the original *threo* stereochemistry was obtained respectively.[7,8)] The retention of configuration on the β-carbon atom is clearly explained with a double inversion mechanism as shown in Fig. 1. Namely, the first inversion occurs at aziridine ring formation through *E*2 reaction of β-tosylate or β-mesylate and the second one at the ring opening through S_N2 substitution with ammonia or carboxylate anion.

In a similar way, we now used thiobenzoic acid for the preparation of β-*S*-acyl compound in order to synthesize *threo*-3-methyl-D-cysteine from D-threonine. The aziridine compound was prepared in the usual manner[9)] as shown in Scheme 1. The reaction of (2*R*,3*R*)-*N*-benzyloxycarbonyl-3-methyl-2-aziridinecarboxylic acid methyl ester (**6**) with thiobenzoic acid in the presence of boron trifluoride gave the desirable *S*-benzoyl-β-mercapto derivative **7** accompanying *O*-thiobenzoyl-β-hydroxy derivative **8** as a by-product. The formation of both *S*-acyl and *O*-acyl compounds might be ascribed as a result of possible equilibrium in thiobenzoic acid, *i.e.*, $C_6H_5COSH \rightleftarrows C_6H_5CSOH$, although the higher reactivity of the former gave the preferable *S*-acyl derivative as a main product. The compounds **7** and **8** were separated by column chromatography and characterized by chemical shifts of their β-methine protons which appeared at 4.34 ppm in **7** and 6.22 ppm in **8**.

Acid hydrolysis of *S*-benzoyl derivative **7** after

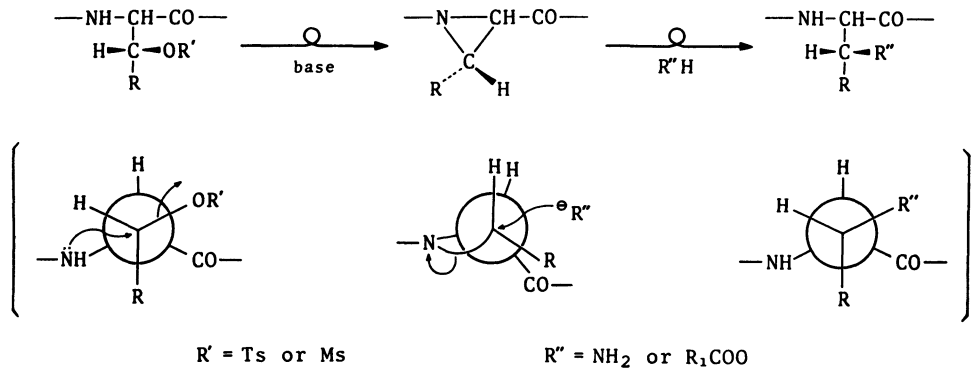
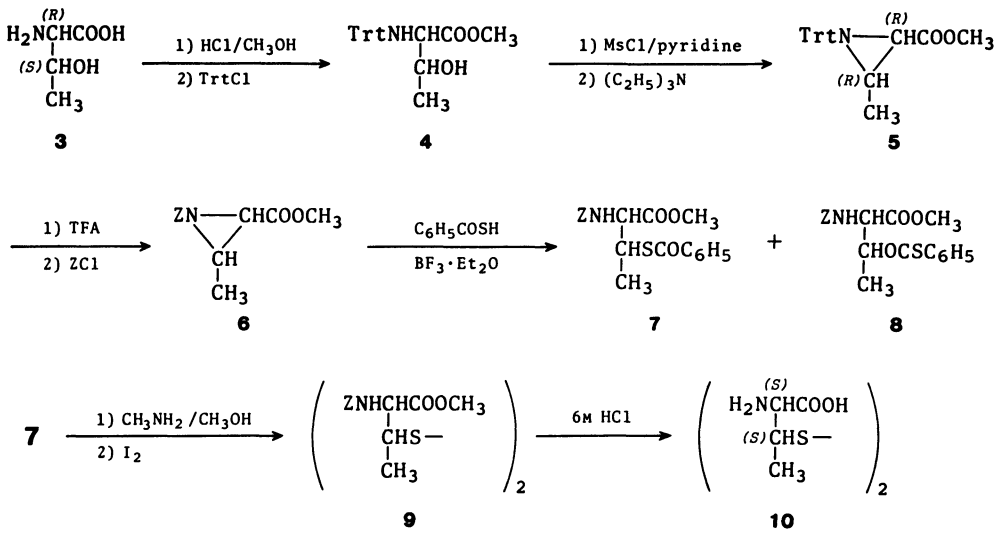
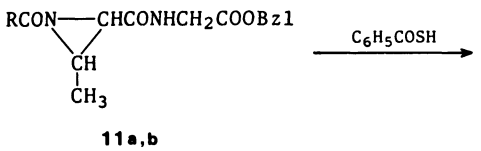


Fig. 1. Double inversion mechanism of the substitution reaction *via* aziridine derivative.

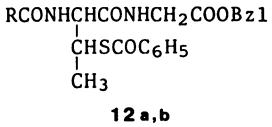


Trt = $(C_6H_5)_3C$, Ms = CH_3SO_2 , Z = $C_6H_5CH_2OCO$

Scheme 1.



11a,b



12a,b

a: R = $C_6H_5CH_2O$; **b**: R = $(CH_3)_3CO$

Bzl = $C_6H_5CH_2$

Scheme 2.

debenzyloxycarbonylation with 30% HBr in acetic acid did not give a good result presumably because of the formation of 5-methyl-2-phenyl-Δ^2-thiazoline-4-carboxylic acid. Therefore, benzyl group was first removed with methylamine in methanol and the product obtained was immediately oxidized to prepare disulfide compound **9**. Hydrolysis of **9** with hydrochloric acid proceeded smoothly and the desired *threo*-3-methyl-D-cysteine was obtained in an oxidized form **10**.

The procedure mentioned above is of course applicable to prepare simple D-cysteine and D-cystine which are commercially very expensive materials. Moreover, threonine residue in peptide chain was next tried to be converted into cysteine residue. A reaction of the peptide **11** containing *N*-acyl-3-methyl-2-aziridinecarboxylic acid derived from threonyl peptide[10] with thiobenzoic acid yielded exclusively *S*-benzoyl-3-methylcysteinyl peptide **12** without contamination with *O*-thiobenzoyl derivative. (Scheme 2) For this reaction, boron trifluoride as a catalyst was not necessary. The sole formation of *S*-acyl derivative may indicate that an amide form of 2-aziridinecarboxylic acid is preferable to an ester form on the opening reaction of the aziridine ring, presumably since the amide form reacts with thiobenzoic *S*-acid much faster than with thiobenzoic *O*-acid. We are now undertaking an application of this method to the synthesis of nisin ring B and the results will be reported soon elsewhere.

Experimental

All melting points are uncorrected. The specific rotations were measured with a Perkin-Elmer 141 polarimeter. NMR spectra were obtained with a Varian XL-100-15 spectrometer and the chemical shifts were given in δ values (ppm) from tetramethylsilane in $CDCl_3$ and from sodium 2,2-dimethyl-2-silapentane-5-sulfonate in DCl. The abbreviations used for coupling patterns were as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad.

N-*Trityl*-D-*threonine Methyl Ester* (**4**). A suspension of D-threonine (10 g, 0.084 mol) in 300 ml of methanol was saturated with hydrogen chloride under stirring in an ice bath to make a clear solution. The solution was allowed to stand overnight at room temperature and then concentrated *in vacuo*. Colorless oily product was obtained in almost quantitative yield by repetition of vacuum concentration with an addition of anhydrous benzene to remove water.

D-Threonine methyl ester hydrochloride (14 g, 0.083 mol) thus obtained was dissolved in 40 ml of chloroform. To the solution was added triethylamine (17 g, 0.17 mol) and then trityl chloride (23 g, 0.083 mol) in 50 ml of chloroform dropwise under stirring in an ice bath. The reaction mixture was concentrated *in vacuo* after stirring for 2 d under cooling. The residue was dissolved in ethyl acetate and the solution was washed successively with saturated sodium chloride solution, 10% citric acid solution, 1 M sodium hydrogencarbonate solution, and saturated sodium chloride solution. Evaporation of the solvent after drying over magnesium sulfate gave 30 g (96%) of **4** as colorless oil. A crop of the product was purified by silica gel column chromatography to remove a slight contaminant of triphenylmethanol. Elution with benzene–ethyl acetate (19 : 1 v/v) gave a crystalline material:

mp 87—89 °C; $[\alpha]_D^{17}$ −6.4° (*c* 1.1, $CHCl_3$).

Found: C, 76.68; H, 6.74; N, 3.71%. Calcd for $C_{24}H_{25}NO_3$: C, 76.78; 6.71; N, 3.73%.

(2R, 3R)-1-Trityl-3-methyl-2-aziridinecarboxylic Acid Methyl Ester (5). To a solution of **4** (4.91 g, 0.0131 mol) in 20 ml of pyridine was added methanesulfonyl chloride (4.5 g, 0.039 mol) under cooling in an ice–salt bath. The mixture was stirred for 8 h at room temperature and the solvent was evaporated *in vacuo*. A solution of the residue in ethyl acetate was washed with saturated sodium chloride solution and dried over magnesium sulfate. The reddish brown oil was obtained by vacuum concentration of the solvent. The oily product was dissolved in 40 ml of anhydrous tetrahydrofuran and triethylamine (3.9 g, 0.039 mol) was added to the solution. The mixture was heated under reflux at 80 °C for 2 d and the reaction mixture was concentrated *in vacuo*. An ethyl acetate solution of the residue was washed, successively, with saturated sodium chloride solution, 10% citric acid solution, saturated sodium hydrogencarbonate solution, and saturated sodium chloride solution. Organic layer was dried over magnesium sulfate and the solvent evaporated *in vacuo*. An oily residue was crystallized by trituration with hexane. Recrystallization of the crude product from methanol gave 3.64 g (78.0%) of colorless needles: mp 111—112.5 °C; $[\alpha]_D^{20}$ +98° (*c* 1.0, $CHCl_3$).

Found: C, 80.66; H, 6.51; N, 3.94%. Calcd for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.48; N, 3.92%.

(2R, 3R)-1-Benzyloxycarbonyl-3-methyl-2-aziridinecarboxylic Acid Methyl Ester (6). To a solution of **5** (3.17 g, 8.88 mmol) in 14 ml of chloroform and methanol (1 : 1 v/v) was added 7 ml of trifluoroacetic acid under an ice cooling. The mixture was stirred for 2.5 h in an ice bath and then concentrated *in vacuo*. Evaporation with ether newly added was repeated several times to remove trifluoroacetic acid as completely as possible. The residue was dissolved in ether again and 3-methyl-2-aziridinecarboxylic acid ester trifluoroacetate was extracted with water three times. To the aqueous extract was added sodium hydrogencarbonate (3.5 g, 0.042 mol) and 30 ml of ethyl acetate. Benzyloxycarbonyl chloride (1.51 g, 8.9 mmol) was added to the mixture under vigorous stirring in an ice bath. After stirring for 1.5 h, ethyl acetate layer was separated from aqueous layer which was extracted with another portion of ethyl acetate once again. Organic layer combined was washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated *in vacuo*. Crude product thus obtained was purified by column chromatography (Merck silica gel 60, 70 g, 1.9 cm × 60 cm). Elution with ethyl acetate and hexane (1 : 5 v/v) yielded 2.12 g (95.9%) of pure **6** as an oily product: $[\alpha]_D^{18}$ +73° (*c* 2.0, $CHCl_3$).

Found: C, 62.75; H, 6.15; N, 5.55%. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62%.

S-Benzoyl-N-benzyloxycarbonyl-threo-3-methyl-D-cysteine Methyl Ester (7). A mixture of **6** (5.2 g, 2.1 mmol) and boron trifluoride etherate (47%, 1.0 ml) in thiobenzoic acid (40 ml) was stirred for 1 d at room temperature. To a reaction mixture was added a large amount of ethyl acetate and the solution was washed with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution. Organic layer was dried over magnesium sulfate and concentrated *in vacuo*. A yellow oily residue was subjected to a silica gel column (Merck, 280 g, 2.7 cm × 110 cm). Elution with benzene and ethyl acetate (19 : 1 v/v) separated thiobenzoic acid, *N*-benzyloxycarbonyl-*O*-thiobenzoyl-D-threonine methyl ester (**8**), and desired **7** in this order. The compound **8** was obtained in a yield of 15% (1.2 g) as pale yellow oil: $[\alpha]_D^{13}$ −183° (*c* 1.44, $CHCl_3$); NMR ($CDCl_3$) δ=1.49 (3H, d, >CH–C<u>H</u>$_3$), 3.68 (3H, s, –OC<u>H</u>$_3$), 4.73 (1H, dd, –NH–C<u>H</u><), 5.14 (2H, s, Ph–C<u>H</u>$_2$–), 5.61 (1H, br. d, –N<u>H</u>–), 6.22 (1H, dq, –O–C<u>H</u>–CH$_3$), 7.3—7.6 (8H, m, aromatic protons), 8.01 (2H, dd, *o*-protons on C_6H_5CS). Found: C, 62.09; H, 5.49; N, 3.50; S, 8.49%. Calcd for $C_{20}H_{21}NO_5S$: C, 62.00; H, 5.4 N, 3.62; S, 8.28%.

Main product **7** was obtained as oil in a yield of 53% (4.3 g); $[\alpha]_D^{18}$ −64° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$) δ=1.48 (3H, d), 3.73 (3H, s), 4.34 (1H, dq, –S–C<u>H</u>–CH$_3$), 4.66 (1H, dd), 5.11 (2H, s), 5.61 (1H, br. d), 7.3—7.6 (8H, m), 7.90 (2H, dd, *o*-protons on C_6H_5CO). Found: C, 61.67; H, 5.47; N, 3.54; S, 8.15%.

N,N′-Bisbenzyloxycarbonyl-threo-3,3′-dimethyl-D-cystine Dimethyl Ester (9). To a solution of **7** (0.59 g, 1.5 mmol) in 20 ml of methanol was added 1 M methylamine in methanol (2.3 ml, 2.3 mmol) during 15 h at 10—15 °C. After an additional stirring overnight, 0.5 M solution of iodine in methanol was added to the reaction mixture until a brown color remained. Oxidation reaction was completed within 30 min and the solution was then concentrated *in vacuo*. Colorless crystalline product **9** formed on addition of ether to the residue was filtered, 0.39 g (91%). The product was recrystallized from ethanol and hexane to obtain an analytical sample, yield 0.35 g (81%): mp 108—110 °C; $[\alpha]_D^{18}$ −167° (*c* 1.00, $CHCl_3$) NMR ($CDCl_3$) δ=1.31 (β-CH_3, d), 3.52 (β-CH, dq), 3.72 (–OCH_3, s), 4.59 (α-CH, dd), 5.12 (Ph–CH_2, s), 5.63 (NH, br. d), 7.34 (C_6H_5, s).

Found: C, 55.50; H, 5.72; N, 4.90; S, 10.92%. Calcd for $C_{26}H_{32}N_2O_8S_2$: C, 55.30; H, 5.71; N, 4.96; S, 11.36%.

threo-3,3′-Dimethyl-D-cystine (10). A suspension of **9** (0.33 g, 0.58 mmol) in 6 M HCl (2 ml) and acetic acid (2 ml) was heated under reflux for 5 h at 110 °C. The hydrolyzat was concentrated *in vacuo* and the residue was dissolved in water. The aqueous solution was once extracted with ether, neutralized with pyridine, and concentrated *in vacuo*. Ethanol was added to the residue and an insoluble material was recrystallized from water and ethanol to obtain pure **10**6; colorless powder: yield 0.13 g (82%); mp 183—193 °C (decomp); $[\alpha]_D^{13}$ −416° (*c* 0.50, 1 M HCl) [lit,[6] $[\alpha]_D^{19}$ −414° (*c* 1, 1 M HCl)]; NMR (2 M DCl) δ=1.43 (β-CH_3, d), 3.96 (β-CH, dq), 4.45 (α-CH, d).

Found: C, 34.32, H, 6.18; N, 10.10; S, 22.70%. Calcd for $C_8H_{16}N_2O_4S_2 \cdot \frac{2}{3}H_2O$: C, 34.27; H, 6.23; N, 9.99; S, 22.87%.

S-Benzoyl-N-benzyloxycarbonyl-threo-3-methyl-L-cysteinylglycine Benzyl Ester (12a). (2*S*, 3*S*)-*N*-Benzyloxycarbonyl-3-methyl-2-aziridinylcarbonylglycine benzyl ester (**11a**)[10] (86 m, 0.23 mmol) was dissolved in 2 ml of dichloromethane and thiobenzoic acid (62 mg, 0.45 mmol) was added to the solution. The mixture was stirred at room temperature for 18 h and at 40 °C for 30 h. The reaction mixture diluted with ethyl acetate (20 ml) was washed once with saturated sodium hydrogencarbonate solution and twice with water. Organic layer dried over magnesium sulfate was concentrated *in vacuo* and an oily residue obtained was treated with hexane to crystallize. Crude product was recrystallized from ethyl acetate and hexane: yield 97 mg (83%); mp 131—133 °C; $[\alpha]_D^{16}$ +1.5° (*c* 0.83, $CHCl_3$); NMR ($CDCl_3$) δ=1.46 (3H, d), 4.03 (2H, dd), 4.21 (1H, m, C_6H_5COS–C<u>H</u>–), 4.46 (1H, t), 5.04 (2H, s), 5.11 (2H, s), 5.91 (1H, d), 6.88 (1H, br. t), 7.2—7.6 (13H, m), 7.80—7.93 (2H, m).

Found: C, 64.50; H, 5.51; N, 5.26; S, 6.26%. Calcd for $C_{28}H_{28}N_2O_6S$: C, 64.60; H, 5.42; N, 5.38; S, 6.16%.

S-Benzoyl-N-t-butoxycarbonyl-threo-3-methyl-L-cysteinylglycine Benzyl Ester (12b). To a solution of (2*S*,3*S*)-*N*-*t*-butoxycarbonyl-3-methyl-2-aziridinylcarbonylglycine benzyl ester (**11b**)[10] (94 mg, 0.28 mmol) in 2 ml of dichrolomethane was added thiobenzoic acid (77 mg, 0.56 mmol) and the mixture

was stirred for 25 h. The reaction mixture diluted with 20 ml of ethyl acetate was washed once with saturated sodium hydrogencarbonate solution and three times with water. Organic layer was dried over magnesium sulfate and concentrated *in vacuo*. An oily residue was crystallized by addition of hexane and a crystalline product was recrystallized from ethyl acetate and hexane: yield 101 mg (75%); mp 125—127 °C; $[\alpha]_D^{16}$ −2.7° (*c* 0.89, CH_3OH); NMR ($CDCl_3$) δ=1.37 (9H, s), 1.47 (3H, d), 4.06 (2H, d), 4.24 (1H, m, C_6H_5COS–C<u>H</u>–), 4.40 (1H, t), 5.12 (2H, s), 5.54 (1H, d), 6.89(1H, br. t), 7.2—7.6 (8H, m), 7.87 (2H, m).

Found: C, 61.70; H, 6.23; N, 5.74; S, 6.73%. Calcd for $C_{25}H_{30}N_2O_6S$: C, 61.71; H, 6.21; N, 5.76; S, 6.59%.

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